

## Geographical variation in agonistic behaviour in a ring species of salamander, *Ensatina eschscholtzii*

ERIKA B. WILTENMUTH & KIISA C. NISHIKAWA  
*Department of Biological Sciences, Northern Arizona University*

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**Abstract.** *Ensatina eschscholtzii* is a plethodontid salamander with several geographical races distributed in a ring-like series throughout the coastal mountains and inland Sierra Nevada mountains of California. Populations of these salamanders show genetic and morphological divergence, and in this study, we examined divergence in overt aggressive, passive aggressive/exploratory, avoidance and sensory behaviours in four populations. Two of the populations represent a zone of secondary contact between coastal and inland lineages of *Ensatina*. We recorded behaviour of resident salamanders paired with same-sex intruders during the non-courtship season. The residents' behaviour was affected independently by the population of the resident and the population of the intruder but not by the interaction between the resident population and the intruder population. Levels of agonistic and sensory behaviour showed a high degree of constraint among the populations. These behaviours also showed divergence among coastal and inland populations, and the geographical variation in behaviour is consistent with the phylogenetic history of *Ensatina*. Convergent evolution of behaviour appears to have occurred at the zone of secondary contact between the coastal and inland lineages. Variation in agonistic and sensory behaviour among the populations observed and between the sexes suggests that there may be geographical and sexual variation in territoriality or competition.

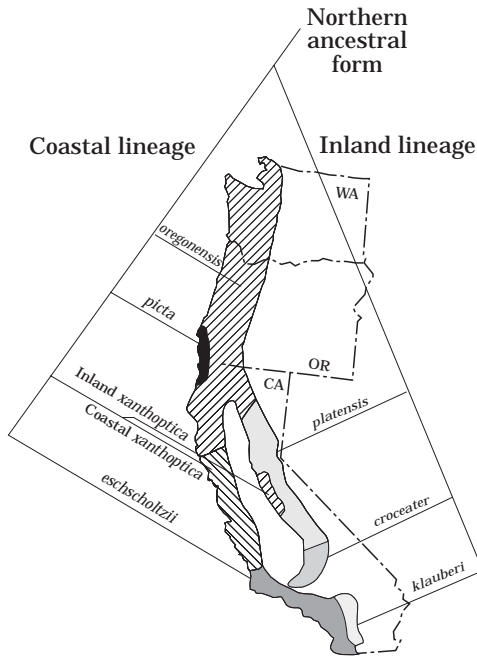
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Geographical variation among closely related populations provides some of the strongest evidence for the role of natural variation in the evolution of species. This link between geographical variation and evolution within a species is especially apparent in ring species, in which intergradation occurs freely between adjacent populations, but those at the terminal ends of the ring do not interbreed and behave as separate species (Mayr 1963). Levels of intergradation may vary throughout the entire ring, resulting in geographical variation among the subspecies. The geographical variation shown by ring species can be used to address questions such as whether behavioural evolution has occurred among closely related and genetically interconnected populations. Behavioural variation may be associated not only with the evolutionary relationships

among subspecies but also with ecological differences among them.

The plethodontid salamander, *E. eschscholtzii*, is one of several species that fit the definition of a ring species (Stebbins 1949; Mayr 1963). In California, there is a contiguous ring distribution of seven geographical races of *Ensatina*, which are recognized as subspecies (Fig. 1). Coastal and inland populations are separated by the Great Valley of California, which is devoid of *Ensatina*. Stebbins (1949) inferred that an ancestral population of *Ensatina* (existing in Oregon or northern California) moved southward and colonized the coastal and inland mountains of California. Because these mountains were separated by the Great Valley, the coastal and inland lineages became isolated reproductively and evolved independently. Different morphological patterns evolved in the coastal and inland lineages. Populations solid in coloration evolved along the coast, while populations blotched in coloration evolved inland. These major steps in the evolution of *Ensatina* are supported by allozyme and

Correspondence: E. B. Wiltenmuth, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640, U.S.A. (email: ebw@nauvax.ucc.nau.edu).



**Figure 1.** Ring-like distribution and ranges of the subspecies of *Ensatina eschscholtzii* throughout Washington (WA), Oregon (OR) and California (CA). Ranges shown are general representations and do not show complex zones of intergradation between subspecies. Phylogenetic relationships shown are not indicative of the complex paraphyly of current populations of some of the subspecies (Moritz et al. 1992; Jackman & Wake 1994). Adapted from Brown (1974).

mtDNA data (Moritz et al. 1992; Jackman & Wake 1994).

Subsequent to the split between the coastal and inland lineages, a zone of secondary contact was established between them during the Pleistocene when the Great Valley of California became temporarily habitable to the salamanders (Stebbins 1949). At this time, coastal *E. eschscholtzii xanthoptica* 'leaked' across the Great Valley and established a population in the Sierra Nevada Mountains sympatric with *E. eschscholtzii platensis*, a subspecies of the inland lineage (Wake et al. 1986). Coastal and inland populations of *xanthoptica* (Fig. 1) show small genetic divergence (Nei's  $D \approx 0.02$ ; Wake & Yanev 1986) since their relatively recent geographical separation.

In addition to its ring distribution, *Ensatina* offers an excellent opportunity for the study of geographical variation and evolution of behaviour

because of the wealth of information available on agonistic and sensory behaviour in other plethodontid salamanders (reviewed by Mathis et al. 1995). Agonistic and sensory behaviour are observed in both laboratory and field settings (Gergits & Jaeger 1990). Aggression may be related to territoriality (Jaeger 1981) and may be important for competition for resources (reviewed by Mathis et al. 1995). Olfactory cues, which are enhanced through nose-tapping behaviour in plethodontid salamanders (Dawley & Bass 1988), may be related to the marking and recognition of territories (reviewed by Mathis et al. 1995) and to the recognition of species and gender (Jaeger & Gergits 1979; Dawley 1984, 1986).

Using the phylogenetic relationships as our framework (Farris 1970; Brooks & McLennan 1991), we investigated the patterns of behavioural divergence among the subspecies of *E. eschscholtzii*. We compared behaviour among two coastal (*E. e. oregonensis* and *E. e. xanthoptica*) and two inland populations (*E. e. platensis* and *E. e. xanthoptica*) to determine whether the behaviour of residents is affected by (1) resident population, (2) intruder population and (3) resident  $\times$  intruder interaction. Based on the phylogenetic history of *Ensatina*, we predicted that coastal populations should differ behaviourally from inland populations, and that coastal and inland *xanthoptica* should differ behaviourally from each other. Having observed a variation in behaviour among the populations, we then asked whether geographical variation in behaviour was associated with the phylogenetic history and/or the geographical distribution of the populations.

## METHODS

We collected adult *E. e. oregonensis* (the Oregon *Ensatina*;  $N=9$  males, 18 females) from Sonoma and Lake Counties, California; *E. e. xanthoptica* (the yellow-eyed *Ensatina*;  $N=9$  males, 20 females) from Sonoma County, California; *E. e. platensis* (the Sierra Nevada *Ensatina*;  $N=10$  males, 20 females) from Calaveras County, California; and *E. e. xanthoptica* (the yellow-eyed *Ensatina*;  $N=10$  males, 10 females) from El Dorado and Tuolumne Counties, California. We avoided collecting animals in areas where hybrid individuals exist.

We housed salamanders individually in plastic boxes ( $28 \times 19 \times 9$  cm) lined with moist,

**Table I.** Behavioural patterns observed in pairs of salamanders (definitions adapted from Nishikawa 1985)

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|  |
|--|
| Overt aggressive behaviour   |
| Bite: contacting an individual with open mouth.  |
| Chase: moving rapidly and directly towards a retreating individual.  |
| Head wrestle: contacting an individual with any part of the body, but usually the head, in a relatively rapid pushing manner; the mouth is not opened as in a biting attack. |
| Passive aggressive/Exploratory behaviour   |
| Approach: moving directly towards another individual.  |
| Turn head toward: turning head directly towards another individual.  |
| Contact: touching another with any part of the body other than the mouth and in a relatively slow manner; includes stepping on an individual.                                |
| Head under: pushing the head under the chin of another individual.   |
| Crawl over: walking over another individual.   |
| Avoidance behaviour  |
| Retreat: quickly moving away from another individual's overt aggressive behaviour.   |
| Avoid: avoiding passive aggressive behaviour by moving away from the other individual.   |
| Sensory behaviour  |
| Nose-tap substrate: touching the nasolabial cirri against the substrate.   |
| Nose-tap individual: touching the nasolabial cirri against another individual.   |

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unbleached paper towels. The boxes were cleaned every 2 weeks during non-experimental periods, and residents' containers were cleaned 3 days prior to introducing an intruder into the container. We kept the salamanders in an environmental room at 15°C on a 12:12 h light:dark cycle (November–May) and a 14:10 h light:dark cycle (June–October). We fed the salamanders crickets and waxworms weekly, but no food was present in the cages during tests.

We paired resident salamanders with intruders of the same sex from each of the four populations. Thus, behaviour of a resident salamander may be influenced by the resident population alone, the intruder population alone or by an interaction effect in which the resident responds differently to intruders from various populations. Salamanders were paired based on similarity in body sizes; 90% of the pairs were within 5% snout–vent length. Some salamanders were used as intruders as well as residents. We randomized the order in which salamanders interacted with individuals from the different populations, as well as the order in which they served as a resident or as an intruder. Although salamanders were used up to three times in the trials, we took the effect of individual variation in behaviour into account in the analyses of the data. We used no salamander in a test more often than every fourth night.

Before we placed a resident and an intruder into the resident's container, we picked up both individuals by hand to equalize handling effects on their behaviour. A divider separated the salamanders visually and physically during 5 min of habituation in the resident's container. We observed behavioural interactions under a blue 25-W light bulb during the dark portion of the light:dark cycle. We observed interactions during the non-courtship seasons of 1991 and 1992, and observed each pair of salamanders for 45 min. We recorded frequencies of agonistic and sensory behaviour for both individuals.

We divided behaviour into four categories (overt aggressive, passive aggressive/exploratory, avoidance and sensory; Table I) based on studies of agonistic and sensory behaviour in plethodontid salamanders (reviewed by Mathis et al. 1995). Behaviours in the second category may be aggressive, exploratory or both. For example, one salamander may approach a second salamander to attack or to investigate, without attacking. During interactions between *Ensatina*, we observed displays such as arching the back and raising the tail too infrequently to analyse.

We used principal component analyses (PCA) to reduce the number of statistical tests conducted on the behaviours observed and hence provide a more powerful test of the data. The PCA resulted in the analysis of four behavioural categories

comprising 12 individual behaviours (Table I). For each behavioural category, we calculated one factor score on the first principal component, that is, the factor score is the sum of the frequency of individual behavioural variables within a behavioural category weighted by the individual behaviour's factor loading (1.0 to  $-1.0$ ) on the first component of the PCA solution.

We analysed only resident behaviour. Because agonistic and sensory behaviour vary with gender (Wiltenmuth 1996), we ran separate PCAs for females and males for each behavioural category, that is, eight PCAs (two sexes  $\times$  four behavioural categories) and eight first principal components. We used StatView II for the Macintosh (Abacus Concepts 1987) to calculate PCA solutions without rotation.

We used ANOVA ( $\alpha=0.05$ ) to determine whether resident behaviour varies with three main effects: resident population, intruder population and the interaction of the two. We performed separate ANOVAs on the first principal component for each of the four behavioural categories and for male-male pairs and female-female pairs, that is, eight ANOVAs (two sexes  $\times$  four behavioural categories) (SuperANOVA for the Macintosh; Abacus Concepts 1989).

We took the effects of individual variation in behaviour into account in the ANOVA model by nesting individual within resident population. We determined appropriate  $F$ -tests using the rules for developing ANOVA models outlined by Neter et al. (1990). To account for individual variation in resident behaviour, the mean square for individual was used as the denominator of the  $F$ -test for the effect of resident population. Because individuals were paired with only one intruder from each population, there was no replication in this model and we could not conduct a separate test for the effect of individual alone. The residual term in this model is represented by the interaction of intruder population  $\times$  individual. The mean square of the residual was used as the denominator in the  $F$ -tests for effect of intruder population and for the effect of the interaction between resident population  $\times$  intruder population. For post hoc comparisons between groups within each main factor, we used Scheffe's  $S$ -tests.

Although we performed analyses on the factor scores, we report frequencies of behaviour as means and standard errors calculated from the raw data, that is, overt aggression is the mean of

frequencies of bite, chase and head wrestle; passive aggression is the mean of approach, contact, head under, crawl over and turn head towards; avoidance is the mean of retreat and avoid; and sensory is the mean of nose-tap substrate and nose-tap other individual.

We investigated patterns of evolutionary changes in the frequencies of agonistic and sensory behaviour along branches of a phylogenetic tree of the subspecies studied. The branching order of our tree was based on the hypothesis that an ancestral population of *Ensatina* diverged into two lineages as it moved southwards throughout the coastal and inland mountains of California (Stebbins 1949; Moritz et al. 1992; Jackman & Wake 1994; Fig. 1). For phylogenetic analyses, we mapped the mean frequency of each behavioural category shown by males and females of the four populations onto the cladogram. Frequencies of behaviours that varied among the populations were denoted as low, medium or high based on differences in post hoc results. We considered these levels of behaviour to represent an ordered change in the frequency of the behaviour and made inferences about the direction of behavioural divergence in the four populations. Evolutionary changes in each behaviour were partitioned along the branches of the tree using the parsimony method of Farris (1970). We determined whether nodes represented a given level of a behaviour by taking the average or median value of the surrounding clades. No outgroup was used, but this does not change our interpretation of the general patterns of behavioural divergence.

## RESULTS

Table II shows the eigenvalues for the first principal component and the proportion of the total variance that is explained by the first component for each of the four behavioural categories. Table III shows correlations between the individual behaviour patterns and the new factors created by the PCA.

### Effect of Resident Population on Resident Behaviour

Among female residents, overt aggressive ( $P=0.0241$ ) and passive aggressive/exploratory

**Table II.** Eigenvalues and proportion of the variance explained by the first principal component in four behavioural categories

|                                | Females (N=68) |            | Males (N=38) |            |
|--------------------------------|----------------|------------|--------------|------------|
|                                | Eigenvalue     | % Variance | Eigenvalue   | % Variance |
| Sensory                        | 1.31           | 65.5       | 1.28         | 64.2       |
| Passive aggression/Exploratory | 1.93           | 38.5       | 2.00         | 40.1       |
| Overt aggression               | 1.37           | 45.6       | 1.61         | 53.8       |
| Avoidance                      | 1.33           | 66.4       | 1.12         | 55.9       |

**Table III.** Correlations between individual behaviour patterns and the new factor created by the first principal component in each of four behavioural categories

|                                | Females (N=68) | Males (N=38) |
|--------------------------------|----------------|--------------|
| Sensory                        |                |              |
| Nose-tap substrate             | 0.809          | 0.801        |
| Nose-tap individual            | 0.809          | 0.801        |
| Passive aggression/Exploratory |                |              |
| Contact                        | 0.815          | 0.775        |
| Approach                       | 0.701          | 0.752        |
| Crawl over                     | 0.676          | 0.450        |
| Turn head towards              | 0.279          | 0.581        |
| Head under                     | 0.485          | 0.544        |
| Overt aggression               |                |              |
| Bite                           | 0.836          | 0.859        |
| Chase                          | 0.701          | 0.816        |
| Head wrestle                   | 0.420          | 0.459        |
| Avoidance                      |                |              |
| Avoid                          | 0.815          | 0.748        |
| Retreat                        | 0.815          | 0.748        |

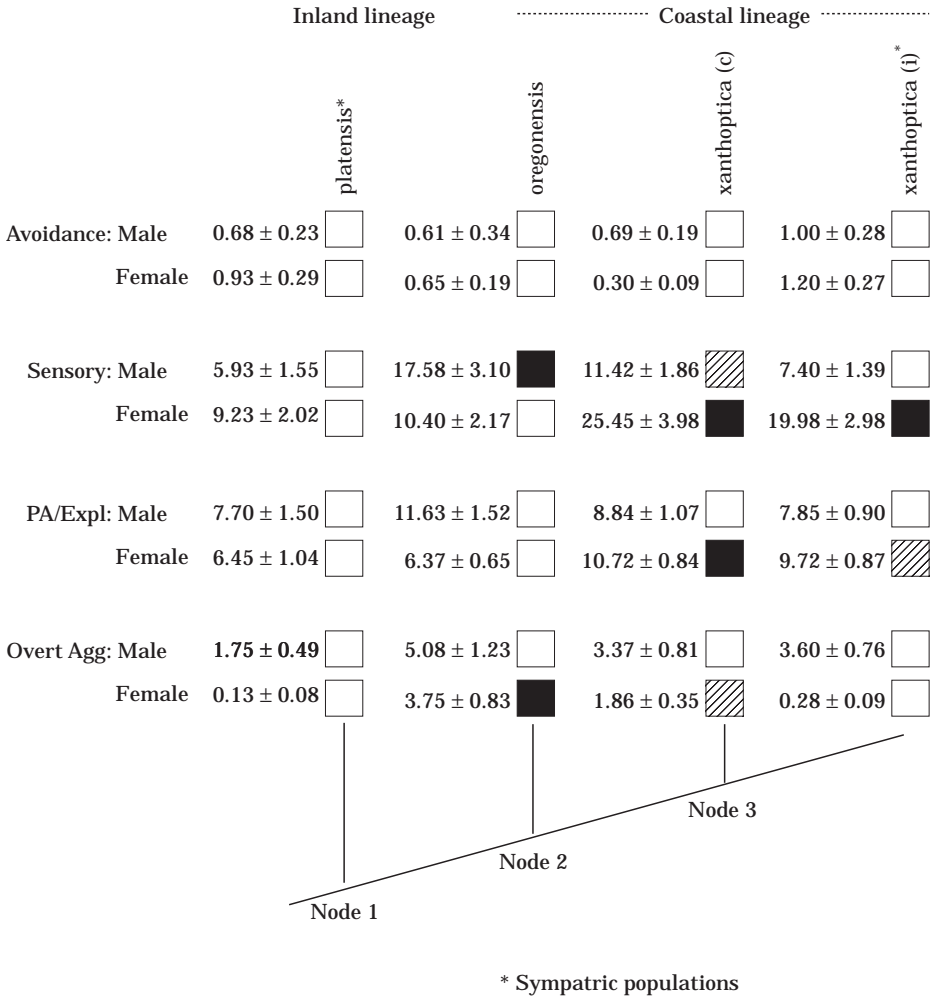
behaviour ( $P=0.008$ ) varied among populations (Fig. 2). Sensory behaviour ( $P=0.0546$ ) showed marginal variation among resident populations, and avoidance behaviour ( $P=0.161$ ) showed no significant variation among resident populations. Based on post hoc tests (Fig. 2), coastal *oregonensis* performed significantly higher levels of overt aggression than did inland *xanthoptica* ( $P=0.0216$ ) and inland *platensis* ( $P=0.0383$ ), but did not differ significantly from coastal *xanthoptica* ( $P=0.1782$ ). Coastal *xanthoptica* performed more passive aggressive/exploratory behaviour than did coastal *oregonensis* ( $P=0.0201$ ) and inland *platensis* ( $P=0.0341$ ), but did not differ significantly from inland *xanthoptica* ( $P=0.8875$ ). Post hoc tests on variation in sensory behaviour of female residents showed that more sensory behaviour was performed by both inland and coastal

*xanthoptica* than by inland *platensis* or coastal *oregonensis* ( $P=0.0546$ ; Fig. 2).

For male residents, overt aggressive ( $P=0.119$ ), passive aggressive/exploratory ( $P=0.415$ ) and avoidance behaviour ( $P=0.609$ ) did not vary with resident population (Fig. 2). Sensory behaviour varied among populations ( $P=0.0075$ ). Post hoc tests revealed that inland *platensis* performed less sensory behaviour than coastal *oregonensis* ( $P=0.0252$ ), but did not differ significantly from inland *xanthoptica* ( $P=0.968$ ) or coastal *xanthoptica* ( $P=0.533$ ). Coastal *xanthoptica* did not differ from coastal *oregonensis* ( $P=0.404$ ). Inland *xanthoptica* did not differ significantly from coastal *oregonensis* ( $P=0.0552$ ), but showed a frequency of sensory behaviour that was more similar to inland *platensis* than to coastal *oregonensis*.

**Effect of Intruder Population on Resident Behaviour**

Among female residents, the population of the intruder did not significantly affect overt aggressive ( $P=0.558$ ), passive aggressive/exploratory ( $P=0.682$ ) or sensory behaviour ( $P=0.0742$ ; Fig. 3). Avoidance behaviour of resident females was significantly affected by the intruder population ( $P=0.0098$ ). Post hoc tests indicated that female residents avoided intruding coastal *xanthoptica* significantly more than intruding inland *platensis* ( $P=0.0116$ ). Avoidance of coastal and inland *xanthoptica* was not significantly different ( $P=0.267$ ), however, nor was avoidance of coastal *xanthoptica* and coastal *oregonensis* ( $P=0.182$ ). Although not significant at  $\alpha=0.05$ , female residents performed more sensory behaviour ( $P=0.0742$ ) towards intruders of inland *platensis* than towards intruders of the other populations.



**Figure 2.** Cladogram showing the plesiomorphic and derived conditions of residents' behaviour in the four populations. Across rows, ■: significantly high frequencies of the behaviours; □: significantly low frequencies of the behaviours; ▨: intermediate levels not significantly different from high or low values. Rows with all □ indicate no significant differences among populations. Means (± SE) are shown adjacent to the boxes. PA/Expl: passive aggressive/exploratory behaviour; Overt Agg: overt aggressive behaviour.

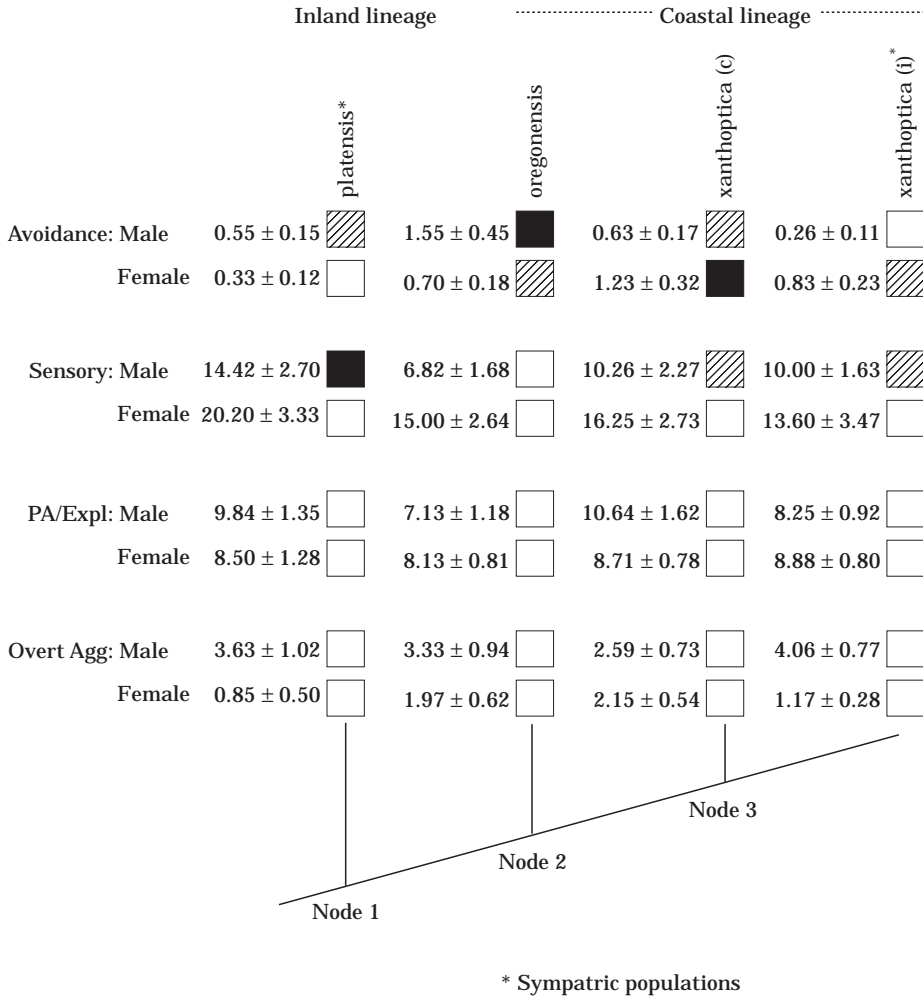
Among male residents, intruder population did not significantly affect frequencies of overt aggressive ( $P=0.608$ ) or passive aggressive/exploratory behaviour ( $P=0.0626$ ), but did significantly affect avoidance ( $P=0.0239$ ) and sensory behaviour ( $P=0.0079$ ; Fig. 3). Based on post hoc tests, male residents avoided intruding coastal *oregonensis* more frequently than intruding inland *xanthoptica* ( $P=0.0329$ ). Male residents performed more sensory behaviour towards intruding inland *platensis* than towards intruding coastal *oregonensis*

( $P=0.0158$ ). Although not significant at  $\alpha=0.05$ , variation in passive aggressive/exploratory behaviour of males ( $P=0.0626$ ) towards inland *platensis* and coastal *oregonensis* intruders showed the same pattern as variation in sensory behaviour.

**Effect of Resident × Intruder Interaction on Resident Behaviour**

Among both males and females, there were no significant effects of the resident × intruder





**Figure 3.** Cladogram showing the plesiomorphic and derived conditions of residents' behaviour as evoked by intruders from the four populations. Across rows, ■, □ and ▨ boxes indicate high, low and intermediate levels of behaviour, respectively. Rows with all □ indicate no significant differences among populations. Means (± SE) are shown adjacent to the boxes. Abbreviations as in Fig. 2.

interaction on behaviour. For female residents, overt aggressive ( $P=0.660$ ), passive aggressive/exploratory ( $P=0.591$ ), avoidance ( $P=0.697$ ) and sensory behaviour ( $P=0.518$ ) did not vary significantly with the interaction of resident population × intruder population. For male residents, overt aggressive ( $P=0.679$ ), passive aggressive/exploratory ( $P=0.144$ ), avoidance ( $P=0.749$ ) and sensory behaviour ( $P=0.0870$ ) did not vary significantly with the resident × intruder interaction.

**Cladistic Analysis (Effects of Resident Population)**

For male residents, only one of the four behavioural categories showed divergence in frequency of behaviour. For female residents, three of the four behavioural categories showed divergence in frequency of behaviour (Fig. 2).

For male sensory behaviour (Fig. 2), we infer from the cladogram that node 3 represents an ancestor with a medium level of sensory

behaviour based on the average condition shown by coastal *oregonensis* and the two sister groups of *xanthoptica*. Using the average for the clades of *platensis*, *oregonensis* and the ancestor of the sister groups of *xanthoptica*, node 2 also represents an ancestor with a medium level of sensory behaviour. Without knowledge of the level of sensory behaviour in the outgroup to these races of *Ensatina*, node 1 is unresolved and may represent an ancestor with low or medium levels of sensory behaviour. If the outgroup shows low levels of sensory behaviour, then node 1 also would represent low levels of sensory behaviour as the primitive condition of the clade. In this case, sensory behaviour of males has increased in the races of the coastal lineage and decreased in inland *xanthoptica*. The decrease in inland *xanthoptica* is convergent with the low levels in inland *platensis*, and coastal *oregonensis* represents the largest increase in sensory behaviour. If the primitive condition in node 1 is a medium level of sensory behaviour, both inland *platensis* and inland *xanthoptica* represent convergent evolution of decreased sensory behaviour, and coastal *oregonensis* shows increased sensory behaviour. Regardless of the ancestral condition in node 1, sensory behaviour of male inland *xanthoptica* is convergent on inland *platensis*, and coastal *oregonensis* shows an increase from the primitive condition.

Overt aggression in females shows a similar pattern of evolution as sensory behaviour in males (Fig. 2). Node 1 of the cladogram is unresolved (either a low or medium level of overt aggression). Both nodes 2 and 3 represent ancestors with medium levels of overt aggression. If the ancestor represented by node 1 had a low level of overt aggression, then overt aggression has increased in the races of the coastal lineage. Overt aggression in coastal *oregonensis* shows the largest increase, and overt aggression has decreased in inland *xanthoptica*, which is convergent on the low levels shown by inland *platensis*. If the ancestor in node 1 had a medium level of overt aggression, both inland *platensis* and inland *xanthoptica* represent convergent evolution of decreased overt aggression, and coastal *oregonensis* represents an increase in overt aggression. Regardless of the ancestral condition in node 1, overt aggressive behaviour of female inland *xanthoptica* is convergent on inland *platensis*, and coastal *oregonensis* shows an increase from the primitive condition.

For female sensory behaviour (Fig. 2), node 3 represents an ancestor with a high level of sensory behaviour. Both nodes 1 and 2 represent ancestors with low levels of sensory behaviour. The primitive condition for the clade is resolved and is a low level of sensory behaviour. High frequency of sensory behaviour is the derived condition and occurs in the two sister groups of *xanthoptica*.

Passive aggressive/exploratory behaviour in females (Fig. 2) shows a similar pattern of evolution as female sensory behaviour. Node 3 represents an ancestor with medium levels of passive aggressive/exploratory behaviour based on the average level exhibited by the three coastal races. Both nodes 1 and 2 represent ancestors with low levels of passive aggressive/exploratory behaviour. The primitive condition of the clade is resolved and is a low level of passive aggressive/exploratory behaviour. The derived condition is an increase of passive aggressive/exploratory behaviour in the sister groups of *xanthoptica* with the greatest increase in coastal *xanthoptica*.

#### Cladistic Analysis (Effects of Intruder Population)

In response to intruders from different populations, male residents showed divergence in frequency of behaviour for two behavioural categories, and female residents showed divergence in frequency of behaviour for one category (Fig. 3).

For avoidance behaviour of males (Fig. 3), nodes 1, 2 and 3 represent ancestors with medium levels of avoidance behaviour. The primitive condition of the clade is resolved and represents salamanders that, as intruders, evoked a medium level of avoidance behaviour. The derived condition represents salamanders that, as intruders, evoke high levels of avoidance behaviour. The derived condition is shown by intruding coastal *oregonensis*. The level of avoidance behaviour evoked by intruding inland *xanthoptica* has decreased from the primitive condition.

For avoidance behaviour of females (Fig. 3), nodes 2 and 3 represent ancestors that, as intruders, evoked medium levels of avoidance. Node 1 is unresolved and may represent an ancestral condition in which intruders evoked either low or medium levels of avoidance. If node 1 represents an ancestral condition in which intruders evoked low or medium levels of avoidance, then the level of avoidance evoked by intruders of the races of



the coastal lineage has increased, especially in coastal *xanthoptica*. Medium levels of avoidance at node 1 would mean that the level of avoidance evoked by inland *platensis* has decreased. Regardless of the ancestral condition in node 1, the derived condition of intruders that evoke increased avoidance behaviour occurs in races of the coastal lineage.

For sensory behaviour of males (Fig. 3), nodes 2 and 3 represent ancestors that, as intruders, evoked medium levels of sensory behaviour. Node 1 is unresolved and may represent an ancestral condition in which intruders evoked either high or medium levels of sensory behaviour. If node 1 represents intruders that evoked high levels of sensory behaviour, then the level of sensory behaviour evoked by intruders among the races of the coastal lineage has decreased, especially in coastal *oregonensis*. If node 1 represents intruders that evoked medium levels of sensory behaviour, then the level of sensory behaviour evoked by intruding inland *platensis* has increased, and the level of sensory behaviour evoked by intruding coastal *oregonensis* has decreased. Regardless of the ancestral condition in node 1, the amount of sensory behaviour evoked by intruding *oregonensis* has decreased.

## DISCUSSION

The results show that agonistic and sensory behaviour vary geographically among populations of *Ensatina* but also show a high degree of constraint. With respect to residents' behaviour independent of the effects of the intruder population, 25% of the observed behavioural categories for males and 75% for females have diverged. With respect to residents' behaviour evoked by intruders from different populations, 50% of the observed behavioural categories for males and 25% for females have diverged. When the amount of divergence is considered per population, males show 6.25% divergence (one category/(four categories  $\times$  four populations)) and females show 18.75% divergence (three categories/(four categories  $\times$  four populations)) in behaviour based on the residents' population. Based on the effects of population of the intruding salamander on residents' behaviour, males show 12.5% divergence and females show 6.25% divergence in behaviour.

Although there is much constraint, divergence in behaviour appears to be associated with the phylogenetic history and/or the geographical distribution of the populations. The phylogenetic history of the family Plethodontidae suggests that aggression is probably the ancestral state for all plethodontid salamanders, including *Plethodon* and *Aneides*, which are the closest relatives of *Ensatina* (Staub 1993). However, much variation exists in reported levels of agonistic and sensory behaviour in plethodontid salamanders. For example, overt aggression varies greatly among species of *Plethodon* as well as among different populations within a species (Nishikawa 1985, 1987). In addition, studies on plethodontid salamanders report behaviours in a variety of terms including means, rates and time spent performing a behaviour, which makes comparisons difficult.

## Interpretations of Cladograms

Three results indicate that some behaviours have diverged between coastal and inland lineages of *Ensatina*. First, female residents of coastal *oregonensis* performed significantly more overt aggression than did female residents of inland *platensis*. Second, male residents of coastal *oregonensis* performed significantly more sensory behaviour than did those of inland *platensis*. Third, males paired with intruders of inland *platensis* performed significantly more sensory behaviour than did males paired with coastal *oregonensis*. Divergence in behaviour between coastal and inland populations is consistent with genetic evidence for the phylogenetic divergence of coastal and inland lineages (Wake & Yanev 1986; Moritz et al. 1992; Jackman & Wake 1994). Analyses of behavioural variation in the other coastal and inland races of *Ensatina* (Fig. 1) would indicate whether patterns of divergence shown in Figs 2 and 3 are maintained throughout the entire coastal and inland lineages.

The pattern of geographical variation in behaviour between coastal and inland *xanthoptica* suggests evolution of some behaviours between these populations with convergent evolution of inland *xanthoptica* on inland *platensis*. For avoidance and sensory behaviours of males and overt aggression and avoidance behaviours of females (Figs 2 and 3), the coastal and inland populations of *xanthoptica* showed divergence even though these populations of *xanthoptica* remain similar

genetically (Nei's  $D \approx 0.02$ , Wake & Yanev 1986): inland *xanthoptica* showed behaviour that is more similar to inland *platensis*, and coastal *xanthoptica* showed behaviour that is more similar to coastal *oregonensis*.

The two populations of *xanthoptica* also showed a high degree of constraint on behaviour. Five of eight behaviours compared between inland and coastal *xanthoptica* were unaffected by resident population, and six of eight behaviours were unaffected by intruder population. The lack of divergence may indicate that ecology has a weak effect on behavioural divergence compared to phylogenetic effects, that not enough time has passed for accumulation of divergent behaviours, or that some behaviours are not under the selective pressures for change.

### Ecological Implications of Geographical Variation in Behaviour

The behaviours that we observed, such as nose-tapping, overt aggression and avoidance behaviour, are associated with recognition of individuals, territoriality, and interference competition in several species of plethodontid salamanders (reviewed by Mathis et al. 1995) and probably also in *Ensatina* (Wiltenmuth 1996). There is both sexual and geographical variation in the divergence of these behaviours. Female residents appear to show more behavioural divergence across populations in overt aggression, passive aggression and sensory behaviour than do males. Males show more divergence in sensory behaviour (Fig. 2). Because different behavioural categories show more variation among females than males, this study supports the hypothesis that territoriality or competition vary between female and male *Ensatina* (Wiltenmuth 1996).

The relationships between the frequencies of overt aggression, avoidance and sensory behaviour are consistent with the probable role of these behaviours in territoriality. Low levels of avoidance of intruding females of inland *platensis* are consistent with the low levels of overt aggression shown by this group. High levels of avoidance of intruding coastal *oregonensis* and coastal *xanthoptica* are consistent with high levels of overt aggression shown by these groups. Divergence in avoidance behaviour of intruding salamanders from populations also suggests that residents can distinguish between intruders on the basis of

behaviour, chemical signals or both. High levels of sensory behaviour in coastal *oregonensis* (Fig. 2) may be perceived as territorial, and, as intruders, such individuals may evoke less sensory behaviour and more avoidance behaviour.

Variation in agonistic and sensory behaviour among populations of *Ensatina* suggests that geographical variation in territoriality or competition may exist among populations. In the plethodontid salamander *Desmognathus ochrophaeus*, divergence in courtship behaviour is associated with geographical isolation and may explain sexual incompatibility among populations (Herring & Verrell 1996). Reduced aggression in females of inland *platensis* and inland *xanthoptica* suggests a reduction of territoriality or competition in the inland populations compared to the coastal populations. We suggest two hypotheses about the selective factors that may be responsible for the convergent reduction of aggression shown by the inland *Ensatina*. A reduction in agonistic behaviour may be associated with reduced competition among inland populations. Alternatively, convergence may result from similar environmental conditions that are unrelated to competition and encountered by the inland populations.

### Ecological versus Genetic Explanations for Geographical Variation in Behaviour

The pattern of geographical variation in behaviour in *Ensatina* may result from differences in individual experience related to ecological differences, the evolution of genetic traits affecting agonistic and sensory behaviour or both. Learned differences in agonistic and sensory behaviour among populations could result from local variation in population density (Nishikawa 1985; Crump 1988), habitat or ecology. Although such local effects alone can explain geographical variation in levels of aggression, they may lead to selective pressures that produce changes in the frequency of genes that affect aggressive behaviour. For example, variation in levels of competition between co-existing populations of salamanders associated with specific localities may result in evolution of increased aggression (Hairston 1980; Nishikawa 1985, 1987). Common garden experiments could resolve whether the major source of behavioural variation is learned or inherited, although such experiments are

difficult to perform with salamanders due to their relatively slow development to reproductive age.

We suggest that the geographical variation in agonistic and sensory behaviour of *Ensatina* may be partly explained by the evolution of genetic traits affecting behaviour. Divergence in behaviour between coastal and inland populations is consistent with genetic evidence for their phylogenetic divergence (Wake & Yanev 1986; Moritz et al. 1992; Jackman & Wake 1994). Convergence in behaviour between inland *xanthoptica* and inland *platensis* is consistent with the secondary contact between these races during the Pleistocene. The constraint on behavioural divergence among the populations is also predictable, given the genetic linkage between populations in a ring species. Whether the observed differences in behaviour are learned or genetic, behavioural divergence may indicate important differences in behavioural ecology between the coastal and inland populations and between male and female *Ensatina*.

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