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Relaxed predation selection on rare morphs of *Ensatina* salamanders (Caudata: Plethodontidae) promotes a polymorphic population in a novel dune sand habitat

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The ring species *Ensatina* represents a classic example of locally adapted lineages. The Monterey ensatina (*Ensatina eschscholtzii eschscholtzii*) is a cryptic subspecies with brown coloration, although a recently discovered polymorphic population within a wind-blown sand region also contains leucistic (pink) and xanthistic (orange) morphs. In the present study, the frequency of leucism/xanthism was mapped across the subspecies' range, revealing that these morphs are generally rare or absent except within regions containing light-coloured substrate. Attack rates were estimated using clay models of the three morphs, deployed only at the crepuscular period and during the night, on both light and dark substrates at a site within the dune sand region. Model selection found that the interaction between morph and substrate colour best predicted attack rates. Typical morphs had equal attack rates on both substrates while xanthistic and leucistic morphs incurred significantly fewer attacks on light vs. dark substrate, and there was no significant difference in attack rates among morphs on light substrates. These results support the idea that xanthistic and leucistic morphs are poorly adapted for dark substrates compared to typical morphs, but they are more or less equally adapted for light substrates. We suggest that this microgeographic island of relaxed selection on light-coloured morphs helps to explain the existence of this polymorphic population.

ADDITIONAL KEYWORDS: clay models – colour polymorphism – microgeographic island – predation – relaxed selection

INTRODUCTION

Species with ranges that overlap multiple distinct habitats and/or substrate types may experience regionally divergent selection pressures (Storfer *et al.*, 1999). In particular, if predation selection pressures differ this can promote localized coloration adaptations such as crypsis, aposematism and mimicry (Darwin, 1859; Cott, 1940; Endler, 1984; Skelhorn *et al.*, 2010), and these adaptations have a higher likelihood of persisting when gene flow between surrounding regions is low (Endler, 1977; Storfer *et al.*, 1999; Nosil *et al.*, 2005). Unique, localized predation selection pressure can either influence the phenotype of an entire population (Marchinko & Schluter, 2007) or promote polymorphism (Endler, 1988). Of evolutionary significance, population-level polymorphism has been shown to be a precursor for rapid divergence and species formation (Gray & McKinnon, 2006; Corl *et al.*, 2010). One way in which polymorphism can be established is when a region's selection pressure is less intense or absent on rare morphs, those typically removed from populations by strong predation selection (Lahti *et al.*, 2009). This removal of selection on a trait, known as 'relaxed selection', has been shown to proceed phenotypic plasticity (Hunt *et al.*, 2011), and when it occurs on islands (including habitat islands) it can influence a population's behaviour (Beauchamp, 2004), promote non-cryptic coloration (Runemark *et al.*, 2014), and increase colourfulness or conspicuousness (Blaird *et al.*, 2020). An alternative process to create a regionally restricted polymorphic population is negative frequency-dependent selection, where rare morphs have a survival advantage (Allen, 1988; Endler, 1988). Thus, by studying predator–prey interactions on a regional scale it is possible to determine if selection pressures are driven by substrate colour, are driven by colour variation within the local population, or contribute to the maintenance

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of coloration polymorphism (Endler,1978; Farallo & Forstner, 2012; Bateman *et al.*, 2017).

One outstanding question concerns the geographical size of a unique substrate patch needed to promote adaptive divergence in coloration within an animal population (Richardson *et al.*, 2014). This question is entirely dependent on the biological qualities of the organism, which are expected to place minimum bounds on the area in which adaptation is possible. These qualities include the population size and density within and surrounding the area in question, the strength of selection within the distinct region, and immigration to/emigration from the distinct region that are influenced by the dispersal abilities of the organism and the presence of barriers to dispersal (Hansen *et al.*, 2002; Urban & De Meester, 2009; Yeaman & Whitlock, 2011; Richardson & Urban, 2013). Adaptive coloration divergence regarding melanin deposition has been documented in small vertebrates inhabiting isolated lava flows and sand dunes (Norris & Lowe, 1964; Rosenblum, 2005; Hoekstra, 2006; Corl *et al.*, 2012; Krohn *et al.*, 2019). These types of landscape features provide uniform backgrounds for organisms to colour-match, and tend to be exposed habitats that increase the visibility of prey to predators and thus increase predator-driven selection (Rosenblum, 2006; Vignieri *et al.*, 2010; Rosenblum & Harmon, 2010). The

focal taxa in the aforementioned studies typically have high vagility (e.g. lizards and mice), but low vagility organisms can presumably diverge in even smaller habitat patches.

The plethodontid salamander *Ensatina eschscholtzii* comprises several locally adapted colour morphs (currently described as subspecies) that occur from British Columbia to northern Baja California forming a classic example of a 'ring species' around the inhospitable Central Valley of California (Stebbins, 1949; Wake, 1997). Six of the seven subspecies use crypsis to avoid predation, each occurring over large regions (Stebbins, 1949; Wake, 1997; Kuchta, 2005). Morphological diversification among subspecies is a result of biogeographical barriers, regional habitat structure and environmental conditions, varying selection pressures, and low vagility of the salamanders (Moritz *et al.*, 1992; Wake, 1997; Kuchta *et al.*, 2009; Pereira & Wake, 2009). The Monterey ensatina (*E. e. eschscholtzii*) typically has a brown dorsal coloration. However, we observed a population containing three morphs: (1) a typical brown-backed morph, although there is variation in the darkness of the brown colour (Supporting Information, Fig. S1a–c), (2) a leucistic morph that lacks dorsal melanin pigment deposition resulting in a pinkish colour (Fig. 1B; Fig. S1d, e), and (3) a xanthistic morph with
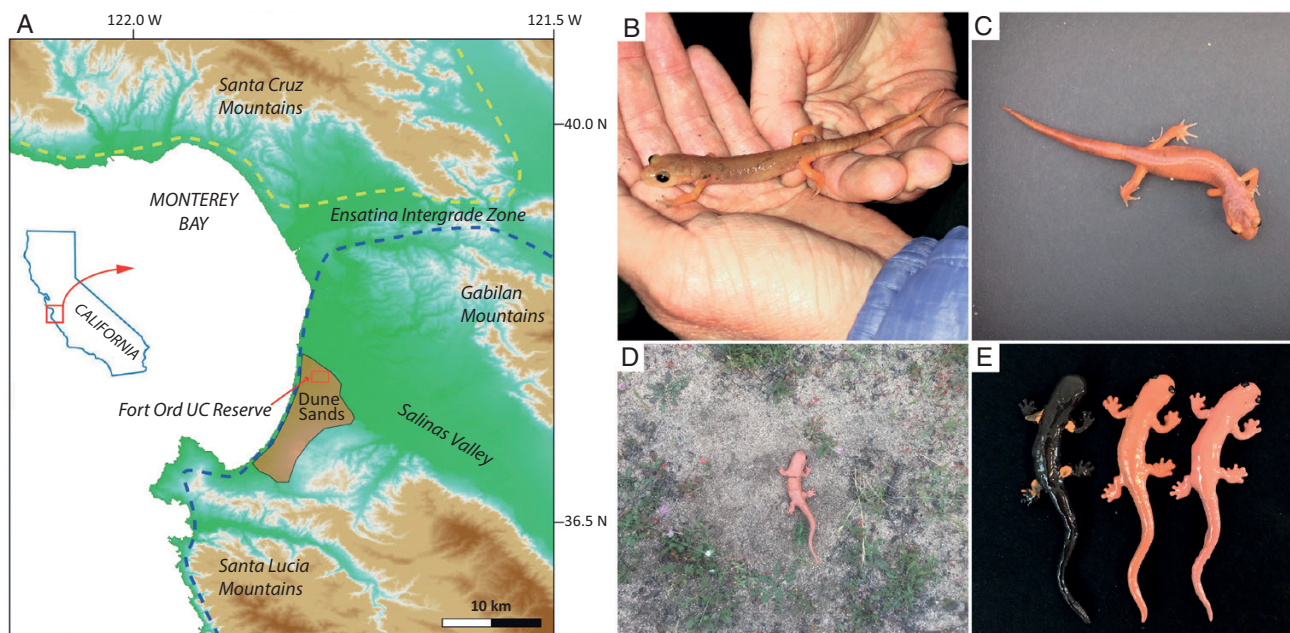

Figure 1. A, map of the Monterey Bay region of California. The blue dashed line depicts the range limits of the cryptically coloured Monterey ensatina (*Ensatina eschscholtzii eschscholtzii*) and the yellow dashed line depicts the range limits of the mimetic coloured yellow-eyed ensatina (*E. e. xanthoptica*). The study site of Fort Ord is indicated. B, a leucistic *E. e. eschscholtzii* found at Fort Ord Reserve. C, a xanthistic *E. e. eschscholtzii* found at Fort Ord Reserve. D, a leucistic clay model deployed on light sand substrate. E, example of the three types of clay model morphs used in the experiment, including (left to right) typical, xanthistic and leucistic.

a prevalence of xanthic pigments over its whole body resulting in an orange colour (Fig. 1C; Fig. S1f). These three morphs occur within an ~30-km² region of predominantly light-coloured dune sand with various stages of habitat (including maritime chaparral and oak woodland) and soil succession occurring in patchy distributions. In general, leucistic salamanders of most species are rare, presumably because this light coloration increases conspicuousness and thereby increases predation risk (Mitchell & Mazur, 1998; Stuart-Fox *et al.*, 2003; Husak *et al.*, 2006). However, the lighter coloration of leucistic and xanthistic Monterey ensatina morphs may provide a background-matching advantage on the light-coloured sand (Norris & Lowe, 1964). While *Ensatina* are nocturnal and crepuscular, cryptic coloration has been shown to effectively reduce attacks by nocturnal predators (Dice, 1947; Kaufman 1974) and the open nature of these dune sand patches would increase the exposure of any organisms within them and possibly increase the strength of predation selection (Kotler *et al.*, 1991).

In this study, we address the potential adaptive value of the leucistic and xanthistic *Ensatina* morphs relative to typical brown forms on the light-coloured dune sands of Fort Ord UC Reserve, a 245-ha plot found in northern Monterey County, California (Fig. 1A). While differences in predator community have been shown to contribute to the maintenance of colour polymorphism between regions (Kraemer *et al.*, 2016), the potential predators at this site are the same or similar to those in the rest of the range of *E. e. eschscholtzii*, including jays, owls, snakes, racoons, skunks, bobcats, coyotes, foxes and rodents (Fitch, 1940; Stebbins, 1954; Wake & Yanev, 1986; Kuchta *et al.*, 2008). First, the frequency of leucistic and xanthistic morphs of the Monterey ensatina were mapped over its range to determine if the dune sand region is unique. To measure relative predation rates among morphs, we deployed soft clay models of each morph on light-coloured sand substrate and darker soil or leaf litter substrates and recorded attack events by documenting predation imprints in the clay (Kuchta, 2005). Our experimental design contains an improvement over previous studies by only deploying these models at just before sunset (crepuscular) and recovering them the next morning, restricting the model exposure to times when these nocturnal/crepuscular salamanders are active at the surface.

Our 'adaptive coloration' hypothesis assumes that divergence in coloration of this population is influenced by predator-driven selection for crypsis by prey to match the light-coloured sandy substrate, in which case we would predict that both leucistic and xanthistic models will have lower predation rates than typical morphs on light backgrounds. However, if xanthistic and leucistic morphs have higher predation rates than typical morphs on dark backgrounds but relatively equal rates on light backgrounds this would support the 'relaxed selection' hypothesis. Finally, if xanthistic and leucistic morphs occur at much lower frequencies than typical morphs, and have lower predation rates than typical morphs on both light and dark backgrounds, this would be consistent with a 'negative frequency-dependent selection' hypothesis.

MATERIAL AND METHODS

MAPPING LEUCISM FREQUENCY

To understand the significance of the Fort Ord population of *E. e. eschscholtzii* relative to the rest of the subspecies' range, we first mapped the frequency of leucistic and xanthistic morph individuals. Scoring of coloration qualities of organisms from citizen science photograph depositories is becoming an increasingly useful tool for biologists (see Moore *et al.*, 2019). We utilized the online database iNaturalist (iNaturalist. org; accessed April 2019) where users upload georeferenced photos of plants and animals. Visual observations were made on 254 *E. e. eschscholtzii* individuals from across the subspecies' range both from our own photos and from iNaturalist (Appendix S1, Rich *et al.*, 2020). Photos were scored by each of the authors through independent visual observations and were ranked by morph type: 0, typical; 1, xanthistic; and 2, leucistic (see Fig. 1B, C for examples of leucistic and xanthistic individuals, respectively). All photos used to map the frequency of leucism, including our own from Fort Ord, can be found at the website: https://www.inaturalist.org/projects/ensatina-eschscholtzii-ssp-eschscholtzii-sinervo-lab. We created a leucism heat map using R (R Core Team, 2020) by using these morph scores, linked to their GPS coordinates, to create a grid cell (raster) matrix at a resolution of 1 × 1 km. The bilinear interpolation method was used to define gradients of estimated morph frequencies between input cells. The map was then used to visualize regions of the range of *E. e. eschscholtzii* that have high frequencies of leucistic/xanthistic individuals, and to confirm that the Fort Ord region of Monterey County has a high proportion of these morphs compared to the rest of the subspecies' range. The R code used to create the frequency map can be found in the Dryad repository (Rich *et al.*, 2020).

EXPERIMENTAL DESIGN

Observing and gathering quantifiable data of natural predation events in the field is difficult. However, success has been found through the use of replacement

methods with clay models mimicking prey (Brodie III, 1993; Irschick & Reznick, 2009; Bateman *et al.*, 2017). Models of prey are deployed in the field where marks are imprinted into the clay by predators' attacks (Bateman *et al.*, 2017). This type of experimental design allows the direct comparison of predation rates on different colour morphs, as well as determining each morph's relative success at avoiding predation (Irschick & Reznick, 2009).

Models were created with plastic salamander casts coated in black non-toxic modelling clay. The models were 55 mm from snout to vent and 105 mm in total length, a size and proportion that is typical of adult *Ensatina*. These models consisted of three colour morphs: (1) light pink resembling leucistic salamanders, (2) orange resembling xanthistic salamanders, and (3) dark brown resembling *Ensatina* typically found across the subspecies' range (Fig. 1E). The leucistic morphs were spray painted with a mixture of tan camouflage Rust-Oleum (Rust-Oleum, Vernon Hills, IL, USA) and gloss coral Ultra Cover paint + primer Rust-Oleum spray paint. The xanthistic morphs were sprayed with a mixture of tan camouflage Rust-Oleum and orange Gloss Protective Enamel Rust-Oleum spray paint. Typical morphs were painted with brown camouflage non-reflective finish Rust-Oleum spray paint and an orange ventral painted with the same orange Rust-Oleum spray paint as the xanthistic morph, with additional orange pigment added to upper leg segments with pure pumpkin orange DecoArt all-purpose paint. The models received a final coat of Rust-Oleum clear enamel gloss finish to appear wet (Kuchta, 2005).

Models were deployed at the University of California Fort Ord Natural Reserve, which has restricted access to the public and represents 245 ha of well-preserved maritime chaparral, oak woodland and coastal scrub habitat. Clay models were placed into 80 clusters of three models (240 models total), each cluster containing one of each morph (Supporting Information, Fig. S2a). These 80 clusters were placed throughout the reserve in roughly equal proportions within each of the four most common habitat types on the reserve: oak forest, chaparral, grassland and open scrub. Each of the three models were tied with Shur Strike (Big Rock Sports LLC, Graham, NC, USA) 2-lb test monofilament fishing line 1 m from a steel tent peg and placed in a triangle formation. Each model was set ~2 m apart and placed on either light-coloured substrate (dune sand; see Fig. 1D) or a dark-coloured substrate (soil or leaf litter). Models were deployed in the field for 14 nights between the dates of 28 November 2018 and 18 January 2019, for a total of 3281 model-nights (one model set out for one night is referred to as a 'model-night'). Single-compartment Nutriboxes (24 oz) were used to cover models during the day to avoid marks from diurnal predators that would not typically come in contact with *Ensatina*, which take shelter during the day (Stebbins, 1949) but begin foraging during the crepuscular period. Nutriboxes were coloured with gloss meadow green Ultra Cover paint + primer Rust-Oleum spray paint to block model visibility from diurnal predators. Models were uncovered each late afternoon between 15:30 and 17:30 h (the beginning of the crepuscular period when we have observed *Ensatina* to be active, pers. obs.) and checked for predation each early morning between 05:30 and 08:30 h. The total number of model-nights for each morph were within 1% variance of each other, with typical morphs deployed for 1098 model-nights, xanthistic morphs for 1085 model-nights and leucistic morphs for 1097 model-nights. Appendix S2 provides raw data from the clay model study (Rich *et al.*, 2020).

PREDATION SCORING AND STATISTICAL TESTS

Predation events were indicated by indentations left by animals on the models (Supporting Information, Fig. S2b, c). We visually inspected the models and recorded any new marks on models from the previous night. We scored each mark as a 'strike'. The total number of strikes on each model were counted every day and the previous night's strike total was subtracted from the new total to calculate the number of new strikes. While quantifying the number of strikes per attack does not best represent real-life attack rates, we analysed strike data (strikes per attack) to assess levels of predatory aggression. Attacks are the primary data for this study and are recorded as a binary character for statistical analyses. Multiple strikes from one night were considered a single 'attack' to avoid misrepresenting multiple marks by a single predator. If a model was missing from a cluster it was counted as a single attack event but was not replaced by another model due to limited model supplies. No cluster had multiple models attacked on the same night. Initial attempts to document the predator type (raptor, rodent, etc.) proved to be difficult and not reliable enough to make any robust inferences, so they are not included here.

All statistics were made using R v.3.6.3 (R Core Team, 2020). To determine if colour morph and/or substrate colour predicted attack rates, we used a generalized linear mixed model (GLMM) using the *glmer* function with a binomial error distribution in the package *lme4* (Bates *et al.*, 2015). We modelled 'Morph', 'Substrate Colour', and the interaction of 'Morph'/'Substrate Colour' as fixed effects, while each night the models were deployed ('Night') and each cluster of models ('Cluster') were treated as random effects to account for any influence of time and place. Nested models and a null model were compared using ANOVAs and a favoured model was selected by examining corrected Akaike information

criterion (AIC_c) and AIC_c weight values (see McElroy, 2016) calculated using the package *bbmle* (Bolker & R Development Core Team, 2020). A log-likelihood ratio test was used to calculate *P*-values to determine if models were a significantly better fit to the data than the null model where attack rates are constant with respect to morph and substrate colour. Once a best-fit model was selected, a Tukey test was implemented to identify significant pairwise comparisons using the *glht* function in the package *multcomp* (Hothorn *et al.*, 2008, 2014). Mean attack rates with standard deviations for each morph across and within a specific substrate colour (light or dark) and type (light sand, dark soil, leaf litter) were calculated using the package *dplyr* (Wickham *et al.*, 2020). Kruskal–Wallis rank sum tests were used to determine if attack rates significantly differed between morphs overall, and between morphs on each substrate colour. Wilcoxon tests were used to calculate *P*-values for both attack rates and strikes-per-attack of morph pairs between and within a substrate colour and plotted using the package *ggpubr* (Kassambara, 2018).

RESULTS

LEUCISM FREQUENCY MAPPING

Of the 254 photos of *E. e. eschscholtzii* scored, we recorded 22 instances of leucistic or xanthistic salamanders with 16 from the dune sand region or the immediate vicinity, two from northern Monterey County just south of the dune sand region, and four from southern California. This correlates to these morphs occurring at a frequency of roughly 0.087 range-wide, which is driven by the high frequency in northern Monterey County (0.35; 16/46). Within the region of northern Monterey County where light morph *Ensatina* are found (the dune sand region plus the area just to the south), leucistic individuals were found at a frequency of 0.11 (5/46), xanthistic individuals at a frequency of 0.28 (13/46) and typical morphs at a frequency of 0.61 (28/46). The results of this coloration trait frequency are shown in Figure 2A. In general, a single leucistic/xanthistic individual is shown as a distinct green or blue dot on the map. The high frequency of leucistic/xanthistic individuals in the dune sand region is seen as a more diffuse green and yellow blob roughly centred over the dune sands area (Fig. 2B).

SCORING PREDATION AND STATISTICAL TESTS

The GLMM that best predicted attack rates was the model with interactions between morph and substrate colour ($AIC_c = 625.38$, AIC_c weight = 0.405) and this model significantly outperformed the null model ($\chi^2 = 13.2$, d.f. = 5, $P = 0.0216$) (Table 1). Including the random effects of 'night' and 'cluster' in the 'morph × substrate colour' model was heavily favoured ($\chi^2 = 81.17$, d.f. = 2, $P = 2e-16$) when compared to excluding random effects
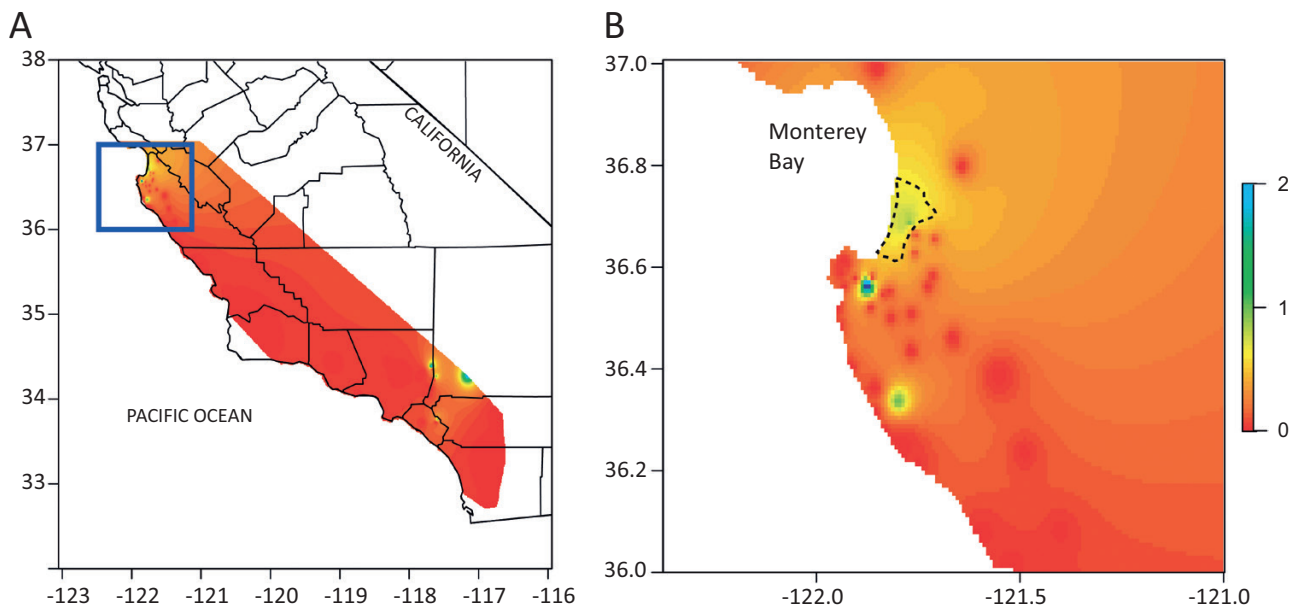

Figure 2. A, heat map of the frequency of leucistic morph individuals across the range of *E. e. eschscholtzii* with red colour indicating their absence (or lack of documentation) and yellow-to-green colours indicating their presence. B, enlargement of the Monterey Bay region showing the relatively high frequency and extent of occurrence of leucistic morph individuals in the region. The black dashed outline depicts the extent of the light-coloured dune sands.

Table 1. Model selection for attacks on clay *Ensatina* models.

Model terms	d.f.	AIC$_c$	ΔAIC$_c$	AIC$_c$ weight	logLik	*P*
Morph × Substrate Colour	8	625.38	0	0.405	−304.69	0.0216*
Morph + Substrate Colour	6	626.66	1.28	0.216	−307.33	0.0476*
Substrate Colour	4	626.73	1.35	0.209	−309.37	0.0498*
Morph	5	628.49	3.11	0.087	−309.24	0.1293
Null	3	628.58	3.2	0.083	−311.29	–

d.f. = degrees of freedom; AIC$_c$ = corrected Akaike Information Criterion; ΔAIC$_c$ = differential AIC$_c$; logLik = log likelihood; *P* = *P*-values for pairwise chi-squared comparisons with the null model where morph and/or substrate colour does not influence attacks on clay models.
*Statistically significant *P*-value.

(AIC = 702.55). The 'morph + substrate colour' and 'substrate colour' models were also favoured over the null model (*P* = 0.0476 and *P* = 0.0498, respectively), and the model with only 'morph' was not significantly better than the null model (*P* = 0.1293). The post-hoc Tukey test indicated a significant difference between the typical and xanthistic morph attack rates (*P* = 0.016).

The mean attack rate across all substrates was highest in xanthistic and lowest in typical morphs but was not significantly different between any of the three morphs (Kruskal–Wallis χ² = 2.96, d.f. = 2, *P* = 0.228) (Fig. 3A; Supporting Information, Table S1). Typical morphs showed no variation in attacks when comparing light and dark substrates (Wilcoxon *P* = 0.994), leucistic morphs incurred significantly less predation on light vs. dark substrates (Wilcoxon *P* = 0.0495), and xanthistic morphs incurred significantly less predation on light vs. dark substrates (Wilcoxon *P* = 0.003) (Fig. 3B). Within dark substrates there were significant differences among morphs (Kruskal–Wallis χ² = 6.09, d.f. = 2, *P* = 0.048) with xanthistic morphs being subject to the highest attack rate and typical morphs the lowest attack rates (Wilcox *P* = 0.014) (Fig. 3C). Within light substrate, typical morphs had the highest attack rate and xanthistic and leucistic morphs had lower attack rates, although these differences were not significant (Kruskal–Wallis χ² = 0.67, d.f. = 2, *P* = 0.710) (Fig. 3C). Further subdividing dark substrates into 'Soil' and 'Leaf Litter' did not reveal any significant attack rate differences between the two dark substrate types within any one morph (Fig. S3). Typical morphs had significantly fewer strikes per attack on light vs. dark substrates (Wilcox *P* = 0.005), and on dark substrates leucistic morphs had significantly fewer strikes per attack than xanthistic morphs (Wilcox *P* = 0.0002) (Fig. S4).

fitness compared to normally pigmented individuals (Mitchell & Mazur, 1998; Mitchell & Church, 2002). Mapping of light morph Monterey ensatina has confirmed that leucism and xanthism are rare or absent over most of the range where this subspecies is associated with forested habitats that typically have a dark substrate consisting of leaf litter, soil and organic matter. The clay model study showed that xanthistic and leucistic morphs have significantly higher predation rates on dark substrates when compared to typical morphs, which may explain their extremely low frequencies across most of the subspecies' range. The mapping data showed that xanthistic and leucistic morphs are most common in the dune sands area of northern Monterey County, CA, and also in the Transverse Range of southern California where patches of light sandstone substrate are found, suggesting that the light substrate somehow allows light morphs to persist in these areas.

Under the adaptation hypothesis, we expect the light-coloured morphs to have lower predation rates than typical morphs on the light sand substrate, which was not supported by our data. While leucistic and xanthistic attack rates were slightly lower than for typical morphs on light substrate, this difference was not significant. However, this relative equality in predation rates due to the relaxed selection of light morphs on light backgrounds is all that is necessary for the xanthistic and leucistic morphs to become established (Lahti *et al.*, 2009). Relaxed predation selection has been linked to both phenotypic plasticity and colour polymorphism, including an increase in the colourfulness or conspicuousness of a population (Endler, 1980; Hunt *et al.*, 2011; Runemark *et al.*, 2014; Blaird *et al.*, 2020), and our results are consistent with the hypothesis that relaxed selection has promoted the maintenance of these rare morphs.

DISCUSSION

THE CASE FOR RELAXED SELECTION

In general, leucistic salamanders tend to be rare and locally distributed, and presumably have decreased

THE POSSIBILITY OF FREQUENCY-DEPENDENT SELECTION

Although our sample size of colour-scored *Ensatina* from the dune sands and immediately surrounding
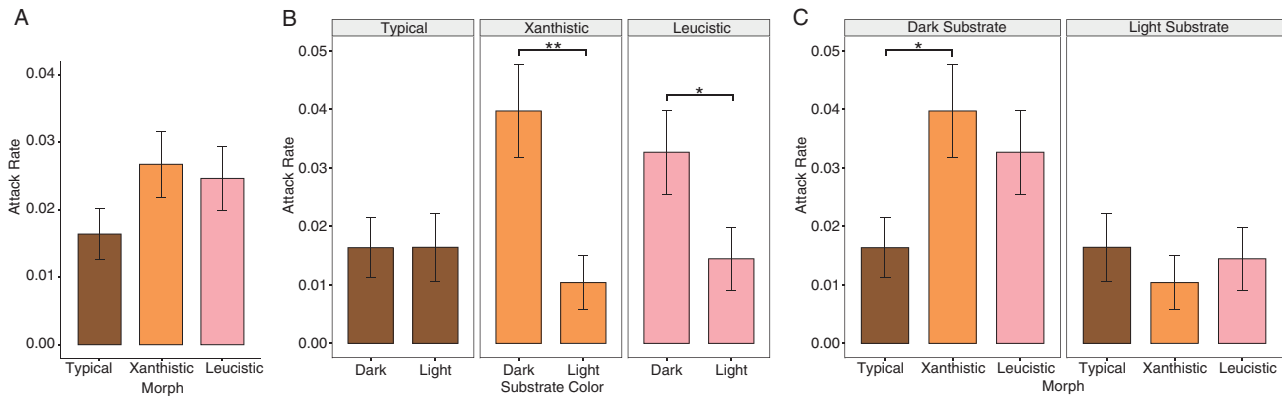
Figure 3. A, overall mean attack rates across all substrates; B, differences in mean attacks per model night for each individual morph between substrate colours; and C, differences in attack rate per model night between different morphs within a shared substrate colour. Error bars represent standard error estimates and horizontal bars indicate statistically significant differences (Wilcoxon test; *$P \leq 0.05$, **$P \leq 0.01$). All pairs without a horizontal bar are not statistically significant.

region is small ($N = 46$), the results suggest that light morphs occur at lower frequencies than typical morphs (leucistic = 0.11, xanthistic = 0.28, typical = 0.61). If our results had shown that light morphs had lower predation on both light and dark substrates this would have supported the negative frequency-dependent hypothesis. However, our experimental design was not optimized to test for frequency-dependent processes. So to understand if frequency-dependent selection is operating to maintain *Ensatina* morphs within the dune sand region we recommend additional clay model studies that manipulate the frequencies of the three morphs, document the specific predators (e.g. motion detection camera traps) and contain sites both in the dune region and outside it.

Frequency-dependent selection has been shown to promote polymorphism in other salamanders with similar ecologies (Fitzpatrick *et al.*, 2009; Hantak & Kuchta, 2018). Although more data are needed for confirmation, we suspect that negative frequency-dependent selection, a form of balancing selection in which the fitness of a phenotype increases as it becomes less common, could be acting to maintain these three morphs (Allen, 1988). In this case, relaxed selection on rare morphs could even be a precursor to frequency-dependent selection processes by allowing the rare morphs to initially co-occur with typical morphs, after which their frequency in the population would dictate their predation rates. Endler (1988) proposed that interactions between background colour, the visual perception of predators and prey colour morphs can allow certain morphs to persist in one region and not another, even when the community of predators is the same between regions. Thus, it may simply be a situation where the rarer morphs are attacked less, a phenomenon seen in many other taxa (see Olendorf *et al.*, 2006; Fitzpatrick *et al.*, 2009).

The complex web of environmental and biological interactions may even create temporal variation in selective forces (Calsbeek *et al.*, 2009), one of which involves the influence of the moon phase on attack rates (Clarke, 1983), which we found not to have an effect on attack rates among morphs (see Appendix S2, Rich *et al.*, 2020).

RELAXED SELECTION ON A MICROGEOGRAPHIC SCALE

The dune sand area is bordered to the west and north by the ocean and to the south and east by the Santa Lucia Mountains (which contain typical dark-coloured *E. e. eschscholtzii*) and there are no physical barriers between the sandy area and the neighbouring forested hills and mountains. This implies that dune sand populations have probably been continuously exchanging migrants with typical morph populations. The dune sand area was ~30 km² originally (most of which has been lost to development and agriculture), which is small compared to the overall range of the Monterey ensatina but large compared to other dune sand regions within this range. The size of this substrate patch, along with the dispersal ability of *Ensatina* (60–120 m; Staub *et al.*, 1995), would qualify it as a 'microgeographic' landscape feature (Stelkens *et al.*, 2012; Richardson *et al.*, 2014). However, rather than driving local adaptation, this microgeographic island differs in its predation selection pressures on light morphs and has essentially provided a level playing field. We suggest that future surveys attempt to find the boundaries of the region where light morph *Ensatina* are found in northern Monterey County and test for an association with the extent of the wind-blown sand substrate patch.

THE INFLUENCE OF PREDATORS

The interactions of predation selection from multiple predators influence the evolution of coloration divergence within a population of prey species (Willink *et al.*, 2014). The potential predators within the Fort Ord Reserve include scrub-jays, a number of owl species, snakes, racoons, skunks, bobcats, coyotes, foxes and large rodents (Fitch, 1940; Stebbins, 1954; Beneski, 1989; Wake & Yanev, 1986). Given that scrub-jays and garter snakes are diurnal, they would have limited opportunities to prey upon *Ensatina* salamaders. We have observed a high density of owls at this site including Great Horned, Barn and Western Screech Owls, which have been known to have excellent vision sharpness and also prey upon salamanders (Allen, 1924; Errington *et al.*, 1940; Mobley & Stidham, 2000). It has also been demonstrated that owls (barn and screech) capture light-coloured prey against a dark background more efficiently than dark prey against a light background (Kaufman, 1974), which may help to explain the difference in predation rates of typical and light morph *Ensatina* on dark backgrounds. The nocturnal mammalian predators at this site have excellent night vision, and although they are dichromatic it has been shown that some species are able to discriminate colours (Denman *et al.*, 2018). After examining faunal records of Fort Ord Reserve (https://fortordreserve.ucsc.edu/maps-habitats-organisms/index.html), we found that the predator composition at Fort Ord is generally representative of the predator community across the range of *E. e. eschscholtzii*. Accordingly, we believe our results are being driven by the unique colour and exposure of the dune sands, rather than by a unique predator community.

THE TEMPORAL SCALE OF SELECTION

Pure dune habitats are not suitable for *Ensatina* salamanders due to the high levels of sun exposure, intense heat and lack of subterranean retreats, so it is likely that *E. e. eschscholtzii* did not colonize the dunes until after larger shrubs and oaks became established. Most estimates show the dunes have existed for roughly 11 000–18 000 years (Dupré, 1990; Thornton *et al.*, 2006), but *Ensatina* would have been inhabiting the region for considerably less time than this as succession of maritime chaparral and oak woodland would have occurred well after initial dune formation. Interestingly, heavy fog (typical of maritime chaparral habitats) during California's dry season allows for nearly year-round nocturnal amphibian activity (S.B.R., pers. obs.) and may have allowed *Ensatina* and other nocturnal amphibians to colonize these dunes before larger plants became established. In either case, this suggests that this polymorphic population is relatively young (~10 000 years or less). The rapid evolution of a novel

coloration has been recorded for various lineages of terrestrial vertebrates, with some occurring on the order of thousands of years (Rosenblum *et al.*, 2010; Manceau *et al.*, 2010). These dunes are not only young, but they are rapidly changing due to both natural (ecological succession) and human-caused processes (development, edge-effects, introduced species, fire suppression, etc.). As vegetation becomes more established in an area, the process of soil succession transforms the light sands into a darker coloured organic humus/sand mixture. The recent human disruption/suppression of natural fire cycles that typically clear out vegetation and dry leaf litter are causing a reduction in exposed light sand. While native Americans burned the region every couple decades from ~11 000 years ago until the late 1700s, the current mean fire interval in this region is now estimated to be >100 years (Greenlee & Langenheim, 1990). We suspect that the recent buildup of darker coloured substrate on the dunes could potentially shift the adaptive advantage back to the typical morph *E. e. eschscholtzii*.

SHORT-TERM COLOUR CHANGE IN *ENSATINA*

Plastic colour change is one final confounding factor for the interpretation of the predation rates of the various morphs within this polymorphic population. Colour change in salamanders, which involves slight lightening or darkening of their coloration, is primarily the result of the redistribution of melanin pigments within the melanophore pigment cells (Duellman & Trueb, 1986). Short-term colour change in some salamanders has been linked to matching their background (Garcia & Sih, 2003; Polo-Cavia & Gomez-Mestre, 2017), and longer-term colour change has been observed by keeping salamanders in captivity (Kraemer *et al.*, 2012). However, leucistic salamanders lack melanin pigments in their skin and therefore it is expected that they are unable to change their colour. The xanthistic salamanders also have little or no melanin, so if any colour change occurs it is expected to be in yellow/orange colours. Given that most colour change in salamanders is associated with melanin, it is expected that typical brown morphs have the greatest ability to darken or lighten themselves, but they will always have some shade of brown on their back. An appropriate follow-up study should examine the degree to which each *Ensatina* morph is able to alter its coloration and if colour change is associated with background matching.

CONCLUSIONS

While our predictions of local adaptation were not supported by our data, these results suggest that a relatively small and young region of unique substrate has promoted the existence of a polymorphic population

of Monterey ensatina by providing a level playing field of predation selection pressure for light morphs. Light morphs of these salamanders probably arise periodically throughout the subspecies range but are quickly removed by higher predation rates, possibly due to their high contrast against dark-coloured backgrounds, or simply that light-coloured objects are easier to see at night compared to dark ones. For some reason, the light-coloured sand appears to relax predation selection on light morphs allowing all three of these morphs to co-occur, at least for now. This study provides evidence that microgeographical islands of relaxed selection pressures can promote the formation of regionally restricted polymorphic populations.

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REFERENCES

Allen AA. 1924. A contribution to the life history and economic status of the screech owl (*Otus asio*). *The Auk* **41:** 1–16.

Allen JA. 1988. Frequency-dependent selection by predators. *Philosophical Transactions of the Royal Society B: Biological Sciences* **319:** 485–503.

Bateman PW, **Fleming PA**, **Wolfe AK. 2017.** A different kind of ecological modelling: the use of clay model organisms to explore predator–prey interactions in vertebrates. *Journal of Zoology* **301:** 251–262.

Bates D, **Mächler M**, **Bolker B**, **Walker S. 2015.** Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67:** 1–48.

Beauchamp G. 2004. Reduced flocking by birds on islands with relaxed predation. *Proceedings of the Royal Society B: Biological Sciences* **271:** 1039–1042.

Beneski TJ Jr. **1989.** Adaptive significance of tail autonomy in the salamander *Ensatina*. *Journal of Herpetology* **23:** 322–324.

Blaird L, **Paquet M**, **Robert A**, **Dufour P**, **Renoult JP**, **Grégoire A**, **Crochet P**, **Covas R**, **Doutrelant C. 2020.** Examining the link between relaxed predation and bird coloration on islands. *Biology Letters* **16:** 20200002.

Bolker B, **R Development Core Team**. **2020.** *Bbmle: Tools for general maximum likelihood estimation. R package version 1.0.23*. Available at: https://cran.r-project.org/web/packages/bbmle

Brodie III ED. 1993. Differential avoidance of coral snake banded patterns by free-ranging avian predators in Costa Rica. *Evolution* **47:** 227–235.

Calsbeek R, **Bonvini L**, **Cox RM. 2009.** Geographic variation, frequency-dependent selection, and the maintenance of a female-limited polymorphism. *Evolution* **64:** 116–125.

Clarke JA. 1983. Moonlight's influence on predator/prey interactions between short-eared owls (*Asio flammeus*) and deermice (*Peromyscus maniculatus*). *Behavioral Ecology and Sociobiology* **13:** 205–209.

Corl A, **Davis AR**, **Kuchta SR**, **Sinervo B. 2010.** Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. *Proceedings of the National Academy of Sciences of the United States of America* **107:** 4254–4259.

Corl A, **Lancaster LT**, **Sinervo B. 2012.** Rapid formation of reproductive isolation between two populations of side-blotched lizards, *Uta stansburiana*. *Copeia* **2012:** 593–602.

Cott HB. 1940. *Adaptive coloration in animals.* London: Methuen and Company.

Darwin C. 1859. *The origin of species by means of natural selection*. London: Murray.

Denman DJ, **Luviano JA**, **Ollerenshaw DR**, **Cross S**, **Williams D**, **Buice MA**, **Olsen SR**, **Reid RC. 2018.** Mouse color and wavelength-specific luminance contrast sensitivity are non-uniform across visual space. *Elife* **7:** e31209.

Dice LR. 1947. Effectiveness of selection by owls of deer-mice (*Peromyscus maniculatus*) which contrast in color with their background. *Contributions from the Laboratory of Vertebrate Biology of the University of Michigan* **34:** 1–20.

Duellman WE, **Trueb L. 1986.** *Biology of amphibians*. New York: McGraw-Hill.

Dupré WR. 1990. Quaternary geology of the Monterey Bay region, California. In: Garrison RE, Greene HG, Hicks KR, Weber GE, Wright TL, eds. *Geology and tectonics of the Central California coastal region, San Francisco to Monterey*. Bakersfield: Pacific Section of the American Association of Petroleum Geologists Guidebook, 185–192.

Endler JA. 1977. *Geographic variation, speciation, and clines*. New York: Princeton University Press.

Endler JA. 1978. A predator's view of animal color patterns. In: Hecht MK, Steere WC, Wallace B, eds. *Evolutionary biology*. Boston: Springer, 319–364.

Endler JA. 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34:** 76–91.

Endler JA. 1984. Progressive background matching in moths, a quantitative measure of crypsis. *Biological Journal of the Linnean Society* **22:** 187–231.

Endler JA. 1988. Frequency-dependent predation, crypsis and aposematic coloration. *Philosophical Transactions of the Royal Society B: Biological Sciences* **319:** 505–523.

Errington PL, **Hamerstrom F**, **Hamerstrom Jr FN. 1940.** The Great Horned Owl and its prey in north-central United States. *Iowa Agriculture and Home Economics Experiment Station Research Bulletin* **24:** 759–850.

Farallo VR, **Forstner MRJ. 2012.** Predation and the maintenance of color polymorphism in a habitat specialist squamate. *PLoS One* **7:** e30316.

Fitch HS. 1940. A biogeographical study of the *ordinoides* artenkreis of garter snakes (genus *Thamnophis*). *University of California Publications in Zoology* **44:** 1–50.

Fitzpatrick BM, **Shook K**, **Izally R. 2009.** Frequency-dependent selection by wild birds promotes polymorphism in model salamanders. *BMC Ecology* **9:** 12.

Garcia TS, **Sih A. 2003.** Color change and color-dependent behavior in response to predation risk in the salamander sister species *Ambystoma barbourin* and *Ambystoma texanum*. *Oecologia* **137:** 131–139.

Gray SM, **McKinnon JS. 2006.** Linking color polymorphism maintenance and speciation. *Trends in Ecology & Evolution* **22:** 71–79.

Greenlee JM, **Langenheim JH. 1990.** Historic fire regimes and their relation to vegetation patterns in the Monterey Bay area of California. *American Midland Naturalist* **124:** 239–253.

Hansen MM, **Ruzzante DE**, **Nielsen EE**, **Bekkevold D**, **Mensberg KLD. 2002.** Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations. *Molecular Ecology* **11:** 2523–2535.

Hantak MM, **Kuchta SR. 2018.** Predator perception across space and time: relative camouflage in a colour polymorphic salamander. *Biological Journal of the Linnean Society* **123:** 21–33.

Hoekstra HE. 2006. Genetics, development and evolution of adaptive pigmentation in vertebrates. *Heredity* **97:** 222–234.

Hothorn T, **Bretz F**, **Westfall P. 2008.** Simultaneous inference in general parametric models. *Biometrical Journal* **50:** 346–363.

Hothorn T, **Bretz F**, **Westfall P**, **Heiberger RM**, **Schuetzenmeister A**, **Scheibe S. 2014.** *Multcomp: simultaneous inference in general parametric models. R package version 1–3*. Available at: https://cran.r-project.org/package=multcomp

Hunt BG, **Ometto L**, **Wurm Y**, **Shoemaker D**, **Yi SV**, **Keller L**, **Goodisman MAD. 2011.** Relaxed selection is a precursor to the evolution of phenotypic plasticity. *Proceedings of the National Academy of Sciences of the United States of America* **108:** 15936–15941.

Husak JF, **Macedonia JM**, **Fox SF**, **Sauceda RC. 2006.** Predation cost of conspicuous male coloration in collared lizards (*Crotaphytus collaris*): an experimental test using clay-covered model lizards. *Ethology* **112:** 572–580.

Irschick DJ, **Reznick D. 2009.** Field experiments, introductions, and experimental evolution. In: Garland T, Rose ME, eds. *Experimental evolution: concepts, methods, and applications of selection experiments*. Berkeley: University of California Press, 173–193.

Kassambara A. 2018. *ggpubr: 'ggplot2' based publication ready plots. R package version 0.1.7*. Available at: https://CRAN.R- project.org/package=ggpubr

Kaufman DW. 1974. Adaptive coloration in *Peromyscus polionotus*: experimental selection by owls. *Journal of Mammalogy* **55:** 271–283.

Kotler BP, **Brown JS**, **Hasson O. 1991.** Factors affecting gerbil foraging behavior and rates of owl predation. *Ecology* **72:** 2249–2260.

Kraemer AC, **Kissner J**, **Adams DC. 2012.** Morphological color-change in the red-backed salamander (*Plethodon cinereus*) while kept in captivity. *Copeia* **2012:** 748–755.

Kraemer AC, **Serb JM**, **Adams DC. 2016.** Both novelty and conspicuousness influence selection by mammalian predators on the colour pattern of *Plethodon cinereus* (Urodela: Plethodontidae). *Biological Journal of the Linnean Society* **118:** 889–900.

Krohn AR, **Diepeveen ET**, **Bi K**, **Rosenblum EB. 2019.** Local adaptation does not lead to genome-wide differentiation in lava flow lizards. *Ecology and Evolution* **9:** 6810–6820.

Kuchta SR. 2005. Experimental support for aposematic coloration in the salamander *Ensatina eschscholtzii xanthoptica*: implications for mimicry of Pacific newts. *Copeia* **2005:** 265–271.

Kuchta SR, **Krakauer AH**, **Sinervo B. 2008.** Why does the yellow-eyed ensatina have yellow eyes? Batesian mimicry of Pacific newts (genus *Taricha*) by the salamander *Ensatina eschscholtzii xanthoptica*. *Evolution* **62:** 984–990.

Kuchta SR, **Parks DS**, **Mueller RL**, **Wake DB. 2009.** Closing the ring: historical biogeography of the salamander ring species *Ensatina eschscholtzii*. *Journal of Biogeography* **36:** 982–995.

Lahti DC, **Johnson NA**, **Ajie BC**, **Otto SP**, **Hendry AP**, **Blumstein DT**, **Coss RG**, **Donohue K**, **Foster SA. 2009.** Relaxed selection in the wild. *Trends in Ecology & Evolution* **24:** 487–496.

Manceau M, **Domingues VS**, **Linnen, CR**, **Rosenblum EB**, **Hoekstra HE. 2010.** Convergence in pigmentation at multiple levels: mutations, genes and function. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365:** 2439–2450.

Marchinko KB, **Schluter D. 2007.** Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. *Evolution* **61:** 1084–1090.

McElroy MT. 2016. Teasing apart crypsis and aposematism – evidence that disruptive coloration reduces predation on a noxious toad. *Biological Journal of the Linnean Society* **117:** 285–294.

Mitchell JC, **Church DR. 2002.** Leucistic marbled salamanders (*Ambystoma opacum*) in Virginia. *Herpetological Review* **29:** 229–230.

Mitchell JC, **Mazur J. 1998.** Leucistic red-backed salamanders (*Plethodon cinereus*) from Maryland. *Northeastern Naturalist* **5:** 367–369.

Mobley JA, **Stidham TA. 2000.** Great Horned Owl death from predation of a toxic California Newt. *The Wilson Journal of Ornithology* **112:** 563–564.

Moore MP, **Lis C**, **Gherghel I**, **Martin RA. 2019.** Temperature shapes the costs, benefits and geographic diversification of sexual coloration in a dragonfly. *Ecology Letters* **22:** 437–446.

Moritz C, **Schneider CJ**, **Wake DB. 1992.** Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. *Systematic Biology* **41:** 273–291.

Norris KS, **Lowe CH. 1964.** An analysis of background color-matching in amphibians and reptiles. *Ecology* **45:** 565–580.

Nosil P, **Vines TH**, **Funk DJ. 2005.** Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* **59:** 705–719.

Olendorf R, **Rodd FH**, **Punzalan D**, **Houde AE**, **Hurt C**, **Reznick DN**, **Hughes KA. 2006.** Frequency-dependent survival in natural guppy populations. *Nature* **441:** 633–636.

Pereira RJ, **Wake DB. 2009.** Genetic leakage after adaptive and nonadaptive divergence in the *Ensatina eschscholtzii* ring species. *Evolution* **63:** 2288–2301.

Polo-Cavia N, **Gomez-Mestre I. 2017.** Pigmentation plasticity enhances crypsis in larval newts: associated metabolic cost and background choice behavior. *Scientific Reports* **7:** 39739.

R Core Team. **2020.** *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. https://www.R-project.org/.

Rich C, **Reilly SB**, **Sinervo B. 2020.** Relaxed predation selection on rare morphs of *Ensatina* salamanders (Caudata: Plethodontidae) promotes a polymorphic population in a novel dune sand habitat. Available at: Dryad, Dataset, https://doi.org/10.5061/dryad.02v6wwq23

Richardson JL, **Urban MC. 2013.** Strong selection barriers explain microgeographic adaptation in wild salamander populations. *Evolution* **67:** 1729–1740.

Richardson JL, **Urban MC**, **Bolnick DI**, **Skelly DK. 2014.** Microgeographic adaptation and the spatial scale of evolution. *Trends in Ecology & Evolution* **29:** 165–176.

Rosenblum EB. 2005. The role of phenotypic plasticity in color variation of Tularosa Basin lizards. *Copeia* **2005:** 586–596.

Rosenblum EB. 2006. Convergent evolution and divergent selection: Lizards at the white sands ecotone. *The American Naturalist* **167:** 1–15.

Rosenblum EB, **Harmon LJ. 2010.** "Same same but different": replicated ecological speciation at White Sands. *Evolution* **65:** 946–960.

Rosenblum EB, **Rompler H**, **Schoneberg T**, **Hoekstra HE. 2010.** Molecular and functional basis of phenotypic convergence in white lizards at White Sands. *Proceedings of the National Academy of Sciences of the United States of America* **107:** 2113–2117.

Runemark A, **Brydegaard M**, **Svensson EI. 2014.** Does relaxed predation drive phenotypic divergence among insular populations? *Journal of Evolutionary Biology* **27:** 1676–1690.

Skelhorn J, **Rowland HM**, **Speed MP**, **Ruxton GD. 2010.** Masquerade: camouflage without crypsis. *Science* **327:** 51.

Staub NL, **Brown CW**, **Wake DB. 1995.** Patterns of growth and movements in a population of *Ensatina eschscholtzii platensis* (Caudata: Plethodontidae) in the Sierra Nevada, California. *Journal of Herpetology* **1995:** 593–599.

Stebbins RC. 1949. Speciation in salamanders of the plethodontid genus *Ensatina*. *University of California Publications in Zoology* **48:** 377–526.

Stebbins RC. 1954. Natural history of the salamanders of the plethodontid genus *Ensatina*. *University of California Publications in Zoology* **54:** 47–124.

Stelkens RB, **Jaffuel G**, **Escher M**, **Wedekind C. 2012.** Genetic and phenotypic population divergence on a microgeographic scale in brown trout. *Molecular Ecology* **21:** 2896–2915.

Storfer A, **Cross J**, **Rush V**, **Caruso J. 1999.** Adaptive coloration and gene flow as a constraint to local adaptation in the streamside salamander, *Ambystoma barbouri*. *Evolution* **53:** 889–898.

Stuart-Fox DM, **Moussalli A**, **Marshall NJ**, **Owens IP. 2003.** Conspicuous males suffer higher predation risk: visual modelling and experimental evidence from lizards. *Animal Behaviour* **66:** 541–550.

Thornton EB, **Sallenger A**, **Conforto J**, **Egley L**, **Mcgee T**, **Parsons R. 2006.** Sand mining impacts on long-term dune erosion in southern Monterey Bay. *Marine Geology* **229:** 45–58.

Urban MC, **De Meester L. 2009.** Community monopolization: local adaptation enhances priority effects in an evolving metacommunity. *Proceedings of the Royal Society B: Biological Sciences* **276:** 4129–4138.

Vignieri SN, **Larson JG**, **Hoekstra HE. 2010.** The selective advantage of crypsis in mice. *Evolution* **64:** 2153–2158.

Wake DB. 1997. Incipient species formation in salamanders of the *Ensatina* complex. *Proceedings of the National Academy of Sciences of the United States of America* **94:** 7761–7767.

Wake DB, **Yanev KP. 1986.** Geographic variation in allozymes in a "ring species," the plethodontid salamander *Ensatina eschscholtzii* of western North America. *Evolution* **40:** 702–715.

Wickham H, **François R**, **Henry L**, **Müller K. 2020.** *dplyr: A Grammar of Data Manipulation. R package version 0.8.4*. Available at: https://CRAN.R-project.org/package=dplyr

Willink B, **García-Rodríguez A**, **Bolaños F**, **Pröhl H. 2014.** The interplay between multiple predators and prey colour divergence. *Biological Journal of the Linnean Society* **113:** 580–589.

Yeaman S, **Whitlock MC. 2011.** The genetic architecture of adaptation under migration–selection balance. *Evolution* **65:** 1897–1911.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Summarized raw data for the number of model-nights, number of attacks and the attack rate with standard deviation for each morph as a whole and within each substrate colour.
Figure S1. Examples of variation within *Ensatina* typical brown morphs (A–C), leucistic morphs (D, E), and xanthistic morphs (F) at Fort Ord UC Reserve, Monterey County, California.
Figure S2. A, an example of one model cluster containing one of each morph, tied by fishing line to an anchor point. This is an example of a light substrate cluster where all models are placed on light-coloured sand. These models have been placed closer to each other in this case for the purposes of the photograph. B, an example of a predation event, a large bite mark, on the head of a typical brown morph. C, an example of a predation event, a small indent, on a leucistic morph model.
Figure S3. A, attack rates of each morph across substrate types. B, attack rates of the three morphs within each substrate type. Error bars represent standard error. *$P < 0.05$, **$P < 0.01$.
Figure S4. A, mean strikes-per-attack of each morph across substrate colours. B, mean strikes-per-attack of the three morphs within each substrate colour. Error bars represent standard error. **$P < 0.01$, ***$P < 0.001$.

SHARED DATA

The R code used to create the frequency map, Appendix S1 (iNaturalist data), and Appendix S2 (clay model data) are available from the DRYAD data repository (Rich *et al*., 2020).