

Natural selection in the origin: how does selection act on snails' shell colour in the source of a diversified population?

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Abstract

The mechanisms of adaptive radiation with phenotypic diversification and further adaptive speciation have been becoming clearer through a number of studies. Natural selection is one of the primary factors that contribute to these mechanisms. It has been demonstrated that divergent natural selection acts on a certain trait in adaptive radiation. However, it is not often known how natural selection acts on the source of a diversified population, although it has been detected in phylogenetic studies. Our study demonstrates how selection acts on a trait in a source population of diversified population using the Japanese land snail *Euhadra peliomphala simodae*. This snail's shell colour has diversified due to disruptive selection after migration from the mainland to islands. We used trail-camera traps to identify the cause of natural selection on both the mainland and an island. We then conducted a mark-recapture experiment on the mainland to detect natural selection and compare the shape and strength of it to previous study in an island. In total, we captured and marked around 1,700 snails, and some of them were preyed on by an unknown predator. The trail-camera traps showed that the predator is the large Japanese field mouse *Apodemus speciosus*, but this predation did not correlate with shell colour. A Bayesian approach showed that the stabilising selection from factors other than predation acted on shell colour. Our results suggest that natural selection was changed by migration, which could explain the ultimate cause of phenotypic diversification in adaptive radiation that was not due to predation.

Original Research

Natural selection in the origin: how does selection act on snails' shell colour in the source of a diversified population?

Short running title: Natural selection in a source population

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ABSTRACT

The mechanisms of adaptive radiation with phenotypic diversification and further adaptive speciation have been becoming clearer through a number of studies. Natural selection is one of the primary factors that contribute to these mechanisms. It has been demonstrated that divergent natural selection acts on a certain trait in adaptive radiation. However, it is not often known how natural selection acts on the source of a diversified population, although it has been detected in phylogenetic studies. Our study demonstrates how selection acts on a trait in a source population of diversified population using the Japanese land snail *Euhadra peliomphala simodae*. This snail's shell colour has diversified due to disruptive selection after migration from the mainland to islands. We used trail-camera traps to identify the cause of natural selection on both the mainland and an island. We then conducted a mark-recapture experiment on the mainland to detect natural selection and compare the shape and strength of it to previous study in an island. In total, we captured and marked around 1,700 snails, and some of them were preyed on by an unknown predator. The trail-camera traps showed that the predator is the large Japanese field mouse *Apodemus speciosus*, but this predation did not correlate with shell colour. A Bayesian approach showed that the stabilising selection from factors other than predation acted on shell colour. Our results suggest that natural selection was changed by migration, which could explain the ultimate cause of phenotypic diversification in adaptive radiation that was not due to predation.

KEYWORDS

Adaptive radiation, Colour diversification, Disruptive selection, Land snail, Mammal predator, Mark recapture, Stabilising selection, Trail camera

INTRODUCTION

Islands are an ideal model to study how phenotypic diversification occurs, including adaptive radiation. Natural selection is a primary influence that favours a certain trait and heightens it according to an adaptive optimum (Schluter, 2000). This influence can be dramatically changed, especially after migration into islands—for example, due to the expansion of habitat resulting from a release from competition (Yoder *et al.*, 2010; Stroud & Losos, 2016). When this occurs, the adaptive trait in a source population might sometimes be located in a “valley” of the fitness surface in an island population, so natural selection could then push it away from the valley to an adaptive peak (Schluter, 2000; Nosil, 2012). In such a case, it is well known that divergent natural selection, such as disruptive selection, acts on a certain trait as natural selection (Gavrilets & Losos, 2009; Martin & Wainwright, 2013; Gillespie *et al.*, 2020). Although stabilising selection should act on a trait in a source population if divergent natural selection acts in a population that has migrated, however, it has not often been unclear how selection acts, but determining it could insight the process of adaptive radiation due to release from stabilising selection (Yoder *et al.*, 2010).

Land snails on islands have also presented cases of trait divergence due to adaptive radiation, such as morphology and shell colour changes (Chiba, 1999a; Chiba & Davison, 2007; Parent & Crespi, 2006, 2009). Studies have demonstrated that character displacement plays a role in preventing interspecific competition (Chiba, 1996, 1999b; Chiba & Davison, 2007; Kimura & Chiba, 2010). Furthermore, land snails have many predators, such as mammals and birds, and it has been shown that some of them could regulate snails' traits (Barker, 2004; Chiba, 2007). Environmental conditions have also been shown to be a cause of trait divergence (Kraemer *et al.*, 2019). Overall, these factors cause disruptive selection and promote diversification by their combined effects, and sometimes, the proportions of effects fluctuate according to the degrees of impacts

such as the age of the island (Hayashi & Chiba, 2004; Kraemer *et al.*, 2019; Ito & Konuma, 2020). In many cases, phenotypic diversification has been a focus in adaptive radiation, but there are almost no studies on how the diversified traits are determined in the source population.

The shell colour diversification of snails has also been studied in the Japanese land snail *Euhadra peliomphala simodae* (Hayashi & Chiba, 2000, 2004; Ito & Konuma, 2020). This snail inhabits the mainland of Japan on the Izu Peninsula, as well as several of the Izu Islands. The mainland populations have monomorphic bright shells, while colour diversification from bright to dark is observed in the peripheral islands' sympatric populations (Hayashi & Chiba, 2004). Ancestral populations distributed on the mainland have migrated to the islands, where shell-colour diversification has rapidly occurred due to disruptive selection (Hayashi & Chiba, 2004; Ito & Konuma, 2020). However, it is unknown what the cause of the natural selection is in this case. Furthermore, it is also unknown what selection pressure has acted on the source population, what the differences are between the mainland and islands, and what the details of the adaptive radiation are among these land snails.

This study examined what species prey on the snails using trail-camera traps and the differences of predation effects between the mainland and islands. We then conducted a mark-recapture experiment with *E. p. simodae* on the mainland and estimated the natural selection pressures from predation and other factors. Finally, we compared the estimated selection pressures between the mainland and a peripheral island, Nijima Island (Ito & Konuma, 2020).

MATERIALS AND METHODS

Mark-recapture experiments

We chose the Izu Peninsula and Nijima Island as sites to study *E. p. simodae* populations on the mainland and an island, respectively (Figure 1). According to a previous study (Hayashi & Chiba, 2004), *E. p. simodae* that was distributed in the southern part of the Izu Peninsula migrated to the Izu Islands including Nijima Island, and then their shell colour diversified. A snail population in Minamiizu-cho on the Izu Peninsula was examined as a mainland ancestral population. A population on Mount Miyatsuka on Nijima Island was examined as an island descendent population. Both sites were narrow forest areas that were about 1 km long.

Mark-recapture surveys were conducted every month on the mainland (February 2019 - February 2020) and the island (March 2017 - October 2018). The details of the mark-recapture approach are described in a previous report (Ito & Konuma, 2020), so we include only an overview here. Each survey was conducted for 2–5 days. When we captured snails, we recorded their growth stage, geographic locations, and shell colour. The growth stage (juvenile or adult) was identified based on the shell lip. The locations where we captured snails were recorded using mobile GPS devices (eTrex LEGEND HCx, Garmin Ltd., Schaffhausen, Switzerland, on the mainland and a Garmin eTrex 10J on the island).

The shell colour was quantified using RGB values of photographs. Photographs of the snail shells were taken using a digital single-lens reflex camera (EOS Kiss X7; Canon, Tokyo, Japan) with an EF-S 60-mm f2.8 USM macro lens (Canon) under a ring light in a light-blocking box. On a computer monitor, a square area of a shell close to the shell aperture was cropped using the software Photos for Mac OS X (Apple Inc., Cupertino, CA, USA). 1000 pixels within cropped images were randomly chosen, and the RGB values of those pixels were automatically measured and averaged using Python 3 code.

The luminance value Y of the shell colour was calculated as follows: $Y = 0.299R + 0.587G + 0.114B$ (Schlick, 1995). The luminance value Y' of the grey scale image (Kodak Color Grey Scale; Eastman Kodak Co., Rochester, NY, USA) photographed with the shell together was also measured in the same way. Y/Y' was calculated to correct minor differences caused by light conditions, and then they were standardized. A higher Y/Y' value implies a brighter shell colour, whereas a lower Y/Y' value implies a darker shell colour.

The captured snails were marked on the shell by gluing on coloured plastic beads and scarifying numbers on them (Pierre-Yves Henry & Jarne, 2007). They were then released at the locations where they were caught.

This study was conducted according to the animal experimentation regulations of Tohoku University and Toho University.

Measuring predation pressures and identification of predators

The modes that predators use to attack gastropods are classified as shell entry, shell crushing, shell boring, and whole-shell swallowing (Vermeij, 1979; DeWitt & Robinson, 2000). Some recaptured snails that were dead showed evidence of shell-crushing, so we considered that they died from attacks by predators rather than old age. We compared the number of predated snails between the mainland and the island using Fisher's exact test. To examine whether the number of predations was associated with shell colour and growth stage, we also analysed them with a generalised linear mixed model (GLMM). Predation (1) and non-predation (0) were used as binary response variables, while the shell colour, growth stage (adults 1; juvenile 0), and their interaction were used as fixed effects. The month when the dead snails were recaptured was used as a random effect. "lme4" (Bates *et al.*, 2007) was used with logit as a link function in the statistical package R (R Core Team, 2020).

To identify the snails' predators, camera-trap experiments were conducted at the mainland study site from December 2019 to July 2020 and at the island study site from April 2017 to February 2018. At these study sites, snails were secured to trees with 5-cm-long strings (Daiso Ltd., Hiroshima, Japan; diameter 0.2 mm) and monitored using trail cameras (TROPHYCAM HD3 ESSENTIAL, Bushnell Ltd., Kansas, USA). Each camera was attached to a tree or on a tripod and operated on a motion trigger system. The camera automatically recorded videos when an animal entered the camera's sensor range. 4 and 5 camera traps were set up at the mainland and island study sites, respectively. The predators were identified from these videos in our laboratory.

Modelling of natural selection of shell colour in mainland

As in a previous study (Ito & Konuma, 2020), we used the survival rate as an index of fitness. First, second-order polynomial regression was used to estimate the selection gradients with Equation 1 (Lande & Arnold, 1983; Blows & Brooks, 2003; Gimenez *et al.*, 2009b). To visualise the survival rate according to shell colour, penalized spline regression (P-spline) was used with Equation 2 (Gimenez *et al.*, 2006a; b).

$$\text{logit}(S_{i,t}) = \mu + \beta \cdot \text{col}_i + \frac{1}{2} \cdot \gamma \cdot \text{col}_i^2 + \epsilon_i + B_t \quad \#(1)$$

$$\text{logit}(S_{i,t}) = f(\text{col}_i) + \epsilon_i + B_t \quad \#(2)$$

$$f(\text{col}_i) = \mu + \beta_1 \cdot \text{col}_i + \dots + \beta_p \cdot \text{col}_i^P + \sum_{k=1}^K b_k (\text{col}_i - \kappa_k)_+^P$$

$$t = 1, 2, \dots, 12; i = 1, 2, \dots, 1714 \text{ (in the Izu Peninsula)}$$

In these equations, $\text{logit}(x)$ means $\log[x/(1-x)]$. μ is the overall average for the survival rate on the logit scale. β is the linear selection gradient, which represents the strength and direction of directional selection. γ is the nonlinear selection gradient, which represents the strength of the disruptive selection when $\gamma > 0$ or that of the stabilizing selection when $\gamma < 0$. ϵ_i represents the individual heterogeneity, which follows a normal distribution with mean 0 and variance σ_ϵ^2 (Royle, 2008). B_t is the monthly effects on the logit scale for month t . P is the degree of freedom in the P-spline, which was set to $P = 3$ in this study. $(\text{col}_i - \kappa_k)_+^P$ is either $(\text{col}_i - \kappa_k)^P$ when $(\text{col}_i - \kappa_k) \geq 0$ or 0 otherwise. κ_k represents k 's fixed knots, with $\kappa_1 < \kappa_2 < \dots < \kappa_K$. The numbers of knots K is decided according to $K = \min(\frac{I}{4}, 35)$, so we set to $K = 35$ (Ruppert, 2002). We calculated $k/(K+1)$ quantiles by using all values of shell colour, with k varying between 1 and 35 (Ruppert, 2002). We assumed that the coefficient b_k of $(\text{col}_i - \kappa_k)_+^P$ follows a normal distribution with mean 0 and

variance σ_b^2 (Gimenez *et al.*, 2009a). The survival rate of this species shows monthly fluctuations (Ito & Konuma, 2020), so we considered the monthly effects t ($t = 1, 2, \dots, 12$).

We constructed a multistate state-space model to estimate the parameters in Equation 1 and 2. Natural selection was estimated while excluding the predation effects by using two stages for the “dead” state: “dead from predation” and “dead from other causes” (Schaub & Pradel, 2004; Marescot *et al.*, 2015). This model was constructed by extending a previous multistate model (Ito & Konuma, 2020). In this model, we defined the state matrix with 12 states (Equation. S1): (1) survival within the study site as a juvenile, (2) dead from a factor other than predation, such as temperature, within the study site as a juvenile, (3) dead from predation within the study site as a juvenile, (4) survival within the study site as an adult, (5) dead from a factor other than predation within the study site as an adult, (6) dead from predation within the study site as an adult, (7) survival outside the study site as a juvenile, (8) dead from a factor other than predation outside the study site as a juvenile, (9) dead from predation outside the study site as a juvenile, (10) survival outside the study site as an adult, (11) dead from a factor other than predation outside the study site as an adult, and (12) dead from predation outside the study site as an adult.

In the matrix, we defined the survival rate of juveniles and adults, the predation rate of juveniles and adults, the site fidelity rate, and the transition rate from juveniles to adults. The survival rate was applied to Equation 1 or 2 separately according to our purpose. The predation rate was assumed to be constant regardless of shell colour and month from comparing the causes of mortality among marked snails (see the results). The transition rate was assumed to have monthly fluctuation, and the site fidelity rate was considered constant according to a previous study (Ito & Konuma, 2020). The transition rate was modelled with a random effects model.

In the observation matrix, we defined seven observations states (Equation. S2): (1) first capture or recapture of a living juvenile snail, (2) recovery of a juvenile snail that died from causes other than predation, (3) recovery of a juvenile snail that died from predation, (4) first capture or recapture of a living adult snail, (5) recovery of an adult snail that died from causes other than predation, (6) recovery of an adult snail that died from predation, and (7) an undiscovered snail or unrecovered marked snail. In this matrix, the recapture rate was for live individuals, and the recovery rate was for marked dead individuals. These parameters were defined as differing between survey months, although they were the same for juveniles and adults. In addition, these parameters could vary according to shell colour (Ito & Konuma, 2020). We modelled the effect of shell colour using a GLMM framework, in which the link function was a logit function. In addition, the same individual heterogeneity $\varepsilon_{rp,i}$ was defined in these parameters. This heterogeneity parameter is normally distributed with mean 0 and variance σ_{rp}^2 . The probabilities model obtained from the state and observation matrices were defined with categorical distributions (Equations S3-S4; Kery & Schaub, 2012).

Estimating parameters in the Bayesian model

We used Markov chain Monte Carlo (MCMC) simulations in the Bayesian framework to estimate parameters in each model. In the model to estimate and visualise natural selection, we also used a non-informative prior distribution with all parameters described in a previous study (Ito & Konuma, 2020). The shell colour used in our models was standardised by the z-score before using the MCMC to avoid numerical instability and to improve the mixing of each chain (Gilks & Roberts, 1996). To obtain the posterior distribution of each parameter in our models, we then generated three Markov chains using 2,000 MCMC simulations that were thinned at a rate of five following the initial burn-in of 1,000 iterations. In the model to visualise natural selection, 2,500 MCMC simulations were required. The convergence with all simulations was confirmed by Gelman and Rubin statistics (Gelman *et al.*, 2011). Then, a 95% Bayesian confidence interval for all parameters was used for the posterior distribution summary. For the MCMC simulations, we used `pystan` (<https://github.com/stan-dev/pystan>) in Python 3.

RESULTS

In the mark-recapture experiment conducted on the mainland, we marked and released 1714 snails in total. We re-encountered 337 of the snails released in the field (257 living and 80 dead snails). We recaptured

some snails more than once, and the maximum number was four times. Most of the snails on the mainland had bright shells, and the distribution of the shell colour was unimodal (Figure 2A), while the shell colour distribution of the island was bimodal (Figure 2B).

Some recaptured dead snails had evidence of shell crushing by predators (Figure 3). There were 37 and 8 such snails on the mainland and island, respectively. More snails were predated on the mainland than the island (Figure S1; Fisher-exact test, odds = 9.46, $P < 0.001$). In the GLMM analysis, the number of predated snails was not significantly correlated with the shell colour in both study sites (Table 1; z -value = 2.86, $P > 0.05$). However, the number of predated snails was correlated with growth stage on the mainland (Table 1). This result implies that predators attacked more adult snails than juvenile ones on the mainland. We conducted trail-camera experiments to identify the predators and filmed eight mammals, four birds, and three reptiles within the monitoring snails' camera range (Table S1). Of these, the only large Japanese field mouse *Apodemus speciosus* was attacking the snails (Figure 3C; Movies S1-S3 in Dryad).

The P-spline regression showed that the survival rates excluding predation effects of mainland adult snails were not bimodal but monomodal in terms of shell colour (Figure 4A). The adult survival rate was estimated using a second-order polynomial function of shell colour in the MCMC simulations based on the Bayesian framework, and the median of the nonlinear selection gradient γ was -1.05 (Table 2). The negative value of γ supports that the P-spline regression curve was concave upward. Although the 95% Bayesian confidence interval (BCI) of γ was [-2.47, 0.33], 94.2% of γ values estimated in the simulation runs were negative. In contrast, the survival rates of mainland juvenile snails were not monomodal (Figure 4A), and the 95% BCI of γ included 0 (Table 2). The other parameters estimated from the Bayesian method are shown in Tables S2-S4.

DISCUSSION

In this study, we first demonstrated only a large Japanese field mouse, *A. speciosus*, preyed on snails, but the predation frequency was not correlated with shell colour (Figure 3; Table 1). To clarify the persistence processes of the diversified shell colour in snails, we then compared the colour variation and selection pressures of the snails between the mainland and the island. The frequency distributions of the shell colour showed that the colour variation was monomorphic on the mainland, while it was dimorphic on the island (Figure 2). The mark-recapture experiments showed that the survival rate excluding predation effects of bright-shell snails was the highest on the mainland, where predation pressure is higher than on the island (Figure 4A; Table 2).

The result of the survival rate of bright-shell snails being the highest suggests that weak stabilizing selection acts on shell colour from a factor other than predation on the mainland. This result differs from a previous study, which showed that disruptive selection acts on the shell colour on the island (Ito & Konuma, 2020). In the previous study, the disruptive selection estimated from the Bayesian method did not exclude predation effects. However, the proportion of dead snail shells from predation was relatively low on the island. Thus, it has been suggested that the cause of disruptive selection is also a factor other than predation. Hence, both stabilising selection and disruptive selection are caused by a factor other than predation. The stabilising selection favours bright snails, which could be why the shell colour variation is monomorphic on the mainland. In contrast, the disruptive selection favours dark and bright snails over intermediate-coloured snails, which could be why the shell colour variation is dimorphic on the island. The difference of the selection pressures would then result in the difference of shell colour diversity between the mainland and the island.

Strong disruptive selection acted only on juveniles on the island, and no natural selection acted on adults (Ito & Konuma, 2020). This pattern is opposite to that of the mainland. The survival rate on the mainland was lower among adults than juveniles, whereas that on the island was higher for adults than juveniles. Therefore, natural selection might only act on shell colour when the survival rate is low. The reason why natural selection acted in juvenile only on the island might be that post-zygotic isolation occurred (Wade, 2002; Coughlan & Matute, 2020). The distribution of shell colour was bimodal, in which darker and brighter snails existed sympatrically. The genetic relationship of the snails was close on the island, and hybrids of

darker and brighter snails showed intermediate colour (Hayashi & Chiba, 2004; Ito & Konuma, 2020). In contrast, there was a unimodal distribution of shell colour on the mainland, in which almost all of snails were a brighter colour. These results implied that the hybrid between dark and bright shells exists only on the island and that they have a lower survival rate since there is a genetic incompatibility between them.

In adults, one of the hypotheses for the cause of natural selection is that shell colour has a function of thermal regulation (Di Lellis *et al.* , 2012; Schweizer *et al.* , 2019). For example, the brighter shell could help to maintain an optimum body temperature when exposed to sunlight in open environments, while a darker shell could be advantageous when exposed to less sunlight in closed environments. The altitude of the distributed area is higher on the islands than the mainland (Figure 1). Thus, this geographical feature of the islands might create complex environmental conditions such as heterogeneity of habitats and lead to shell colour diversification (Ozgo & Schilthuizen, 2012). In contrast, predation from birds is an alternatives hypothesis because bird predators promote shell colour diversification (Allen *et al.* , 1988; Kraemer *et al.* , 2019). In a predation scenario, for example, a brighter shell colour has an advantage of camouflage in an open habitat, while a darker shell would have the opposite effect. Although no birds preyed on the snails that we studied, some birds were filmed in our trail-camera experiments. Hence, this possibility is lower than the environmental hypothesis for this snail, as in the case of polymorphism in *Cepaea nemoralis* (Schilthuizen, 2013).

The high predation pressure would be a primary factor in reducing the survival rates on the mainland. Our trail-camera experiments showed that field mice were the main predators of the snails. The number of the predated snails was not correlated with the shell colour in the mark-recaptured experiments, so the field mice are unlikely to predate snails based on dark or bright shells. In fact, rodents like this mouse do not recognise colour because they are colour blind with two cone types (Conway, 2007), so predation pressure from mice could occur randomly regardless of shell colour (Rosin *et al.* , 2011). Our results are consistent with this idea, so it is suggested that predation from field mice can be only a factor that reduces the survival. In addition, previous studies reported that field mice are poor at climbing trees and dwell on the ground all year (Imaizumi, 1978; Nishikata, 1981). Thus, the mainland snails would face constant predation risk from field mice on the ground. The adult snails need to come down from trees to lay eggs on the ground (Inoue & Nakada, 1981), so the evidence of predation would have been more frequently observed among adults than juveniles on the mainland.

However, predation could be an indirect driver of persistence in shell-colour monomorphism. Constant predation can result in restricted habitat or microhabitat use to avoid predation (Losos *et al.* , 2004; Lapiedra *et al.* , 2018). In such a case, a trait is fitted to the adaptive optimum according to the habitat (Eklöv & Svanbäck, 2006). In contrast, a restricted trait is diversified when a release from predation occurs (Stroud & Losos, 2016). In the Izu Islands, a release from predation could have triggered the use of a variety of habitats or microhabitats and then shell colour diversification.

In conclusion, our findings suggested that monomorphic shell colour persisted in the source population due to stabilising selection. In contrast, disruptive selection acted on shell colour on the island, which was diversified in derived population. Although it is unknown whether natural selection could be changed without predation effects, the direct cause of changing natural selection was a factor other than predation, such as environmental effects. In some land snails on islands, adaptive radiation can occur, and then shell colour is rapidly diversified (Davison & Chiba, 2006; Kraemer *et al.* , 2019). The change of natural selection from stabilising selection to disruptive selection that we have presented in this study could explain the ultimate factor of such shell colour diversification in the process of adaptive radiation, especially in an initial stage.

DATA AVAILABILITY STATEMENT

Supporting Movie S1-S3, source code and data used in the study are available from the Dryad Digital Repository. The stan codes used in mark-recapture are available from the GitHub.

REFERENCES

- Allen, J.A., Raymond, D.L. & Geburtig, M.A. 1988. Wild birds prefer the familiar morph when feeding on pastry-filled shells of the landsnail *Cepaea hortensis* (Müll.). *Biological journal of the Linnean Society*. **33** : 395–401. Oxford University Press. <http://dx.doi.org/10.1111/j.1095-8312.1988.tb00452.x>
- Barker, G.M. 2004. *Natural Enemies of Terrestrial Molluscs*. CABI, Wallingford, UK.
- Bates, D., Sarkar, D., Bates, M.D. & Matrix, L. 2007. The lme4 package. *R package version 2* : 74. <https://github.com/lme4/lme4>
- Blows, M.W. & Brooks, R. 2003. Measuring nonlinear selection. *The American naturalist*. **162** : 815–820. <http://doi.org/10.1086/378905>
- Chiba, S. 1999a. Accelerated Evolution of Land Snails *Mandarina* in the Oceanic Bonin Islands: Evidence from Mitochondrial DNA Sequences. *Evolution* **53** : 460–471. <http://doi.org/10.2307/2640782>.
- Chiba, S. 1999b. Character displacement, frequency-dependent selection, and divergence of shell colour in land snails *Mandarina* (Pulmonata). *Biological journal of the Linnean Society*. **66** : 465–479. <https://doi.org/10.1111/j.1095-8312.1999.tb01921.x>.
- Chiba, S. 1996. Ecological and morphological diversification within single species and character displacement in *Mandarina*, endemic land snails of the Bonin Islands. *Journal of evolutionary biology*. **9** : 277–291. <http://doi.org/10.1046/j.1420-9101.1996.9030277.x>
- Chiba, S. 2007. Morphological and ecological shifts in a land snail caused by the impact of an introduced predator. *Ecological research*. **22** : 884–891. <http://doi.org/10.1007/s11284-006-0330-3>
- Chiba, S. & Davison, A. 2007. Shell shape and habitat use in the North-west Pacific land snail *Mandarina polita* from Hahajima, Ogasawara: current adaptation or ghost of species past? *Biological journal of the Linnean Society*. **91** : 149–159. <http://doi.org/10.1111/j.1095-8312.2007.00790.x>
- Conway, B.R. 2007. Color vision: mice see hue too. *Current biology*. **17** : R457–60. <http://doi.org/10.1016/j.cub.2007.04.017>
- Coughlan, J.M. & Matute, D.R. 2020. The importance of intrinsic postzygotic barriers throughout the speciation process. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*. **375** : 20190533. <https://doi.org/10.1098/rstb.2019.0533>
- Davison, A. & Chiba, S. 2006. Labile ecotypes accompany rapid cladogenesis in an adaptive radiation of *Mandarina* (Bradybaenidae) land snails. *Biological journal of the Linnean Society*. **88** : 269–282. Narnia. <http://doi.org/10.1111/j.1095-8312.2006.00624.x>
- DeWitt, T.J. & Robinson, B.W. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. *Evolutionary Ecology Research*.
- Di Lellis, M.A., Seifan, M., Troschinski, S., Mazzia, C., Capowiez, Y., Triebkorn, R., *et al.* 2012. Solar radiation stress in climbing snails: behavioural and intrinsic features define the Hsp70 level in natural populations of *Xeropicta derbentina* (Pulmonata). *Cell Stress and Chaperones* **17** : 717–727. <http://doi.org/10.1007/s12192-012-0344-4>
- Eklöv, P. & Svanbäck, R. 2006. Predation risk influences adaptive morphological variation in fish populations. *The American naturalist*. **167** : 440–452. <https://doi.org/10.1086/499544>
- Gavrilets, S. & Losos, J.B. 2009. Adaptive radiation: contrasting theory with data. *Science* **323** : 732–737. <http://doi.org/10.1126/science.1157966>
- Gelman, A., Shirley, K. & Others. 2011. Inference from simulations and monitoring convergence. In: *Handbook of markov chain monte carlo* (S. Brooks, A. Gelman, G. L. Jones, & X. L. Meng, eds.), pp. 163–174. CRC Press Boca Raton, FL.

- Gilks, W.R. & Roberts, G.O. 1996. Strategies for improving MCMC. In: *Markov chain Monte Carlo in practice* (W.R., Gilks, S. Richardson, & D. J. Spiegelhalter, eds.), pp. 89–114. Chapman and Hall, New York, USA.
- Gillespie, R.G., Bennett, G.M., De Meester, L., Feder, J.L., Fleischer, R.C., Harmon, L.J., Hendry A.P., Knope, M.L., Mallet, J., Martin, C., Parent C.E., Patton, A.H., Pfenning K.S., Rubinoff, D., Schluter, D., Seehausen, O., Shaw, K.L., Stacy, E., Stervander, M., Stroud, J.T., Wagner, C. & Wogan, G.O.U. 2020. Comparing Adaptive Radiations Across Space, Time, and Taxa. *The Journal of heredity*. **111** : 1–20. <http://doi.org/10.1093/jhered/esz064>
- Gimenez, O., Bonner, S.J., King, R., Parker, R.A., Brooks, S.P., Jamieson, L.E., *et al.* 2009a. WinBUGS for Population Ecologists: Bayesian Modeling Using Markov Chain Monte Carlo Methods. In: *Modeling Demographic Processes In Marked Populations* (D. L. Thomson, E. G. Cooch, & M. J. Conroy, eds), pp. 883–915. Springer US, Boston, MA.
- Gimenez, O., Covas, R., Brown, C.R., Anderson, M.D., Brown, M.B. & Lenormand, T. 2006a. Nonparametric estimation of natural selection on a quantitative trait using mark-recapture data. *Evolution* **60** : 460–466. <http://doi.org/10.1111/j.0014-3820.2006.tb01127.x>
- Gimenez, O., Crainiceanu, C., Barbraud, C., Jenouvrier, S. & Morgan, B.J.T. 2006b. Semiparametric regression in capture-recapture modeling. *Biometrics* **62** : 691–698. <http://doi.org/10.1111/j.1541-0420.2005.00514.x>
- Gimenez, O., Grégoire, A. & Lenormand, T. 2009b. Estimating and visualizing fitness surfaces using mark-recapture data. *Evolution* **63** : 3097–3105. <https://doi.org/10.1111/j.1558-5646.2009.00783.x>
- Hayashi, M. & Chiba, S. 2004. Enhanced colour polymorphisms in island populations of the land snail *Euhadra peliomphala*. *Biological journal of the Linnean Society*. **81** : 417–425. <http://doi.org/10.1111/j.1095-8312.2003.00292.x>
- Hayashi, M. & Chiba, S. 2000. Intraspecific diversity of mitochondrial DNA in the land snail *Euhadra peliomphala* (Bradybaenidae). *Biological journal of the Linnean Society*. **70** : 391–401. <http://dx.doi.org/10.1111/j.1095-8312.2000.tb01230.x>
- Imaizumi, Y. 1978. Climbing Behavior of *Apodemus argenteus* and *Apodemus speciosus* (Rodentia : Muridae). *Applied entomology and zoology*. **13** : 304–307. <http://doi.org/10.1303/aez.13.304>
- Inoue, T. & Nakada, I. 1981. Electrophoretic Studies of Proteins in Egg and Albumen Gland of *Euhadra peliomphala*. *Seibutsu Butsuri Kagaku* **25** : 155–160.
- Ito, S. & Konuma, J. 2020. Disruptive selection of shell colour in land snails: a mark-recapture study of *Euhadra peliomphala* simodae. *Biological journal of the Linnean Society* **129** : 323–333. <http://dx.doi.org/10.1093/biolinnean/blz168>
- Kery, M. & Schaub, M. 2012. Estimation of survival and movement from capture-recapture data using multistate models. In: *Bayesian Population Analysis using WinBUGS: A Hierarchical Perspective* (M. Kery & M. Schaub, eds.). pp. 263–313. Elsevier, Amsterdam, Nederland.
- Kimura, K. & Chiba, S. 2010. Interspecific interference competition alters habitat use patterns in two species of land snails. *Evolutionary ecology*. **24** : 815–825. <https://doi.org/10.1007/s10682-009-9339-8>
- Kraemer, A.C., Philip, C.W., Rankin, A.M. & Parent, C.E. 2019. Trade-offs direct the evolution of coloration in Galápagos land snails. *Proceedings of the Royal Society B*. **286** : 20182278. <http://doi.org/10.1098/rspb.2018.2278>
- Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37** : 1210–1226. <http://dx.doi.org/10.1111/j.1558-5646.1983.tb00236.x>
- Lapiedra, O., Schoener, T.W., Leal, M., Losos, J.B. & Kolbe, J.J. 2018. Predator-driven natural selection on risk-taking behavior in anole lizards. *Science* **360** : 1017–1020. <http://doi.org/10.1126/science.aap9289>

- Losos, J.B., Schoener, T.W. & Spiller, D.A. 2004. Predator-induced behaviour shifts and natural selection in field-experimental lizard populations. *Nature* **432** : 505–508. <https://doi.org/10.1038/nature03039>
- Marescot, L., Forrester, T.D., Casady, D.S. & Wittmer, H.U. 2015. Using multistate capture–mark–recapture models to quantify effects of predation on age-specific survival and population growth in black-tailed deer. *Population Ecology*. **57** : 185–197. <http://doi.org/10.1007/s10144-014-0456-z>
- Martin, C.H. & Wainwright, P.C. 2013. Multiple fitness peaks on the adaptive landscape drive adaptive radiation in the wild. *Science* **339** : 208–211. <http://dx.doi.org/10.1126/science.1227710>
- Nishikata, S. 1981. Habitat Preference of *Apodemus speiosus* and *A. argenteus*. *Journal of the Japanese Forestry Society* **63** : 151–155.
- Nosil, P. 2012. *Ecological Speciation* . Oxford University Press, Oxford, UK.
- Ozgo, M. & Schilthuizen, M. 2012. Evolutionary change in *Cepaea nemoralis* shell colour over 43 years. *Global change biology*. **18** : 74–81. <http://doi.org/10.1111/j.1365-2486.2011.02514.x>
- Parent, C.E. & Crespi, B.J. 2009. Ecological opportunity in adaptive radiation of Galapagos endemic land snails. *The American naturalist*. **174** : 898–905. <https://doi.org/10.1086/646604>
- Parent, C.E. & Crespi, B.J. 2006. Sequential colonization and diversification of Galapagos endemic land snail genus *Bulimulus* (Gastropoda, Stylommatophora). *Evolution* **60** : 2311–2328. <http://doi.org/10.1111/j.0014-3820.2006.tb01867.x>
- Pierre-Yves Henry & Jarne, P. 2007. Marking Hard-Shelled Gastropods: Tag Loss, Impact on Life-History Traits, and Perspectives in Biology. *Invertebrate biology*. **126** : 138–153. <http://dx.doi.org/10.1111/j.1744-7410.2007.00084.x>
- R Core Team. 2020. R: A Language and Environment for Statistical Computing. <https://www.r-project.org/>
- Rosin, Z.M., Olborska, P., Surmacki, A. & Tryjanowski, P. 2011. Differences in predatory pressure on terrestrial snails by birds and mammals. *Journal of biosciences*. **36** : 691–699. <http://doi.org/10.1007/s12038-011-9077-2>
- Royle, J.A. 2008. Modeling individual effects in the Cormack-Jolly-Seber model: a state-space formulation. *Biometrics* **64** : 364–370. <https://doi.org/10.1111/j.1541-0420.2007.00891.x>
- Ruppert, D. 2002. Selecting the Number of Knots for Penalized Splines. *Journal of computational and graphical statistics*. **11** : 735–757. <https://doi.org/10.1198/106186002853>
- Schaub, M. & Pradel, R. 2004. Assessing the relative importance of different sources of mortality from recoveries of marked animals. *Ecology* **85** : 930–938. <http://doi.org/10.1890/03-0012>
- Schilthuizen, M. 2013. Rapid, habitat-related evolution of land snail colour morphs on reclaimed land. *Heredity* **110** : 247–252. <http://doi.org/10.1038/hdy.2012.74>
- Schlick, C. 1995. Quantization Techniques for Visualization of High Dynamic Range Pictures. In: *Photorealistic Rendering Techniques* (G. Sakas, P. Shirley, & S. Muller, eds.), pp. 7–20. Springer, Berlin, Germany.
- Schluter, D. 2000. *The ecology of adaptive radiation* . Oxford University Press, Oxford, UK.
- Schweizer, M., Tribskorn, R. & Köhler, H.-R. 2019. Snails in the sun: Strategies of terrestrial gastropods to cope with hot and dry conditions. *Ecology and evolution*. **9** : 12940–12960. <https://doi.org/10.1002/ece3.5607>
- Stroud, J.T. & Losos, J.B. 2016. Ecological Opportunity and Adaptive Radiation. *Annual review of ecology, evolution, and systematics* . **47** : 507–532. <http://doi.org/10.1146/annurev-ecolsys-121415-032254>
- Vermeij, G.J. 1979. Shell architecture and causes of death of micronesians reef snails. *Evolution* **33** : 686–696. <http://doi.org/10.1111/j.1558-5646.1979.tb04721.x>

Wade, M.J. 2002. A gene's eye view of epistasis, selection and speciation: A gene's eye view of epistasis. *Journal of evolutionary biology*. **15** : 337–346. <http://doi.org/10.1046/j.1420-9101.2002.00413.x>

Yoder, J.B., Clancey, E., Des Roches, S., Eastman, J.M., Gentry, L., Godsoe, W., Hagey, J., Jochimsen, D., Oswald, B.P., Robertson, J., Sarver, B.A.J., Schenk, J.J., Spear, S.F. & Harmon, L.J. 2010. Ecological opportunity and the origin of adaptive radiations. *Journal of evolutionary biology*. **23** : 1581–1596. <https://doi.org/10.1111/j.1420-9101.2010.02029.x>

Table 1. Results of generalized linear mixed model on the association of cause of death in marked land snails (0: the others, 1: predated) with shell colour and growth stage (juvenile and adult) in the Izu Peninsula and Niiijima Island.

	Fixed effect	Coefficient $\pm$ SE	Z-value	P-value
Izu Peninsula	Intercept	-1.24 $\pm$ 0.48	-2.56	< 0.05
	Shell Colour	0.28 $\pm$ 0.43	0.64	0.52
	Growth Stage	1.61 $\pm$ 0.56	2.86	< 0.001
	Interaction	0.09 $\pm$ 0.55	0.16	0.87
Niiijima Island	Intercept	-3.17 $\pm$ 0.93	-3.41	< 0.001
	Shell Colour	-0.15 $\pm$ 0.83	-0.18	0.86
	Growth Stage	0.49 $\pm$ 0.87	0.57	0.57
	Interaction	0.55	0.60	0.55

Table2. Estimated linear selection gradient β and nonlinear selection gradient γ and individual heterogeneity σ^2 using the Bayesian model on each growth stage.

Stage	Parameter	Median	SD	95% BCI	95% BCI
				2.5%	97.5%
Juvenile	β	-0.16	0.54	-1.35	0.73
	γ	-0.34	0.54	-1.35	0.73
	σ^2	3.68	3.63	0.21	12.9
Adult	β	0.01	0.66	-1.17	1.47
	γ	-1.05	0.70	-2.47	0.33
	σ^2	9.77	3.32	3.11	14.8

SD: Standard Deviation

Figure 1. A) Maps of study sites in the Izu Peninsula. *Euhadra peliomphala simodae* is distributed on the Izu Peninsula (mainland) and the Izu Islands including Niiijima Island. The survey was conducted on both the Izu Peninsula and Niiijima Island. B, C) The cross-section of the Izu Peninsula and Niiijima Island from the west side. In the Izu peninsula, *E. p. simodae* is inhabited only in southern part of it.

Figure 2. Frequency shell colour distribution on marked snails in A) the Izu Peninsula and B) Niiijima Island (modified from Ito and Konuma, 2020). Shell colour was quantified using the luminance value Y using a photograph of the snail, with higher values representing brighter colours. In the Izu Peninsula, the distribution showed unimodal and mainly bright shell colour existed whereas it showed bimodal, dark and bright shell colour existed in Niiijima Island.

Figure 3. Images of predated empty shell and the large Japanese field mouse *Apodemus speciosus*. A) the scene that *A. speciosus* predated a living snail which was tethered using sting in front of a trail camera. B) the shell left after a trail camera captures a scene that *A. speciosus* preyed on a snail. C) empty shells predated by *A. speciosus* found in mark-recapture survey. It has characteristics the splitting of the apex or

the shattering of the shell.

Figure 4. The relationship between survival rate and shell colour in A) the Izu Peninsula and B) Niijima Island (modified from Ito and Konuma, 2020); I) is juvenile, II) is adult. This shape represents the natural selection worked on shell colour. In the Izu Peninsula, the natural selection was estimated after excluding predation effects. The solid and dashed lines indicate the median and the 95% Bayesian confidence interval, respectively, in the posterior distribution obtained from Markov chain Monte Carlo simulations.





