Chemical Signaling In An Inducible Offense

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Abstract:

Inducible phenotypic plasticity is the ability of an organism's genotype to express different observable traits or phenotypes, such as morphology, over and individual's lifespan. The snails *Lacuna vincta* and *L. variegata* produce two different tooth morphologies, blunt or pointed, depending on their diet. They are found in two habitats, eelgrass and macroalgae. We tested whether there are different chemical cues from macroalgae, a kelp in this case, diatoms, or *Zostra marina*, eelgrass, that trigger different tooth morphologies in these two species of *Lacuna* to understand what induces the change in phenotype. Changes in tooth morphology during the experiment was different for snails from two source locations, False Bay, an eelgrass bed, and Pile Point, which is dominated by macroalgae, which may reflect different prior environmental variability or stability. Most animals of both species produced pointed teeth on the neutral diet of lettuce, as well as when fed kelp or eelgrass, suggesting this is a default morphology, and blunt tooth production requires a cue for production. Our data suggest that alginate is not a neutral carrier for food, and affected the rate of tooth change. Further experimentation is needed to determine if diatoms produce chemical cues that trigger blunt teeth.

Project: Chemical Signaling In An Inducible Offense

Introduction

Phenotypic plasticity is the ability of an organism's genotype to express different observable traits or phenotypes, such as morphology. A wide range of species, terrestrial to marine and plants to animals, have expressed phenotypic plasticity in response to their environment (West-Eberhard 1989; Padilla and Savedo 2013). Traits expressing phenotypic plasticity may or may not have adaptive value (Padilla and Adolph 1996). In patchy environments, these traits can be adaptive when different phenotypes function differently in different environments (reviewed in Padilla and Savedo 2013). Costs of phenotypic plasticity can include the mismatching of phenotype and environment, cost of the change, and the lag time between environments (Padilla and Adolph 1996).

Two types of phenotypic plasticity are inducible defenses and inducible offenses. Inducible defenses are phenotypic changes within an individual's lifetime that are triggered by the presence of a consumer. This type of phenotypic plasticity is effective at reducing the risk or consequences of predation; for example, the snail, *Nucella lamellosa*, produces a thick shell in the presence of their crab predator and a thin shell with the absence of the predator (Appleton and Palmer 1988). Inducible offenses are phenotypic changes within an individual's lifetime that are triggered by the presence of competitors or prey for a consumer. Inducible offenses enhance competitive ability for a limited resource (Harvell and Padilla 1990, Padilla et al. 1996), or enhance the ability of a consumer to obtain or consume food (Padilla 2001). For example, the brachyuran crab, *Cancer productus,* develops larger and stronger claws when feeding on hard prey (Smith and Palmer 1994).

Inducible defenses and offenses allow organisms to respond to environmental change throughout their life. They allow better phenotypic matching between habitats and organisms in patchy environments and when organisms are expected to encounter different environmental conditions and different phenotypes provide fitness advantage in each of the habitat types. An organism without inducible phenotypic plasticity can have a phenotype that matches the dominant habitat type or a phenotype that performs well on average in a heterogeneous environment. However, not all traits are phenotypically plastic. A fixed phenotype would be advantageous if there are costs to having a plastic phenotype that outweigh the benefits. For plasticity to be advantage, there must be reliable, detectable cues that an organism can respond to when the environment changes; furthermore, the lag time needed to produce a different, useable, phenotype must be less than rate of environmental change (Padilla and Adolph 1996). In addition, any regulatory or sensory machinery needed to produce different phenotypes, or the opportunity cost of having one phenotype versus another, needs to be taken into account to determine if a plasticity will be advantageous (Dewitt et al. 1998).

The Radula

The radula is a feeding organ found in most gastropods and can be highly specialized depending on diet (Peile 1937; Padilla et al. 1998). It is a long chitinous ribbon consisting of repeated rows of teeth. These rows of teeth are continually produced and shed when worn throughout a snail's lifetime (Padilla et al. 1996). Two species of snails, *Lacuna vincta* and *L. variegata*, can change tooth morphology depending on their food source (Padilla 1998). Both species of *Lacuna* will produce blunt teeth when raised in an eelgrass bed environment, where they feed on diatom epiphytes on the seagrass, and

pointed teeth when raised in a macroalgal environment, where they feed on fleshy macroalgae, including kelp. Blunt shaped teeth are more effective at scraping diatoms off of the surface of eelgrass blades and pointy teeth are more effective when consuming soft, fleshy macroalgae (Padilla 1998). After metamorphosis from the larval stage, *Lacuna* can settle in a macroalgal or seagrass environment, therefore is advantageous to be able to switch tooth morphology to match their diet. Additionally, *Lacuna* can float using mucus threads and move between habitats when food is degraded, or depleted, when predators are present, or they are disturbed (Martel and Chia 1991, Martel and Diefenbach 1993, Padilla unpublished data). Inducible phenotypic plasticity is advantageous for this lifestyle, because snails can switch between these two phenotypes throughout their life.

Cues to Phenotypic Plasticity

Understanding the mechanism or mechanisms behind phenotypic plasticity are important because cues must be detectable and reliable indicators of the environment (reviewed in Padilla and Savedo 2013). Chemical cues are useful because they can indicate environmental change more quickly rather than direct exposure. Additionally, an organism needs to discriminate cues to avoid mistakes. An organism that expresses a phenotype that does not match its environment may have a lower fitness and be less advantageous. In marine systems chemical cues are common and are used for navigation, to locate food, and to identity suitable habitats (reviewed in Padilla and Savedo 2013). In inducible defenses, chemical cues are often used to minimize consumption; for example some species of subtropical green algae produce toxic chemicals that deter herbivory (reviewed in Paul and Fenical 1986, Padilla and Savedo 2013). In some species of algae,

chemical defenses are produced at higher concentrations in areas of high herbivory (Paul and Fenical 1986).

Chemical cues have not yet been shown in inducible offences or in marine plant-herbivore interactions. In laboratory experiments, *Lacuna* will change tooth morphology when switched from a kelp habitat where it is fed kelp, to an eelgrass habitat where it is fed diatom covered eelgrass, and vice versa (Padilla 1996). However, *L. variegata* will produce blunt teeth when in an experimental eel grass environment regardless of a diet of kelp or diatoms (Padilla 2001). Because *L. variegata* will produce blunt teeth while feeding on kelp in an eelgrass environment, this indicates that the cue for the inducible blunt morphology is likely due some sort of chemical signal.

Question

The cues/signals that induce the switching between phenotypes for this phenotypically plastic trait are unknown. Here, we test whether there are different chemical cues from macroalgae, a kelp in this case, diatoms, or *Zostra marina*, eelgrass, that trigger different tooth morphologies in two species of *Lacuna* to understand what induces the change in phenotype.

Methods and Materials

Lacuna are common snails along the low intertidal and shallow subtidal shores ranging from Alaska to California. They consume micro and macro algae, and live either on macroalgae, which they consumer, or eelgrass and surfgrass, where they consume epiphytes growing on the grass. They live approximately 6 months to one year, are

dioecious, and are reproductively active year-round. In the San Juan Arichipleago there are two sympatric species of *Lacuna, L. vincta* and *L. variegata.*

Snails were collected at Pile Point (48.29N and 123.06W; PP) and False Bay (48.28N, -123.04W; FB), WA. At PP, *L. vincta* was collected from a low intertidal zone kelp habitat; snails from this site were expected to have pointed shaped teeth (Padilla 2001). Both *L. vincta* and *L. variegata* were collected from an eelgrass habitat in False Bay, and snails from this site were expected to have blunt shaped teeth (Padilla 2001). 60% of the *Lacuna* collected at FB were *L. vincta*, and 40% were *L. variegata*.

Treatments

L. vincta from PP and FB, and *L. variegata* from FB were given one of 5 food treatments with 10 replicates each. Food treatments were created using lyophilized kelp (*Saccharina latissima*), eelgrass (*Zostera marina*), or a mixture of diatom species that are common epiphytes on eelgrass (*Entomoneis* sp., *Melosira* sp. and *Odontella* spp.) combined with alginate. Food for the control treatments were alginate with lyophilized lettuce (organic romaine), and fresh lettuce.

Alginate foods were made by mixing 1.5 g of sodium alginate with 50 ml of tap water with a MiniMixer. Then 50 ml of lyophilized food was mixed into the alginate and the mixture was left still until the lyophilized food rehydrated. After rehydration, the mixture was poured onto 20.32 x 20.32 cm glass plate and submerged in 0.25M CaCl. A second plate was used to compress the mixture until it formed a flat layer and hardened. The test food was then cut into smaller pieces (~25x25 mm) and stored in a -20C freezer until used in the experiment.

Experimental Design

Three snails, one species from each location, were placed into cylindrical screened cages (90 ml volume, interior dimensions – 43.4 mm diameter, 61.4 mm height) with the designated food treatment. The cages were placed in individual 1.32 L containers with flow through seawater. *L. variegata* used in the experiment were from a single site and the shell color pattern of this species is distinct from *L. vincta*. To identify PP and FB *L. vincta* in each cage, shell color morphs, either solid color without contrasting stripes or bands, or shells with bands were used to contrast locations; 5 snails of each shell variation was used for each replicate within a treatment. Previous studies suggest that there are no differences between banded and solid shell variations except appearance (Padilla unpublished data).

The 50 containers were allotted to 3 sea tables for this experiment. A current of flowing sea water was delivered to each container individually to prevent any crossing of chemical cues. To get equal flow through each container, a header take with 8-9 ports fed into 7.93 mm diameter line that split with a T-connector to 4.76 mm diameter line. Each line produced a flow rate of 6.36 L/hr in each container.

Snails had access to the same food treatment throughout the experiment and excess food was always present. The food for the alginate treatments was replaced two times after the initial feeding. For the lettuce only treatment, the lettuce was replaced every other day or as needed as it degraded quickly in the seawater. During food changes, containers were rinsed two times with seawater to remove old food and fecal pellets. Containers used during food changes were washed after each treatment to prevent the transfer of chemical cues. Dead snails found during the experiment were

recorded and replaced. Snails were exposed to the food treatment for 18 days. After 18 days, animals were frozen and remained frozen until dissection.

Radula Dissection

Before dissection, snails were defrosted and the shell length (from the apex to the base of the shell along the coiling axis) and total damp weight and sex were recorded. The radula was removed by dissection and rinsed with one drop commercial bleach in 5 ml of RO water to clean tissue from the radula. The cleaned radula was mounted on a glass slide using a water soluble mounting medium (polyvinyl lactophenol). Fifteen snails of each species from each site were frozen after collection and later dissected to determine the initial shapes of the teeth of snails used in the experiment.

Lacuna replaces 3 rows of teeth per day (Padilla et al. 1996). Therefore, to determine which day during the experiment individual teeth were produced, the number of tooth rows from the youngest part of the radula to the tooth row of interest can be counted and divided by 3, producing the number of days after the initiation of the experiment that tooth row was produced. We recorded the initial tooth morphology, those teeth produced prior to the initiation of the experiment, and categorized the tooth as blunt or pointed based on the shape of the central cusp of the rachidian (Figure 1). Final tooth morphology was recorded, as well as any tooth shape produced during the experiment. If there was a change in tooth shape during the course of the experiment, we determined the day that the change occurred by counting the row where a change took place divided by 3, the average number of tooth rows produced each day (Padilla et al. 1996).

Statistics

We used a G-test to determine if different frequencies of snails responded to different treatments by changing the shapes of their teeth when given different treatments. Analysis of variance (ANOVA) was used to test for differences among treatments in the number of days until a morphological change was seen.

Results

Due to mortality, 47 *L. variegata* (from False Bay FB), 47 FB *L. vincta*, and 50 PP *L. vincta* (from Pile Point) snails were dissected at the end of the experiment. FB *L. variegata* ranged in size from 4.18-8.80 mm shell length with a mean of 6.32 ± 0.22 mm. All *L. vincta* used in this experiment ranged from 3.15-9.22 mm in shell length with a mean of 5.90 ± 0.11 mm. *L. variegata* had a mean weight of 6.34 ± 0.22 g, and *L. vincta* had a mean weight of 5.90 ± 0.11 g. There was no significant difference in size between the *L. vincta* snails from FB and PP (two-sample t-test, P = 0.18). Furthermore, there was no significant difference in shell length between *L. variegata* and *L. vincta* used (two-sample t-test, P = 0.053).

The sex of snails in the experiment could not be determined until they were dissected at the end of the experiment. 46.8% of *L. variegata* at the end of the experiment were female, 38.3% male, and sex was not determined for 14.9% of snails used. 55% of *L. vincta* were female, 33.0% male, and sex was not determined for 11.3% of snails used.

Initial Tooth Morphology

Because the experiment was ended before the full radula was replaced, we were able to determine the shape of teeth animals were producing at the start of the experiment. All *L. variegata* from the FB site had blunt shaped teeth at the start of the experiment. For the *L. vincta* from the FB site, 46.8% had blunt and 53.2% had pointed shaped teeth at the start of the experiment. Only 2.0% (one animal) of *L. vincta* from the PP site had blunt shaped teeth, and 98.0% had pointed shaped teeth at the start of the experiment.

Effect of Test Diets

There was difference between the number of snails that changed tooth morphology during the experiment for the two source locations (G-test, df = 1, G=29.19, $P < 0.0005$). FB snails changed tooth morphology more frequently than PP snails. There was also a significant difference in the number of snails that changed teeth for the *L. vicata* and *L. variegata* from FB (G-test, df = 1, G = 6.58, $P < 0.01$). FB *L. vincta* changed tooth morphology more frequently than FB *L. variegata.* Additionally, there was a significant difference between the lag time of phenotypic change for the two species (two-sample t-tests, unequal variances, P = 0.023). When tooth shape changed during the experiment, FB *L. vincta* responded more rapidly than the FB *L. variegata*. The average lag time between exposure to the test food and morphology change was 3.7 ± 1.1 days (N = 22) for FB *L. vincta* , and 7.9 ± 1.3 days (N = 12) for FB *L. variegata*.

Alginate + Lettuce (A+L)

In the A+L treatment, all FB *L. vincta* that started with blunt teeth (n = 5, 100%) switched to pointed teeth, and all the FB *L. vincta* that started with pointy teeth (n = 5,

100%) continued to produce pointy teeth. All *L. vincta* from the PP site started with pointy teeth and continued to produce pointed teeth throughout the experiment (Figure 1). All *L. variegata* in this treatment started with blunt teeth. One *L. variegata* (11%) switched from blunt to pointed teeth, the remaining 8 snails (89%) continued to produce blunt teeth and did not change their tooth morphology over the course of 18 days (Figure 2).

In the alginate + lettuce treatment, there was a significant difference between the number of snails that changed tooth morphology for the two populations (G-test, df = 2, G = 10.28, P < 0.01). Snails from False Bay changed morphology, whereas snails from Pile Point did not. Here and elsewhere, the day of tooth morphology change was calculated by the number of days of the experiment (18) minus the number of newly produced tooth rows divided by 3 (snails produce 3 rows of teeth per day, Padilla et al. 1996). To account for small differences in tooth production rate and counting error, we included all animals that had changed morphology within 2 days of the start and end of the experiment. 6 snails in total changed tooth morphology during the experiment with a mean of 2.6 ± 2.8 days. Of the FB *L. vincta* that changed tooth morphology, the average change was at 2.5 ± 3.40 days and ranged from day -2 – day 16 (Figure 7a). The one *L. variegata* that changed morphology, from blunt to pointed shaped teeth, changed morphology on day 3 of the experiment.

Alginate + Kelp (A+K)

In the A+K treatment, 55.6% of the FB *L. vincta* started with blunt teeth (N = 5) and the other 44.4% started with pointy teeth (N = 4). Of the FB *L. vincta* that started with blunt teeth, all switched to pointy teeth over the course of this experiment, and all of

the FB *L. vincta* that started with pointy teeth continued to produce pointy teeth. All PP *L. vincta* started with pointy teeth and produced only pointy teeth in this treatment. All FB *L. variegata* in this treatment started with blunt teeth. 22.2% of the *L. variegata* switched to pointy teeth (N = 2), and 77.8% continued to produce blunt teeth (N = 7, Figure 3).

There was a significant difference between the number of snails that changed tooth morphology for the two populations (G-test, df = 2, G = 10.28, P < 0.01). FB snails changed morphology, whereas PP snails did not. Of the *L. vincta* and *L. variegata* that changed tooth morphology in this treatment (N = 7), the average number of days to morphological change was 4.5±2.0 days and ranged from day -0.33 to day 16 (Figure 6b.).

Alginate + Zostra (A+Z)

In the A+Z treatment, 44.4% of the FB *L. vincta* started with blunt teeth (N = 4), while 55.6% started with pointed teeth (N = 5). All the FB *L. vincta* that started blunt teeth switched to producing pointy teeth. All the PP *L. vincta* started with pointy teeth continued to produce pointy teeth during this experiment. All FB *L. variagata* began with blunt teeth. 20.0% switch to producing pointy teeth (N = 2), and 80% continued to produce blunt teeth (N = 8, Figure 4).

In the alginate + zostra treatment, there was a significant difference between the the number of snails that changed tooth morphology and source site (df = 2, G = 7.28, P = < 0.05). The snails from FB changed tooth morphology more frequently than those from

PP, which did not change. The mean day that snails switched tooth morphology was 4.1±1.9 days (N=5) into the experiment and ranged from day 0 to day 9.7 (Figure 7c).

Alginate + Diatoms (A+D)

In the alginate + diatom treatment, 50% (N = 4) of the FB *L. vincta* started with blunt teeth, while the other 50% started with pointy teeth (N = 4). 71.4% changed tooth morphology over the course of this experiment. Of the 71.4% of snails that changed tooth morphology, 20% change from blunt to pointy teeth (N =1), and 40% changed from pointy to blunt teeth (N = 2). 2 of the FB *L. vincta* snails (40%) switched their tooth morphology from blunt-pointy-blunt or blunt-pointy-blunt-pointy over the 18 day experiment (Figure 5). 100% of the PP *L. vincta* started with pointy teeth and remained pointy. All FB *L. variegata* snails started with blunt teeth. 20% FB *L. variegata* snails (N = 2), switched tooth morphology; however, both snails alternated tooth morphology from blunt to pointed to blunt to pointed over the course of the experiment (Figure 5).

In the A+D treatment, there was a statistically significant difference between the number of snails that changed tooth morphology for snails from the two source populations (G-test, df = 2, G = 11.94, $P < 0.005$). FB snails changed more frequently. Snails changed tooth morphology with a mean of 8.3 ± 2.5 (N = 7) and ranged from day -1.3 to day 14 of the experiment (Figure 6d).

Lettuce (L)

Of the snails fed fresh romaine lettuce, 70% (N = 7) of the FB *L. vincta* started with pointed teeth, while 30% started with blunt teeth (N = 3). All 3 of the FB *L. vincta* that started with blunt teeth switched to pointed teeth. 90% PP *L. vincta* snail started with

pointy teeth (N = 9), and 10% started with blunt teeth (N = 1). The one PP snail that started with blunt teeth switched to pointed during the experiment. All FB *L. variegata* started with blunt teeth (N = 9), and 55.6% changed from blunt to pointed (N = 5), while 44.4% of FB L. variegata continued to produce blunt teeth (N = 4, Figure 6).

There was no significant difference between the numbers of animals that changed morphology for the two source populations (G-test, df = 2, G = 5.04, P > 0.10). On average, *Lacuna* switched tooth morphology after 5.0 ± 1.1 days (N = 9), and ranged from day 1 to 10.7 days (Figure 7e).

Lettuce and Alginate + Lettuce

There was a significant difference in response of FB *L. variegata* and FB *L. vincta* to the two different treatments with the neutral diet of lettuce, the A+L and fresh lettuce treatments, in response for these two species (G-test, G = 4.27, df = 1, P < 0.05). *L. variegata* responded more to the fresh lettuce than the A+L treatment, while FB *L. vincta* responded similarly to the two diets (Figure 2, 6).

Mortality

There was a significant difference in mortality between the species during the experiment (G-test, G =4.52, df = 1, P < 0.05). *L. variegata* had a higher mortality rate in this experiment than *L. vincta*. There was no significant in difference mortality among the 5 treatments (G-test, G = 3.34, df = 4, P > 0.05).

Discussion

We found a difference in the number of snails that changed tooth morphology during the experiment for the two source locations, suggesting that prior environmental history can affect the likelihood that a snail will respond phenotypically to a new environment within a short period of time. FB snails changed tooth morphology more frequently than PP snails. Furthermore, FB *L. vincta* change more frequently than FB *L. variegata*. We predicted that animals collected from FB would express the blunt phenotype. While all FB *L. variegate* snails began with blunt teeth, 46.8 % of the FB *L. vincta* snails began with blunt teeth and 53.2% began with pointy teeth. This original miss-matched phenotype suggests that some of the FB *L. vincta* recently migrated from another location and had not switched their phenotype. We suggest that animals that have experienced patchy or a more variable environment are more likely to switch phenotypes in a shorter period of time. This is consistent with the lag times we found for morphological change; for animals that switched morphology during the experiment, FB *L. vincta* changed phenotype quickly, 3.7 ± 1.1 days, whereas FB *L. variegata* changed in 7.9 ± 1.3 days.

Results from the lettuce treatment suggest that the pointed tooth phenotype is the default tooth morphology. All snails that change phenotype produced pointy teeth, and no individuals switched from pointed to blunt. Because there is a default phenotype, there is likely only one signal to induce the blunt phenotype.

Padilla (2001) demonstrated that *Lacuna* raised on kelp in an eelgrass environment will produce the blunt phenotype, which suggests that the cue for the inducible offense in this system is a chemical response. In this experiment, alginate + eelgrass did not produce a chemical signal to induce the blunt phenotype. Because

eelgrass does not trigger the change, we would expect the diatoms to induce the blunt phenotype. We observed mixed results in our alginate + diatom treatment. Of the FB *L. vincta* in this treatment, 40% of animals responded as predicted, however 20% did the opposite, and 20% alternated between phenotypes. Additionally, we found animals that produced deformed cusps and strange tooth shapes that are not seen in nature. Mixed results may be because the concentration of chemical signals was not strong enough or consistent enough to induce change, or the alginate altered or masked the chemical signal in some way. We suggest it is the later because of the difference between the fresh lettuce and alginate + lettuce treatment. Because more FB *L. variegata* snails changed phenotype in the fresh lettuce than the alginate + lettuce treatment, alginate is not a neutral medium. Future studies need to be completed in order to develop a better delivery method.

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References

Appleton RD, and Palmer AR. 1988. Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. *Proc Natl Acad Sci* 85: 4387-4391.

DeWitt TJ, Sih A, and Wilson DS. 1998. Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* 13:77–81.

Harvell CD, and Padilla DK. 1990. Inducible morphology, heterochrony, and size hierarchies in a colonial invertebrate monoculture. *Proc. Nati. Acad. Sci. USA.* 87: 508-12.

Martel A, and Chia FS. 1991. Foot-raising behaviour and active participation during the initial phase of post-metamorphic drifting in the gastropod *Lacuna* spp. *Mar Ecol Progr Ser* 72: 247–254.

Martel A, and Diefenbach T. 1993. Effects of body size, water current and microhabitat on mucous-thread drifting in post-metamorphic gastropods *Lacuna* spp. *Mar Ecol Prog Ser* 99: 215-220.

Padilla DK. 1998. Inducible phenotypic plasticity of the radula in *Lacuna* (Gastropoda: Littorinidae). *Veliger* 41: 201-204

Padilla DK. 2001. Food and environmental cues trigger an inducible offence. *Evol. Ecol. Res.* 3: 15–25.

Padilla DK, and Adolph SC. 1996. Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. *Evol Ecol* 10: 105-117

Padilla DK, Dittman DE, and Franz J, Sladek R. 1996. Radular production rates in two species of *Lacuna* Turton (Gastropoda: Littorinidae). *J Molluscan Stud.* 62: 275-280.

Padilla DK, and Savedo MM. 2013. A systematic review of phenotypic plasticity in marine invertebrate and plant systems. *Adv. Mar. Biol*. 65: 67-94.

Paul VJ, and Fenical W. 1986. Chemical defense in tropical green algae, order Caulerpales. *Mar. Ecol. Prog. Ser.* 34: 157-169.

Piele AJ. 1937. Some radula problems. *J. Conchol.* 20(10): 192-304.

Smith LD, and Palmer AR. 1994. Effects of manipulated diet on size and performance of brachyuran crab claws. *Science.* 264: 710–712.

West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* 20:240-78.

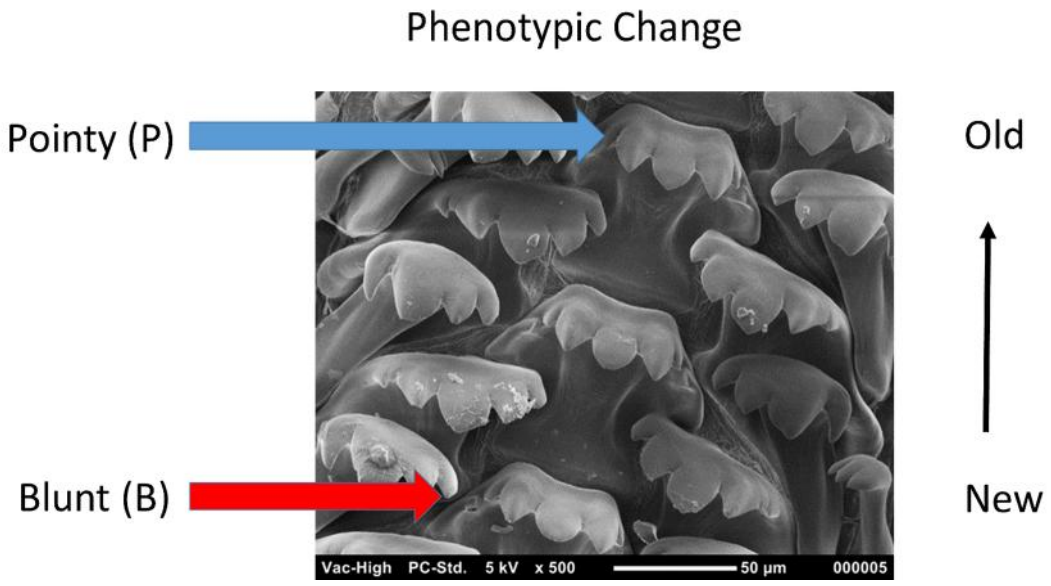

Figure 1. Scanning electron micrograph of the phenotypic change in tooth morphology in *Lacuna vincta*. Tooth shape can be pointed (blue arrow) or blunt (red arrow). The blunt tooth in this image was produced within one day of the older pointed tooth. The scale bar is 50 µm.

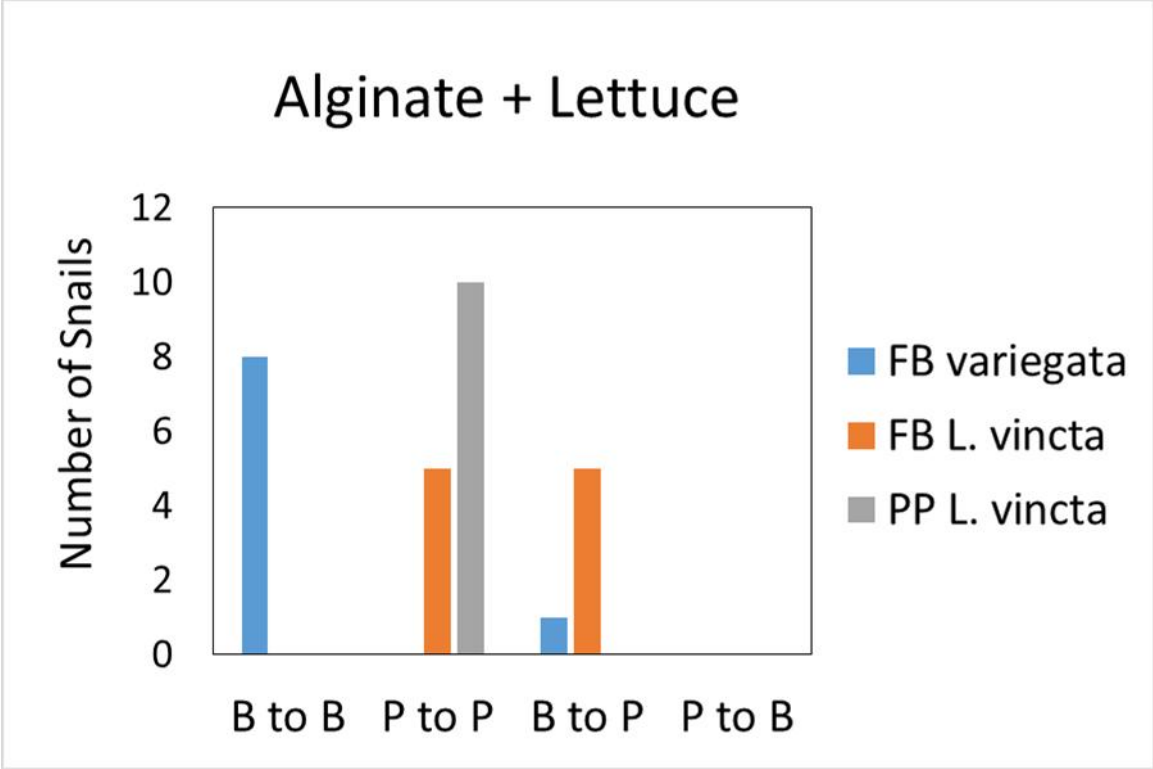

Figure 2. The number of snails that changed tooth morphology when fed alginate + lettuce. B to B = no change from a blunt morphology, P to P = no change from a pointed morphology. B to P = change from a blunt to a pointed morphology, and P to B = change from a pointed to blunt morphology. In the alginate + lettuce treatment, all animals that change phenotype switched to the pointy tooth morphology. No snails switched from pointed to blunt. FB *L. vincta* (red bar) changed phenotype more frequently than FB *L. variegata* (blue bar) and PP *L. vincta* (gray bar).

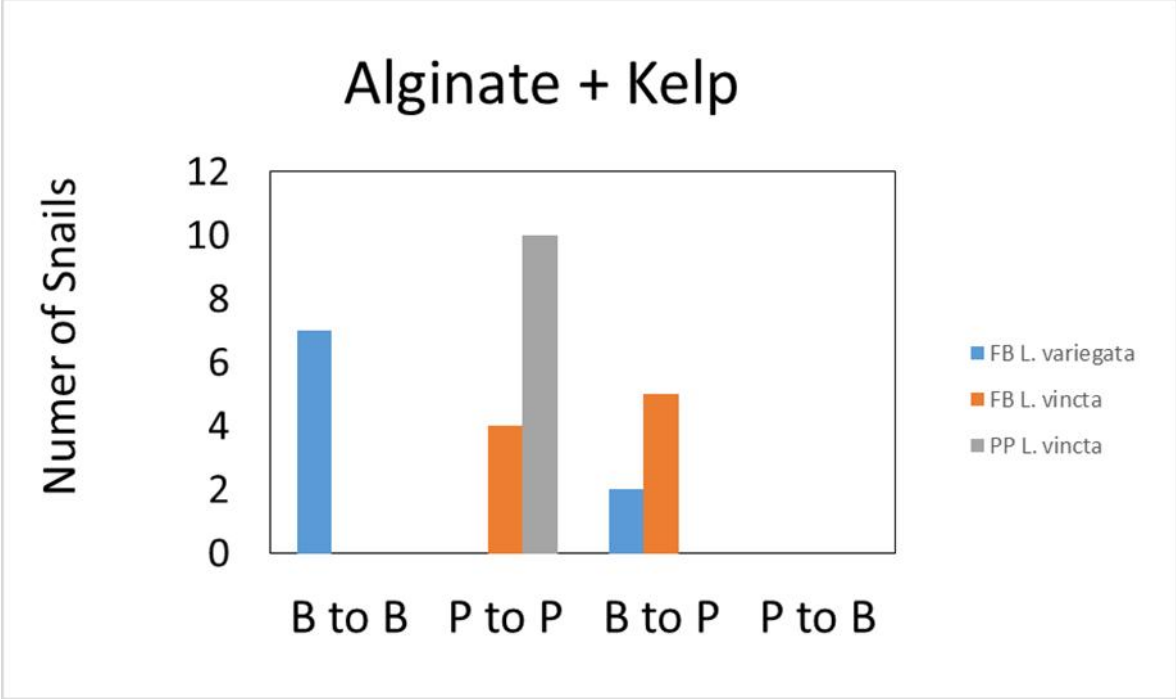

Figure 3. The number of snails that changed tooth morphology when fed alginate + kelp. B to B = no change from a blunt morphology, P to P = no change from a pointed morphology. B to P = change from a blunt to a pointed morphology, and P to B = change from a pointed to blunt morphology. All animals that changed phenotype switched to the pointy tooth morphology. No snails switched from pointed to blunt. FB *L. vincta* (red) changed phenotype more frequently than FB *L. variegata* and PP *L. vincta*.

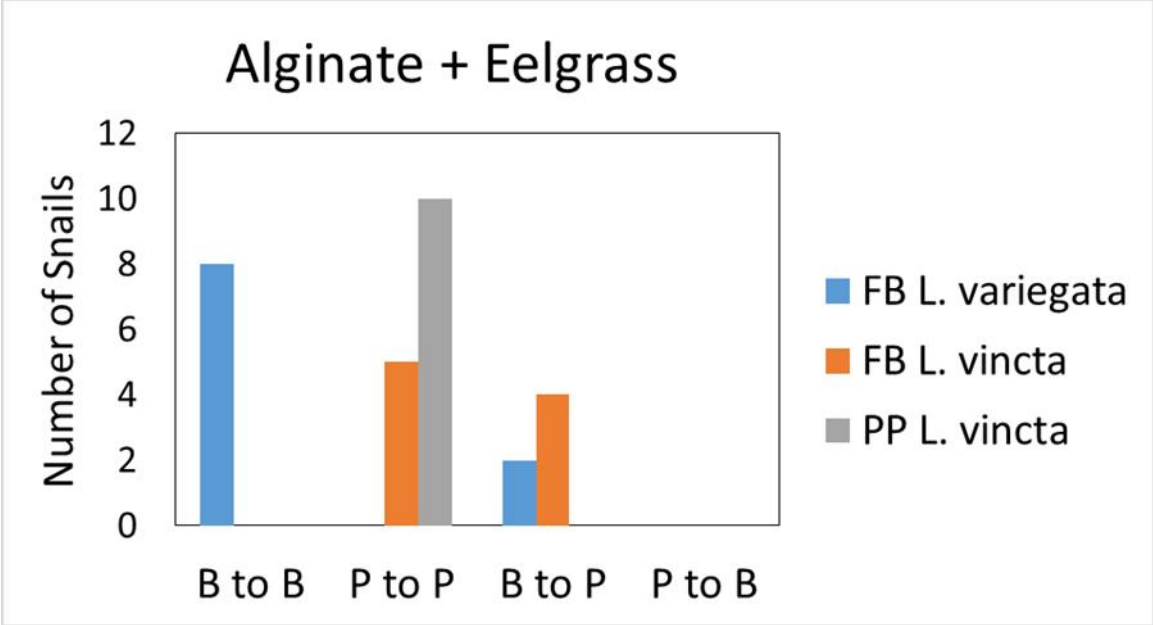

Figure 3. The number of snails that changed tooth morphology when fed alginate + eelgrass. B to B = no change from a blunt morphology, P to P = no change from a pointed morphology. B to P = change from a blunt to a pointed morphology, and P to B = change from a pointed to blunt morphology. All animals that changed phenotype switched to the pointy tooth morphology. No snails switched from pointed to blunt. Eelgrass did not produce a chemical signal inducing the blunt tooth morphology. FB *L. vincta* changed phenotype more frequently than FB *L. variegata* and PP *L. vincta*.

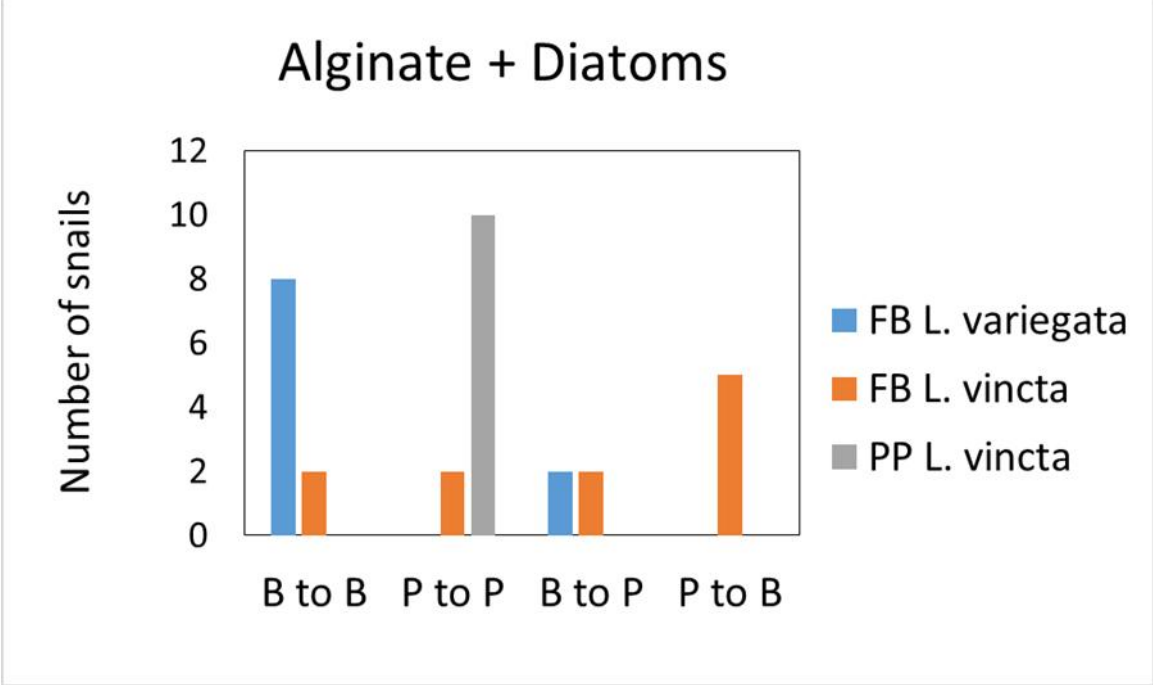

Figure 4. The number of snails that changed tooth morphology when fed alginate + diatoms. B to B = no change from a blunt morphology, P to P = no change from a pointed morphology. B to P = change from a blunt to a pointed morphology, and P to B = change from a pointed to blunt morphology. Snails that changed tooth morphology switched from pointed to blunt and blunt to pointed. FB *L. vincta* changed phenotype more frequently than FB *L. variegata* and PP *L. vincta.*

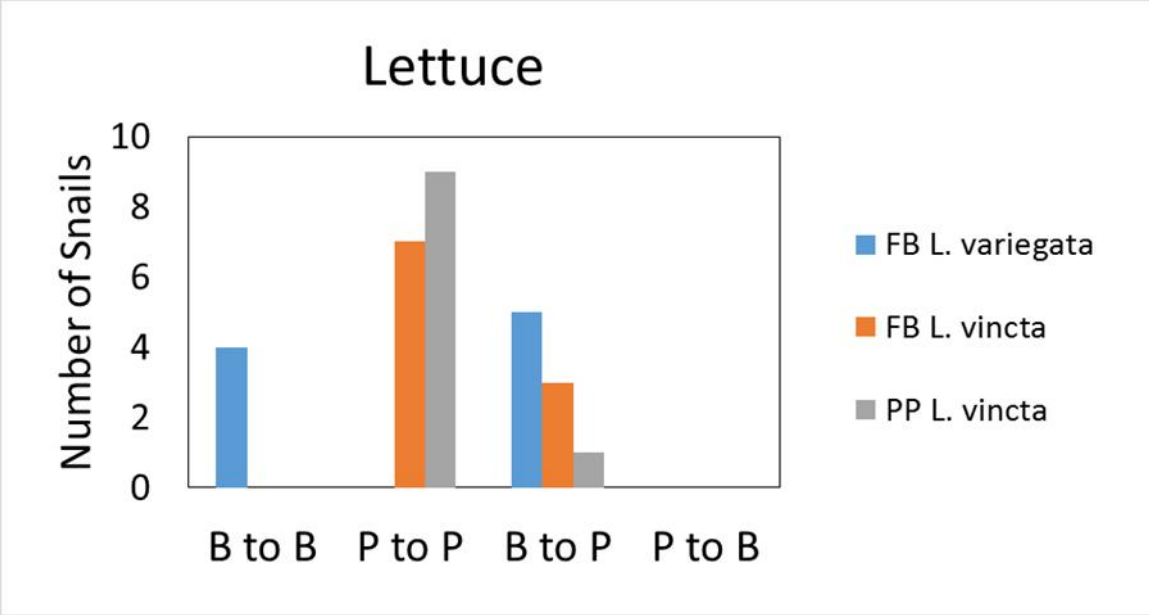

Figure 5. The number of snails that changed tooth morphology when fed fresh lettuce. B to B = no change from a blunt morphology, P to P = no change from a pointed morphology. B to P = change from a blunt to a pointed morphology, and P to B = change from a pointed to blunt morphology. All animals that changed phenotype switched to the pointy tooth morphology. No snails switched from pointed to blunt. Results suggest that the pointed phenotype is the default tooth morphology.

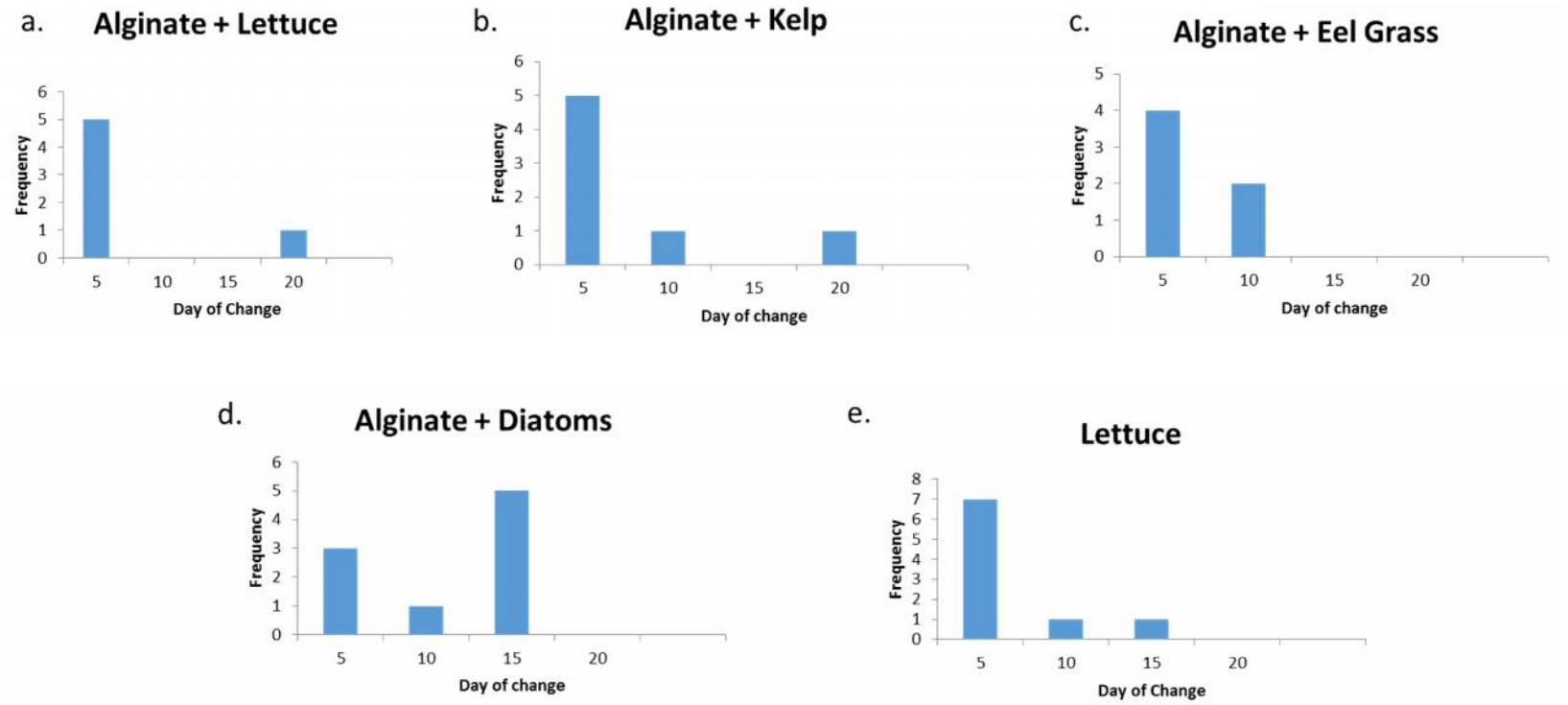

Figure 6. The lag time to morphological change for snails fed different diets. (**a**) 6 snails fed alginate + lettuce changed tooth morphology during the experiment with a mean time to change of 2.6 ± 2.8 days. (**b**) On average, snails fed alginate + kelp changed after 4.5 ± 2.0 days, and the lag time ranged from day -0.33 to day 16. (**c**) The mean day that snails switched tooth morphology when fed alginate + eelgrass was 4.1 ± 1.9 days into the experiment and the lag time ranged from day 0 to 9.7

days. (**d**) For snails fed alginate plus diatoms, FB snails changed more frequently. Snails changed tooth morphology on average on day 8.3 ± 2.5 (N = 7), and the lag time ranged from 1.3 to14 days. (**e**) On average, *Lacuna* fed fresh lettuce switched tooth morphology after 5.0 ± 1.1 days (N = 9), and the lag time ranged from 1 to 10.7 days.