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ABSTRACT

Inducible defenses are phenotypically plastic traits in which individuals or colonies develop a predator/herbivore-resistant trait in response to a cue. The barnacle Chthamalus fissus, commonly found in the upper intertidal of southern California and Baja California, Mexico, exhibits three morphs: one with an oval operculum, one with a narrow, slit-like operculum, and a relatively uncommon bent form with the operculum opening on one side. A previous study suggested that the narrow and bent morphs are defended from attack by the predatory snail Mexacanthina lugubris lugubris. In the present study, predator exposure and predator exclusion experiments revealed that operculum morphology of C. fissus is a plastic trait: individuals exposed to M. lugubris lugubris develop into the narrow operculum morph or, less commonly, the bent morph. While some species of marine invertebrates exhibit either a generalized plasticity response to various predators or plastic traits that are specific to the type of predator to which they have been exposed, the observation in this study appears to be the first demonstration of the occurrence of alternative inducible defenses to the same predator within a single species of marine invertebrate.

KEY WORDS: barnacles, Chthamalus fissus, inducible defense, Mexacanthina lugubris lugubris, phenotypic plasticity

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INTRODUCTION

Temporal and spatial variation in morphological, behavioral, and life history traits of species is common. Variation in natural selection among habitat patches may lead to local adaptation when specific genotypes (and corresponding phenotypes) differ in fitness depending on the habitat patch occupied (Turesson, 1922; Williams, 1966; Endler, 1977; Lively, 1999; Kawecki and Ebert, 2004). For marine invertebrates with limited dispersal abilities (direct developers), genetic differentiation and character divergence among populations often result when there is relatively large environmental heterogeneity (Endler, 1977; Day and Bayne, 1988; Johannesson et al., 1993). In contrast, gene exchange among populations of species with wide dispersal abilities can limit opportunities for genetic differentiation and character divergence among populations inhabiting different environments (Ehrlich and Raven, 1969; Slatkin, 1985). Under the latter condition, phenotypic plasticity, in which morphological types can result from modification of a phenotype in response to specific cues in the local environment, may be a suitable alternative adaptive response (Wright, 1931; Bradshaw, 1965; Pigliucci, 2001).

Inducible defenses are examples of such phenotypically plastic traits: individuals or colonies develop a predator/herbivore-resistant trait in response to a cue (Harvell, 1990). Some species of marine molluscs develop thicker shells in response to crab predators (Appleton and Palmer, 1988; Trussell, 1996; Leonard et al., 1999; Caro and Castillo, 2004). The colonial bryozoan Membranipora membranacea Linnaeus, 1767 exhibits cue-specific responses developing stolons in the presence of competing colonies (Harvell and Padilla, 1990) and defensive spines in the presence of nudibranch predators (Harvell, 1984; Harvell, 1990).

Chthamalus fissus Darwin, 1854 is a barnacle commonly found in the upper intertidal zone of the Pacific coast of the United States and Baja California, Mexico. Like the barnacle Chthamalus anisopoma Pilsbry, 1916 in the Gulf of California, C. fissus has an oval operculum morph and a rare bent morph in which the operculum opens to one side. However, unlike C. anisopoma, C. fissus also has a third morph with a narrow, slit-like operculum (Miller et al., 1989), which can be the dominant morph in some locations (Jarrett, personal observation). Jarrett (2008) found that juvenile C. fissus transplanted among two populations, one in which the oval morph is most common and one in which only the narrow and bent morphs occur, developed the morphology associated with the site to which they were transplanted. On these grounds, he suggested that operculum morphology is a phenotypically plastic trait for this species. Because the population of the predatory snail Mexacanthina lugubris lugubris Sowerby, 1821, was larger where the narrow morph was more common, Jarrett (2008) suggested that M. lugubris may be the inducing factor for this barnacle and that the narrow morph may represent an alternative, defensive strategy. Indeed, Jarrett (2008) demonstrated that the narrow morph of C. fissus is significantly more vulnerable to attack by M. lugubris lugubris than the oval morph. In the present study, I tested the hypothesis that operculum morphology of C. fissus is a phenotypically plastic trait and that exposure of juvenile C. fissus to M. lugubris lugubris induces development of the narrow operculum morphology.

MATERIALS AND METHODS

All field studies were conducted in La Jolla, CA, USA (32°48′N, 115°16′W). As described by Fotheringham (1974), the site consists of a
6000 m$^2$ boulder field bounded on the landward side by an igneous dike and with a tidal range of 0.6 m to +2.4 m around MLLW. All experiments were conducted between 20 August 2005 and 14 April 2006 at ~1.5 m above MLLW within the zone normally occupied by adult C. fissus.

Transect Survey

Similar to the foraging behavior of Mexacanthina lugubris angelica Oldroyd, 1918, described by Lively (1986a), M. lugubris lugubris retreats into cracks and crevices during high tide but forages out from these safe havens on the receding tide. Barnacles close to these refugia would therefore be exposed to the predator more frequently; therefore exposure to this predator induces a defensive morphology, the narrow and bent morphs should be more common closer to these refugia. To determine whether barnacle morphology differs as a function of distance from crevices inhabited by M. lugubris lugubris, five 15 cm transects perpendicular to crevices occupied by M. lugubris lugubris were randomly selected on the igneous dike and photographed. The width and length of the operculum of each barnacle were measured from the digital photographs using Sigmascan Pro 5™ Version 5.0 and the data were averaged within each 1 x 5 cm section of the transect.

Predator Exclusion Experiment

To determine whether barnacle morphology varies as a function of exposure to M. lugubris lugubris under field conditions, quadrats were established adjacent to crevices occupied by M. lugubris lugubris on the igneous dike at 1.5 m above MLLW. Six quadrats were scraped bare and then assigned randomly to one of three treatment groups: 1) predator exclusion consisting of two quadrats covered with stainless steel cages (0.25 cm$^2$ mesh, ~16 cm L x 10 cm W x 5 cm H) attached to the bare substrate using stainless steel screws and plastic anchors; 2) a cage control consisting of two quadrats covered with stainless steel cages with the central 9 cm of each 16-cm side removed to allow M. lugubris lugubris access into the cage; 3) and a control consisting of two quadrats (16 cm x 10 cm) with no cages but boundaries marked with 4 stainless steel screws and plastic anchors. After approximately 8 months, each quadrat was photographed and barnacle operculum width and length were measured as described previously.

Predator Induction Experiment

To determine whether direct exposure to M. lugubris lugubris induces changes in operculum morphology of juvenile C. fissus, 6 quadrats (15 x 15 cm) of similar juvenile density on a single large sandstone boulder at 1.3 m above MLLW were randomly assigned to either a control or predator exposure group. The sandstone boulder was chosen because M. lugubris lugubris is rarely found on sandstone boulders in this study area and therefore the control group would likely not be exposed to naturally occurring M. lugubris lugubris over the course of the study. Juvenile C. fissus in the predator exposure quadrats were completely covered by hand-placed M. lugubris lugubris (snail shell length approximately 20 mm, 20 snails per quadrat) during six low tides over 3 d while juvenile C. fissus in control quadrats were not manipulated. Barnacle operculum width and length and basal diameter were measured from digital photographs taken after the third day of treatment and at the end of the experiment (8 months) as described previously. These measurements were used to characterize operculum morphology and estimate growth.

Statistical Analyses

To detect changes in operculum morphology as a function of distance from a crevice, data for barnacles within 1 x 5 cm increments were pooled along the 15 cm transect and mean operculum widths were compared among these increments. To examine barnacle operculum morphology in the predator exclusion experiment, operculum width was compared among the three groups using a nested ANCOVA with treatment (cage, uncaged, cage control) as a fixed factor and replicate quadrats as a random factor nested within the treatment (SYSTAT™ Version 11, San Jose, CA, USA). Operculum length was the covariate because of the irregular shape of the base. To examine barnacle operculum morphology in the predator exposure experiment, operculum width was compared among the two groups using a nested ANCOVA with treatment (predator exposure, control) as a fixed factor and replicate quadrats as a random factor nested within the treatment (ibid). Operculum length was the covariate. It is predicted that, for both the predator exclusion and predator exposure experiments, the slopes of the regressions of operculum width as a function of operculum length for the treatments and controls will be heterogeneous, indicating differences among these groups in barnacle operculum morphology. Growth of control and exposed barnacles was compared using a nested ANOVA.

RESULTS

Transect Survey

Although mean operculum width varied among the five transects, mean barnacle operculum width tended to increase with increasing distance from crevices for all five transects (Fig. 1). In general, barnacles within 6 cm of crevices inhabited by M. lugubris lugubris tended to have narrow operculum openings compared to barnacles found between 7 and 15 cm from such crevices (Fig. 1). Variation in mean operculum width among transects could be due to variation among transects in barnacle age and/or predator abundance. Very few (between 1 and 11) bent morphs were found within each entire transect.

Predator Exclusion

Barnacles within caged quadrats developed wider operculum openings than barnacles within the cage control and uncaged treatments (Table 1, Figs. 2-4). Barnacle operculum width varied as a function of operculum length and treatment but there were no significant differences in operculum width among replicates within treatments (Table 1). In caged quadrats, barnacle operculum width increased as a function of operculum length and there was no difference in this relationship between the two replicates (Fig. 2). For both the cage control and uncaged treatments, barnacles in only one replicate exhibited a slight, yet significant, positive relationship between operculum width and length (Figs. 3 and 4). These differences among replicates within quadrats may be due to differences in the degree of natural exposure to the predator. No bent morphs were found in replicates of any of the three treatments.
Predator Induction

The relationship between operculum width and length was similar among replicates within control and predator-exposure treatments and among the two treatments in general at the start of the predator induction experiment (Table 2a, Fig. 5). Barnacles exposed to the predatory snail developed narrow operculum openings after 8 months (Mean = 0.52 mm, SE = 0.011), with no significant positive relationship between operculum width and length, while barnacles in the control quadrats developed oval operculum openings (Mean = 0.76 mm, SE = 0.016) and exhibited a significant positive relationship between operculum width and length (Table 2b, Fig. 6). No bent morphs were found in the control quadrats and two and three bent morphs were found respectively in two predator-exposure quadrats.

Growth did not differ among replicates within the control and predator exposed quadrats ($F_{4, 117} = 0.52, P = 0.72$) but control barnacles (Mean = 0.057 mm/month) grew significantly faster than barnacles exposed to the predator (Mean = 0.030 mm/month) ($F_{1,4} = 220.5, P < 0.0001$).

**Table 1.** Nested ANCOVA for operculum width in predator exclusion experiment. Operculum length was used as the covariate ($df$ = degrees of freedom, MS = mean square, $F = \text{value of } F\text{-statistic}, P = \text{p-value}$). *Error term: (a) = Replicate (Treatment); (b) = Residual.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>$df$</th>
<th>MS</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>0.311</td>
<td>11.96</td>
<td>0.04 (a)</td>
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<tr>
<td>Operculum Length</td>
<td>1</td>
<td>3.81</td>
<td>97.7</td>
<td>&lt; 0.0001 (b)</td>
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<tr>
<td>Treatment $\times$ Length</td>
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<td>50.7</td>
<td>0.005 (a)</td>
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<tr>
<td>Replicate (Treatment)</td>
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<td>Error</td>
<td>342</td>
<td>0.039</td>
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**DISCUSSION**

Results of this study suggest that juveniles of *C. fissus* have the capacity to develop a defensive morphology in response to the snail *M. lugubris lugubris*. Most barnacles exposed to *M. lugubris lugubris* developed operculum openings that were significantly narrower than those of barnacles not receiving the predator cue, while a few barnacles developed the bent morph. Plasticity in operculum morphology may allow juvenile barnacles, too small to be preyed upon by *M. lugubris*, the opportunity to develop a defensive morphology before they become suitably sized prey. These morphs have been shown previously to be more resistant than
Table 2. Nested ANCOVA for operculum width in predator exposure experiment. Operculum length was used as the covariate. Abbreviations as in Table 1.

<table>
<thead>
<tr>
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<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.0005</td>
<td>0.46</td>
<td>0.53</td>
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<tr>
<td>Operculum Length</td>
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<td>&lt; 0.0001</td>
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<tr>
<td>Treatment × Length</td>
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<td>1.19</td>
<td>0.37</td>
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<tr>
<td>Replicate (Treatment)</td>
<td>4</td>
<td>0.001</td>
<td>1.69</td>
<td>0.15</td>
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<tr>
<td>Error</td>
<td>172</td>
<td>0.0007</td>
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<td></td>
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</tbody>
</table>

B) End of experiment

<table>
<thead>
<tr>
<th>Source of variation</th>
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<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
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<td>0.066</td>
<td>6.36</td>
<td>0.06</td>
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<tr>
<td>Operculum Length</td>
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<td>0.137</td>
<td>18.8</td>
<td>&lt; 0.0001</td>
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<td>19.9</td>
<td>0.03</td>
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<tr>
<td>Replicate (Treatment)</td>
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<td>0.010</td>
<td>1.5</td>
<td>0.20</td>
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<tr>
<td>Error</td>
<td>116</td>
<td>0.007</td>
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Fig. 6. Linear regression of operculum width as a function of operculum length for control barnacles and barnacles exposed to M. lugubris, after 8 months in the field. Open circles identify control data and closed squares identify data from exposure quadrats.

oval morphs to predation by M. lugubris lugubris, which feeds by ramming its shell spine into the barnacle operculum (Jarrett, 2008). It is not known if narrow morphs can revert back to the oval morph in the absence of predators.

These results also appear to be the first demonstration of the occurrence of two alternative plastic responses to a single predator within a marine invertebrate population.

Other marine invertebrates, such as bryozoans and mollusks, exhibit more generalized responses to a variety of predators (Iyengar and Harvell, 2002; Freeman and Byers, 2006). For example, Caro and Castillo (2004) recently demonstrated that the mussel Semimytilus algosus Gould, 1850 exhibits a generalized shell-thickening response to crab and snail predators, although the magnitude of the response is predator-specific. Jarrett (2008) found that in Las Olas, Baja California (32°00’N 116°52’W), a site where M. lugubris lugubris is approximately 50 × more abundant than in La Jolla, California, 16.6% of the C. fissus population consisted of bent morphs and the remaining 83.4% exhibited the narrow morph. Although all three morphs of C. fissus are found in La Jolla, the bent form is rare even close to refugia where predator exposure would be maximal, and so it is possible that the lack of induction of bent morphs in the present study is due to few individuals having the capacity to develop this morph. Alternatively, the degree of predator exposure in the present study may have been below some threshold level required to induce the bent morph. Since M. lugubris lugubris is much less abundant in La Jolla compared to Las Olas, it is possible that selection for the ability to develop into the bent morph has been relatively weak in La Jolla. The narrow morphology may represent a successful compromise between the need to avoid predation and the costs associated with developing the bent morph. Lively (1986b) found that for bent and oval morphs of the barnacle C. anisopoma in the Gulf of Mexico, the bent morph grew more slowly and was less fecund than the oval morph. The cost of being bent may be similar for C. fissus, but what remain to be determined are the costs associated with the narrow morph. It is possible that cirral feeding may be impaired in the bent morphs to a greater degree than in the narrow morphs but that the oval morphs are most efficient at feeding. The low growth rate of the narrow morph compared with the oval morph in the present study suggests that under predator-free conditions, the oval morph may better compete for space on intertidal substrata.

M. lugubris lugubris has, in the last few decades, extended its northern limit along the Southern California coast (public communication: 4 April 2007, Conservation and Biodiversity of the Rocky Intertidal of Southern California, http://www.biology.ucsd.edu/labs/roy/CBRISC/CBhome.html, Muhs et al., 2002). This situation is similar to that described for northern populations of the mussel Mytilus edulis Linnaeus, 1758 along the east coast of the United States, which have only recently been encountering the invasive Asian shore crab Hemigrapsus sanguineus de Haan, 1853 whose northern limit has extended into the Gulf of Maine (McDermott, 1998). Freeman and Byers (2006) found that mussels from northern populations did not develop thicker shells while mussels from southern populations did develop thicker shells when exposed to H. sanguineus suggesting that: 1) populations in the south have undergone rapid evolution of an inducible defense, and 2) prey populations differ in the occurrence of inducible defenses and it should not be assumed that prey populations will evolve an inducible defense when faced with a new predator. What remain to be determined for C. fissus are the extent to which populations along the Southern California coast newly exposed to M. lugubris lugubris differ in the occurrence of inducible defenses, and the extent to which there exists variation in the degree of plasticity in operculum morphology among these populations.

Acknowledgements

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References

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