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Barnacle appendage plasticity: Asymmetrical response time-lags, developmental mechanics and seasonal variation

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ABSTRACT

Examples of morphological plasticity now exist across a wide range of taxa and experimental systems. Much of what we know about such plasticity comes from field and laboratory manipulations assessing the magnitude of plastic responses. However, we know much less about how – and how quickly – such changes occur, nor about how changes in form track natural environmental variation. Knowing more about plastic responses under natural conditions is important to help identify developmental costs and limits that may restrict the evolution of plasticity in variable environments. In this study, I measured leg and penis form plasticity in the common Pacific acorn barnacle *Balanus glandula*. A ten-week transplant experiment under natural conditions in the intertidal revealed: i) barnacles took longer to change leg form than previously thought, ii) response rates depended on transplant direction, and iii) barnacles changed leg length through a combined response of growing longer leg segments and adding more leg segments. Furthermore, a two-year survey of barnacle leg and penis form in natural populations revealed that barnacle leg and penis form varied in a manner consistent with adaptation to seasonal variation in wave height. Together, these results suggest that resource acquisition under natural conditions may be an important and underappreciated limit on morphological response times in natural systems, and shed light on how such changes occur during development.

1. Introduction

The capacity of a single genotype to produce different forms in response to different environmental cues, known as developmental plasticity, occurs across a wide range of taxa and experimental systems (Piersma and Van Gils, 2011; Pigliucci, 2001): plasticity in leaf, stem, and root form enable plants to better tolerate abiotic conditions and reduces competition (Callaway et al., 2003); plasticity in body form provides plants and animals with some defense against would-be predators (reviewed in Tollrain and Harvell, 1999); and changes to the parental environment can even induce adaptive changes to the form and behaviour of offspring (Agrawal et al., 1999; Marshall, 2008).

Most past studies have focused primarily on field and laboratory manipulations to assess the magnitude of plastic responses. However, much less is known about the timing of responses under natural conditions (but see Piersma and Drent, 2003). Such information is important because, to be adaptive, changes in form must be fast enough to yield a body form matched to current conditions (Moran, 1992; Tufto, 2000). If responses are too slow, the environment which induced a certain phenotype may already have changed; if responses are too quick, the costs associated with producing a new phenotype may outweigh the benefits of the new form (Gabriel et al., 2005; Padilla and Adolph, 1996). Although response time-lags are well-studied theoretically, much less is known about time-lags in natural systems (Gabriel et al., 2005; Harvell and Padilla, 1990; Vanalstyne, 1988), nor about the developmental mechanics that produce changes in form. Importantly, because development ultimately determines the speed of plastic responses, understanding how – and how quickly – organisms can change body form will help us understand the costs and limits that may restrict the evolution of developmental plasticity in variable environments (Auld et al., 2009; Gabriel et al., 2005).

In this study, response time-lags, developmental mechanics, and patterns of seasonal variation were investigated in the common Pacific acorn barnacle *Balanus glandula* Darwin. Barnacles are famously plastic in many aspects of shell and body form; some species alter shell form when exposed to cues from a potential predator (Lively, 1986a,b), and many dramatically modify shell form in response to many other environmental cues (Bourget and Crisp, 1975; Crisp and Bourget, 1985). Barnacle feeding legs and penises are also highly plastic (Hoch, 2009; Marchinko, 2003; Neufeld and Palmer, 2008). In *B. glandula*, individuals moved to wave-exposed shores grow feeding legs and penises that are 25–50% shorter and 40–50% stouter than their protected-shore counterparts (Marchinko, 2003; Neufeld and Palmer, 2008). Variation in conspecific density is also associated with variation in leg and penis form in the three species where it

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has been explicitly studied (Hoch, 2010; Lopez et al., 2007; Neufeld, 2011).

The first goal of the current study was to investigate leg form changes under natural conditions in the intertidal, using a reciprocal transplant experiment between wave-exposed and wave-protected shores. Although barnacles grow longer feeding legs quickly (within 18 days) when transplanted from a wave-exposed shore to a subtidal site in a quiet harbour (Marchinko, 2003), the speed of response under natural conditions is unknown. Importantly, most barnacles occur on the high shore where immersion time – and thus access to food – is greatly reduced. Therefore, if food supply limits developmental plasticity, response times may be significantly slower under these food-limited conditions. Also, by reciprocally transplanting barnacles between sites that differed in water velocity, this experiment could investigate whether response times were asymmetrical depending on transplant direction (from low- to high flow and vice versa).

A second goal was to examine the developmental mechanisms that produce changes in barnacle leg length. Specifically, barnacle feeding legs are comprised of a short two-segment protopodite that then divides into two rami, each of which is made up of many jointed segments that allow the feeding legs to curl into the shell when not in use. Given this jointed construction, this study investigated whether leg length changes are facilitated through changes in the number of ramus segments, the length of each segment, or through a combination of mechanisms. Varied terminology has been used to describe the individual units that make up the jointed paired rami of barnacles (Boxshall, 2004; Darwin, 1854); however, here the term “segment” will refer to an individual element of the jointed ramus, following Boxshall (2004). Although seemingly inconsequential, whether barnacle rami indeed contain true segments is important in the context of what barnacles can tell us about the evolution and development of segmentation (see Discussion).

A final goal was to ask whether populations of barnacles modify leg and penis form at multiple sites over time. Past studies have compared barnacle leg and penis form among environments at a single time interval (Arsenault et al., 2001; Marchinko and Palmer, 2003; Neufeld and Palmer, 2008), or have relied on the results of short-term transplant experiments (Hoch, 2009; Marchinko, 2003; Neufeld and Palmer, 2008) However, barnacles experience variation in water velocity both in space and in time: larvae may travel long distances before settlement, and must also cope with variation in water velocity after settlement due to seasonal variation in wave height. Knowing the extent to which barnacles modify Leg form at a single site over time will shed light on whether barnacle leg form varies with temporal as well as spatial variation in water velocity.

2. Methods

2.1. Transplant experiment

Barnacles were transplanted from two source populations to each of four destination sites in Barkley Sound, British Columbia, Canada. On September 12, 2006, adult *B. glandula* were collected on mussel shells (*Mytilus californianus* and *M. trossulus*) from two source populations chosen for a substantial difference in wave force between sites and for a sizeable supply of adult barnacles growing on mussels: a moderately-protected shore (Ross Islets (RI) (Arsenault et al., 2001)) and an exposed shore (Seppings Island (SI) (Arsenault et al., 2001)) in Barkley Sound. Mussel shells were cut using a rotary tool so that one barnacle occupied each mussel shell fragment. 20 barnacles on mussel shell fragments were randomly selected to determine initial leg lengths from each source population. The remainder of mussel shell fragments were spaced approximately 13 mm apart and glued to twelve 10 by 13 cm Plexiglas plates using marine epoxy putty (Z-spar™ Splash Zone Compound) in a 10 by 7 grid alternating between protected- and exposed-shore source populations (yielding 35 barnacles from each source on each of three plates per site). Plates were kept overnight in flowing seawater and then three plates were bolted to the rock in the middle of the *B. glandula* zone at each of four outplant locations chosen for a more than five-fold variation in wave force: a protected shore (Bamfield Inlet (BMSC); Marchinko and Palmer, 2003), a moderately-protected shore (Ross Islets (Ross); Arsenault et al., 2001), and two exposed shores (Seppings Island (Seppings); Arsenault et al., 2001; Prasiola Point (Prasiola); Neufeld and Palmer, 2008). At two-week intervals, two rows of barnacles were selected from each of three plates at each site and frozen prior to processing. Due to some mortality at each site, bi-weekly samples from each plate contained 3–7 barnacles (mean = 6) from each source population.

2.2. Seasonal variation survey

To determine whether barnacles respond to seasonal variation in wave force, leg and penis form were measured in three populations of adult barnacles (quiet-water, moderately-exposed, and exposed-shore) every 2-3 months over approximately two years. Barnacles were collected from natural populations near three of the four transplant destination sites in Barkley Sound, British Columbia, Canada: BMSC, Ross, and Prasiola (all described above). Sites were chosen to span a range of wave-exposures and for ease of access during stormy winter months. At each site and sampling interval, 10–15 barnacles were collected from the middle of the *B. glandula* zone. To minimize the effect of conspecific density on leg length (Neufeld, 2011), sampled barnacles touched other barnacles on all sides and at least four adjacent barnacles had opercula within 1.5 cm. Offshore wave height data was obtained from an offshore buoy (National Data Buoy Centre, station C46206 – La Perouse Bank). A past study in California found a significant positive correlation between measured offshore wave height and onshore wave force in the majority of sites, suggesting such data are at least a reasonable proxy for onshore data where direct measurements are unavailable (Helmuth and Denny, 2003).

2.3. Sample processing and measurement

Samples were frozen at −20 °C and processed as time permitted. Barnacles were thawed in seawater and the soma was removed, blotted dry and weighed to the nearest 0.1 mg following Arsenault et al. (2001). The 6th thoracic leg, from the left side, was wet mounted in seawater and photographed under a dissecting microscope at 15× with a 6mp digital camera. Photographs were measured to the nearest 10 μm using ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997–2010). Feeding-leg length and setae length were measured following Marchinko and Palmer (2003). Penis form was measured following Neufeld and Palmer (2008). Segment length was calculated as total ramus length divided by the number of segments (i.e., the mean segment length for each ramus).

2.4. Statistical analyses

All statistics were calculated using R 2.10.1 (R Development Core Team, 2010) on $\log_{10}$-transformed data. However, for ease of interpretation, plots display back-transformed values plotted on a log scale wherever possible. For the transplant experiment, individual leg length measurements from the entire experiment were first adjusted to a common body size (soma mass = 0.0052 g; approximate basal diameter = 6 mm) using ANCOVA (Table S1). Mean size-adjusted leg length was then calculated for each plate at each time interval and these plate means were used as independent replicates in all subsequent analyses. This size-standardization technique has been used in many past studies of barnacle form (Arsenault et al., 2001; Li and Denny, 2004; Marchinko and Palmer, 2003; Miller, 2007; Neufeld and Palmer, 2008) and is appropriate here because