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Phenotypic variation for adhesive tenacity in the barnacle *Balanus amphitrite*

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ABSTRACT

Silicone fouling-release coatings represent a non-toxic alternative to biocide-containing ship hull paints. These coatings allow fouling organisms to attach to the hull surface, but prevent firm adhesion. Adhesive tenacity to fouling-release materials varies both among and within species. We quantified broad-sense genetic and environmental sources of intraspecific variation in tenacity to two silicone substrata, for the barnacle *Balanus amphitrite*. For both materials tenacity varied over an order of magnitude; however, the partitioning of this variation differed between the substrata. For International Veridian, a commercially-available fouling-release coating, removal stress varied significantly among maternal families and replicate barnacle cultures. Variation among the maternal families was associated with previously observed differences among these families in the condition of the adhesive plaque. Additional experiments suggested that variation among the replicate cultures arose from heterogeneity between replicate coatings in properties that affect tenacity. We could not attribute variation in removal stress for Dow Corning Silastic T-2, a silicone rubber used for mold-making, to any of the genetic or environmental sources tested. Instead, variation may have been due to measurement error or heterogeneity within replicate coatings in properties affecting tenacity. Differences among maternal families in removal stress may stem from variation in the interaction between the adhesive and the substratum, or in the viscoelastic properties of the adhesive plaque.

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1. Introduction

The accumulation on ship hulls of biofilms, algae, and sessile invertebrates (that is, 'fouling') generates significant environmental costs. Fouled hulls can serve as a vector for nonindigenous species (Carlton and Hodder, 1995; Gollasch, 2002; Godwin, 2003; Minchin and Gollasch, 2003). In addition, a fouled hull incurs a drag penalty, requiring the vessel to consume more fuel to travel a particular distance or speed and resulting in increased emission of exhaust gases (Woods Hole Oceanographic Institution, 1952; Abbott et al., 2000; Townsin, 2003; Schultz, 2007). Protection of a hull from fouling, however, also carries an environmental cost. Paints containing broad-spectrum biocides are widely used to control fouling. The biocides have significant detrimental effects on non-target species, and can remain in the environment for extended periods (see Champ, 2000; Omae, 2003; Konstantinou and Albanis 2004, for reviews). Despite the considerable disagreement as to the environmental costs and benefits of the current toxic antifouling paint formulations, there is a significant regulatory impetus to develop environmentally-friendly coatings (Champ, 2000; Konstantinou and Albanis, 2004).

Silicone fouling-release coatings represent a non-toxic alternative to the biocide-containing hull paints now in use. Fouling-release coatings allow organisms to accumulate on the hull surface, but prevent their firm attachment (Swain and Schultz, 1996; Schultz et al., 1999). The adhesive tenacity of attached fouling is reduced by surface and bulk properties of the coating, including low surface energy and elastic modulus and increased coating thickness, that affect the fracture of the bond between the organism and the coating (see Brady and Singer, 2000, for review). In addition, uncrosslinked silicone polymer chains or silicone oils may affect the bond by interacting with organismal adhesives as they cure (Berglin and Gatenholm, 1999; Meyer et al., 2006). The coating properties render attached organisms more susceptible to disturbance by hydrodynamic forces generated by the movement of the ship through the water. Ideally tenacity is decreased such that fouling is sloughed from the hull at routine operating speeds ('hydrodynamic self-cleaning,' Schultz et al., 1999).

Adhesive tenacity to fouling-release materials varies among and within species. Interspecific variation takes two forms; the magnitude of tenacity or removal stress varies among species for a particular material (for example, Kavanagh et al., 2001; Holm et al., 2006), as does the ranking of fouling-release materials by mean removal stress (Stein et al., 2003; Holm et al., 2006). All species of encrusting organisms tested to

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date, for all materials examined, exhibit substantial intraspecific variation (for example, Kavanagh et al. 2001, 2003; Holm et al., 2006), with ranges for adhesive tenacity occasionally exceeding an order of magnitude (Holm, personal observation).

The sources of inter- and intraspecific variation in tenacity have not been completely characterized. The composition of the adhesives of fouling organisms differs among species (Naldrett and Kaplan, 1997; Urushida et al., 2007). Interspecific variation in tenacity may arise from phylogenetic differences in adhesive structure and the effects these differences have on the interaction between the adhesive and the substratum and on the physical properties of the cured adhesive. Similarly, intraspecific variation in tenacity may be due to genetic or environmental effects on adhesive properties and the adhesive/substrate interaction, but may also arise from irregularities in the bulk and surface properties of the test substrata or from measurement error.

We quantified broad-sense genetic and environmental sources of variation in adhesive tenacity for the barnacle *Balanus amphitrite* on two silicone substrata, including a commercially-available fouling-release coating. Our results differed between the two materials, but suggested that heritable genetic or maternal environmental variation influences removal stress, primarily by affecting the characteristics of the adhesive plaque.

2. Methods

Balanus amphitrite Darwin (= *Amphibalanus amphitrite* Pitombo, 2004) is a common member of intertidal and shallow subtidal fouling communities of tropical to warm temperate marine environments (Zullo, 1979). Along the southeastern coast of North America, *B. amphitrite* occurs on a diversity of substrata including oyster (*Crassostrea virginica*) shells, plant material both living and dead, rocks, seawalls, pilings, and boat and ship hulls. Adults are iteroparous simultaneous hermaphrodites. Outcrossing in *B. amphitrite* requires pseudocopulation, and fertilized eggs are brooded in the mantle cavity of the maternal parent until release as nauplius larvae.

2.1. Larval culture, attachment to test materials, and rearing of spat

Collection of maternal parents and larvae, and larval culture, followed Holm et al. (2000, 2005). Larvae from 47 maternal families were cultured over 3 trials, commencing 13 May 2003 (16 families), 24 May 2003 (15 families), and 5 June 2003 (16 families). Cypris (settlement stage) larvae were held for 1 day at 6 °C until use in experiments.

We obtained settlement on the test substrata (coated panels, see Section 2.2 below) by placing 10–15 cyprids in each of 3 1 mL (approximately) drops on the material surface and incubating at 27 °C to 28 °C and a 12:12 L:D cycle. Details of the procedure can be found in Holm et al. (2005). After 3 days the majority of larvae in each drop had attached to the surface of the test materials and metamorphosed. Attachment typically occurred around the circumference of the water drop.

We reared barnacles settled on the test substrata in plastic containers (8×8×14 cm) in 725 mL of aged filtered seawater. Each container corresponded to a single larval culture (3 containers/cultures per family, see Holm et al., 2005), and barnacles on both test substrata from a given culture were reared in the same container. Barnacle spat were fed daily a mixture of varying volumes (20–40 mL total) of the microalgae *Skeletonema costatum* and *Dunaliella tertiolecta*; after 7 weeks brine shrimp *Artemia salina* (10 mL) were added to the diet. The particulars of the feeding regimen (volume and type of food offered each day) varied among the trials but not among the families or spat cultures within a trial. Water in the containers was changed twice per week. To prevent overgrowth, densities of barnacles on the panels were reduced several times during the course of the rearing by removing haphazardly-chosen individuals with a blunt probe. We did not attempt to maintain a fixed density of barnacles across all containers.

2.2. Silicone substrata

Two silicone materials were employed for the experiments: International Veridian, a translucent, commercially-available fouling-release coating for propellers, outboard motors, and outdrives; and Dow Corning Silastic® T-2, a transparent silicone rubber typically used for mold-making. Details regarding the preparation of the silicone substrata can be found in Holm et al. (2005). Target thicknesses for application of the silicone materials were chosen to minimize any effect of substratum thickness on barnacle tenacity. For the Veridian coating, the application process yielded a coating system with mean dry film thicknesses, measured beneath barnacle attachment points (see Section 2.4 below), ranging from 100 μm to 620 μm. For the Silastic T-2 material, mean dry film thicknesses, measured beneath barnacle attachment points (see Section 2.4 below), ranged from 460 μm to 820 μm.

Before they were used in experiments we soaked the coated panels in flowing seawater for 5 days (Silastic T-2) or 35 days (Veridian), in order to remove any compounds toxic to barnacle larvae or spat under the static conditions of culture. After soaking we rinsed the panels in deionized water and allowed them to air dry.

2.3. Measurement of contact angle

In an effort to better understand how surface properties of the silicone substrata varied within and among replicate coated panels, we measured water contact angles for a sample of the panels prepared in 2003. Ten panels of each test material were chosen at random, and contact angle measured for 20 random points on each panel. The measurements were taken on panels that had not been soaked in seawater, and all surfaces were rinsed with isopropanol and dried with nitrogen immediately prior to testing.

Contact angle was determined by goniometry from sessile drops (approximately 1 μL) of HPLC grade water, using a KSV Instruments CAM 100 Optical Contact Angle Meter (KSV Instruments Ltd., Helsinki). The meter's software fit the Young–Laplace equation to a captured image of each drop, and calculated the contact angle (for both sides of the drop) where the fitted curve intersected the surface of the silicone substratum. For statistical tests the mean value of the right and left angles was used as the datum for each drop.

2.4. Measurement of adhesive tenacity

We quantified adhesive tenacity, or removal stress, following the method described in ASTM D 5618-94 (Anonymous, 1997). This method measures only tenacity in shear. For barnacles greater than 5 mm in basal diameter, force was applied to the base parallel to the substratum using a handheld mechanical force gauge (Shimpo MF-5lb, #93953-10, Cole-Parmer, Vernon Hills, IL), at a rate of approximately 4.5 N s^{-1}, until the barnacle detached from the surface. If on detachment greater than 10% of the barnacle's base plate remained adhered to the test material, the datum was discarded. Otherwise, the diameter of the barnacle was measured using calipers and the basal area estimated after standard methods (Anonymous, 1997). Adhesive tenacity (or removal stress) in shear was then calculated by dividing the measured shear force required to remove the barnacle by the basal area. For the size range specified in the ASTM method, removal stress measured in this way is typically independent of the attachment area (size) of the barnacle (Kavanagh et al., 2001). Tenacity was measured for up to five barnacles, chosen haphazardly, on each panel.

Immediately after removal of each barnacle we measured the thickness of the silicone substratum and panel at the barnacle's attachment point, using a Mitutoyo Electronic Thickness Gauge (#547-520, McMaster-Carr Supply Company). Thickness of the silicone