Journal of Experimental Marine Biology and Ecology 454 (2014) 49–54

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Where even a long penis can't help: Evidence of long-distance spermcast mating in two acorn barnacles

Marjan Barazandeh [a,b,*], Corey S. Davis [a], A. Richard Palmer [a,b]

[a] Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada
[b] Bamfield Marine Sciences Centre, Bamfield, British Columbia V0R 1B0, Canada

ARTICLE INFO

Article history:
Received 6 October 2013
Received in revised form 5 February 2014
Accepted 7 February 2014
Available online 3 March 2014

Keywords:
Cirripedia
Copulation
Crustacea
Fertilization
Mating system
Penis size

ABSTRACT

Free-living barnacles are sessile as adults, mostly hermaphroditic, and generally thought to mate either by pseudo-copulation, using their famously long penises, or by self-fertilization. However, the recent discovery of spermcast mating in a stalked barnacle, *Pollicipes polymerus* — which has a relatively short penis — raises questions about whether spermcast mating might occur in the more familiar acorn barnacles as well. To assess how widespread spermcast mating is in barnacles with longer penises, we studied two common intertidal acorn barnacles, *Balanus glandula* Darwin, 1854 and *Chthamalus dalli* Pilsbry, 1916, whose penises can extend up to seven times their body length. We sampled fertilized isolated individuals, as well as isolated pairs where at least one parent carried fertilized eggs, and genotyped both adults and embryo masses using seven single-nucleotide-polymorphism loci for each species. 100% and 70% of embryo masses in isolated individuals had at least one non-maternal allele in *B. glandula* and *C. dalli*, respectively. Even in isolated pairs, 7.7% of *B. glandula* and 9.1% of *C. dalli* had alleles not present in either parent. This incidence of spermcast mating is lower, and over smaller spatial scales, than reported for the stalked barnacle, *P. polymerus*. Nonetheless, these observations provide strong evidence that spermcast mating does occur — at least occasionally — in acorn barnacles too. This possibility therefore needs to be considered in studies of barnacle mating systems, sex allocation and population genetics.

1. Introduction

Barnacles are famous for their exceptionally long penises that, in extreme cases, can extend up to eight times their body length (e.g., *Cryptophialus minutus* Darwin, 1854). Such long intromittent organs are crucial for barnacle reproduction because: 1) unlike other arthropods, they are sessile as adults, and 2) despite being typically hermaphroditic, self-fertilization rarely happens in barnacles. Most species are presumed to transfer sperm only by pseudo-copulation (Darwin, 1851, 1854), where a functional male extends its penis into a partner's mantle cavity to release sperm that induce the release of eggs (Anderson, 1994; Buhl-Mortensen and Høeg, 2006). Such long penises also imply that within a clump of barnacles, a functional female could potentially be fertilized by more than one functional male (Charnov, 1982, 1987).

However, even a long penis cannot ensure mating success in isolated individuals that lie outside the penis range of any neighbors. A few species are thought to overcome this problem by self-fertilization, which is usually assumed when fertilized embryo masses are found inside the mantle cavities of isolated barnacles (Barnes and Barnes, 1958; Barnes and Crisp, 1956; Furman and Yule, 1990). But the recent discovery of spermcast mating in a stalked barnacle, *Pollicipes polymerus* (Barazandeh et al., 2013), raises the possibility that eggs in the mantle cavity of isolated acorn barnacles could also be fertilized by sperm released by distant individuals.

Spermcast mating occurs in many sessile marine invertebrates including sponges, hydroids, bryozoans, pterobranch hemichordates and ascidians (Bishop and Pemberton, 2006), but has not been reported in any barnacles other than *P. polymerus* (Barazandeh et al., 2013). In the acorn barnacle, *Tetraclita rubescens* Darwin, 1854, although some remote individuals were fertilized, 87% of the many broods studied were nonetheless fertilized by only one father, even when multiple mates were nearby (Kelly et al., 2012). Sperm capture may therefore occur only rarely, if at all, in this species. However, individuals of other acorn barnacles, *Amphibalanus amphitrite* and *Chirona hameri* (formerly *Balanus hameri*), sometimes eject sperm into the water when a copulation attempt fails (Walker, 1980; W.A. Newman, personal communication, 2013), and "alternative mechanisms of outcrossing" other than pseudo-copulation have been suggested as a source of sperm in isolated fertilized *Chthamalus montagui* (Pannacciulli and Bishop, 2003). Therefore, whether spermcast mating occurs at all in acorn barnacles remains an open question.

* Corresponding author at: Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada. Tel.: +1 780 492 3633.
E-mail address: barazand@ualberta.ca (M. Barazandeh).

http://dx.doi.org/10.1016/j.jembe.2014.02.003
0022-0981/

Among barnacles, spermcast mating might have evolved only in species like *Pollicipes polymerus* that have relatively short penises (e.g., maximum extension up to 0.7 body lengths), and live in extreme environments (highly wave-exposed shores) that could facilitate the process. If so, the presence of fertilized eggs in isolated individuals of other species that have longer penises and hence have many more potential mates within penis range must be due to self-fertilization if spermcast mating is not a possibility. We therefore tested for spermcast mating in two acorn barnacles, *Balanus glandula* Darwin, 1854 and *Chthamalus dalli* Pilsbry, 1916 that possess relatively long penises and that live in a wide range of wave exposures (calm to highly exposed shores). These barnacles are common on the rocks and mussels of northeast Pacific intertidal shores. Mating occurs mostly in winter and spring in *B. glandula* and from spring through fall in *C. dalli* (Newman and Abbott, 1980; Strathmann, 1987).

The penis length of many barnacles depends on their reproductive status. Indeed, some species lose their penis altogether at the end of the mating season and grow a new one as the next breeding season begins, with the maximum size occurring at the peak of the mating season (Barnes, 1992). In *B. glandula*, relaxed and artificially extended penis lengths are around 1.4 and 3 times the body length, respectively (Barazandeh et al., 2013) and the maximum penis length is achieved from December to June (Barnes, 1992). The average penis length for *C. dalli* may depend on the number of fertile individuals nearby, among other factors. For instance, the relaxed penis is as long as the feeding legs when only 25% of the population is fertilized (near the beginning of the breeding season) but twice that long when 75% of the population is fertilized (near the peak of the breeding season; Barnes, 1992). *B. glandula* is believed to mate only by pseudo-copulation with neighbors (Kado, 2003; Neufeld and Palmer, 2008). *C. dalli*, however, is thought to self-fertilize when neighbors are out of reach (Barnes and Barnes, 1958; Newman and Abbott, 1980).

A number of interesting questions remain to be addressed here. Do species with long penises also sometimes capture sperm from distant individuals? How valid are reports of self-fertilization in *C. dalli* — and therefore possibly other acorn barnacles — where spermcast mating is a possibility? Do individuals with an immediate neighbor but far away from other barnacles (isolated pairs) copulate only with each other or is neighbor-mating supplemented with captured sperm as well?

2. Materials and methods

2.1. Barnacle sampling and measurements

To test whether spermcast mating, if it happens, is not limited only to shores with high wave action (e.g., as in *P. polymerus*\Barazandeh et al., 2013), barnacles were sampled from sites with different flow regimes. *B. glandula* were collected from rocks, mussels (*Mytilus* sp.) and *Pollicipes* capitular plates of high (Seppings Island, 48.841° N, 125.209° W), intermediate (Wizard Islet, 48.866° N, 125.169° W) and low (Grappler Inlet, 48.833° N, 125.119° W) wave-exposed shores (Arsenault et al., 2001) in Barkley Sound (near Bamfield, British Columbia, Canada) in August 2010 and May 2012. *C. dalli* were sampled from rocks and mussels on shores of high (Seppings Island) and intermediate (Helby Island, 48.847° N, 125.168° W) wave-exposure (Arsenault et al., 2001) in August 2010.

A total of 430 *B. glandula* and 110 *C. dalli* were scored for fertilization incidence, body length (=opercular length: length of the aperture parallel to the gape) of the focal individual and its nearest neighbor, and the distance to the nearest neighbor (shortest possible distance between the nearest margins of two barnacles' opercula). Isolation was defined as the ratio of nearest-neighbor distance to opercular length of the nearest neighbor.

Isolated individuals in both species were identified as individuals separated by more than seven body lengths from their nearest neighbor. Isolated pairs consisted of two adjacent barnacles, each of which was more than seven body lengths away from any conspecifics (see Penis measurements below).

A total of nine and 23 isolated individuals bearing fertilized eggs were collected for *B. glandula* and *C. dalli*, respectively. In addition, 26 fertilized *B. glandula* and 22 fertilized *C. dalli* from isolated pairs were also collected. For barnacles on mussels, we avoided sampling those close to the shell margin to reduce the possibility that barnacles might have mated with individuals on adjacent shells that had shifted position. We also did not collect any barnacles close to the remnant basal plates (*B. glandula*) or attachment halos (*C. dalli*) of recently dead barnacles to ensure that sampled individuals had not been near another individual that had recently died.

The whole body of each adult and whole embryo mass were removed from the shell and put separately in labeled 1.5 ml microfuge tubes containing 70% ethanol and stored at −20 °C until DNA extraction.

2.2. Penis measurements

To estimate the length of fully extended penises in actively mating barnacles, and to assess the true extent of erect penis-length variation among populations from different wave-exposure regimes, small rocks and mussels carrying *B. glandula* and *C. dalli* were collected from two highly wave-exposed (Seppings Island, Helby Island), two moderately exposed (Dixon Island, Brady's Beach) and three very calm sites (Grappler Inlet, Bamfield Inlet, Ross Islets) in Barkley Sound in May 2013 and taken to the laboratory. We held them in running-seawater tanks with aeration under a 18:6 h light:dark regime and watched them for mating activity each day following 2–3 h exposure to air. Any penis movement or extension, regardless of success, was videotaped. Full penis extension of spontaneously mating individuals was recorded for 123 *B. glandula* and 186 *C. dalli*. The opercular length and the total curved length of the extended penis of each target individual was measured on screen from different angles and the highest ratio of total penis length to opercular length was recorded for each individual to estimate the maximum possible reach.

2.3. Marker development, genotyping and simulation

Attempts to cross-amplify existing microsatellite markers developed for *Balanus amphitrite* and *C. montagui* (Pannacciulli et al., 2005; Robson et al., 2009) in *B. glandula* and *C. dalli* were unsuccessful. Consequently, we developed Single Nucleotide Polymorphism (SNP) markers.

Library construction involved total genomic DNA extraction from the whole body of five *B. glandula* and five *C. dalli* using a DNeasy® Blood and Tissue Kit (QIAGEN). For each species, we digested 5 μl of pooled DNA with XbaI, extracted 400–650 bp fragments from an agarose gel and ligated them to SuperSNX linkers (Glenn and Schable, 2005). Inserts were amplified by PCR and cloned using the TOPO TA cloning kit (Invitrogen). A total of 63 *B. glandula* and 15 *C. dalli* clones were amplified and sequenced using BigDye® Terminator v3.1 (Applied Biosystems, ABI) and resolved on a 3730 ABI DNA analyzer. By aligning and trimming the resulting sequences using SeqMan® (DNASTAR), we obtained 28 *B. glandula* and 11 *C. dalli* contigs, for which primers were designed using Primer3 (Version 0.4.0) (Rozen and Skaletsky, 2000). Seven individuals of each species were amplified and those primer sets that resulted in expected product sizes and amplified in all individuals (13 primer sets in *B. glandula* and 8 primer sets in *C. dalli*) were resequenced. Five *B. glandula* and four *C. dalli* contigs contained a total of nine putative SNP markers in each species. Amplification and SNaPshot interrogation primers were designed for the SNP loci using Primer3 (Version 0.4.0) ((Rozen and Skaletsky, 2000), electronic supplementary material, Table S1, Table S2) and seven individuals were amplified using the ABI PRISM® SNaPshot™ Multiplex kit. Seven polymorphic SNP loci were identified for each species, where each had two alleles, the rare allele was observed at least twice among seven individuals, and there was