Journal of Experimental Marine Biology and Ecology 396 (2011) 224–232

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

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

Contrasting population makeup of two intertidal gastropod species that differ in dispersal opportunities

Kirsten M. Donald [a,*], Devon B. Keeney [b], Hamish G. Spencer [a]

[a] Allan Wilson Centre for Molecular Ecology and Evolution, Department of Zoology, University of Otago, PO Box 56, Dunedin, 9054, New Zealand
[b] Department of Biological Sciences, Le Moyne College, 1419 Salt Springs Road, Syracuse, NY 13214-1301, USA

ARTICLE INFO

Article history:
Received 1 March 2010
Received in revised form 27 October 2010
Accepted 29 October 2010

Keywords:
Diloma nigerrima
Diloma subrostrata
Genetic structure
Microsatellite
Monodontinae
Rafting
Trochidae

ABSTRACT

This study of population structure of two intertidal monodontine species: *Diloma nigerrima* and *Diloma subrostrata*, revealed the level of genetic connectedness among populations. Despite their markedly different geographic ranges (*D. nigerrima* is a geographically widespread species, inhabiting both Chile and New Zealand, including its subantarctic islands, whereas *D. subrostrata* is endemic to New Zealand), both species are believed to possess short-lived lecithotrophic larval stages. Polymorphic DNA microsatellite sequences were used to reveal the level of genetic connectedness among populations, thus inferring the two species' relative effective dispersal abilities. For each species *F* statistics, AMOVA values and the strength of the relationship between geographic and genetic distance were calculated. We observed a higher within-species level of genetic variation ($\Phi_{ST}=0.099$ vs. $\Phi_{ST}=0.016$) and a higher proportion of variance (11.15% vs. 0%) among populations of *D. nigerrima* than of *D. subrostrata*. A larger fraction of significant F_{ST} values was observed among *D. nigerrima* population pairs (65%) than among *D. subrostrata* population pairs (33%). Significant correlation between genetic and geographic distance was observed for *D. nigerrima* but not for *D. subrostrata*, but this relationship was not consistent among pairwise *D. nigerrima* population comparisons and PCA analysis confirms that, for each species, population structure does not follow a consistent pattern of increasing with geographic distance. The lack of population structure among *D. subrostrata* populations is probably due to its ubiquitous distribution, meaning little opportunity exists for genetic structure. *D. nigerrima*, by contrast has a patchier distribution, which allows for greater opportunities for genetic differentiation to occur. We argue that, despite the probable short larval stage in this species, the lack of geographical pattern in the genetic structure found in *D. nigerrima* is best explained by occasional dispersal over relatively short distances around the coast of New Zealand, over longer distances from New Zealand to the subantarctic islands and even across the Pacific Ocean from New Zealand to Chile.

1. Introduction

The open oceans are often regarded as a geographic barrier to the dispersal of intertidal animals that have no larval stage or possess relatively short-lived, non-feeding larvae. Nevertheless, there is a large body of literature detailing examples of lecithotrophs, brooders, and direct developers, with biogeographic ranges spanning oceans (e.g., Jackson, 1986; Johannesson, 1988; Ó Foighil, 1989; Davenport and MacAlister, 1996; Collin, 2003; Waters and Roy, 2004a; Donald et al., 2005b). This commonly observed phenomenon in marine invertebrates is often believed to be the result of adult rafting (Ó Foighil, 1989; Donald et al., 2005b; Waters, 2008) and, despite a general emphasis in the past on larvae as a means of dispersal, the importance of rafting in the marine environment is now recognized (Jackson, 1986; Davenport and MacAlister, 1996; Waters, 2008). Indeed Jackson (1986) argued that rafting, either on natural substrates, such as seaweed or pumice, or attached to ships, may be the only possible dispersal mechanism across wide stretches of open ocean for marine invertebrates whose larval stage lasts less than 1 month. Rafting has probably acted as an alternate method of long-distance dispersal for numerous intertidal organisms that do not possess long-lived, feeding larvae, such as oysters (Ó Foighil et al., 1999), gastropods (Smith and Simpson, 1995), sponges and ascidians (Jackson, 1986), and topshells (Donald et al., 2005b).

Members of the trochid genus *Diloma* Philippi, 1845 are broadcast spawners, releasing eggs and sperm into the water column in stormy weather (Grange, 1976). Although the feeding behavior of only a minority of species of trochacean larvae has been studied (Hickman, 1992), the absence of velar structures and a ciliated food groove argues against effective suspension feeding among these molluscs, meaning that larvae are most likely short-lived lecithotrophs. In her review article, Hickman (1992) reported trochacean larvae stages of between 2 and 28 days for a diverse range of trochids. However, the larval stage of *Diloma* is most likely to be at the shorter end of this time

* Corresponding author.
E-mail address: kirsten.donald@stonebow.otago.ac.nz (K.M. Donald).

0022-0981/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2010.10.028

scale: larvae of the congeners *Diloma aethiops* and *Diloma zelandica* were estimated to begin to settle after 5 days (K. Grange, personal communication) and the closely related *Austrocochlea constricta*, which recent phylogenetic analysis places with *Diloma* in the subfamily Monodontinae (Williams et al., 2010), is believed to have a larval stage lasting only a few days (Underwood, 1974). During this time, larvae would be unable to disperse more than a few hundred kilometers and probably much less (Moran, 1997).

In this report, we use microsatellite analysis to contrast population structure of two species of *Diloma*: *Diloma subrostrata* (Gray, 1835) and *Diloma nigerrima* (Gmelin, 1791). The endemic New Zealand *D. subrostrata* predominantly inhabits mudflats and is abundant in estuaries around the coast of New Zealand's main islands (Willan, 1979). It is, however, absent from New Zealand's more far-flung islands, including the Chathams and the subantarctic islands. By contrast, *D. nigerrima* is patchily distributed over a large geographic range: the main and subantarctic islands of New Zealand, the Chatham Islands, and southern Chile (Donald et al., 2005b). In New Zealand, there are large stretches of coastline where *D. nigerrima* is absent, as it requires the presence of decaying wracks of bullkelp, *Durvillaea antarctica*, at and above the high tide level. Where the species occurs, however, it is often found in vast numbers (Powell, 1979). Analyses by Donald et al. (2005a) of >2 kb of the mitochondrial genes 16S and COI and the nuclear gene actin confirmed that populations from New Zealand and Chile are indeed the same species. Nevertheless, the divergence between New Zealand and Chilean populations was estimated at 0.2 to 0.6 mya, which precludes any possible anthropogenic dispersal.

We have previously argued (Donald et al., 2005b) that one of these species, *D. nigerrima*, disperses chiefly as an adult via rafting, whereas the other, *D. subrostrata*, which does not live on kelp, presumably relies on larval dispersal. The claim for dispersal by rafting of adult *D. nigerrima* is based on several circumstantial strands of evidence, namely its life cycle, geographic distribution, and position within the topshell phylogeny and prevailing currents in the Southern Ocean. The inferred length of the larval stage (see above) precludes larval dispersal across the open ocean between New Zealand and southern Chile; transport via seabirds or other animal vectors is also most improbable.

One possible rafting candidate is *Durvillaea antarctica*. We suggest bullkelp as a potential raft for four reasons: (i) it is extremely tough and buoyant, capable of surviving many months in the water column (Morton and Miller, 1973); (ii) it is abundant in the Southern Ocean, with millions of kelp rafts estimated to be circulating in the Southern Ocean (Smith, 2002); (iii) both bullkelp and members of the bullkelp holdfast community (e.g., the flat oyster, *Ostrea chilensis*) have a similar distribution (Ó Foighil et al., 1999; Fraser et al., 2009); (iv) *D. nigerrima* lives directly on the hypothesized raft, beach-cast bullkelp.

Owing to their widely differing habitats, ubiquity, and geographic range, one would expect these two species to exhibit differing levels of genetic structure. We hypothesize that *D. subrostrata*, with its smaller geographic range and ubiquitous presence around the coast of New Zealand, will exhibit a lower level of intraspecific population structure than the more patchily distributed, widely dispersed *D. nigerrima* because there is less opportunity for geographic isolation among *D. subrostrata* populations. We also hypothesize that, if adult rafting is an important mechanism of *D. nigerrima* dispersal, we would expect to observe some geographically distant *D. nigerrima* populations with little genetic differentiation.

2. Materials and methods

2.1. Sample collection

D. subrostrata and *D. nigerrima* were collected from intertidal sites around the coast of mainland New Zealand, and *D. nigerrima* was also collected from Chile and two sites in the subantarctic Auckland Islands (Auckland Island itself and Enderby Island) south of mainland New Zealand (see Table 1 and Fig. 1 for sampling sites and numbers sampled). Live snails were immediately crushed upon collection, preserved in 70% ethanol, and returned to the laboratory where they were stored at 4 °C.

2.2. Isolation and characterization of polymorphic loci

Five polymorphic microsatellite loci were isolated and characterized from *D. subrostrata* using the method described in Donald and Spencer (2006). As none of the 5 loci identified from *D. subrostrata* were shared with *D. nigerrima*, it was necessary to identify and examine novel loci from *D. nigerrima*. The same method was employed for isolation and characterization of three novel polymorphic microsatellite *D. nigerrima* loci. Following isolation and characterization of all polymorphic loci, PCR conditions were then optimized and used in all subsequent genotyping (see legend in Table 2). While the number of loci used in this study was small, if analyses of loci reveal population structure, then the conservative

Table 1
Topshell species collected and their sampling sites.

Topshell species	Sampling site	Sample code	Map reference	Number analyzed
Diloma subrostrata (Gray, 1835)	Beachlands, South Auckland, New Zealand	BL	36°53′S 174°59′E	28
	Cornwallis, South Auckland, New Zealand	CW	37°00′S 174°36′E	32
	Maketu, South Auckland, New Zealand	MK	37°45′S 176°27′E	30
	Raglan, South Auckland, New Zealand	RG	37°48′S 174°52′E	28
	Porirua Harbour, Wellington, New Zealand	PH	41°08′S 174°50′E	30
	Nelson, Nelson, New Zealand	NL	41°18′S 174°48′E	22
	Heathcote Estuary, Canterbury New Zealand	HE	43°33′S 172°45′E	30
	Purakaunui Bay, Otago, New Zealand	PB	45°44′S 170°37′E	27
	Bluff Harbour, Southland, New Zealand	BH	46°37′S 168°18′E	29
Diloma nigerrima (Gmelin, 1791)	Maunganui Bluff, Northland, New Zealand	MG	35°44′S 173°32′E	24
	O'Neills Beach, South Auckland, New Zealand	ON	36°53′S 174°26′**E**	24
	Raglan, South Auckland, New Zealand	RG	37°48′S 174°52′E	24
	Wellington, Wellington, New Zealand	WL	41°18′S 174°48′E	24
	Kaikoura, Marlborough, New Zealand	KK	42°24′S 173°41′E	24
	Little Akaloa, Canterbury, New Zealand	LA	43°40′S 172°59′E	24
	St Clair, Otago, New Zealand	SC	45°55′S 170°29′E	24
	Bluff Harbour, Southland, New Zealand	BH	46°37′S 168°18′E	24
	Enderby Island, subantarctic island, New Zealand	EI	50°30′S 166°17′E	24
	Auckland Island, subantarctic island, New Zealand	AI	50°47′S 166°00′E	24
	Concepcion, Chile	CO	36°60′S 73°00′W	19
	Puelche, Chile	PU	41°44′S 72°39′W	5