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Invasion success of a habitat-forming marine invertebrate is limited by lower-than-expected dispersal ability

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ABSTRACT: Species that disperse by means of planktonic larvae are typically not genetically structured along environmentally homogeneous coastlines. In contrast, those that lack a planktonic dispersal phase, or species with a short (<12 h) pelagic propagule duration (PPD), tend to show population genetic structure at small spatial scales, with dispersal often taking place by means of a stepping-stone process. These general patterns emerged in the literature after decades of studies based on relatively poorly resolving genetic markers (e.g. allozymes and DNA sequences). However, recent evidence based on more informative genetic markers (microsatellites) suggests that stepping-stone dispersal is not uncommon in species with a PPD of days to weeks. Here, we used microsatellite data to investigate genetic structure in a non-native population of the solitary ascidian *Pyura doppelgangera* in southern Australia. This species is part of a group of marine invertebrates with great potential to become invasive, whose 1 day PPD was considered to be sufficiently long to drive genetic homogeneity along continuous coastlines. We identified genetic structure at scales of a few kilometres, with clear signatures of larval retention at natal sites. This limited dispersal potential may explain why the species has not yet established itself throughout the invaded region. Our results add to the growing evidence that many previous studies may have over-interpreted the dispersal potential of this group, likely because of insufficient resolution of the more slowly evolving DNA markers used to make inferences at ecological time-scales.

KEY WORDS: Ascidia · Approximate Bayesian Computation · Connectivity · Genetic structure · Microsatellites · *Pyura doppelgangera* · Sea squirt

INTRODUCTION

Sessile or sedentary marine invertebrates disperse primarily by means of propagules, but considerable differences in the offspring's mode of development may manifest itself in different patterns of population genetic structure (Strathman 1985). Species with direct development (i.e. those whose offspring hatch fully developed and remain in the parent habitat) tend to be highly structured, with genetic exchange between sites reflecting a stepping-stone model of dispersal (Teske et al. 2007). In contrast, the extent of population structuring in species whose life history includes a planktonic dispersal phase is far from consistent. With the possible exception of species whose pelagic propagule duration (PPD) is <12 h (e.g. Shanks 2009), the magnitude of population genetic structure is usually not correlated with species' PPD (Banks et al. 2007, Piggot et al. 2008, Weersing & Toonen 2009), possibly because behavioural mechanisms and local currents can strongly influence the propagules' realised dispersal (Taylor & Hellberg, 2003). Although most studies on species that disperse actively or by means of planktonic propagules have

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identified panmixia in coastal regions that lack dispersal barriers, there are also numerous examples of stepping-stone dispersal in such species (e.g. Pogson et al. 2001, Polato et al. 2010, Teske et al. 2015).

Sea squirts (Urochordata: Ascidiaceae) are a group of marine invertebrates that includes several highly invasive species (Lambert & Lambert 2003, Coutts & Forrest 2007, Wallentinus & Nyberg 2007). Of particular concern are habitat-forming species of the genus *Pyura*, a group of solitary ascidians that can radically alter invaded habitats by overgrowing native habitat-forming species (Castilla et al. 2004, Rius & Teske 2013). Most solitary ascidians have free-swimming embryos, while those of colonial ascidians are brooded (Stewart-Savage et al. 2001, Brown & Swalla 2012). Subsequent larval development takes a few minutes in colonial forms, whereas the lecithotrophic larvae of solitary forms settle within 24 h (Griffiths 1976, Svane & Young 1989, Clarke et al. 1999). This difference in larval duration has been used to explain why colonial ascidians are genetically distinct at each site along a continuous habitat (Ayre et al. 1997, Yund & O'Neil 2000), whereas solitary forms are often genetically homogeneous over hundreds or even thousands of kilometres of coastline (Nóbrega et al. 2004, Ordóñez et al. 2013, Teske 2014, but see David et al. 2010). Solitary ascidians may pursue various strategies of propagule retention that include negatively buoyant eggs and mucous strings (Svane & Havenhand 1993), foam (Castilla et al. 2007) and larval preference for shade (Svane & Young 1989). High dispersal potential can thus not be taken for granted on the basis of a propagule duration that exceeds half a day (Shanks 2009). Given the recent evidence from rapidly mutating microsatellite DNA markers that genetic differentiation in marine species with much longer larval duration can be affected by geographic distance (e.g. Coleman et al. 2013, Teske et al. 2015), it is possible that the markers most commonly used to study solitary ascidians (e.g. allozymes, mitochondrial DNA and nuclear introns) provide insufficient resolution to detect genetic structure.

Here, we used microsatellites to study the genetic structure of a non-native South Australian population of the solitary ascidian *Pyura doppelgangera* Rius & Teske, 2013, with the aim of explaining why this species has so far failed to establish itself beyond the immediate vicinity of its point of introduction. The finding that recruitment is mostly local adds to the growing evidence that slowly evolving DNA markers fail to identify marine species' true dispersal potential, and stresses the necessity of using highly variable markers particularly when studying genetically impoverished introduced species. As such, this study reconciles the discrepancies between direct estimates of solitary ascidians' dispersal potential (Bingham & Young 1991) with those inferred by means of genetic data.

MATERIALS AND METHODS

Study species and sampling

The solitary ascidian *Pyura doppelgangera* is native to Tasmania and a non-indigenous species in mainland Australia, where its range is very limited (Teske et al. 2014), and in New Zealand, where it has become invasive (Hayward & Morley 2009, Jones 2011). As it was previously synonymised with the mainland species *P. praeputialis* and has only recently been described as a distinct species (Rius & Teske 2013), there are no historical records of its colonisation history in mainland Australia. Nonetheless, the evidence for its alien status in this region is very strong and includes (1) its exclusive presence near harbours (Teske et al. 2014), i.e. typical points of introduction for alien marine species (Carlton & Geller 1993); (2) its exclusive presence on artificial structures due to a lack of natural substrate suitable for settlement, suggesting that this species has only established itself in habitats in mainland Australia in which its native sister taxon *P. praeputialis* was not represented; (3) recent genetic divergence from its inferred source population in northern Tasmania; and (4) lower genetic diversity than in Tasmania (Teske et al. 2014).

Samples of *P. doppelgangera* were collected at 6 sites along the metropolitan coast of Adelaide in South Australia (Fig. 1). Details on DNA extraction and microsatellite data generation are described elsewhere (Molecular Ecology Resources Primer Development Consortium et al. 2013, Teske et al. 2014). We used 7 of the 8 microsatellite loci described in Teske et al. (2014) because 1 (*Pysp*02) was not variable in the population residing in Adelaide, which is consistent with its proposed non-native status. Some of the data used here were previously used to investigate historical colonisation scenarios throughout temperate Australia and New Zealand (Teske et al. 2014), rather than addressing fine-scale dispersal as in the present study. To improve geographic cover, genetic data from 2 additional sites (Sites 1 and 6; Fig. 1, Table 1) were added to the original data set from 4 sites. An extensive survey along ~500 km of

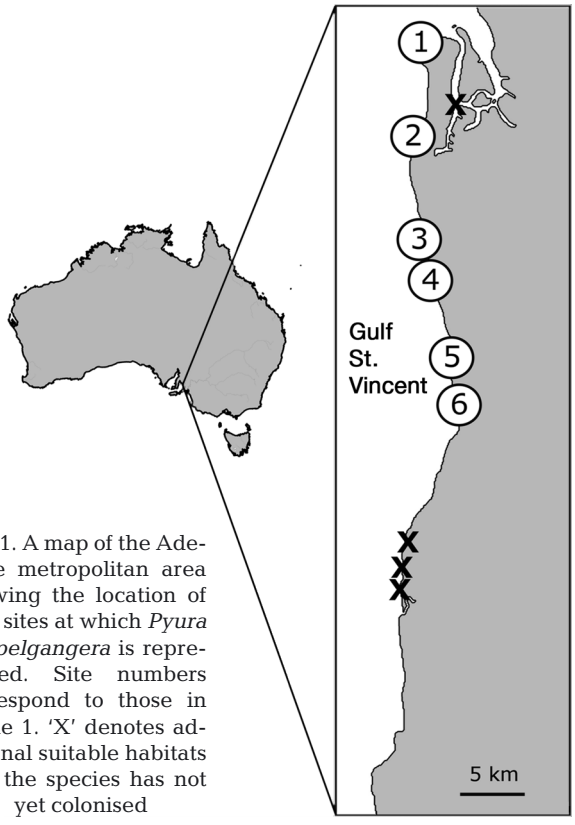

Fig. 1. A map of the Adelaide metropolitan area showing the location of all 6 sites at which *Pyura doppelgangera* is represented. Site numbers correspond to those in Table 1. 'X' denotes additional suitable habitats that the species has not yet colonised

coastal South Australia confirms that the sampling area thus encompasses the species' entire regional distribution range.

Descriptive statistics

Allele frequencies per locus were calculated in GenAlEx v6.5 (Peakall & Smouse 2012). Allelic richness (AR, the number of alleles in a sample; Kalinowski 2004) for all microsatellite loci was calculated using HP-Rare v1.1 (Kalinowski 2005). Rarefaction was applied to account for differences in sample sizes (the smallest number of individuals at a particular site was 30 at Site 2, and this number of individuals was sub-sampled at all other sites to allow for direct comparisons, Table 1). As sea squirts often mate with close kin (Dupont et al. 2009), we calculated observed heterozygosity and expected heterozygosity for each locus and site using Genetix v4.05.2 (Belkhir et al. 1996–2004), and used the same programme to estimate the overall inbreeding coefficient (F_{IS}) at each site, with confidence intervals of F_{IS} determined using 1000 bootstrap replications.

Tests for genetic structure

Tests for genetic structure among pairs of sites were conducted in GenAlEx using the genetic structure statistics F_{ST} (Wright 1943) and G''_{ST} (Meirmans & Hedrick 2011). G''_{ST} is the unbiased estimator of G'_{ST} (Hedrick 2005), which is an equivalent of F_{ST} standardised by the maximum value it can obtain among populations when no alleles are shared. In multiallelic loci such as microsatellites, this maximum value is often considerably lower than the maximum value of 1 in biallelic markers (Meirmans & Hedrick 2011). Significance was tested using 999 permutations, and the B-Y false discovery rate method (Benjamini & Yakutieli 2001) was applied to account for multiple comparisons.

Analyses of spatial genetic structure

In species whose dispersal potential is limited, and in which gene flow occurs primarily among adjacent demes, genetic differentiation is positively correlated with genetic distance (Wright 1943, Slatkin 1993). Tests for correlations between genetic and geographic distances are problematic when multiple genetic clusters were sampled (Meirmans 2012). A

Table 1. Sampling sites along the metropolitan coast of Adelaide, South Australia (arranged from north to south), and population genetic statistics for *Pyura doppelgangera* microsatellite DNA data. AR: allelic richness (calculated for 30 individuals); F_{IS}: inbreeding coefficient; CI: confidence interval

Site no.	Site name	No. of samples	Coordinates	AR	F_{IS} (95% CI)
1	Outer Harbour	31	34° 46′ 47.98″ S, 138° 28′ 50.24″ E	1.55	0.12 (−0.10, 0.30)
2	Semaphore Beach	30	34° 50′ 15.14″ S, 138° 28′ 35.99″ E	1.72	−0.02 (−0.17, 0.10)
3	Grange Beach	49	34° 54′ 09.47″ S, 138° 29′ 14.24″ E	1.65	−0.01 (−0.22, −0.08)
4	Henley Beach	46	34° 55′ 11.21″ S, 138° 29′ 31.42″ E	1.59	−0.11 (−0.23, 0.00)
5	Glenelg Beach	37	34° 58′ 49.75″ S, 138° 30′ 35.27″ E	1.68	−0.12 (−0.28, 0.01)
6	Brighton Beach	39	35° 01′ 02.91″ S, 138° 30′ 48.12″ E	1.75	−0.07 (−0.20, 0.05)

cluster analysis showed that this is not an issue in this particular system, as all sites are part of the same cluster, and individuals are closely related and likely descend from a small number of founder individuals (Teske et al. 2014). A Mantel test (Mantel 1967) was run in GenAlEx to test for statistically significant correlations between genetic and geographic distance matrices comprising data from individuals. Geographical distances among sites were measured as the shortest along-coast distances in Google Earth, and significance was tested using 999 random permutations. We also plotted geographic versus genetic distances, in this case with data from sites rather than individuals, using G''_{ST} as the genetic distance measure, and tested for a relationship between the 2 variables using a linear regression analysis in Sigma Stat 1.0 (Systat Software).

Spatial autocorrelation analysis, an approach that allows identifying the spatial scale at which genetic discontinuities occur, was used to infer larval retention at natal sites. We computed the autocorrelation coefficient r in GenAlEx for distance classes of 1 km. This coefficient is related to Moran's I (bounded by 0 and 1; Moran 1948) but can account for both positive and negative autocorrelation (i.e. bounded by +1 and −1; Smouse & Peakall 1999). Statistical significance was tested by estimating 95% confidence intervals for the null distribution under panmixia using 1000 random permutations, an approach that eliminates spatial structure. We also estimated the confidence intervals of r by specifying 1000 bootstrap replications. When estimates of r are significantly greater than expected under conditions of panmixia at lower distance classes, then this suggests that most larvae settle close to their natal habitat. In contrast, a value of r that does not differ from expectations under panmixia indicates that larvae have an equal chance of settling at other sites.

Testing colonisation scenarios

The approximate Bayesian computation (ABC) approach implemented in DIYABC v2.0 (Cornuet et al. 2014) was used to determine whether there was stronger support for stepping-stone dispersal (Scenario 1) compared to a scenario in which the population at the point of introduction seeded all other populations (Scenario 2). Harbours represent important points of introduction for alien marine species (Cariton & Geller 1993, Lambert & Lambert 2003), and, in this case, the ferry terminal at Outer Harbour (Site 1) represents the most likely point of introduction in Adelaide. In both scenarios, newly established populations experienced a short genetic bottleneck (Fig. 2). We specified default parameters for microsatellites and selected the following summary statistics: mean size variance (1 sample), mean size variance (2 samples) and $(\delta\mu)^2$ distance (Goldstein et al. 1995). Pre-evaluation of scenario-prior combinations indicated that these statistics produced simulated data sets that did not deviate significantly from the observed data set. We simulated 2 million data sets and determined posterior probabilities of each scenario using a logistical regression estimate based on 20 000 data sets (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m536p221_supp.pdf for details on priors and mutation models).

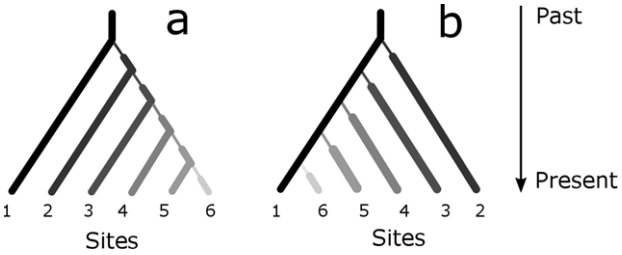

Fig. 2. Colonisation scenarios tested using approximate Bayesian computation analyses. (a) Scenario 1 depicts a stepping-stone model of colonisation, in which Site 1 (black) gives rise to Site 2 (dark grey), which in turn gives rise to Site 3 (lighter grey), etc. (b) In Scenario 2, Site 1 colonises all other sites. All newly established populations go through a brief genetic bottleneck

RESULTS

Pyura doppelgangera in Adelaide is characterised by very low genetic diversity, with the overall number of alleles per locus ranging from 3 (in *Pysp*12, *Pysp*13, *Pysp*15, *Pysp*25 and *Pysp*26) to 5 (in *Pysp*03). We identified no latitudinal trends in allelic richness. Even though observed heterozygosity was lower than expected heterozygosity in numerous instances (see Table S2 in the Supplement), there was no evidence for inbreeding, as F_{IS} values were not significantly greater than zero at any of the 6 sites (Table 1). Genetic structure was found among 7 out of 15 pairs of sites (Table 2). Results were congruent irrespective of the statistic used.

The Mantel test identified significant correlations between genetic and geographic distances at the level of individuals (R_{xy} = 0.07, p = 0.01), and a linear regression analysis of geographic distance versus G''_{ST} was also highly significant (F = 9.13, p = 0.01;

Table 2. Estimates of 2 genetic structure statistics calculated for pairs of sites at which *Pyura doppelgangera* was collected. Below diagonal: F_{ST}; above diagonal G''_{ST}. Significance (after correction for multiple comparisons) is indicated as: *p < 0.05 (corrected: 0.020); **p < 0.01 (corrected: 0.004)

	1	2	3	4	5	6
1		0.041*	0.005	0.023	0.032	0.079**
2	0.024*		0.028*	0.033*	0.042**	0.027
3	0.008	0.017*		0.008	0.020*	0.038**
4	0.016	0.019*	0.008		0.000	0.013
5	0.020	0.023**	0.013*	0.004		0.009
6	0.038**	0.017	0.019**	0.010	0.009	

the data passed the tests for normality and homogeneity of variances required for this test with p = 0.49 and p = 0.12, respectively). A positive slope of the plot of geographic versus genetic distance among sites (see Fig. S1) confirms this result, as does a spatial autocorrelation plot (with 5 km distance classes), in which spatial autocorrelation at the lowest distance class was significantly positive, and that at the highest distance class was significantly negative (Fig. 3). A spatial autocorrelation plot using 1 km distance classes (Fig. S2) shows that this trend was largely due to individuals from the same artificial structure being significantly more closely related to each other than they were to individuals from other structures (1 km distance class; smallest distance between sites [3 and 4]: 1.95 km) and Sites 1 and 6 being highly distinct (see also Table 2).

The ABC analysis strongly supported a stepping-stone model of colonisation (Scenario 1: posterior probability: 0.89, 95% confidence interval: 0.87 to 0.91) over a scenario in which the population at Site 1 (Outer Harbour) colonised all jetties to the south (Scenario 2: posterior probability: 0.11, 95% confidence interval: 0.10 to 0.13).

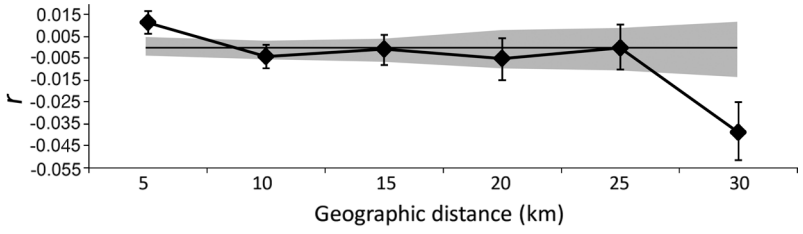

Fig. 3. Spatial autocorrelation correlogram for *Pyura doppelgangera* in the Adelaide metropolitan area. The correlogram depicts the autocorrelation parameter *r* at a scale of kilometres comparing all 6 sites. Geographic distances represent the end point of a particular 5 km distance class. The shaded areas represent the 95% confidence interval under the assumption of panmixia, beyond which the hypothesis of no spatial autocorrelation is rejected, and the error bars represent confidence intervals around *r*

DISCUSSION

Most genetic studies on solitary ascidian populations residing on continuous coastlines did not identify genetic structure (e.g. Nóbrega et al. 2004, Ordóñez et al. 2013, Teske 2014, but see David et al. 2010), even though direct estimation of dispersal distances suggests limited dispersal potential at a scale of hundreds of metres (Bingham & Young 1991). The developmental biology of the recently described *Pyura doppelgangera* has not yet been studied, but the species' PPD can be expected to exceed 12 h, as shown for all the Australian and African congeners with which it shares a recent evolutionary origin (Anderson 1976, Griffiths 1976, Clarke et al. 1999), and much longer than that of colonial ascidians (Ayre et al. 1997, Yund & O'Neil 2000). The present study adds to the growing evidence that the simple rule that populations of colonial ascidians being genetically structured while those of solitary ascidians are not (Ayre et al. 1997) does not apply universally (e.g. Demarchi et al. 2008, Dupont et al. 2009, David et al. 2010).

This study suggests that the spread of a newly established solitary ascidian population from the point of introduction can be expected to be slow when substrata suitable for colonisation are spaced a few kilometres apart. Our results potentially explain the present distribution of *P. doppelgangera* in the metropolitan area of Adelaide: even though there are additional piers south of Site 6, and ample habitat for settlement in the protected Inner Harbour (sites denoted with X in Fig. 1), the species is not yet represented there, despite having been present in the region at least since the middle of the previous century (Kott 1952, Teske et al. 2014). If the species was indeed introduced in the Outer Harbour, the only sampling site that receives shipping traffic from Tasmania (the native habitat of *P. doppelgangera*), then it has been spreading southwards at a very slow rate. As with all non-indigenous species that may become introduced to new habitats by attaching themselves to artificial structures, it is not possible to determine conclusively whether *P. doppelgangera* spread by means of planktonic propagules or whether its southward range extension was facilitated by the attachment of adults to small vessels. The pattern of isolation by geographic distance identified for this population, although weak, supports the former, as

genetic differentiation in solitary ascidians that are primarily dispersed through human activities is unrelated to geographic distance (Dupont et al. 2009). While the colonisation scenarios tested using ABC analyses represent 2 extremes, and the true colonisation process was likely intermediate, the very strong support for stepping-stone dispersal further corroborates occasional dispersal over short distances by means of propagules. This conclusion is further supported by the fact that the ferry terminal at Outer Harbour is not accessible to local boat traffic, that the Inner Harbour has by far the most boat traffic but lacks any settlement by *P. doppelgangera*, and that none of the piers in the area are suitable for the mooring of vessels.

The distribution range of *P. doppelgangera* in mainland Australia is limited to Adelaide (South Australia) and Corner Inlet (Victoria) (Teske et al. 2011, 2014). Both regions lack natural substrate suitable for settlement, which may also explain why there are no natural populations of the native mainland species *P. praeputialis* in these areas. Prior to European settlement, the coast of the Adelaide metropolitan area was a continuous Holocene sand dune system in which ascidians were unable to settle (Bowman & Harvey 1986). Initiatives to stabilise the coastline primarily involve the pumping of sand to erosion locations, and the number of artificial structures that could serve as habitat for *P. doppelgangera* is limited (Coastal Management Branch, Department of Environment and Planning 1984). Because of this, a management program that involves physical removal (Jones 2011) may be successful in eliminating this species before it spreads to other sites in South Australia, such as Port Lincoln, to whose aquaculture industry it presents a significant economic threat. The exclusive presence of *P. doppelgangera* on artificial structures, and the long stretches of sandy beach between them, may explain its limited range. In areas where habitat is more continuous, invasion success can be expected to be significantly greater. In a study on an invasive population of the solitary ascidian *Microcosmus squamiger*, Ordóñez et al. (2013) did not find evidence for larval retention on the basis of microsatellite data, possibly because of the presence of ample natural and artificial habitat for settlement. For *P. doppelgangera*, a similar situation may exist in North Island, New Zealand, where this species was already well established when it was discovered in 2007 (Hayward & Morley 2009). Rocky shore habitat suitable for settlement in New Zealand is extensive, and the species' range is increasing rapidly (Jones 2011).

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LITERATURE CITED

Anderson DT, White BM, Egan EA (1976) The larval development and metamorphosis of the ascidians *Pyura praeputialis* (Heller) and *Pyura pachydermatina* (Herdman) (Pleurogona, Family Pyuridae). Proc Linn Soc New South Wales 100:205–217

Ayre DJ, Davis AR, Billingham M, Llorens T, Styan C (1997) Genetic evidence for contrasting patterns of dispersal in solitary and colonial ascidians. Mar Biol 130:51–61

Banks SC, Piggott M, Williamson J, Bové U, Holbrook N, Beheregaray LB (2007) Oceanic variability and coastal topography shape local genetic structure in a long-dispersing marine invertebrate. Ecology 88:3055–3064

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996–2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier (France)

Benjamini Y, Yekutieli D (2001) The control of false discovery rate under dependency. Ann Stat 29:1165–1188

Bingham RL, Young CM (1991) Larval behaviour of the ascidian *Ecteinascidia turbinata* Herdman; an *in situ* experimental study of the effects of swimming on dispersal. J Exp Mar Biol Ecol 145:189–204

Bowman G, Harvey N (1986) Geomorphic evolution of a Holocene beach–ridge complex, LeFevre Peninsula, South Australia. J Coast Res 2:345–362

Brown FD, Swalla BJ (2012) Evolution and development of budding by stem cells: ascidian coloniality as a case study. Dev Biol 369:151–162

Carlton JT, Geller JB (1993) Ecological roulette: the global transport of nonindigenous marine organisms. Science 261:78–82

Castilla JC, Guiñez R, Caro AU, Ortiz V (2004) Invasion of a rocky intertidal shore by the tunicate *Pyura praeputialis* in the Bay of Antofagasta, Chile. Proc Natl Acad Sci USA 101:8517–8524

Castilla JC, Manríquez PH, Delgado AP, Gargallo L, Leiva A, Radic D (2007) Bio-foam enhances larval retention in a free-spawning marine tunicate. Proc Natl Acad Sci USA 104:18120–18122

Clarke M, Ortiz V, Castilla JC (1999) Does early development of the Chilean tunicate *Pyura praeputialis* (Heller, 1978) explain the restricted distribution of the species? Bull Mar Sci 65:745–754

Coastal Management Branch, Department of Environment and Planning (1984) Adelaide Coast Protection Strategy Review, March 1984. Adelaide.

Coleman MA, Feng M, Roughan M, Cetina-Heredia P, Connell SD (2013) Temperate shelf water dispersal by Australian boundary currents: implications for population connectivity. Limnol Oceanogr 3:295–309

Cornuet JM, Pudlo P, Veyssier J, Dehne-Garcia A and others (2014) DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. Bioinformatics 30:1187–1189

Coutts ADM, Forrest BM (2007) Development and application of tools for incursion response: lessons learned from the management of the fouling pest *Didemnum vexillum.* J Exp Mar Biol Ecol 342:154–162

David GK, Marshall DJ, Riginos C (2010) Latitudinal variability in spatial genetic structure in the invasive ascidian, *Styela plicata.* Mar Biol 157:1955–1965

Demarchi M, Chiappero M, Laudien J, Sahade R (2008) Population genetic structure of the ascidian *Sytela rustica* at Kongsfjorden, Svalbard, Arctic. J Exp Mar Biol Ecol 364: 29–34

Dupont L, Viard F, Dowell MJ, Wood C, Bishop JDD (2009) Fine- and regional-scale genetic structure of the exotic ascidian *Styela clava* (Tunicata) in southwest England, 50 years after its introduction. Mol Ecol 18:442–453

Goldstein DB, Linares AR, Cavalli-Sforza LL, Feldman MW (1995) An evaluation of genetic distances for use with microsatellite loci. Genetics 139:463–471

Griffiths R (1976) The larval development of *Pyura stolonifera* (Tunicata). Trans R Soc S Afr 42:1–9

Hayward BW, Morley MS (2009) Introduction to New Zealand of two sea squirts (Tunicata, Ascidiacea) and their subsequent dispersal. Rec Auckland Mus 46:5–14

Hedrick PW (2005) A standardized genetic differentiation measure. Evolution 59:1633–1638

Jones EJ (2011) *Pyura* treatment programme, stage two report. MAF Biosecurity, Wellington

Kalinowski ST (2004) Counting alleles with rarefaction: private alleles and hierarchical sampling designs. Conserv Genet 5:539–543

Kalinowski ST (2005) hp-rare 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5:187–189

Kott P (1952) Ascidians of Australia. I. Stolidobranchiata and Phlebobranchiata. Aust J Mar Freshwater Res 3:205–334

Lambert CC, Lambert G (2003) Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. Mar Ecol Prog Ser 259: 145–161

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27: 209–220

Meirmans PG (2012) The trouble with isolation by distance. Mol Ecol 21:2839–2846

Meirmans PG, Hedrick PW (2011) Assessing population structure: F_{ST} and related measures. Mol Ecol Resour 11: 5–18

Molecular Ecology Resources Primer Development Consortium et al. (2013) Permanent Genetic Resources added to Molecular Ecology Resources Database 1 October 2012–30 November 2012. Mol Ecol Resour 13:341–343

Moran PAP (1948) The interpretation of statistical maps. J R Stat Soc B 10:243–251

Nóbrega R, Sole-Cava AM, Russo CAM (2004) High genetic homogeneity of an intertidal marine invertebrate along 8000 km of the Atlantic coast of the Americas. J Exp Mar Biol Ecol 303:173–181

Ordóñez V, Pascual M, Rius M, Turon X (2013) Mixed but not admixed: a spatial analysis of genetic variation of an invasive ascidian on natural and artificial substrates. Mar Biol 160:1645–1660

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research — an update. Bioinformatics 28:2537–2539

Piggott MP, Banks SC, Tung P, Beheregaray LB (2008) Genetic evidence for different scales of connectivity in a marine mollusc. Mar Ecol Prog Ser 365:127–136

Pogson GH, Taggart CT, Mesa KA, Boutilier RG (2001) Isolation by distance in the Atlantic cod, *Gadus morhua*, at large and small geographic scales. Evolution 55:131–146

Polato NR, Concepcion GT, Toonen RJ, Baums IB (2010) Isolation by distance across the Hawaiian Archipelago in the reef–building coral *Porites lobata*. Mol Ecol 19: 4661–4677

Rius M, Teske PR (2013) Cryptic diversity in coastal Australasia: a morphological and mito-nuclear genetic analysis of habitat-forming sibling species. Zool J Linn Soc 168:597–611

Shanks AL (2009) Pelagic larval duration and dispersal distance revisited. Biol Bull 216:373–385

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity 82:561–573

Stewart-Savage J, Phillippi A, Yund PO (2001) Delayed insemination results in embryo mortality in a brooding ascidian. Biol Bull 201:52–58

Strathman AE (1985) Feeding and non-feeding larval development and life-history evolution in marine invertebrates. Ann Rev Ecol Syst 16:330–361

Svane IB, Havenhand JN (1993) Spawning and dispersal in *Ciona intestinalis* (L.). Mar Ecol 14:53–66

Svane I, Young CM (1989) The ecology and behaviour of ascidian larvae. Oceangr Mar Biol Rev Camb Philos Soc 27:45–90

Taylor MS, Hellberg ME (2003) Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299:107–109

Teske PR (2014) Connectivity in solitary ascidians: is a 24-h propagule duration sufficient to maintain large-scale genetic homogeneity? Mar Biol 161:2681–2687

Teske PR, Papadopoulos I, Zardi GI, McQuaid CD, Edkins MT, Griffiths CL, Barker NP (2007) Implications of life history for genetic structure and migration rates of southern African coastal invertebrates: planktonic, abbreviated and direct development. Mar Biol 152:697–711

Teske PR, Rius M, McQuaid CD, Styan CA and others (2011) 'Nested' cryptic diversity in a widespread marine ecosystem engineer: a challenge for detecting biological invasions. BMC Evol Biol 11:176

Teske PR, Sandoval-Castillo J, Waters JM, Beheregaray LB (2014) Can novel genetic analyses help to identify low-dispersal marine invasive species? Ecol Evol 4: 2848–2866

Teske PR, Sandoval-Castillo J, van Sebille E, Waters JM, Beheregaray LB (2015) On-shelf larval circulation limits population connectivity in a coastal broadcast spawner. Mar Ecol Prog Ser 532:1–12

Wallentinus I, Nyberg CD (2007) Introduced marine organisms as habitat modifiers. Mar Pollut Bull 55:323–332

Weersing K, Toonen RJ (2009) Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser 393:1–12

Wright S (1943) Isolation by distance. Genetics 28:114–138

Yund PO, O'Neil PG (2000) Microgeographic genetic differentiation in a colonial ascidian (*Botryllus schlosseri*) population. Mar Biol 137:583–588

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