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Genetic differentiation in the barnacle *Catomerus polymerus* despite migration across a biogeographic barrier

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ABSTRACT: Biogeographic barriers can set range limits for marine organisms by restricting migration, or because subsequent recruitment may be prevented by post-settlement selection. We used microsatellite and mtDNA data for adults and juveniles of the barnacle *Catomerus polymerus* to test the hypothesis that persistent differentiation of populations occurring to the northeast as compared with south and west of the southeast Australian biogeographic barrier (SEABB) is maintained by selection rather than dispersal limitation. We also explored the dispersal of *C. polymerus* along uninterrupted linear stretches of coastline (1440 km) to the east and west (120 km) of the SEABB. Within the regions flanking the SEABB, we found little genetic differentiation (F_{ST} < 0.006), implying strong gene flow. In contrast, adults to the northeast were significantly differentiated from those to the south and southwest of the SEABB (F_{ST} = 0.10), confirming some restriction of gene flow by the SEABB. Similarly, Bayesian analyses revealed eastern and western clusters of adults and juveniles, with the great majority assigning strongly to their region of collection. Nevertheless, 5 of 556 adults, and 10 of 537 juveniles, had genotypes that aligned more strongly with the genetic cluster of the opposite side of the SEABB, implying that migration does occur. Mitochondrial DNA sequence data (n = 71) revealed one additional eastern adult with an immigrant western haplotype. Our data imply that differentiation of eastern and western lineages reflects both restricted dispersal and possibly regional-scale selection acting on immigrant genotypes.

KEY WORDS: Australia · Gene flow · Marine invertebrate · Recruitment · Rocky intertidal shores · Range limits

INTRODUCTION

Biogeographic barriers can set range limits for marine invertebrates either because they prevent dispersal between biogeographic regions (Wares et al. 2001, Patarnello et al. 2007, Ayre et al. 2009, Galarza et al. 2009, Glor & Warren 2011) or separate regions that display contrasting environmental conditions, and hence different regimes of selection (Bell 2008, Sherman et al. 2008, Ayre et al. 2009). However, perhaps because most phylogeographic studies have focussed on patterns of differentiation of adults, there has been disproportionate emphasis on the role of dispersal limitation rather than selection in setting limits. Indeed, in cases where there is evidence that barriers separate populations of adults that are genetically differentiated (York et al. 2008), it is possible that larval supply, local oceanographic currents and geographical isolation can result in retention of larvae in either regional or local scales, leading to distinct assemblage structure and geographic distribution (Pannacciulli et al. 1997, Kojima et al. 2000, Wethey 2002, Dawson et al. 2010, Reece et al. 2010). Alternatively, however, genetic differentiation of lin-

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eages may result because immigrant offspring comprising of early settlers or juveniles, even if relatively common, may be removed by selection before they become reproductive adults. Few studies have tested for post settlement selection in maintaining the genetic separation of lineages (Schmidt & Rand 2001) of marine invertebrates at a major biogeographic barrier. There are, however, many studies that have shown that within marine populations allele frequencies differ significantly between adults and juveniles (Johnson & Black 1982, Watts et al. 1990, Hedgecock 1994, Moberg & Burton 2000), and in some cases such changes in allele frequencies reflect post settlement mortality as reported for the barnacle *Semibalanus balanoides* (Schmidt & Rand 2001) and the limpet *Siphonaria jeanae* (Johnson & Black 1984).

The most obvious but rarely implemented approach that allows assessment of the roles of selection and dispersal in maintaining genetic differentiation across marine biogeographic barriers is the comparison of the genotypes of adults and early life stages. The barnacle *Catomerus polymerus* provides an intriguing case study because mitochondrial DNA (mtDNA) sequence data have revealed reciprocal monophyly and, at a minimum, a 200 000 yr separation of lineages, found either side of the southeast Australian biogeographic barrier (SEABB) (Ayre et al. 2009; see York et al. 2008 for a report of one putative west to east migrant as judged by its mtDNA haplotypes). Nevertheless, variation at nuclear microsatellite markers reveals significant moderate genetic differentiation (York et al. 2008), which implies that differentiation is opposed by at least limited migration.

The SEABB represents a complex zone of disjunction for 2 major oceanographic currents, and is the apparent cause of both species range limits and the separation of genetic lineages for many intertidal rocky shore specialists (Knox 1963, Waters et al. 2005, Hidas et al. 2007, Ayre et al. 2009, McWilliam et al. 2013). On the Australian mainland, the barrier is typically considered to separate populations to the northeast of Ninety Mile Beach and to the west of Wilson's Promontory (at the southernmost tip of mainland Australia) (Ayre et al. 2009, see our Fig. 1). For rocky shore invertebrates, the SEABB represents the convergence zone of the southward flowing Eastern Australian Current (EAC), the westward flowing Zeehan Current and an extensive area of uninhabitable sandy shore that includes Ninety Mile Beach and that extends to Wilson's Promontory (see Ayre et al. 2009). Within this region, the EAC is typically erratic and often forms large offshore eddies. Surprisingly, however, the effectiveness of the SEABB in setting range limits or maintaining patterns of genetic differentiation varies widely, even within sets of related taxa (Ayre et al. 2009). Moreover, there is accumulating evidence that, in particular for subtidal species, the effectiveness of the barrier may be eroded by the increased southward penetration of the EAC as a result of climate change (Ling 2008, Ling et al. 2009). Strikingly, the southeast Australian coastline supports a suite of barnacle species considered to have similarly moderate pelagic larval duration and, according to available genetic data, are excellent dispersers within biogeographic regions (York et al. 2008, Ayre et al. 2009). Nevertheless, the barrier-region seemingly influences the population structures of these taxa differentially, because several lines of evidence imply that, while barnacle larvae are able to cross the SEABB, some of these taxa experience powerful effects of regional selection (described in the following paragraphs). The Bass Strait is also thought to limit dispersal to Tasmania which lies further to the south (see 'Materials and methods' for a more detailed explanation).

Most barnacle species on the east coast have described ranges that span the SEABB (Dakin 1952, Edgar 2008), but this may mask the effects of contrasting historical and contemporary processes. In addition to the case of *C. polymerus*, which arguably has lineages with long established range limits to either side of the SEABB but may be experiencing contemporary migration, 2 other well studied barnacle species display evidence of contemporary migration with contrasting consequences. *Tetraclitella purpurascens* spans the SEABB and, based on mtDNA, displays no genetic differentiation with a wide range of haplotypes occurring on both sides of the barrier (Ayre et al. 2009). These data imply that there is strong connectedness between regions and that *T. purpurascens* exhibits wide environmental tolerances, because the ocean conditions (for example, sea surface temperature and salinity) found on either side of the SEABB vary (Cresswell 2000, Ridgway 2007).

In contrast, *Tesseropora rosea*'s described southern range limit is immediately to the northeast of the SEABB, but there are several reports of ephemeral populations on the southwest side of this barrier (Jones 1990, Edmunds et al. 2004). This implies that while *T. rosea* larvae are able to cross the barrier and recruit, resultant populations are episodically eliminated by selection before reaching reproductive age or are simply not maintained because recruitment across the barrier occurs too infrequently.

Here, we use microsatellite and mtDNA genetic surveys of adult and juvenile *C. polymerus* to test for

evidence that migration across the SEABB occurs, but that the differentiation of adult populations is maintained by regional selection. Our approach was to characterise adult populations spread across widely separated mainland sites to the northeast of the SEABB, both southern mainland sites to the west of the SEABB and northeastern Tasmanian sites to the south of the SEABB (see Fig. 1). We compared these with populations of juveniles collected from mainland sites to the northeast and southwest of the SEABB. These data also extended existing genetic surveys of *C. polymerus* (York et al. 2008, Ayre et al. 2009) to include more sites further north that are expected to be strongly interconnected by the EAC, which transports warm water originating in the tropics in a southerly direction along Australia's east coast. We then used these data to estimate migration across the barrier in our samples of both adults and juveniles. The unpredictability and typically reduced strength of the EAC in far southeastern Australia might influence the current's capacity to produce along-shore larval transport (Hamon et al. 1975, Cresswell & Legeckis 1986). Indeed, there was evidence of reduced recruitment and altered environmental conditions for populations of several species of marine invertebrates found on rocky shores bordering the SEABB (Hidas et al. 2007, 2010). We therefore tested the hypothesis that levels and patterns of genetic diversity vary throughout the eastern and western regions, with populations adjacent to the barrier displaying reduced diversity and more divergent allele frequencies.

MATERIALS AND METHODS

Study species, study area and collection of adults and juveniles

Catomerous polymerus occurs within the low-to-mid-intertidal zone on exposed rocky shores, and both adults and early settlers are sessile (Dakin 1952, Edgar 2008). As for many other barnacles, larvae are brooded within the mantle until eyed nauplii are released into the water column where, because larval culture studies indicate that minimum pelagic larval duration is approximately 16 d (Egan & Anderson 1989), they have the potential to be widely dispersed by ocean currents. *C. polymerus* is distributed between southern Queensland (northern range edge) and the Great Australian Bight in South Australia, including Tasmania (Edgar 2008).

To assess patterns of genetic variation within and among biogeographic regions, we sampled *C. polymerus* on 23 rocky intertidal shores along a 1560 km stretch of coast that spanned the SEABB. Based on known biogeographic regions (Bennett & Pope 1953, Knox 1963), genetic structuring of marine invertebrate populations across the southeast coastline (Ayre et al. 2009) and results by York et al. (2008), we divided the study area into 4 sampling regions. Two of these lie to the northeast of the SEABB and comprise contiguous stretches of the eastern mainland coast: (1) the northern region ~907 to ~1422 km to the northeast of the SEABB and (2) the eastern (southeast coastal) region extending from Region 1 to the northeast border of the SEABB at Ninety Mile Beach. The other 2 regions lie to the south and west of the SEABB: (3) the region to the west extends from Wilson's Promontory to Phillip Island, Victoria, and (4) the region to the south comprised sites on the northeast coast of Tasmania (Fig. 1). The biogeographic affinity of the Tasmanian sites is less clear, as Bennett & Pope (1953) considered them a region of overlap of the Maugean and Peronian Provinces, with the Maugean Province considered to include Tasmanian and Victorian sites to the west of the SEABB and the Peronian in the northwest corner of Tasmania and mainland sites to the northeast of the SEABB (Fig. 1). We sampled *C. polymerus* on 6, 7, 5 and 4 rocky shores (hereafter referred to as locations) within each of these 4 regions, respectively. Within each region, locations were separated by 10 to 50 km, and the intervening coastline included areas of habitat unsuitable for *C. polymerus*. The majority of locations were either moderately or highly wave exposed, and with 2 exceptions rock platforms were comprised of sandstone. At each location, we sampled within a 60 m^2 area and attempted to collect 30 adult and 30 juvenile *C. polymerus*. The density and, hence, availability of juveniles was highly variable among locations and no juveniles were collected from the Tasmanian shores in the south. Specimens were collected by prising intact barnacles contained in their tests from the rocky shore with the aid of a chisel, placing them in sterile containers and storing them in liquid nitrogen for transfer back to the laboratory. All barnacles were stored at −80°C until required for DNA extraction and examination to confirm their reproductive status. As for other barnacle species, reproductively mature adults can be recognised by the presence of gonads that range in colour from cream to bright orange or pink, or by the presence of brooded nauplii (Egan & Anderson 1989, Raimondi & Martin 1991). There was slight overlap in the size range of the small and always reproductively immature individuals (2.8 to 7.7 mm) that we classified as

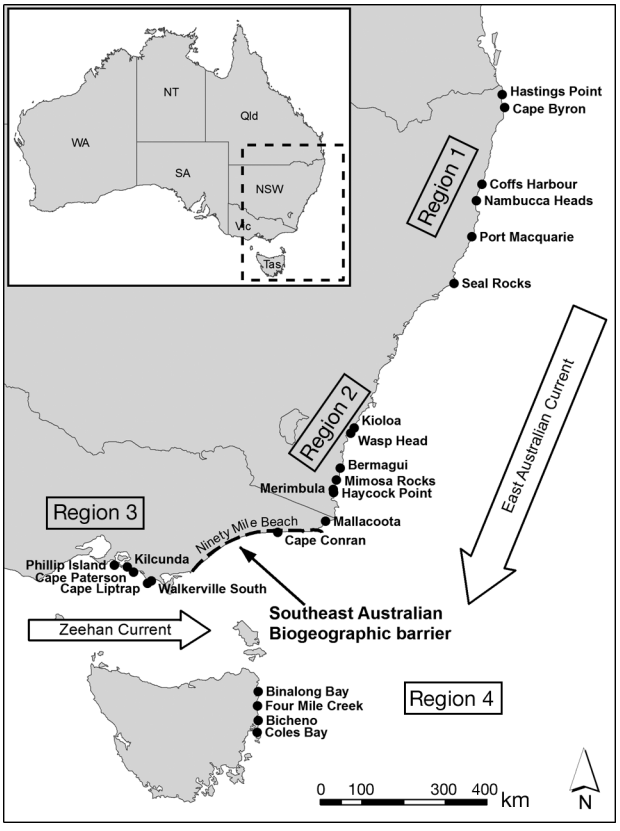

Fig. 1. Sampling locations across the southeastern Australian biogeographic barrier (SEABB, black dashed line) were divided into 4 regions: Region 1 from Hastings Point to Seal Rocks in New South Wales (NSW), Region 2 from Kioloa (NSW) to Cape Conran (Victoria; Vic), Region 3 from Phillip Island to Walkerville South (Vic) and Region 4 from Binalong Bay to Coles Bay (Tasmania; Tas). Arrows with black outline depict the direction of movement of oceanographic currents

juveniles and the larger typically mature individuals that we classified as adults (6.2 to 15.5 mm). Nevertheless, we considered all individuals ≥6.0 mm to be adult regardless of reproductive status.

Microsatellite genotyping

We genotyped a total of 556 adult and 537 juvenile *C. polymerus* using 4 microsatellite loci developed for *C. polymerus* by York et al. (2008). To obtain DNA, <5 mm of the cirri of each animal was placed in a 1.5 ml tube containing 5% Chelex resin in 500 µl of sterile double distilled H_2O and 10 µl of Proteinase K (10 mg ml^{-1}). Tubes were placed on a heating block at 65°C and left overnight, vortexed for 10 s, reheated at 100°C for 10 min, vortexed for another 10 s and centrifuged at 13 000 *g* for 3 min (Walsh et al. 1991), and stored in the freezer at −20°C. The polymerase chain reaction (PCR) conditions were modified from those in York et al. (2008). Optimal PCR conditions for each locus comprised of an initial denaturation of 10 min at 95°C, 30 cycles of 30 s at 94°C and 30 s at the annealing temperature 54°C, and 0.1 units of Ampli *Taq* Gold® (Applied Biosystems) was used in each 15 µl reaction with the buffer supplied by the manufacturer. Reactions comprised of 2.5 mM $MgCl_2$, 0.17 mM of each dNTP, 0.25 mM each of primer (C1 to C4) 5′-labelled with 1 of 3 fluorescent dyes (FAM for C1 and C3, VIC for C2, and NED for C4) and approximately 20 to 200 ng of DNA. All primers were combined into a single reaction cocktail, and negative controls were included in each PCR to verify the absence of contaminating DNA. Refer to Appendix 1 for product size ranges.

Genetic diversity within populations

We used the program GENALEX v.6.1 (Peakall & Smouse 2006) to determine whether the number and types of alleles differed within and among all 4 study regions, and to determine whether allele frequencies differed between adults and juveniles. We also used a combination of GENALEX and FSTAT to estimate standard population genetic parameters for each location (within each region) and for the overall collection of barnacles for each region. We obtained the following measures: number of alleles per locus, number of private alleles per locus (alleles unique to a particular site or sampling location), and observed and expected heterozygosity. Estimates of allelic richness (the standardised number of alleles per locus, based on the location with the lowest sample size of barnacles) and the inbreeding coefficient (F_{IS}) were estimated with FSTAT v.2.9.3.2 (Goudet 1995).

Using data for each location, we tested for linkage disequilibria among all pairs of loci to ensure that the loci appear to assort independently. Tests for linkage disequilibria were carried out for the pooled adult and juvenile data for populations with sample sizes ≥30 using the default settings in GENEPOP v.4.2.1 (Raymond & Rousset 1995). These analyses provided little evidence of linkage disequilibria and certainly do not imply physical linkage as only 1 of the 120 tests carried out for the 4 pairs of loci within each of the 20 populations yielded a significant outcome ($p < 0.05$). Therefore, all 4 loci were treated as independent tests of our hypotheses.

We tested for evidence of heterozygous deficiencies as compared to expectations of Hardy-Weinberg

equilibria (H) for the combined adult and juvenile data for each locus and location using GENEPOP v.4.2.1 (Raymond & Rousset 1995). The Markov chain method was used to estimate exact p-values for each combination. Only 1 of the 4 loci, Locus C2, showed no evidence for deficits in heterozygotes as only 2 of the 20 locations included in the test were significant. The remainder of the loci had between 12 and 20 locations that did not meet expectations for each test from Hardy-Weinberg equilibria ($p < 0.05$).

Genetic population structure

Using STRUCTURE v.2.3.4 (Pritchard et al. 2000), we performed Bayesian analysis to confirm the genetic differentiation of adult populations on either side of the SEABB and to determine our ability to assign individuals (i.e. juveniles) to each of our 4 sampling regions (see Appendix 2 relating to our initial STRUCTURE analyses). We also used more conventional hierarchical F-statistics to quantify levels of differentiation within and among regions, and estimated pairwise F_{ST} analyses (Wright 1949) to provide a matrix of genetic distances to accompany a geographic distance matrix. This allowed us to test for isolation by distance (IBD) for the within-regional pooled datasets that included both adults and juveniles, using Mantel tests (GENALEX v.6.41, Peakall & Smouse 2006). Hierarchical F-statistics were estimated using TFPGA (Miller 1997) and statistical significance was determined by generating 95% confidence (probability) intervals (CI) by bootstrapping across loci.

Detection of juvenile immigrants using microsatellite data

Following a preliminary investigation to determine the number of well supported clusters present within the adult datasets, we proceeded to examine the probability of assignment of all juvenile specimens to their region of collection and to the alternate side of the SEABB. Our analyses of *C. polymerus* adult microsatellite data together with existing evidence that eastern and western lineages can be distinguished by their mtDNA haplotypes (York et al. 2008, Ayre et al. 2009) imply that either marker could be used to identify immigrants that have crossed the SEABB. However, because they are biparentally inherited, microsatellite data offer the advantage of being able to detect migrants of either sex and to distinguish individuals that assign more strongly to the opposite side of the barrier (genuine migrants) from those that assign poorly to either side and are likely hybrids. We therefore used our microsatellite survey data as our primary means of investigating the immigrant status of all juveniles. Nevertheless, for all putative migrants (and a random sample of other individuals), we further investigated their immigrant status using mtDNA sequence data. We used the USEPOPINFO subroutine implemented in STRUCTURE and set $K = 2$ (corresponding to the major regional division of the adults; K is the number of populations). We varied the migration prior ($m = v = 0.001$ to 0.1) in a series of additional analyses (refer to Pritchard et al. 2000 for detailed description of terms). Varying the level of migration from the default prior value of $v = 0.05$ to $v = 0.1$ (i.e. high migration rates) made no substantive difference to the results and our subsequent interpretations and conclusions. Even at relatively low to moderate rates of migration ($v = 0.005$ to 0.01), we were still able to detect at least one putative immigrant in each region. Nevertheless, we report the results for $v = 0.05$ with the remainder of parameters set to default. Immigrants were defined by the STRUCTURE program as individuals falling outside the 95% CI ($p < 0.05$) of belonging to their sampled region. This enabled us to determine whether or not there were putative immigrants in the collection of adults and provided a basis for comparison with our collection of juveniles. A greater number of immigrants among the juveniles as compared to the adults may be an indication that dispersal occurs but selection acts to eliminate or reduce numbers of recruits before they reach adulthood.

Immigrant identification using mtDNA COI amplification and sequencing

York et al. (2008) and Ayre et al. (2009) examined mitochondrial cytochrome oxidase I (COI) sequence variation for collections of adult *C. polymerus* spanning the SEABB and uncovered strong genetic subdivisions, with the greatest differentiation occurring between locations northeast and southwest of the barrier. Indeed, the pattern of evolution of COI haplotypes is consistent with reciprocal monophyly. Moreover, F_{ST} calculated for samples of adults collected to the northeast and southwest of the barrier for mtDNA sequence data was 0.951, implying that there has been no recent gene flow. Calibrated sequence divergence of up to 3.1% between northeastern and southwestern lineages indicates a substantial period of evolution in isolation, dating back perhaps to as early as the

Pleistocene (Ayre et al. 2009). Notably, York et al. (2008) found one individual with a haplotype not in agreement with its sampling location, implying that at least some migration does occur (see their Fig. 3).

Importantly, this genetic backdrop provides the opportunity for mtDNA to be used as a marker for the detection and confirmation of migrant ancestry in all life stages. Indeed, if individuals with putatively immigrant ancestry (i.e. individuals unassigned to their region of collection) also display COI haplotypes characteristic of immigrants, this would confirm that they are true immigrants, whereas the presence of a local haplotype would imply that they have some migrant ancestry but are introgressed. Similarly, individuals whose microsatellite genotypes assign with equal probability to either side of the SEABB, but display immigrant COI haplotypes, would be consistent with introgression. Because mtDNA haplotypes are maternally inherited, however, first and later generation hybrids or back crosses may not display immigrant haplotypes. Comparing the frequency of adults and juveniles with immigrant ancestry (based on the criteria above) will provide insight into whether immigrants are able to survive to reproductive age or experience post settlement selection. Here, we generated COI sequence data for 15 adults and 20 juveniles collected at the northeastern sites, and 7 adults and 4 juveniles from southwestern sites.

PCR reaction conditions are detailed in Ayre et al. (2009). The sequence data for all 46 individuals were generated by Macrogen using Big Dye^{TM} DNA sequencing (Applied Biosystems). We inspected the resulting electropherograms and then aligned the sequences, also incorporating 48 sequences from the earlier study of Ayre et al. (2009) on this species. The alignment used package Clustal W (Thompson et al. 1994), implemented in the software Bioedit (Hall 1999). We constructed a network of the *C. polymerus* COI haplotypes, allowing visual inspection of their spatial distribution, and hence identification of immigrants, using NetWork v.4.2.0.1 (Bandelt et al. 1999).

RESULTS

Genetic diversity measures

Within each region, levels of genetic diversity were very similar as judged by the number of alleles, allelic richness (corrected for sample size) and expected heterozygosity, although in most cases values were slightly lower for the eastern and western regions, for both the adults and juveniles. The 4 microsatellite loci displayed an average of 4.8 ± 0.6 (SE) to 8.3 ± 2.4 alleles per locus within each sampled region (Table 1). Private alleles were rare in each region and allelic patterns were consistent between the 2 life stages (Table 1). Importantly, there was little variation in levels of diversity within regions, with sites abutting the barrier displaying similar diversity to those closer to range centres.

F_{IS} estimates for almost all locations and all loci in both the adults and juveniles were consistently positive, ranging from 0.02 to 0.33 (Tables 1 & 2); however, after application of a sequential Bonferroni correction (Holm 1979), heterozygous deficits were significant in only 46 and 39% of cases for adults and juveniles, respectively. Regardless of the genetic markers used, heterozygous deficiencies are common in marine invertebrates, and a range of explanations have been proposed (Pudovkin & Zhivotovsku 1980). It is possible that heterozygous deficits detected using microsatellite markers may reflect the presence of null alleles, but the complex nature of these markers prevented formal tests of this hypothesis using programs such as Microchecker (Van Oosterhout et al. 2004).

Genotypic structure of populations

As predicted, *F*-statistics using our microsatellite survey data revealed minimal and non-significant genetic structuring of samples of adults or juveniles collected from populations within each of the 4 regions, again with no suggestion of greater differentiation of range edge sites (F_{SR} ranged from 0.0 to 0.023), but moderate and significant differentiation ($p < 0.05$) among regions on either side of the barrier (F_{RT}), and among populations within regions (F_{ST}; Table 2). The Mantel test was highly significant for combined northern and eastern regions ($R^2 = 0.0008$, $p = 0.0001$), but not significant for either the western ($R^2 = 0.00$, $p = 0.49$) or southern ($R^2 = 0.005$, $p = 0.069$) regions. Similarly, Bayesian analysis of these data for both adults and juveniles using STRUCTURE confirmed the presence of distinct eastern (Cluster 1 representing Regions 1 and 2) and western (Cluster 2 representing Regions 3 and 4) clusters (see Appendix 2 for a more detailed description of this approach).

Identification of immigrants

Both our microsatellite and mtDNA data revealed a clear separation of 2 groups comprised of Regions 1

Table 1. Genetic diversity measures in (a) adults and (b) juveniles of *Catomerus polymerus* for populations and regions across the southeastern Australia biogeographic barrier (SEABB). *N*: number of individuals genotyped; N_a: mean number of alleles per locus; *AR*: allelic richness, corrected for sample size; *PA*: number of private alleles (alleles unique to a particular population or environment); H_e: expected heterozygosity; F_{IS}: fixation index, $F_{IS} = 1 - (H_o/H_e)$, where H_o is observed heterozygosity. Significance levels for the inbreeding coefficients: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Data for N_a, *AR*, H_e and F_{IS} are mean ± SE

Region	Population	*N*	N_a	*AR*	*PA*	H_e	F_{IS}
(a)							
North	Cape Byron	34	8.00 ± 2.65	6.25 ± 1.78	0	0.62 ± 0.12	0.19 ± 0.05***
	Nambucca Heads	35	8.25 ± 2.39	5.68 ± 1.80	1	0.66 ± 0.11	0.13 ± 0.12*
	Coffs Harbour	35	7.50 ± 2.33	6.22 ± 1.71	0	0.63 ± 0.11	0.15 ± 0.05***
	Port Macquarie	33	7.50 ± 2.63	5.74 ± 1.71	0	0.63 ± 0.10	0.26 ± 0.05***
	Seal Rocks	34	7.75 ± 2.32	6.09 ± 1.81	0	0.64 ± 0.11	0.19 ± 0.09***
	Mean	34.2	7.80 ± 0.15	6.00 ± 0.12		0.64 ± 0.01	
East	Kioloa	28	7.50 ± 1.94	6.27 ± 1.63	0	0.65 ± 0.09	0.15 ± 0.11*
	Wasp Head	26	6.25 ± 1.70	5.46 ± 1.36	0	0.66 ± 0.09	0.09 ± 0.11*
	Bermagui	22	7.00 ± 2.04	6.24 ± 1.68	0	0.68 ± 0.08	0.09 ± 0.01*
	Mimosa Rocks	26	7.50 ± 2.33	6.04 ± 1.65	2	0.67 ± 0.07	0.15 ± 0.13
	Merimbula	22	6.25 ± 0.85	5.28 ± 0.70	0	0.65 ± 0.06	0.25 ± 0.08**
	Haycock Point	16	7.25 ± 2.10	6.82 ± 1.88	0	0.68 ± 0.13	0.16 ± 0.05**
	Mallacoota	21	6.75 ± 1.89	5.82 ± 1.43	0	0.64 ± 0.10	0.25 ± 0.17***
	Cape Conran	12	5.75 ± 1.80	5.60 ± 0.71	0	0.65 ± 0.09	0.24 ± 0.08**
	Mean	20	6.78 ± 0.23	5.94 ± 0.18		0.66 ± 0.01	
West	Walkerville South	23	7.00 ± 1.15	5.40 ± 1.05	1	0.63 ± 0.05	0.14 ± 0.07**
	Cape Paterson	21	6.50 ± 1.50	5.30 ± 0.43	0	0.60 ± 0.07	0.25 ± 0.08***
	Kilcunda	25	6.00 ± 0.58	4.39 ± 0.69	0	0.61 ± 0.04	0.17 ± 0.11**
	Phillip Island	24	5.25 ± 1.11	4.81 ± 0.64	0	0.54 ± 0.06	0.16 ± 0.11**
	Mean	23.3	6.19 ± 0.37	4.98 ± 0.23		0.60 ± 0.02	
South	Binalong Bay	33	6.75 ± 1.44	4.65 ± 1.00	0	0.55 ± 0.07	0.10 ± 0.11*
	Four Mile Creek	33	6.50 ± 1.04	5.00 ± 0.84	0	0.51 ± 0.12	0.18 ± 0.04**
	Bicheno	31	7.50 ± 1.55	5.27 ± 0.94	2	0.56 ± 0.10	0.06 ± 0.10
	Coles Bay	22	7.00 ± 1.35	6.27 ± 1.46	2	0.57 ± 0.11	0.33 ± 0.07
	Mean	29.8	6.94 ± 0.21	5.30 ± 0.35		0.55 ± 0.01	
Total		556			8		
Total mean		26.8	6.93 ± 0.17	5.65 ± 0.10		0.62 ± 0.01	0.18 ± 0.01**
(b)							
North	Hastings Point	33	7.00 ± 2.74	5.87 ± 2.13	1	0.61 ± 0.11	0.16 ± 0.11***
	Cape Byron	32	6.50 ± 1.94	5.62 ± 1.53	1	0.61 ± 0.10	0.15 ± 0.07**
	Nambucca Heads	40	7.75 ± 2.63	6.04 ± 1.90	0	0.63 ± 0.09	0.06 ± 0.08
	Coffs Harbour	38	7.50 ± 2.63	6.12 ± 1.72	1	0.65 ± 0.08	0.22 ± 0.09***
	Port Macquarie	36	7.00 ± 2.74	5.72 ± 1.85	0	0.62 ± 0.10	0.05 ± 0.12
	Seal Rocks	35	7.50 ± 2.63	6.35 ± 1.99	0	0.67 ± 0.09	0.09 ± 0.11*
	Mean	35.7	7.21 ± 0.19	5.95 ± 0.11		0.63 ± 0.01	
East	Kioloa	27	6.75 ± 1.80	5.99 ± 1.64	0	0.65 ± 0.09	0.15 ± 0.04**
	Wasp Head	29	7.00 ± 1.87	6.02 ± 1.60	0	0.61 ± 0.09	0.14 ± 0.12**
	Bermagui	26	6.50 ± 1.94	5.85 ± 1.66	0	0.64 ± 0.09	0.14 ± 0.08**
	Mimosa Rocks	25	7.00 ± 2.42	6.45 ± 2.00	0	0.67 ± 0.09	0.18 ± 0.07***
	Merimbula	27	7.25 ± 2.50	6.46 ± 2.11	1	0.63 ± 0.10	0.14 ± 0.04**
	Haycock Point	29	7.00 ± 2.42	6.28 ± 1.87	0	0.67 ± 0.09	0.16 ± 0.07**
	Mallacoota	30	7.50 ± 2.33	6.64 ± 1.99	0	0.67 ± 0.09	0.02 ± 0.11
	Mean	27.6	7.00 ± 0.12	6.24 ± 0.11		0.65 ± 0.01	
West	Walkerville South	15	4.75 ± 0.63	4.75 ± 0.63	0	0.56 ± 0.06	0.16 ± 0.10**
	Cape Liptrap	29	6.75 ± 1.18	5.78 ± 0.73	0	0.61 ± 0.05	0.27 ± 0.07***
	Cape Paterson	33	6.25 ± 1.31	5.17 ± 0.91	0	0.56 ± 0.08	0.16 ± 0.05**
	Kilcunda	18	5.75 ± 0.63	5.47 ± 0.58	1	0.60 ± 0.09	0.20 ± 0.18**
	Phillip Island	35	6.75 ± 0.63	5.37 ± 0.52	1	0.56 ± 0.06	0.13 ± 0.11**
	Mean	26	6.05 ± 0.37	5.31 ± 0.17		0.58 ± 0.01	
Total		537			6		
Total mean		29.8	6.81 ± 0.17	5.89 ± 0.12	0	0.62 ± 0.01	0.14 ± 0.01**

Table 2. Hierarchical *F*-statistics at 4 microsatellite loci for *Catomerus polymerus* adults and juveniles across the southeastern Australian biogeographic barrier (SEABB). Genetic variation was partitioned among: all individuals (F_{IS}), populations (F_{ST}), regions within the total population (F_{RT}), samples within regions (F_{SR}) and individually for northern, eastern, western and southern samples (for adults only). Significant *F* values for α = 0.05 are in **bold**

Locus	F_{IS}	F_{ST}	F_{RT}	Region (F_{SR})			
				F_{ST} north	F_{ST} east	F_{ST} west	F_{ST} south
Adults							
C1	0.242	0.044	0.060	0.006	0.016	−0.008	−0.003
C2	0.034	0.155	0.200	−0.009	0.010	−0.006	0.016
C3	0.172	0.103	0.135	0.003	−0.015	0.008	−0.004
C4	0.244	0.024	0.029	0.000	0.010	0.023	−0.005
95% CI	0.093–0.243	0.030–0.131	0.047–0.170	−0.005 – 0.005	−0.009 – 0.015	−0.008 – 0.016	−0.005 – 0.010
Mean ± SD	**0.188** ± 0.047	**0.076** ± 0.029	**0.102** ± 0.038	0.002 ± 0.003	0.006 ± 0.007	0.000 ± 0.008	0.001 ± 0.005
Juveniles							
C1	0.215	0.047	0.082	0.008	−0.003	−0.001	
C2	0.006	0.014	0.014	−0.006	0.002	0.004	
C3	0.076	0.082	0.135	0.000	0.005	−0.007	
C4	0.106	0.005	0.010	−0.006	−0.001	−0.009	
95% CI	0.035–0.189	0.009–0.071	0.012–0.112	−0.006–0.006	−0.003–0.004	−0.009–0.000	
Mean ± SD	**0.123** ± 0.050	**0.040** ± 0.016	**0.069** ± 0.027	0.002 ± 0.004	0.000 ± 0.002	−0.006 ± 0.003	

and 2, and Regions 3 and 4 (hereafter referred to as eastern and western regions), but each revealed the presence of a small percentage of individuals with putatively introgressed migrant ancestry.

In our surveys, the microsatellite genotypes of the majority of adults collected from both east and west of the SEABB (>88%) could be assigned with ≥95% confidence to their region of collection (i.e. individuals collected from east and west of the SEABB assigned to Clusters 1 and 2, respectively). Moreover, the vast majority of adults (>98%) could be assigned with ≥65% confidence. Of the 13 individuals that could not be assigned with ≥65% confidence to either cluster and, hence, represent potential immigrants, STRUCTURE identified only 5 individuals as immigrants (*p* of correct assignment ≤41%). Of these 5 potential immigrants, 2 displayed microsatellite genotypes characteristic of first generation migrants (i.e. *p* assignment to local cluster <0.05) with a probability of ≥95%. The remaining individuals had slightly higher probabilities of assignment to the cluster characteristic of their region of sampling (*p* = 9.3, 22.1 and 40.3%), suggesting that their genotypes reflected one or more generations of introgression requiring that migrants have both established and interbred with local *C. polymerus* (Fig. 2). These data for adults imply bidirectional migration, with 2 putative immigrants detected in the east (Regions 1 and 2) and 3 in the west (Regions 3 and 4).

Examination of the data for juveniles revealed a strikingly similar pattern of assignment to that seen in adult *C. polymerus*, i.e. 85% of juveniles assigned strongly to their pre-defined eastern and western clusters with a probability of ≥95% (Fig. 3), and in total, STRUCTURE identified 10 juveniles as putative migrants. These putative migrants again appeared to represent admixed genotypes as would be expected for second or later generation hybridisation and backcrossing (Fig. 3). As for adults, these data suggest bidirectional migration with 5 putative migrants detected in both the west and in the east (Fig. 3).

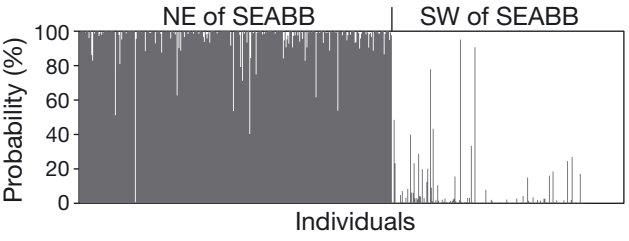

Fig. 2. Bayesian assignment for *Catomerus polymerus* adults from the most northern (Cape Byron, New South Wales; left side of graph) to the most western and southern (Coles Bay, Tasmania; right side of graph) locations sampled within regions east and west of the southeastern Australian biogeographic barrier (SEABB). Each bar represents each individual's posterior probability (*y*-axis) of its multilocus genotype (4 loci) pertaining to each of 2 genetic clusters corresponding to eastern (Regions 1 and 2) or western (Regions 3 and 4) sides of the SEABB. Five putative immigrant adults (i.e. misassigned to their site of collection) are depicted in the plot as the individuals with highest probability of belonging to the alternate sampling region (i.e. grey bars on white background)

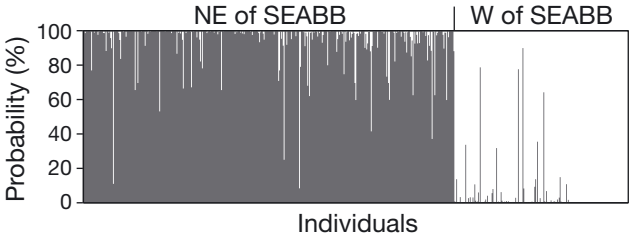

Fig. 3. Bayesian assignment for *Catomerus polymerus* juveniles, including those unassigned to their region of collection, from the most northern (Hastings Point, New South Wales; left side of graph) to the most western locations (Phillip Island, Victoria; right side of graph) sampled within regions east and west of the southeastern Australian biogeographic barrier (SEABB). Each bar plot represents an individual's assignment score as a posterior probability (*y*-axis) of its genotype across 4 loci pertaining to eastern (Regions 1 and 2) or western (Region) sides of the SEABB

Our mtDNA genotyping (n = 120) and analysis produced haplotype networks consistent with reciprocal monophyly and with the range of haplotypes reported by Ayre et al. (2009), again confirming the persistent separation of eastern and western lineages. Importantly, 1 of 120 individuals had a genotype consistent with immigrant ancestry.

Interestingly, none of the 16 adults or juveniles identified as potential migrants, on the basis of either their microsatellite genotypes or mtDNA haplotypes, displayed both nuclear genotypes and mtDNA haplotypes consistent with a migrant origin. The individual sampled in the east with a western mtDNA haplotype had a microsatellite genotype with an 82.7% probability of assignment to its sampled region. All 15 individuals with microsatellite genotypes characteristic of migrants had local mtDNA haplotypes. These data again imply that most individuals with migrant ancestry are the products of introgression and have inherited local mtDNA haplotypes. Indeed, these data may indicate that selection favours individuals with local mtDNA haplotypes regardless of their microsatellite genotypes, although the detection of a single eastern individual with a western mtDNA haplotypes demonstrates that such individuals are viable.

DISCUSSION

Our survey of nuclear and mtDNA genetic variation in *Catomerus polymerus* along the southeast Australian coastline confirms that the SEABB is an important barrier to gene flow for this widely dispersing barnacle. Indeed, our data for both adults and juveniles extend the limits of sampling for this species within the northeastern region by up to ~400 km to include Hastings Point, New South Wales, and support the presence of the highly differentiated east and west genetic lineages and the virtual absence of genetic differentiation of populations within bioregions described by York et al. (2008) and Ayre et al. (2009). However, our surveys of variation in nuclear DNA on either side of the SEABB imply that extant populations of both adults and juveniles contain low proportions of individuals with putatively immigrant ancestry, implying either that effects of the barrier are breaking down under current conditions or that the differentiation of lineages is maintained by episodic rather than continuous effects of selection. Interestingly, our data also indicate that samples from Tasmanian sites (Region 4) cluster with those collected from Victoria (Region 3), emphasizing the role of the Bass Strait in restricting gene flow from the northeast mainland. For *C. polymerus* at least, the SEABB therefore separates both the northeast and southwest mainland coasts and the northeast and Tasmanian coasts.

Microsatellite and mitochondrial variation among and within regions

Our microsatellite and mtDNA data for both adult and juvenile *C. polymerus*, like that of York et al. (2008) and Ayre et al. (2009), support the presence of a phylogeographic break between eastern and western populations and provides more compelling evidence of high levels of gene flow within both the eastern and western regions. Indeed, there was no significant differentiation of samples within regions as judged by hierarchical *F*-statistics or tests for isolation by distance. Bayesian analysis of our microsatellite data with STRUCTURE revealed the presence of eastern and western clusters, and, while hierarchical *F*-statistics revealed no significant variation within regions with mean F_{SR} values ranging from –0.006 ± 0.003 to 0.006 ± 0.007 (Table 2) for adults and juveniles, respectively, mean values for F_{RT} (indicating variation among regions) ranged from 0.069 ± 0.027 to 0.102 ± 0.038 (Table 2). This overall pattern of F_{ST} variation is similar to that reported using both mtDNA and nuclear DNA variation for several other species of planktonically dispersed intertidal and subtidal marine invertebrates occurring to the east and west of the SEABB (Billingham & Ayre 1996, Ayre et al. 2009, Miller et al. 2013). Similarly, mtDNA haplotype data revealed a pattern of east and west

differentiation consistent with studies using mtDNA in other species, such as *Cellana tramoserica*, *Meridiastra calcar*, *Plaxiphora albida* (Ayre et al. 2009), the seastar *Coscinasterias muricata* (Waters & Roy 2003) and the littoral gastropod *Nerita atramentosa* (Waters et al. 2005), the latter now considered to form cryptic species pairs distributed east and west of the SEABB (Spencer et al. 2007).

Evidence of migration across the SEABB

Our microsatellite dataset provided sufficient power to assign the vast majority of individuals to the genotypic cluster associated with their site of origin on either side of the SEABB. Indeed, in our study, 87% of all adults and juveniles assigned strongly to these clusters (≥95% probability) and 97% of individuals could be assigned with $p \geq 65\%$. Moreover, our smaller sample of adult and juvenile mtDNA haplotypes together with those reported by York et al. (2008) and Ayre et al. (2009) demonstrates the presence of eastern and western lineages suggestive of reciprocal monophyly. Nevertheless, both datasets reveal small numbers of individuals with potentially migrant origin (see also York et al. 2008), although in our study all individuals appeared to be later generation migrants generated through introgression. This suggests that, even though lineages are differentiated, migrants both settle and interbreed, and hybrids between the lineages persist for multiple generations. The detection of fewer apparently migrant individuals using mtDNA and the finding that individuals inferred to have migrant ancestry (based on microsatellite data) displayed local mtDNA haplotypes may indicate stronger selection against foreign mtDNA. Exploration of these issues would require experimental transplantations or long term monitoring of populations and more accurate assessment of the extent of introgression using genomic techniques.

CONCLUSIONS

Taken together, the patterns of microsatellite and mtDNA variation presented here and in earlier studies (York et al. 2008, Ayre et al. 2009) imply that migration across the SEABB erodes the current strong separation of eastern and western lineages or alternatively that present patterns have been maintained by strong but episodic selection. Populations of several other planktonically dispersing species, including the barnacle *Tetraclitella purpurascens*, display relatively homogeneous eastern and western populations, indicating that their populations are well connected across the SEABB (Ayre et al. 2009). In contrast, however, bouts of selection have apparently prevented the persistent southward range extension of the barnacle *Tesseropora rosea*, which is reported to form ephemeral populations to the west of the SEABB that lie beyond its described range limit to the northeast of the SEABB (Jones 1990). Our data provide a valuable baseline dataset that can be used to test for evidence of increasing gene flow across the SEABB given predictions of strengthening and a greater southern and western penetration of the EAC during warmer months (Ridgway & Godfrey 1997, Bowen et al. 2005, Ridgway 2007).

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Appendix 1. Details of microsatellites for life histories per locus, including total number of alleles, mean (± SE) and allelic size range across loci in base pairs

Life history	Locus	Total no. of alleles	Allele size range (bps)
Adults	C1	21	286–336
	C2	6	220–230
	C3	14	272–300
	C4	11	174–202
	Mean	8.25 ± 3.14	
Juveniles	C1	20	298–336
	C2	5	222–230
	C3	11	272–298
	C4	11	174–204
	Mean	11.75 ± 3.09	
Total	C1	41	286–336
	C2	11	220–230
	C3	25	272–300
	C4	22	174–204
	Mean	6.19 ± 6.20	

Appendix 2. Using STRUCTURE to determine the number of regional genetic clusters based on adult *Catomerus polymerus*

We used the program STRUCTURE (v.2.3.4, Pritchard et al. 2000) to infer the most likely number of genetic clusters within the set of adult *C. polymerus* microsatellite genotypes. STRUCTURE uses the observed allele frequency data to estimate the likelihood of K genetic clusters within the data, by simultaneously estimating the ancestry of every individual (i.e. q-values representing the proportional membership of an individual's genotype to K clusters, where K = 1, 2, 3…n), under the assumptions of linkage and Hardy-Weinberg equilibria within clusters. We used STRUCTURE's admixture subroutine to calculate q-values, as well as the log probability or likelihood of K clusters, for values of K between 1 and 12. We originally planned to estimate the above for all K, up to K = 23 (the number of locations sampled); however, inspection of the plots depicting the q-values and used to visualise genetic structure during the course of the analysis revealed no biologically interpretable genetic patterns beyond K = 2. Default program parameters were used for all settings. Our initial analysis considered only genotype data, i.e. sampling locations were not included in the analysis. We used the correlated allele frequency model. We collected preliminary data for 10^4 iterations in a burn-in, and then proceeded with a run of 10^5 iterations. There were 10 replicate runs for each value of K (1 to 12). The ΔK statistic of Evanno et al. (2005) was used as a formal estimator of the most likely number of genetic clusters detectable by STRUCTURE. Evanno's test, which is implemented in the online program STRUCTURE HARVESTOR (Earl & Vonholdt 2012), uses a stepwise procedure to evaluate the change in likelihood with respect to K (K = 1, 2…) or ΔK, with the highest value of ΔK corresponding to the most likely number of genetic clusters within the data. Calculation of ΔK was based on the mean ± SD 'log probability of the data' (or likelihood value) outputted by STRUCTURE. We proceeded to use the USEPOPINFO subroutine implemented in STRUCTURE (i.e. the analysis made use of both genotype data and the regional clusters) (refer to 'Materials and methods').

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