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Seascape genetics of the New Zealand greenshell mussel: sea surface temperature explains macrogeographic scale genetic variation

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ABSTRACT: Polymorphic microsatellite markers were employed to assay genetic variation in 14 populations of the endemic New Zealand greenshell mussel *Perna canaliculus*, which is continuously distributed across ~11.5° of latitude. Previous population genetics assessments of this species have identified a pronounced and ~1.3 million year old genetic discontinuity at ~42°S, just south of Cook Strait, the major break between the North and South islands. In addition to this genetic structuring, the present seascape genetics analysis of 7 environmental and 3 geospatial variables revealed that sea surface temperature (SST) explains far more genetic variation (among populations and individuals) than any other variable. Genetic variation across the full distributional range of *P. canaliculus* is, therefore, best explained by SST, which suggests that a contemporary temperature-related selective force is acting on greenshell mussels from the subtropical north to the cold temperate south. The identification of this association between environmental and genetic variation highlights how previously unrecognised patterns of genetic structure may be revealed by the examination of environmental variation and provides important leads for new research directions.

KEY WORDS: *Perna canaliculus* · Microsatellite markers · Population genetic variation · Environmental variation · Coastal processes

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INTRODUCTION

Historically, much of the marine population genetics research has focussed on 3 interconnected areas: (1) describing population genetic structure and quantifying connectivity (gene flow) in 'open' marine coastal systems (e.g. Lessios et al. 1998, Apte & Gardner 2001, Cowen et al. 2006, Addison et al. 2008); (2) identifying phylogeographic structure, often associated with pronounced genetic discontinuities (e.g. Hare & Avise 1996, Barber et al. 2000, Apte & Gardner 2002, Star et al. 2003, Waters & Roy 2004, Goldstien et al. 2006, Saarman et al. 2010, Sivasundar & Palumbi 2010); and (3) the importance of self-recruitment in explaining population genetic structure (e.g. Jones et al. 1999, Swearer et al. 1999, Taylor & Hellberg 2003, Wood & Gardner 2007, Wei et al. 2013). While the contribution of selection to maintaining both protein polymorphisms and fitness differences among individuals has been highlighted at single loci (e.g. DiMichele & Powers 1982, Hilbish et al. 1982, Hilbish & Koehn 1985, Powers et al. 1986, Gardner & Kathiravetpillai 1997, Gardner & Palmer 1998), until recently it has been difficult, if not impossible, for researchers to assess the contribution of selection arising from environmental variation to the generation and maintenance of multi-locus genotypic variation.

Environmental variability has long been recognised as a contributing factor to population genetic

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structuring, and recent advances in genetic marker technology and computing provide new opportunities for assessing how environmental variation may contribute to the genetic structuring of populations — the new discipline of landscape genetics (e.g. Manel et al. 2003, Foll & Gaggiotti 2006, Holderegger & Wagner 2006). In the sea, this new discipline is often called seascape genetics, but to date examples of its application are few in comparison to terrestrial studies. Seascape genetics offer the possibility of increasing our understanding of the role of ocean currents in promoting and/or preventing gene flow, locus-specific responses to environmental variability such as salinity fluctuation, macrogeographic patterns of multi-taxa genetic structuring, stock structure of commercial fisheries, and marine protected areas network design and implementation (e.g. Kenchington et al. 2006, Selkoe et al. 2008, Gaggiotti et al. 2009, Gonzalez-Wanguemert et al. 2009, 2010, McInerney et al. 2009, Pampoulie et al. 2009, Galindo et al. 2010, Kelly & Palumbi 2010, White et al. 2010).

New Zealand (NZ) presents an unusual opportunity to test the possible effect of multi-factorial environmental variation on genetic variation. The coastline is approximately orientated north to south, such that the main islands span a range of environments, from the subtropical north to the cold temperate south (see Fig. 1). Many species have distributions that encompass the range of these environments, and are, thus, amenable to quantification of the effect of environmental variation on genetic variation and population genetic structuring. The endemic greenshell mussel *Perna canaliculus* is distributed throughout NZ's 3 main islands, from ~35° S to ~47° S (Fig. 1). Due to its substantial biomass on intertidal and shallow subtidal substrates, the greenshell mussel is of considerable ecological, cultural, and economic importance (Morton & Miller 1968, Gardner 2000, 2002, Apte et al. 2003, Whyte et al. 2009). This mussel is used here as a model organism to test hypotheses regarding the influence of environmental variation on the genetics of a species with a planktonic larval dispersive stage lasting 3 to 4 wk (Hayden 1994) and which is distributed over ~11.5° of latitude. Significantly, pronounced genetic structuring has already been reported for this species (Apte & Gardner 2002, Star et al. 2003, Wei et al. 2013) and several others such as brittle-stars, sea-stars, and limpets (Waters & Roy 2004, Ayers & Waters 2005, Goldstien et al. 2006). Estimated divergence times between northern and southern mitochondrial DNA lineages range from ~1.3 million years (Myr) in the greenshell mussel *P. canaliculus* to ~0.2 to 0.3 Myr in the limpet *Cellana ornata* (Apte & Gardner 2002, Goldstien et al. 2006). These divergence times coincide with global fluctuations in sea level throughout the Pleistocene, and also with the complex geological and hydrodynamic processes that gave rise to the establishment of Cook Strait (Apte & Gardner 2002, Goldstien et al. 2006, Wei et al. 2013). While not reported for all coastal taxa (e.g. the blackfoot abalone *Haliotis iris*, Will et al. 2011; and the blue mussel *Mytilus galloprovincialis*, Westfall 2010), this general phenomenon of a genetic break just south of Cook Strait at ~42° S is reported for many taxa (reviewed by Ross et al. 2009, Gardner et al. 2010). The present day genetic discontinuity may, therefore, reflect a balance between a number of different issues, including historical processes (phylogeography), connectivity (gene flow within and among populations), and environmental variation (contemporary selection pressure). While there is no indication that this multi-taxon genetic structuring is attributable to local or regional adaptation, the recognition of its existence as a pre-existing condition is important. This does not preclude the existence of other structuring, perhaps at a different spatial scale, that results from environmental variation from the north to the south.

The present paper describes the application of 10 polymorphic microsatellite markers (MacAvoy et al. 2008) to examine the seascape genetics of 14 populations of *Perna canaliculus* in the context of nearshore coastal oceanographic and geospatial data across 11.5° of latitude. As pointed out by Selkoe et al. (2008), molecular tools used most often for population genetic analyses perform best when integrated with complementary techniques/analyses. This is the approach that we take in the present paper, using microsatellite data and nearshore oceanographic and geospatial data to better understand the seascape genetics of a widely distributed coastal marine invertebrate. Our aim is to assess the influence of environmental variability on macrogeographic genetic variation (e.g. Holderegger & Wagner 2006, Storfer et al. 2007, Gaggiotti et al. 2009), and as such we test the null hypothesis that genetic variation for *P. canaliculus* across its full distribution is not correlated with or explained by environmental or geospatial variables. In a wider context, we seek to understand if and how environmental variation may contribute to and explain genetic variation among coastal taxa and how this may be superimposed on the known patterns of population genetic variation that exist.

MATERIALS AND METHODS

Unless otherwise stated, the methods below follow those described by Wei et al. (2013).

Sample collection

Mussels were collected from the intertidal and shallow subtidal zones at 14 sites from North, South, and Stewart islands, from 1996 to 2008 (Fig. 1). Individuals were a range of sizes and presumed ages. Mussels from 10 sites were collected between 1996 and 1998, and are a subset of the samples analysed by Apte & Gardner (2002), Apte et al. (2003), and Star et al. (2003). To address the question of genetic discontinuity in central NZ, mussels from 4 additional populations (CAM, FLE, KAI, and WEST; Fig. 1) were collected between 2002 and 2008. Tissues of all mussels were preserved in 95% ethanol and stored at −20°C until DNA extraction.

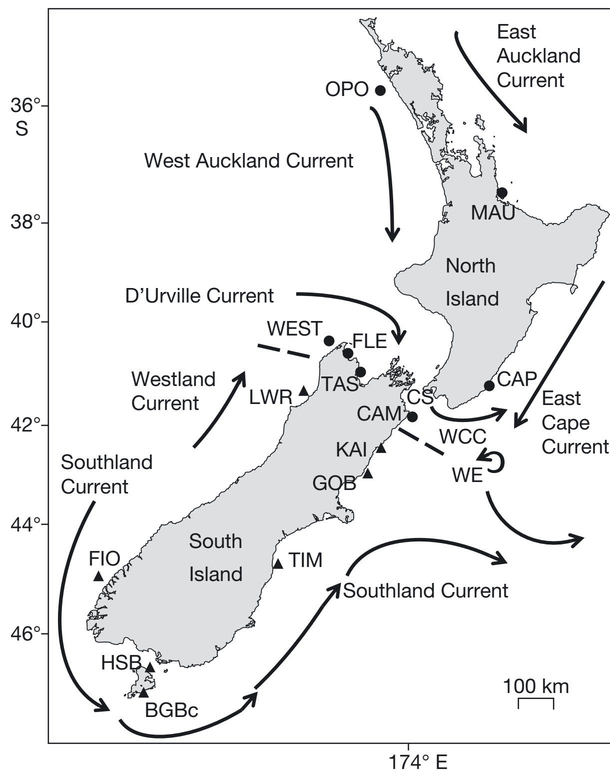

Fig. 1. *Perna canaliculus*. Collection sites and hydrographic patterns around New Zealand (from Wei et al. 2013). Opononi (OPO, n = 27); Maunganui (MAU, n = 29); Castlepoint (CAP, n = 30); Westhaven (WEST, n = 13); Fletchers Beach (FLE, n = 14); Tasman Bay (TAS, n = 20); Cape Campbell (CAM, n = 30); Little Wanganui River (LWR, n = 26); Kaikoura (KAI, n = 16); Gore Bay (GOB, n = 28); Timaru (TIM, n = 22); Fiordland (FIO, n = 19); Horseshoe Bay (HSB, n = 22); Big Glory Bay (BGB, n = 20). ●: northern group; ▲: southern group; — —: areas of coastal upwelling; c: cultured mussels; WE: Wairarapa Eddy; WCC: Wairarapa Coastal Current. Hydrographic data from Stanton (1976), Bowman et al. (1983), Barnes (1985), Heath (1985), Harris (1990), Vincent & Howard-Williams (1991), Stanton & Moore (1992), Chiswell (2000)

DNA extraction and polymerase chain reaction (PCR) amplification

A total of 311 mussels (mean of 22 mussels per population; Fig. 1) were genotyped at 10 polymorphic microsatellite loci using the primers and PCR conditions of MacAvoy et al. (2008). In all cases, DNA from a single individual was used as a positive control for genotyping. Genotyping was performed on an Applied Biosystems Instruments 3730 Genetic Analyzer (Allan Wilson Centre Genome Service, Massey University). DNA fragment sizes were identified by comparison with an internal size standard (GeneScan-500) using GENEMAPPER version 3.5 and PEAK SCANNER version 1.0 (Applied Biosystems).

Population genetic variation

As described by Wei et al. (2013), basic population genetics indices were calculated for all 14 populations. In addition, all 14 populations were examined for the presence of loci which may be under selection (i.e. are possibly not selectively neutral). Where appropriate, the false discovery rate method of correcting for multiple testing was employed (Verhoeven et al. 2005).

Data analyses

Population and spatial genetics analyses of the microsatellite data set are described by Wei et al. (2013). This same data set (14 populations, 10 loci) for seascape genetics analyses was employed in the present work: see Tables A1 & A2 in the Appendix for summary information. Allelic frequencies were calculated using the software package GenePop v3.4 (Raymond & Rousset 1995, 2004), Wright's fixation index (F_{ST}) values were calculated using FSTAT v2.9.3 (Goudet 1995, 2000), and modified phi-statistics (Φ'_{ST}) values were calculated using GenoDive (Meirmans & Van Tienderen 2004, Meirmans 2006). The Φ'_{ST} index is specifically designed for use with multi-allelic data and, as such, performs better with microsatellite data than F_{ST}, but because F_{ST} is so widely employed and understood, we follow the recommendation of Meir-

mans & Hedrick (2011) by analyzing and presenting both F_{ST} and Φ'_{ST} values. Based on earlier recognition of genetic differences between northern (OPO, MAU, CAP, WEST, FLE, TAS, CAM) and southern (KAI, LWR, GOB, TIM, FIO, HSB, BGB) populations (Fig. 1; Wei et al. 2013), we conducted separate analyses as detailed below for all 14 populations, the 7 northern and 7 southern populations.

We obtained site-specific data for 10 environmental factors: (1) annual mean solar radiation (W m^{-2}), (2) winter solar radiation (W m^{-2}), (3) wintertime sea surface temperature (SST_{winter}) (°C), (4) annual amplitude of SST ($SST_{an\ amp}$) (°C), (5) spatial gradient annual mean SST (SST_{grad}) (°C km^{-1}), (6) summertime SST anomaly (SST_{anom}) (°C), (7) mean orbital water velocity (Orb-v-mean) (m s^{-2}), (8) extreme orbital water velocity (m s^{-2}), (9) tidal current (Tidal) (m s^{-2}), and (10) freshwater fraction (FW) (proportion). These are 10 of the 11 different variables provided by the New Zealand Marine Environmental Classification (NZMEC) scheme (the last being sediment type, not used here because we only collected mussels from rocky reefs), and each has a spatial resolution of ~1 km. Variables represent long term averages of different duration and from different periods of time depending on the variable in question, but typically are drawn from multiple years between 1983 and 2000, or derived from NZ-specific models (New Zealand Ministry for the Environment 2005). Three geospatial variables were also obtained or calculated: (11) latitude, (12) longitude (both in decimal degrees; obtained from Google Earth), and (13) an index of geographic distance defined as the sum of all shortest possible coastal distances (km) among pairs of populations (low values indicate greater proximity of the one population to all other populations via coastal routes, high values indicate greater distance from that one population to all other populations). The software package STATISTICA (StatSoft 2004) was employed to test for independence among the 10 environmental variables and among the 3 geospatial variables. Following correlation analyses and principal component analysis (PCA), the 10 environmental variables were reduced to a subset of 7 independent (at $\alpha = 0.05$) variables (annual mean solar radiation, winter solar radiation, and extreme orbital water velocity were removed from the analyses because of their correlation with other variables). The 3 geospatial variables were treated as independent.

A generalized linear model (GLM approach; calculated using the GLZ routine in STATISTICA v7.0) was employed to test for the effect of the 7 environmental and 3 geospatial variables on F_{ST} or Φ'_{ST} values calculated for all 14 populations, as well as for the 7 northern and 7 southern populations (F_{ST} and Φ'_{ST} values were recalculated for northern-only and southern-only analyses). We calculated a mean multilocus F_{ST} or Φ'_{ST} value for each population (derived from 13 pairwise F_{ST} or Φ'_{ST} values for all 14 populations, or derived from 6 pairwise F_{ST} or Φ'_{ST} values for the southern or northern populations) to be used as a dependent variable (e.g. Foll & Gaggiotti 2006, Selkoe et al. 2010). GLM analyses were performed using the Akaike information criterion (AIC) to rank all possible models (combinations of independent variables). The AIC identifies the best subset of variables to include in the model: low AIC values reflect a better fit of the variables in terms of explaining variation in the model. Best fit models (lowest AIC values) are presented, as are results of a test of all effects. The null hypothesis is that none of the environmental or geospatial variables explain significant variation in the F_{ST} or Φ'_{ST} values.

The routine BEST (Biological Environmental Stepwise) from the software package PRIMER v6.0 (Clarke & Gorley 2005) was used to explore associations between population-specific genetic variation (allele frequencies per locus) and site-specific environmental/geospatial variation. A Bray-Curtis resemblance matrix was calculated for the genetic data (alleles being in the same 'units') and a Euclidean distance resemblance matrix was calculated for the normalized environmental/geospatial data (variables being in different 'units'). The BIOENV subroutine was used to test for an association between the 2 matrices using the Spearman correlation coefficient (r_S). All possible models were tested and then ranked according to which subset of environmental/geospatial variables best explained variation in the genetic data set (i.e. the model with the largest Spearman's r_S). The null hypothesis is that environmental/geospatial variables do not explain significant variation in population-specific allele frequencies. In addition, we also used the BEST routine to test for locus-specific responses to environmental variation. In this series of analyses, we tested locus-specific allele frequencies (one locus at a time) for all populations against environmental variation for all populations. The difference between the GLM and the BEST procedures, as employed here, is that the former is conducted on mean F_{ST} or Φ'_{ST} values and the latter on raw allele frequency data (both use the same set of environmental and geospatial variables). These 2 analyses are different but complementary in their approach to determining if and in what way the observed genetic variation is explained by the environmental/geospatial variables.

RESULTS

Population genetic variation

As described by Wei et al. (2013), observed heterozygosity (H_o) and expected heterozygosity (H_e) over all 10 loci varied from 0.60 ± 0.18 (±SD) to 0.70 ± 0.18 and from 0.68 ± 0.28 to 0.77 ± 0.16, respectively. H_o, H_e, and average heterozygosity (H_{ave}) for each locus across all populations ranged from 0.50 to 0.77, from 0.49 to 0.92, and from 0.47 to 0.89, respectively. Genetic diversity was high across all loci with mean H_{ave} = 0.71 ± 0.16. After correction for multiple testing, deviations from Hardy-Weinberg equilibrium (HWE) at 3 of 10 loci (*Pcan*6–17, *Pcan*1–29 and *Pcan*10–44) were significant (p < 0.001), and 13 of 14 populations did not conform to HWE across all loci (Appendix Table A1). However, on a locus per population basis, and after correction for multiple testing, only 26 of 139 tests (~19%) revealed significant heterozygote deficiencies.

GLM analyses

Among all populations, the GLM and the Akaike information criterion (AIC) tests identified a 5-factor model as explaining most variation in F_{ST} (Table 1). The best fit model contained 3 variables related to SST. SST_{winter} was identified as the single most significant factor, and occurred as an explanatory variable in the first 64 models. For Φ'_{ST} variation among all populations, the best fit model contained 3 factors: SST_{winter}, SST_{grad}, and Orb-v-mean. For the F_{ST} analysis, 45 models had p < 0.01, whereas for the Φ'_{ST} analysis only 3 models had p < 0.01. Among the northern populations, a 6-factor model provided the best fit for variation in F_{ST} and also in Φ'_{ST}. These 2 models were very similar in terms of their explanatory variables (Table 1). Among southern populations, a 6-factor model provided the best fit for variation in F_{ST} and also in Φ'_{ST}. SST variation explained more variation in F_{ST} than it did in Φ'_{ST} (Table 1). GLM testing did not identify a significant model

Table 1. *Perna canaliculus*. Results of generalized linear model analyses (GLZ routine in STATISTICA v7.0; StatSoft 2004) employing Akaike information criterion (AIC) to identify best fit model (as judged by lowest AIC value) for 7 environmental and 3 geospatial variables as explanation for genetic variation (mean F_{ST} and mean Φ'_{ST} values). SST_{winter}: wintertime sea surface temperature (°C); $SST_{an\ amp}$: annual amplitude of sea surface temperature (°C); SST_{grad}: annual mean sea surface temperature spatial gradient (°C km^{-1}); SST_{anom}: summertime sea surface temperature anomaly (°C); Orb-v-mean: mean orbital water velocity (m s^{-2}); Tidal: tidal current (m s^{-2}); FW: freshwater fraction (proportion). Data for these 7 variables were derived from the New Zealand Marine Environment Classification scheme (New Zealand Ministry for the Environment 2005). Latitude and longitude (in decimal degrees) were obtained from Google Earth; Geographic distance: an index defined as the sum of all coastal distances (km) among pairs of populations (lower values indicate greater proximity among populations, whereas higher values indicate greater distance among populations)

GLM test	Populations	Best fit model	p	Test of all effects: Variable	p
F_{ST} vs. 7 environmental variables	All 14	SST_{winter} + SST_{grad} + SST_{anom} + Tidal + FW	<0.0001	SST_{winter}	<0.0001
				$SST_{an\ amp}$	0.554
				SST_{grad}	0.198
				SST_{anom}	0.035
				Orb-v-mean	0.529
				Tidal	0.016
				FW	0.004
Φ'_{ST} vs. 7 environmental variables	All 14	SST_{winter} + SST_{grad} + Orb-v-mean	0.0067	SST_{winter}	0.037
				$SST_{an\ amp}$	0.568
				SST_{grad}	0.007
				SST_{anom}	0.944
				Orb-v-mean	0.506
				Tidal	0.151
				FW	0.489
F_{ST} vs. 7 environmental variables	7 Northern	$SST_{an\ amp}$ + SST_{grad} + SST_{anom} + Orb-v-mean + Tidal + FW	<0.0001	SST_{winter}	0.759
				$SST_{an\ amp}$	0.399
				SST_{grad}	<0.0006
				SST_{anom}	0.014
				Orb-v-mean	0.011
				Tidal	<0.0001
				FW	<0.0001

(continued on next page)

Table 1. (continued)

GLM test	Populations	Best fit model	p	Test of all effects Variable	p
Φ'_{ST} vs. 7 environmental variables	7 Northern	$SST_{winter} + SST_{grad} + SST_{anom}$ + Orb-v-mean + Tidal + FW	<0.0001	SST_{winter}	0.617
				$SST_{an\ amp}$	0.709
				SST_{grad}	0.010
				SST_{anom}	0.877
				Orb-v-mean	0.038
				Tidal	<0.0001
				FW	<0.0001
F_{ST} vs. 7 environmental variables	7 Southern	$SST_{winter} + SST_{an\ amp} + SST_{anom}$ + Orb-v-mean + Tidal + FW	<0.0001	SST_{winter}	0.113
				$SST_{an\ amp}$	0.136
				SST_{grad}	0.012
				SST_{anom}	0.032
				Orb-v-mean	0.405
				Tidal	<0.0001
				FW	1.000
Φ'_{ST} vs. 7 environmental variables	7 Southern	$SST_{winter} + SST_{grad} + SST_{anom}$ + Orb-v-mean + Tidal + FW	<0.0001	SST_{winter}	0.577
				$SST_{an\ amp}$	0.870
				SST_{grad}	0.128
				SST_{anom}	0.161
				Orb-v-mean	0.008
				Tidal	<0.0001
				FW	<0.0001
F_{ST} vs. 3 geospatial variables	All 14	Geographic distance	0.147	Latitude	0.119
				Longitude	0.346
				Geographic distance	0.269
Φ'_{ST} vs. 3 geospatial variables	All 14	Geographic distance	0.097	Latitude	0.370
				Longitude	0.611
				Geographic distance	0.043
F_{ST} vs. 3 geospatial variables	7 Northern	Geographic distance	0.104	Latitude	0.602
				Longitude	0.861
				Geographic distance	0.374
Φ'_{ST} vs. 3 geospatial variables	7 Northern	Geographic distance	0.054	Latitude	0.852
				Longitude	0.537
				Geographic distance	0.470
F_{ST} vs. 3 geospatial variables	7 Southern	Latitude + Geographic distance	0.037	Latitude	0.188
				Longitude	0.474
				Geographic distance	0.055
Φ'_{ST} vs. 3 geospatial variables	7 Southern	Latitude	0.323	Latitude	0.027
				Longitude	0.023
				Geographic distance	0.012

among the 3 geospatial variables for all 14 populations based on variation in either F_{ST} or Φ'_{ST} (Table 1). Among northern populations, the best fit models contained only one variable (geographic distance) for both F_{ST} and Φ'_{ST}, but in both cases were not statistically significant. Among southern populations, the best fit model for F_{ST} was statistically significant and contained 2 geospatial variables, but for Φ'_{ST} all models were not statistically significant (Table 1). Overall, and as expected, there was a substantial amount of concordance in terms of response between F_{ST} and Φ'_{ST}. Significant results tended to highlight the contributory role of some aspect of SST in explaining genetic variation among the populations, whereas the geospatial variables were generally not important in this regard.

BEST analyses

The analysis of multi-locus allele frequency variation for all 14 populations identified 6 different models with Spearman's $r_S > 0.450$, and a single best fit model containing 7 of the 10 variables with $r_S = 0.475$. $SST_{gradient}$, freshwater component, and longitude occurred in all top 10 models, whilst other variables such as SST_{winter}, $SST_{an\ amp}$, Orb-v-mean, and latitude all occurred in 7 or more of the top 10 models. Variables such as SST_{anom}, tidal range, and geographic distance did not generally explain variation in the genetics data set (Table 2). Within the northern group of 7 populations, 5 models had $r_S > 0.400$, the highest rating model having $r_S = 0.501$. $SST_{an\ amp}$ occurred in 9 of the top 10 models, whilst variables

Table 2. *Perna canaliculus*. Results from BEST analyses linking environmental and genetic variation: the best fit 10 top models for each analysis are presented (X indicates that given variable is included in the model). See Table 1 for definitions of variables

Data set (populations)	No. of variables in model	r_S	SST_{winter} (°C)	$SST_{an\ amp}$ (°C)	SST_{grad} (°C km^{-1})	SST_{anom} (°C)	Orb-v-mean (m s^{-1})	Tidal (m s^{-1})	FW (proportion)	Latitude (°S)	Longitude (°E)	Geographic distance (km)
All 14	7	0.475	X	X	X		X		X	X	X	
	5	0.474			X		X		X	X	X	
	6	0.468		X	X		X		X	X	X	
	6	0.467	X	X	X		X		X		X	
	6	0.457	X		X		X		X	X	X	
	5	0.452	X		X		X		X		X	
	6	0.449	X	X	X				X	X	X	
	8	0.449	X	X	X	X	X		X	X	X	
	5	0.445	X	X	X				X		X	
	7	0.445		X	X		X		X	X	X	X
7 Northern	4	0.501		X			X		X			X
	5	0.441		X	X		X		X			X
	5	0.436		X	X		X		X		X	
	5	0.417		X			X		X		X	X
	6	0.405		X	X		X		X		X	X
	3	0.399		X					X			X
	3	0.394		X			X					X
	3	0.378					X		X			X
	1	0.340		X								
	4	0.338		X	X		X					X
7 Southern	3	0.283	X	X	X							
	5	0.279	X	X	X				X	X		
	3	0.269			X	X				X		
	3	0.244		X	X	X						
	3	0.243	X	X		X						
	2	0.239		X	X							
	4	0.234	X	X	X							X
	6	0.227	X	X	X			X	X	X		
	2	0.226			X					X		
	4	0.226	X	X	X					X		

such as Orb-v-mean, freshwater input, and coastal distance between populations were all identified as being explanators of genetic variation. Variables such as SST_{winter}, SST_{anom}, tidal range, and latitude did not contribute to explaining genetic variation (Table 2). As judged by r_S values for the southern group of 7 populations, environmental variation did not explain as much genetic variation as reported for the previous 2 analyses. Only 3 models exhibited $r_S > 0.250$, the best fit model having $r_S = 0.283$. Generally, a small subset of variables was important in explaining variation in the genetic data set, with factors such as SST_{winter}, $SST_{an\ amp}$, and SST_{grad} being present in most of the top 10 rated models. Variables such as Orb-v-mean, tidal range, freshwater input, longitude, and coastal distance did not contribute much to explaining genetic variation (Table 2).

BEST analyses of locus-specific data (Table 3) revealed a wide range of r_S values for the single best fit model in each case (range of −0.056 to 0.554), suggesting that genetic variation at some loci (e.g. *Pcan*1–27 and *Pcan*10–36) may be affected to a greater extent than genetic variation at other loci (e.g. *Pcan*2–60 and *Pcan*1–25). Qualitative assessment of the impact of each environmental and geospatial variable (the number of times that any given variable occurred in the top 10 models for each locus) on locus-specific genetic variation did not reveal any particular patterns of effect. All variables except SST_{anom} occurred 10 times at least once, but no particular variable occurred 10 times more than twice (SST_{winter}, SST_{grad}, FW). Overall, these results suggest that if individual locus effects are occurring then they are relevant to a small subset of loci.

Table 3. *Perna canaliculus*. Results from BEST analyses linking environmental and genetic variation for each locus and for all 14 populations. Top part of table shows single best-fit model for each locus: X indicates a variable that is included in the model. Bottom part of table shows number of times that each variable occurred in the top 10 best fit models (larger numbers indicate greater contribution of that variable). See Table 1 for definitions of variables

Locus	r_S	SST_{winter} (°C)	$SST_{an\ amp}$ (°C)	SST_{grad} (°C km^{-1})	SST_{anom} (°C)	Orb-v-mean (m s^{-1})	Tidal (m s^{-1})	FW (proportion)	Latitude (° S)	Longitude (° E)	Geographic distance (km)
*Pcan*1–25	0.178			X		X				X	
*Pcan*1–27	0.554	X		X		X		X	X	X	
*Pcan*1–29	0.298					X		X			
*Pcan*2–17	0.185			X							
*Pcan*2–20	0.320	X		X		X	X	X			
*Pcan*2–60	−0.056		X								
*Pcan*6–17	0.294		X	X					X	X	
*Pcan*10–36	0.481							X		X	X
*Pcan*10–44	0.330	X	X	X	X				X		
*Pcan*22–11	0.277			X							
*Pcan*1–25	–	0	3	7	4	9	0	0	0	5	0
*Pcan*1–27	–	10	5	6	0	6	0	10	10	10	2
*Pcan*1–29	–	6	7	0	0	9	0	7	0	3	0
*Pcan*2–17	–	1	9	10	4	0	0	0	0	4	4
*Pcan*2–20	–	2	0	8	3	10	10	9	3	0	3
*Pcan*2–60	–	0	6	0	4	6	8	3	0	0	0
*Pcan*6–17	–	0	10	6	3	2	1	0	9	8	0
*Pcan*10–36	–	1	0	1	2	2	0	10	3	7	10
*Pcan*10–44	–	10	8	8	7	0	0	0	4	0	3
*Pcan*22–11	–	0	7	10	2	5	0	3	0	1	0

DISCUSSION

The earliest population genetic surveys of *Perna canaliculus* employed allozymes (biochemical markers) and re-

ported partial isolation between northern and southern populations at ~38° S (Smith 1988), or an isolation-by-distance structure (Gardner et al. 1996). At the time, Smith (1988) noted that his results were consistent with a warm water adapted northern group and a cool or cold water adapted southern group of populations. Generally, the results of Gardner et al. (1996) tended to support Smith's suggestion that greenshell mussel populations may be differentiated, possibly as a consequence of local adaptation to regional temperature differences. However, the next and largest allozyme survey found no evidence for regional differences or localised adaptation, but instead reported minimal genetic subdivision as a consequence of high levels of gene flow, suggesting that a single panmictic model best explained population genetic homogeneity over the entire range (Apte & Gardner 2001). Subsequently, the application of a suite of molecular markers to the analysis of greenshell mussel populations (Apte & Gardner 2002, Star et al. 2003, Wei et al. 2013) revealed the existence of a pronounced genetic break just south of Cook Strait. Reports of similar genetic structure for many other taxa (Ross et al. 2009, Gardner et al. 2010) confirm the spatially explicit nature of this genetic discontinuity at ~42° S.

In the present study, both sets of seascape analyses have identified one or more indices of SST as being an important explanator for the observed genetic variation. The results point to a macrogeographic level of genetic structuring that is in large part associated with variation in SST, and is, therefore, very different in form from the genetic discontinuity described above. In all 3 cases (all 14 populations; 7 northern; 7 southern), the GLM analyses highlighted the importance of SST (defined more specifically as SST_{winter}, SST_{anom}, or SST_{grad}) as explaining more genetic variation (population-specific mean F_{ST} or Φ'_{ST} values) than the other environmental variables. While variables such as tidal current, freshwater proportion, and Orb-v-mean were identified as being statistically significant in different models, by far the greatest influence across all models was observed for indices of SST. None of the geospatial variables (latitude, longitude, geographic distance among populations) explained genetic variation in all 14 populations or in the 7 northern populations. However, latitude and geographic distance were identified as explaining genetic variation among the 7 southern populations for the F_{ST} test only. Consistent with the GLM results, in all cases the BEST analyses identified at least one index of SST as explaining allelic variation among individuals. Although other environmental variables also contributed to explaining the genetic variation, their contributions were less than that of SST. All 3 of the geospatial variables (longitude, latitude, and geographic distance among populations) were variously identified as being important explanatory variables in the different analyses of allelic variation.

The locus-specific BEST analyses highlight the differential contribution of the environmental and geospatial variables when explaining individual locus genetic variation. Genetic variation (allelic frequencies) at some loci appear to be explained by variation in the environmental variables, whereas variation at other loci is apparently almost independent of environmental variation. At the single locus level, there is little evidence that any one environmental or geospatial variable contributes to genetic variation across all loci: some environmental variables are important explanators for allelic variation at some loci, but not at others. Overall, these findings are consistent with a body of published work that points to the neutral role of most assayed genetic variation, but can highlight the key role of a small number of loci (sometimes as few as one) that may be under selective pressure (e.g. Gardner & Kathiravetpillai 1997, Gaggiotti et al. 2009, Wei et al. 2013). The approach of testing individual loci for evidence of selection is becoming increasingly feasible with new molecular markers and new analytical techniques, meaning that there is now an opportunity to identify the genetic basis and the mechanism of action of natural selection (Balkenhol et al. 2009, Luttikhuizen et al. 2012, Núnez-Acuna et al. 2012).

A potential limitation of the seascape genetics approach as employed here is that the environmental and geospatial variables being tested in the different models are unknown quantities in the sense that there is no *a priori* reason to expect any one of them, or any combination of them, to be important in explaining genetic variation. As such, the model testing is best viewed as an exploratory approach to identifying key environmental variables that are associated with genetic variation. Beyond this, the variables tested are those that can be drawn from existing data sets (e.g. the NZMEC scheme) or those such as latitude, longitude, and geographic distance among populations which can be obtained easily or calculated and which might reasonably be viewed as being of possible importance (e.g. latitude is a surrogate for NZ's north–south geographic orientation). We recognise that the variables we tested are but a subset of a much larger potential suite of variables, but we are presently limited to the use of variables that are compiled using a standard methodology and

are readily available. The NZMEC scheme is due to be updated in the near future, at which time new variables may be available for further testing. Initial testing of 10 variables from the NZMEC scheme indicated that 7 were statistically independent (annual mean solar radiation and winter solar radiation were correlated with SST winter; extreme orbital water velocity was correlated with mean orbital velocity). Of these 7 independent environmental variables, 4 are related to an index of SST, so it might not be surprising that at least one index of SST was identified as being an important explanator of genetic variation. However, the reasonably consistent contribution within analyses of some indices and the absence of contribution of others highlights the association between environmental and genetic variation. The mechanistic basis of such a relationship cannot be inferred from the present analyses, but these results do highlight new avenues for research.

The contribution of several different factors, both genetic and logistical, may influence the interpretation of the results; below, we consider 4 such points. First, 19% of all locus-by-population tests exhibited significant heterozygote deficiencies. Heterozygote deficiencies are very common in bivalve populations across a range of genetic marker types (e.g. Zouros & Foltz 1984, Addison & Hart 2005, Addison et al. 2008, Diz & Presa 2009, Ong et al. 2009). Whilst the cause of these heterozygote deficiencies is usually unknown, several different explanations have been proposed including inbreeding, null alleles, selection, aneuploidy, maternal imprinting, and the Wahlund effect (Zouros & Foltz 1984, Gardner 1992, Teixeira de Sousa et al. 2012). In the present study, most loci were in HWE across most populations, but loci such as *Pcan*6–17 and *Pcan*1–29 were not in HWE for many populations (Wei et al. 2013). For our analyses, there is no compelling reason that all loci must be in HWE, thus we do not consider that the heterozygote deficiencies reported here contribute to the association between genetic and environmental variation. Second, one key aim of this research was specifically to better understand if genetic variation of populations in central NZ (at the genetic discontinuity near ~42° S) is explained by environmental variation. Our sampling design means that we effectively have 6 of 14 sampled populations in a small range of latitude in central NZ. It might be argued that a more even spacing of populations across the 11.5° of latitude would be more informative, but such a sampling design would not provide good spatial cover of the genetic discontinuity. Ultimately, we found no evidence of an association between genetic and environmental variation at a small spatial scale (10s to 100s of km) in central NZ, but we did find it at a larger scale (100s to 1000s of km) across NZ in both sets of analyses, which used different data sets (genetic distance estimates and multilocus genotypes) and different analytical approaches. Third, there is a temporal component to our population sampling, with new sites in central NZ having been sampled subsequent to the identification of the genetic discontinuity. To see if this temporal variability in sampling contributed significantly to population genetic variability (e.g. Gardner & Palmer 1998, Prakoon et al. 2010, Poulsen et al. 2011), we used AMOVA testing to reveal no significant difference between time periods 1996 to 1998 versus 2002 to 2008, or among time periods 1996 to 1998 versus 2002 to 2004 versus 2008 (Wei et al. 2013). Thus, year of sample collection does not contribute significantly to genetic variation. Fourth, and finally, we note the possible disproportionate contribution of a single locus as an explanator of an association between genetic and environmental variation. For example, in other studies, a single locus has been identified as being under strong selection pressure and as such its genetic signal will not reflect neutral demographic processes (e.g. Gaggiotti et al. 2009). In the BEST single locus tests, we did see evidence that one locus contributed substantially to the overall association between genetics and environmental variation. Locus *Pcan*1–27 had $r_S = 0.554$, the next highest $r_S = 0.481$, and then after that several loci had $r_S \approx 0.3$. This finding agrees with the identification of *Pcan*1–27 as being an outlier based on locus-specific F_{ST} values, and, therefore, possibly under selection pressure (Wei et al. 2013). Both sets of findings suggest that *Pcan*1–27 is a locus that warrants further investigation, in particular as a candidate locus for selection.

Elsewhere, coastal currents have been shown to play a key role in explaining seascape genetics (e.g. Galindo et al. 2010, Selkoe et al. 2010). In the NZ situation, this does not appear to be the case, at least not in the same way. Multi-species genetic discontinuities (Ross et al. 2009, Gardner et al. 2010) do not correspond with obvious surface flow, nor with obvious surface temperature features, but do correspond with regions of upwelling. The most pronounced oceanographic feature related to temperature is the subtropical convergence, which tends to sit half way up the South Island (at about 44° S) during the winter, and wraps around the bottom of the South Island (at about 46 to 47° S) during the summer. However, this large body of warm water, which exhibits pronounced seasonal shifts in its location, is apparently unrelated

to the SST variation that we have observed in our analyses as an explanation for population-level genetic variation. Of greater relevance are localised environmental conditions — SST_{winter}, SST_{grad}, and SST_{anom} in particular — that are either unrelated to latitude (NZ's natural north–south gradient) or to the known patterns of coastal surface circulation around NZ. In other comparable work, the 2 most important variables influencing genetic variation of the Atlantic herring *Clupea harengus* from the western North Sea to the inner Baltic Sea were salinity at spawning sites and feeding migrations (Gaggiotti et al. 2009). Those authors identified 2 of 8 microsatellite loci that were non-neutral and one that showed evidence of being under directional selection with regard to salinity variation, and concluded that any historical genetic signal derived from the demographic history of the herring populations had been lost as a consequence of the interaction and influence of contemporary demographic and environmental processes. The present study of greenshell mussels and the study of herring (Gaggiotti et al. 2009) highlight the relationships between historical and contemporary factors in explaining genetic variation of marine taxa, and also illustrate how single loci may be under directional selection (or at least tightly linked to other loci that may be under selective pressure).

Despite the presence of a genetic discontinuity at ~42° S, no environmental variable, or combination of variables, explains the narrow geographic range of the north–south split. Instead, the variables tested are associated with larger spatial scale genetic variation and structuring, which has not previously been recognised. Wei et al. (2013) highlighted the existence of high levels of self-recruitment within populations and within regional groups (northern and southern) of greenshell mussels. This latter point may prove to be important for seascape genetics, given that extensive gene flow tends to have an homogenizing effect, whereas localised self-recruitment may promote and maintain the development of regional genetic differences possibily reflecting localised selection pressures. While our seascape analyses do not allow us to ascribe a cause and effect linkage between environmental and genetic variation, we can suggest possible mechanisms of action. For example, one mode of action of selection may be via thermal stress acting directly on heat shock proteins (e.g. Buckley et al. 2001, Dutton & Hofmann 2009). This would require that one or more of the microsatellite markers which we employed is linked to heat shock protein genes, given that microsatellite markers are thought to be selectively neutral and are, therefore, unlikely to respond directly to selection (Chambers & MacAvoy 2000, Luikart et al. 2003, Holderegger & Wagner 2006). Whilst speculative, this putative mechanism is worthy of further investigation, and provides an illustration of how mussels may be responding to environmentally-driven selection, just as herring are responding to salinity variation at their spawning grounds (Gaggiotti et al. 2009). As suggested by Smith (1988) more than 20 yr ago, there may indeed be warm-adapted and cold-adapted populations of greenshell mussels. The pattern of genetic variation that we have observed in greenshell mussels is consistent with this, and its association with SST may be reflected in other coastal taxa. If so, further examination will advance our understanding of the patterns and processes of genetic structuring in coastal biota.

CONCLUSIONS

The application of seascape genetics to various systems has provided new insights into how and why marine coastal populations are structured. One approach is the development and application of coupled biological and physical oceanographic models to study connectivity. This approach has proven to be powerful in its ability to predict population structure of Caribbean corals and marine larval dispersal along the central California coast (e.g. Galindo et al. 2006, 2010). Not surprisingly, the power and utility of such models is derived at least in part from data-intensive studies and detailed knowledge of the taxa and the coastal system in question. Since the requisite level of data to implement this approach is not available for most species in most coastal systems, these examples highlight what may be achieved for other taxa and systems at some stage in the future. An alternative approach to trying to explain genetic structuring of populations, and patterns of connectivity that maintain such structure, may involve determination of the role of environmental variables and the role of life-history and behavioural responses of the taxa in question. When applied to a multi-species dataset, this approach can reveal common patterns of taxon genetic structuring, as well as the influence of key environmental drivers (e.g. Selkoe et al. 2010) and can help to disentangle the roles of historical (phylogeographic) and contemporary (selection) processes in generating and maintaining genetic variation (e.g. Gaggiotti et al. 2009).

Our seascape analyses have revealed a new level of genetic structure in a widely distributed taxon

that already displays a pronounced genetic discontinuity. Thus, we can now superimpose a macrogeographic scale of genetic variation associated with SST on top of the existing genetic break at 42° S. Our findings strongly suggest that different processes (phylogeographic for the genetic break and contemporary selection for the association with SST) are influencing the genetic strucuture of *Perna canaliculus* populations.

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Appendix

Table A1. *Perna canaliculus*. Hardy-Weinberg equilibrium (HWE) probability tests for all loci and all populations. *Significant at $\alpha = 0.05$ after false discovery rate correction for multiple testing (Verhoeven et al. 2005)

Locus	HWE for 10 loci across all 14 populations		Pop.	HWE for 14 populations across all 10 loci	
	χ^2	p		χ^2	p
*Pcan*2–60	38.2	0.095	TAS	55.8	<0.0001*
*Pcan*10–36	41.4	0.050	CAP	31.5	0.049
*Pcan*6–17	∞	<0.0001*	MAU	38.3	0.008*
*Pcan*2–20	38.8	0.084	OPO	∞	<0.0001*
*Pcan*1–25	42.6	0.038	CAM	∞	<0.0001*
*Pcan*1–29	∞	<0.0001*	FLE	65.7	<0.0001*
*Pcan*2–17	24.7	0.642	WEST	∞	<0.0001*
*Pcan*1–27	23.8	0.590	KAI	46.3	0.0007*
*Pcan*22–11	48.8	0.090	LWR	33.3	0.031*
*Pcan*10–44	∞	<0.0001*	TIM	35.4	0.008*
			BGB	46.9	0.0006*
			HSB	∞	<0.0001*
			FIO	45.4	0.0010*
			GOB	∞	<0.0001*

Table A2. *Perna canaliculus*. Pairwise estimates of Φ'_{ST} (below diagonal) and F_{ST} (above diagonal) between populations. See Fig. 1 for collection site information. *Testing of F_{ST}: significant at $\alpha = 0.05$ after false discovery rate correction for multiple testing (Verhoeven et al. 2005)

Pop.	TAS	CAP	MAU	OPO	CAM	FLE	WEST	KAI	LWR	TIM	BGB	HSB	FIO	GOB
TAS	–	0.004	0.008	0.007	0.013	0.011	−0.001	0.010	0.024*	0.054*	0.042*	0.019*	0.028*	0.014
CAP	0.004	–	0.022*	0.002	0.006	0.009	−0.007	0.017*	0.028*	0.055*	0.043*	0.024*	0.036*	0.019*
MAU	0.019	0.075	–	0.014*	0.019*	0.019	0.008	0.026*	0.037*	0.081*	0.071*	0.038*	0.038*	0.030*
OPO	0.013	−0.002	0.042	–	0.005	0.000	−0.010	0.019*	0.033*	0.070*	0.052*	0.027*	0.039*	0.021*
CAM	0.033	0.015	0.058	0.009	–	0.006	−0.008	0.019*	0.029*	0.074*	0.063*	0.026*	0.048*	0.031*
FLE	0.015	0.022	0.059	−0.017	0.004	–	−0.008	0.000	0.020*	0.053*	0.038*	0.007	0.036*	0.010
WEST	−0.035	−0.045	0.019	−0.053	−0.048	−0.062	–	0.005	0.021	0.058*	0.040*	0.013	0.033*	0.012
KAI	0.018	0.056	0.085	0.063	0.058	−0.021	0.001	–	0.009	0.021*	0.020*	0.006	0.017*	−0.001
LWR	0.088	0.106	0.132	0.120	0.101	0.067	0.072	0.027	–	0.036*	0.030*	0.000	0.001	0.008
TIM	0.180	0.190	0.260	0.231	0.236	0.169	0.187	0.061	0.120	–	0.011	0.020*	0.021*	0.017*
BGB	0.147	0.157	0.241	0.182	0.216	0.128	0.138	0.064	0.104	0.030	–	0.013	0.028*	0.017*
HSB	0.058	0.087	0.131	0.094	0.083	0.003	0.030	0.005	−0.011	0.056	0.035	–	0.013	0.008
FIO	0.091	0.127	0.123	0.132	0.155	0.115	0.108	0.051	−0.001	0.058	0.088	0.032	–	0.001
GOB	0.038	0.064	0.098	0.070	0.101	0.020	0.029	−0.018	0.022	0.046	0.052	0.016	−0.008	–

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