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ORIGINAL PAPER

Low to moderate levels of genetic differentiation detected across the distribution of the New Zealand abalone, *Haliotis iris*

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Abstract Two regions of the mitochondrial genome (cytochrome oxidase I and ATPase 8–ATPase 6) were used to examine the population genetic structure of New Zealand's endemic abalone (*Haliotis iris*). Samples were collected from 28 locations around New Zealand between January 2005 and February 2008. At least four phylogeographic breaks were present and occurred across the Chatham rise, in the western Cook Strait region, along the southeast coast of the South Island, and at East Cape in the North Island. Gene flow across the Chatham rise is probably limited due to infrequent dispersal across large geographic distances (~850 km), while factors limiting gene flow around the North and South Islands are less clear, and understanding these may require intense temporal and spatial sampling in complex hydrographic regions. High genetic diversity and weak genetic structure may be a general feature of abalone potentially reflecting large and/or ancient populations.

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Introduction

The interaction between a variety of extrinsic and intrinsic factors has been proposed to shape the genetic structure of marine invertebrates with bipartite life histories (reviewed in Palumbi 1994; Hellberg et al. 2002; Sponaugle et al. 2002). The biology of a species can help predict how it will respond to a particular environment, while past and present environmental features can help predict potential barriers and corridors to gene flow. However, neither biology nor New Zealand's oceanography offer clear-cut predictions for the genetic structure of New Zealand's black-foot abalone or paua (*Haliotis iris*).

Paua are long-lived marine gastropods that inhabit the intertidal and subtidal rocky reefs surrounding mainland and offshore islands and the Chatham Islands (Fig. 1). The commercial and cultural importance of paua has warranted much research regarding its biology. Paua are highly fecund broadcast spawners with a larval duration under 10 days (Tong et al. 1992). Spawning events, larval survival, and juvenile settlement and recruitment are variable and influenced by many abiotic and biotic factors (Poore 1973; Sainsbury 1982; Hooker and Creese 1995; McShane and Naylor 1995; Naylor and McShane 1997; Naylor and McShane 2001; Roberts et al. 2004; Phillips and Shima 2006).

The numerous factors affecting spawning, larval survival, and juvenile settlement and survival result in variable recruitment over time and space, as observed by Sainsbury (1982). Potentially, this could result in patterns of genetic patchiness. On the other hand, the larvae are considered passive and as a result can be influenced by the local hydrodynamic environment (McShane 1992; Bohonak 1999). In general, population genetic research on abalone species has found either panmixia or slight

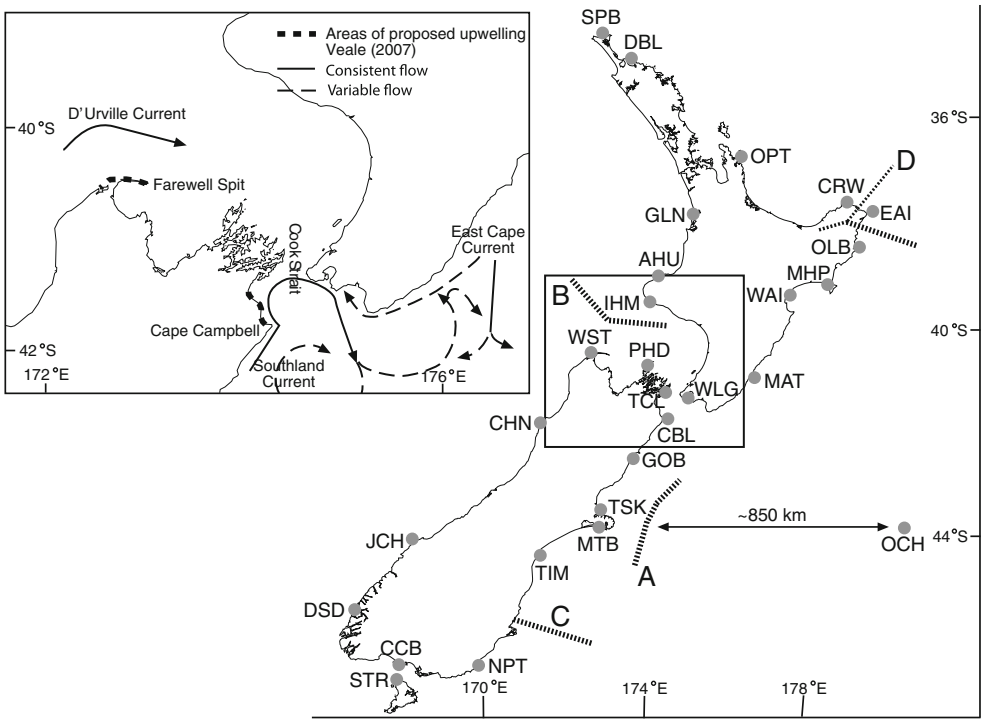

Fig. 1 Cook Strait region and sampling locations. The *box* is an enlargement of the Cook Strait region. Shown are areas of upwelling proposed by Veale (2007), locations mentioned in text, and currents around the Cook Strait region. The flow and direction of the East Cape and Southland Currents are from Fig. 4 in Barnes (1985). The map of New Zealand shows the 28 sampling localities listed in Table 2. The location of the Chatham Islands sample (OCH; 44.01° S, 176.21° W) is not to scale and lies about 850 km off the east coast of New Zealand. The *dashed lines* labeled *A*, *B*, *C*, and *D* mark areas of genetic discontinuities identified using Barrier 2.2 (Manni et al. 2004). *Thickness of the dashed lines* denotes the percentage of resampled matrices identifying the barrier (supplementary information)

differentiation, which was detectable with highly variable markers or a number of independent loci (e.g., Withler et al. 2003; Hara and Sekino 2005; Gruenthal et al. 2007). In several cases where pronounced differentiation was detectable with mitochondrial markers, phylogeographic breaks corresponded with present and past oceanography (e.g., Evans et al. 2004; Imron et al. 2007).

New Zealand's marine environment is complex. Although New Zealand has six major offshore currents (summarized in Laing and Chiswell 2003), coastal circulation patterns are complicated with features like coastal topography, upwelling, eddies, and river plumes that could inhibit dispersal and/or promote local retention of larvae (Sponaugle et al. 2002; Schiel 2004). Arguably, the two most persistent features associated with genetic patterns of coastal marine invertebrates around New Zealand are (1) the large geographic distance (about 850 km) between the Chatham Islands and the mainland (North and South Islands; e.g., Goldstien et al. 2009) and (2) the Cook Strait region, an area encompassing the waterway separating the North and South Islands (e.g., Apte and Gardner 2002; Waters and Roy 2004; Goldstien et al. 2006; Veale 2007).

Although gene flow between the Chatham Islands and the mainland is most likely limited due to isolation by distance (Chiswell 2009), factors limiting gene flow across the Cook Strait region are unknown. Cook Strait was submerged 10,000–5,000 ya with the rise of sea levels at the end of the last ice age (Stevens et al. 1995). The present-day hydrography around the Cook Strait region (Fig. 1) is complex and involves the convergence of three offshore currents that vary in temperature and salinity (Heath 1970; Heath 1985), river discharge (Harris 1990), strong tidal flows and large amounts of tidal mixing (Heath 1978; Hume et al. 1992), and upwelling (Heath 1972; Bowman et al. 1983; Barnes 1985; Shirtcliffe et al. 1990). Potentially, present-day upwelling could limit larval dispersal and, therefore, gene flow (Star et al. 2003; Waters and Roy 2004; Veale 2007). Unfortunately whether upwelling really limits larval dispersal is debatable (Roughgarden et al. 1988; Poulin et al. 2002; Shanks and Brink 2005), and the upwelling hypothesis also does not reconcile well with attempts to date genetic disjunctions across the Cook Strait region (Apte and Gardner 2002; Goldstien et al. 2006).

Previous genetic studies on paua support differentiation between the Chatham Islands and the mainland (Frusin 1982; Smith and McVeagh 2006). However, genetic structure around the mainland remains inconclusive, in part

due to a limited number of samples (<4), which do not span the geographic range of paua, and small sample sizes (<11). To elucidate paua genetic structure further, this study analyzed variation in two regions of the mitochondrial genome (COI and ATPase8–ATPase6) for samples from 28 locations around New Zealand to identify if genetic structure exists and, if it does, to determine the pattern, specifically genetic differentiation between the Chatham Islands and the mainland and between the North and South Islands.

Materials and methods

Samples

Between January 2005 and February 2008, foot or mantle tissue was collected from 13–24 paua from 28 locations (Fig. 1, Table 1). Paua were collected by a variety of means, including confiscated illegal catch, commercial catch, recreational catch, and scientific catch, and as a result, different tissues were available for different samples. Individual paua varied in size and therefore age (Naylor et al. 2007). All individuals were mature (i.e. had shell lengths ≥44 mm; Hooker and Creese 1995); however, not all individuals were larger than the minimum legal catch size (≥125 mm).

DNA extraction, PCR amplification, and sequencing

DNA was extracted from individuals collected at 27 of the 28 sites using Qiagen's DNEasy® Blood & Tissue Kit. Abalone DNA from the remaining site (OPT) was extracted using a modified LiCl protocol (Gemmell and Akiyama 1996). Two regions of the mitochondrial genome were amplified: cytochrome oxidase I (mtCOI) and ATPase8–ATPase6. These

Table 1 Locations, collection dates, and sample sizes for collection sites shown in Fig. 1

Sample	Location	Latitude[a]	Longitude[a]	Collection date	*N*
AHU	Ahu Ahu Rd	−39.117159°	173.929819°	16 Oct 2005	22
CBL	Cape Campbell	−41.741920°	174.275685°	15 Oct 2005	12
		−41.734349°	174.276258°		
		−41.725371°	174.273675°		
CCB	Colac Bay	−46.422131°	167.836311°	13 Jan 2005	20
CHN	Charleston	−41.912494°	171.423681°	28 Nov 2007	20
				5 Dec 2007	
CRW	Cape Runaway	−37.547890°	178.166130°	2 Nov 2005	19
DBL	Doubtless Bay	−34.849000°	173.470007°	2 May 2005	15
DSD	Doubtful Sound	−45.269575°	166.889359°	12 Jan 2005	14
EAI	East Island	−37.690435°	178.577756°	19 Dec 2005	24
GLN	Raglan	−37.822233°	174.800314°	26 Jun 2006	20
GOB	Goose Bay	−42.482263°	173.529256°	8 Jan 2005	19
IHM	Pihama	−39.521503°	173.913828°	16 Oct 2005	20
JCH	Cascade Point	−44.008238°	168.365705°	6 Jul 2007	20
MAT	Castle Point	−40.882115°	176.224620°	18 Jan 2005	21
MHP	Mahia Beach	−39.085833°	177.865000°	14 Feb 2008	20
MTB	Magnet Bay	−43.841655°	172.738753°	6 Feb 2005	22
NPT	Nugget Point	−46.481505°	169.755918°	13 Jan 2005	20
OCH	Chatham Island	−45.005556°	176.455556°	6 Dec 2005	13
OLB	Tolaga Bay	−38.378430°	178.342005°	19 Jan 2006	20
OPT	Opito Point	−36.716311°	175.817936°	10 Apr 2006	19
PHD	Port Hardy	−40.750326°	173.887572°	29 Nov 2005	20
SPB	Spirits Bay	−34.417460°	172.855740°	16 Apr 2006	21
STR	Stewart Island	−46.705408°	167.716418°	14 Nov 2006	15
TCL	Tory Channel	−41.205550°	174.305633°	27 Sep 2006	20
TIM	Timaru	−44.375875°	171.252545°	7 Jan 2006	23
TSK	Taylor's Mistake	−43.585165°	172.789056°	11 Dec 2005	20
WAI	Waipatiki	−39.300333°	176.978333°	14 Feb 2008	20
WLG	Wellington	−41.337139°	174.792826°	19 Jul 2006	20
WST	West Haven	−40.565063°	172.553310°	29 Nov 2005	15

[a] Geographic coordinates are approximations

are separated by 1526 bp in *H. rubra* (Maynard et al. 2005). Initially, a 581-bp fragment of mtCOI was amplified with primers F1 and R1 (Metz et al. 1998). However, due to inconsistent amplifications, two new internal mtCOI primers (mtCOI_F2 (5′-TTTAGGGGACGACCAACTGTA-3′) and mtCOI_R2 (5′-TACGGTCGGTTAGGAGCATT-3′)) were designed for paua using Primer3 (Rozen and Skaletsky 2000). These modified mtCOI primers amplified a 540-bp fragment. A 723-bp fragment of the ATPase8–ATPase6 region was amplified using primers COIIcons-F and H22-R1 (Maynard et al. 2005).

Both mtCOI and ATPase8–ATPase6 were amplified in 25 μL reaction volumes containing 1–40 ng of genomic DNA, 200 μM of dNTPs, 0.4 μM of each primer, 1.5 mM $MgCl_2$, 1X NH_4 Reaction Buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris–HCl (pH 8.8 at 25°C), and 0.1% Tween-20), and 0.5 units BIOTAQ™ (Bioline). Thermal cycling profiles consisted of denaturation at 96°C for 2 min, 35 cycles of 96°C/20 s, 55°C (mtCOI) and 60°C (ATPase8–ATPase6)/30 s, 72°C/30 s (mtCOI) and 45 s (ATPase8–ATPase6), and a final cycle elongation step at 72°C for 7 min. Successful amplifications were purified according to manufacturer's instructions using either a vacuum method with Eppendorf Perfectprep® PCR Cleanup 96 plates or a centrifugation method with PALL® AcroPrep™ 96-well Filter Plates.

Purified amplicons were directly sequenced with ABI Prism® Big Dye® Terminator v. 3.1 Cycle Sequencing Kit as per the manufacturer's instructions but used at 0.125 the suggested volume of Big Dye® Terminator. Sequence products were purified using Sephadex™ GS-50 gel filtration (Amersham Bioscience) and run on an ABI3100 Genetic Analyzer at the University of Canterbury. Sequences were edited with Sequencher™ 4.2.2 (Gene Codes Corporation). Sequence alignment was done by hand using Se-Al v2.0a11 (Rambaut 2002), and all variable sites were confirmed by visual inspection of chromatograms. A total of 459 bp of mtCOI and 597 bp of ATPase8–ATPase6 were obtained from 534 out of 538 individuals.

Analyses

The mitochondrial regions were concatenated giving a total of 1056 bp for analyses. Sequence variation within samples was assessed with standard molecular indices calculated in Arlequin 3.5 (Excoffier et al. 2005). To evaluate similarity and differences among haplotypes for each fragment, percent divergences between haplotype pairs were calculated using maximum likelihood settings in Paup* (Swofford 1998). Maximum likelihood parameters were established separately for each mitochondrial region in jModelTest (Guindon and Gascuel 2003; Posada 2008). According to Akaike information criterion (Posada and Buckley 2004), the most appropriate model of sequence evolution was the Tamura and Nei (1993) model (TrN) with the proportion of invariant sites (I) equal to 0.7800 for the mtCOI region and the general time reversible model (GTR) with I equal to 0.5560 for ATPase 8–ATPase 6 region.

Haplotype networks were constructed to visually examine similarities and differences among haplotypes (Posada and Crandall 2001). Due to differences among network-building algorithms (Cassens et al. 2005), relationships between haplotypes were inferred with three frequently used network-building algorithms: median-spanning (Excoffier and Smouse 1994; implemented in Arlequin 3.5, Excoffier et al. 2005), median-joining (Bandelt et al. 1999; implemented in Network 4.2.0.1, Fluxus Technology Ltd.), and statistical parsimony (Templeton et al. 1992; implemented in TCS, Clement et al. 2000). Differences among haplotype networks were minor, and thus, only the statistical parsimony network is presented.

To test for the presence of a genetic split between (1) the Chatham Islands and the mainland (North and South Island), (2) the North Island and the South Island, and (3) the areas north and south of the upwelling regions (Fig. 1), analyses of molecular variance (AMOVAs), based on the number of pairwise differences, were employed (Excoffier et al. 1992). AMOVAs were calculated in Arlequin 3.5 (Excoffier et al. 2005), and significance tests used 16002 permutations. To identify alternative patterns, associations between genetic distance (Φ_{ST}) and both geographic distance and spatial geometry were examined. First, isolation by distance was tested using a Mantel test (Mantel 1967) calculated in Arlequin 3.5 (Excoffier et al. 2005). Coastal distances between locations were determined using ArcMap™ 9.1 (Environmental Systems Research Institute, Inc.) and Google™ Earth 5.2. Second, barriers representing areas of large genetic discontinuities between sampling locations were identified using Monmonier's (1973) maximum difference algorithm implemented in Barrier 2.2 (Manni et al. 2004). Barrier 2.2 connects adjacent sampling locations using Delaunay triangulation. The default triangulation was manipulated as much as possible to reflect sampling along the entire coast of the North and South Islands (supplementary information). To evaluate the robustness of the predicted barriers, Monmonier's (1973) maximum difference algorithm was also run on 100 resampled distance matrices. SEQBOOT (Felsenstein 2004) was used to generate 100 bootstrap replicates of the original data set, and these replicates were used to create 100 genetic distance matrices in Arlequin 3.5 (Excoffier et al. 2005).

Additional information about processes affecting genetic structure was inferred through mismatch distributions and neutrality tests (implemented in Arlequin 3.5,

Excoffier et al. 2005). Mismatch distributions compared the observed numbers of pairwise differences between haplotypes with simulated data under two models of expansion: pure demographic expansion (Slatkin and Hudson 1991; Schneider and Excoffier 1999) and spatial expansion with migration (Ray et al. 2003; Excoffier 2004). The significance of the sum of squared deviations between the observed and expected mismatch distributions and the raggedness index (Harpending et al. 1993) were assessed with 10,000 parametric bootstraps. The presence of geographic structure within all samples and within groups limited the application of mismatch distributions to only the sampling locations. Tajima's D (Tajima 1989) and Fu's F_s (Fu and Li 1993) were used to assess sequence neutrality and mutation-drift equilibrium. Tajima's D examines the relationship between the number of segregating sites and nucleotide diversity to test for deleterious alleles and balancing selection under the assumption of population equilibrium (Tajima 1989), while Fu's F_s tests for the excess of recent mutations (indicative of population growth, hitchhiking, and background selection) via the relationship between the mean number of nucleotide differences and the number of alleles (Fu 1997).

Results

A 459-bp fragment of mtCOI and a 597-bp fragment of ATPase8–ATPase6 were amplified in 534 paua from the 28 locations (GenBank accession numbers for unique mtCOI sequences: JF441275-JF4411316 and unique ATPase8–ATPase6 sequences: JF4411317-JF4411424). The mtCOI fragment and the ATPase8–ATPase6 fragment corresponded to base pairs 3504–3612 and base pairs 5582–6178, respectively, in the *H. rubra* mitochondrial genome (ACCN: NC_0059400). As separate fragments, ATPase8–ATPase6 was more variable than mtCOI: it had a larger number of polymorphic sites, higher haplotype diversity, and greater nucleotide diversity (supplementary information). The mtCOI contained 33 polymorphic sites of which 13 were parsimony informative, while ATPase8–ATPase6 contained 96 polymorphic sites of which three had indels and 40 were parsimony informative. Percent pairwise divergence between haplotypes calculated using a maximum likelihood approach ranged from 0.22–1.31% for mtCOI and 0.17–2.02% for ATPase8–ATPase6.

The concatenated sequences (bp = 1056) contained 129 polymorphic sites (122 transitions, 9 transversions, 3 indels) of which 53 were parsimony informative. The overall haplotype diversity was 0.900 ± 0.008; otherwise, haplotype diversity ranged from 0.5824 (DSD)–0.9810 (SPB) within sampling locations (supplementary information). The overall nucleotide diversity was 0.004 ± 0.002, while nucleotide diversity ranged from 0.001 (MTB)–0.006 (IHM) within sampling locations (Table 2).

A total of 147 haplotypes were identified (Fig. 2). Only 23 haplotypes were shared among locations, and the remaining 124 haplotypes were private. Four haplotypes (numbered 9, 11, 18, and 19, Fig. 2) were identified in 50 or more individuals. In general, haplotypes were closely related as expected according to the low nucleotide diversity.

Table 2 AMOVA results

Groups	Φ_{ST}	Φ_{SC}	Φ_{CT}
[All]	0.039		
	$p < 0.001$*		
[Mainland]	0.155	0.032	0.127
[OCH]	$p < 0.001$*	$p < 0.001$*	$p = 0.033$
[Mainland]	0.033		
	$p < 0.001$*		
Split across Cook Strait	0.046	0.018	0.028
[North Island]	$p < 0.001$*	$p = 0.012$	$p < 0.001$*
[South Island + PHD + TCL]			
Split across upwelling regions	0.046	0.019	0.028
[North Island + PHD + TCL]	$p < 0.001$*	$p = 0.011$	$p < 0.001$*
[South Island]			
Split according to Barrier 2.2 results	*0.058*	*0.009*	*0.050*
[AHU, IHM, GLN, SPB, DBL, OPT, CRW]	*$p < 0.001$**	*$p = 0.136$*	*$p < 0.001$**
[EAI]			
[OLB, MHP, WAI, MAT, WLG, CBL, TCL, PHD, WST, CHN, JCH, DSD, CCB, STR, NPT, TIM, MTB, TSK, GOB]			

* Indicates significance after Bonferroni correction ($p = 0.008$)

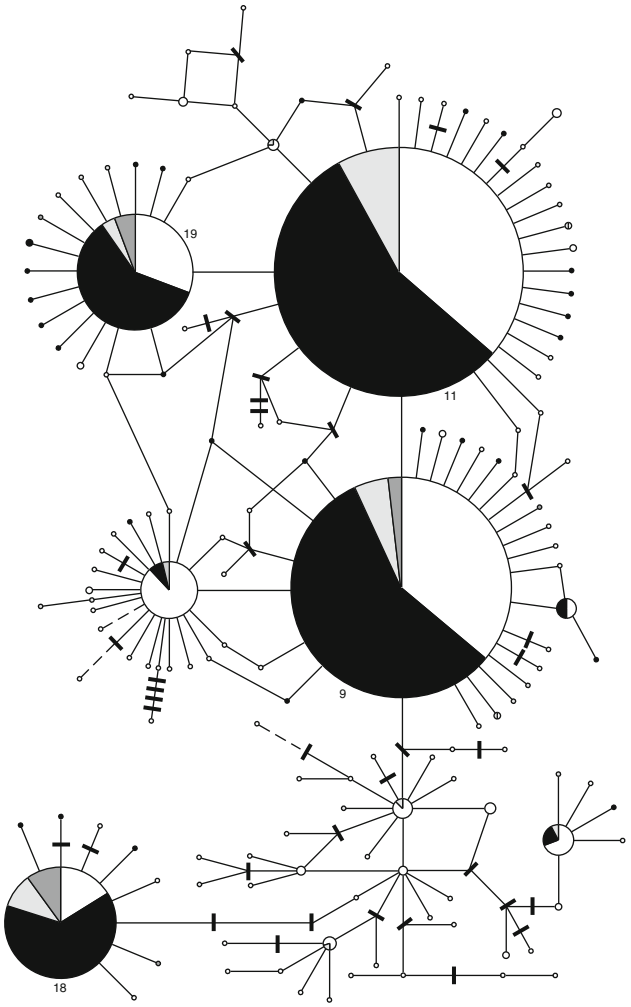

Fig. 2 Statistical parsimony network. The network was constructed in TCS (Clement et al. 2000). *Black* represents South Island samples. *White* represents North Island samples. *Light gray* represents South Island samples (TCL and PHD) that are north of the upwelling areas (Fig. 1). *Dark gray* represents Chatham Island (OCH) individuals. *Dashed lines* represent indels and *black tick* marks represent missing haplotypes

Significant population genetic structure existed among all samples ($\Phi_{ST} = 0.039$, $p < 0.001$). Grouping the locations according to structures proposed by Smith and McVeagh (2006) showed that Chatham Islands paua were distinct from North and South Island paua ($\Phi_{CT} = 0.127$, $p = 0.033$; Table 2). In addition, the frequency of the most common shared haplotypes (9, 11, 18, and 19) differed between the Chatham Islands and the mainland samples. Haplotypes 9, 11, and 19 were common, and haplotype 18 was rare in the mainland samples, while haplotype 18 was as common as haplotype 9, 11, and 19 in the Chatham Islands sample (Fig. 2).

After removing the Chatham Islands sample, significant population genetic structure still existed among the mainland samples ($\Phi_{ST} = 0.033$, $p < 0.001$; Table 2). To test whether this mainland genetic structure was related to the Cook Strait region, further AMOVAs tested two different divisions around Cook Strait: (1) a split across Cook Strait and (2) a split across upwelling regions as proposed in Veale (2007). Both scenarios resulted in similar significant Φ_{CT} indices (Table 2). However, significant differentiation still occurred between samples within groups (Φ_{SC}). In both cases, the variance within sampling locations was around 95%.

To interpret the AMOVA results better, molecular indices for the above groupings and pairwise comparisons of Φ_{ST} were inspected (Table 3, Fig. 3). Noticeably, the number of haplotypes, the number of private haplotypes, and the haplotype diversities were larger for groups that contained North Island samples (Table 3). In fact, haplotype diversities between northern and southern groups differed by more than two standard deviations. The proposed groups also differed in the frequency of the most common shared haplotypes 9, 11, 18, and 19 (Fig. 2). For example, haplotype 18 was absent at nine North Island sampling locations, while it was only absent at one South Island sampling location. The pairwise Φ_{ST} showed that MTB and TIM (South Island), IHM and GLN (North Island), and Chatham Islands were the most divergent samples (Fig. 3). A larger proportion of significant comparisons (32%) occurred between North and South Island samples than between samples within either island (19% for the North Island and 18% for the South Island).

A Mantel test indicated that there was isolation by distance among all samples ($r^2 = 0.221$, $p = 0.019$). This was most likely due to the inclusion of the Chatham Islands sample, and removing the Chatham Islands sample from the test resulted in no significant relationship between genetic divergence and geographic distance among mainland samples ($r^2 = 0.141$, $p = 0.081$). Among mainland samples, isolation by distance was identified for North Island paua ($r^2 = 0.280$, $p = 0.027$) but not for South Island paua ($r^2 = -0.131$, $p = 0.963$).

At least four areas of genetic differentiation were consistently found across the original data set and the 100 resampled matrices using Barrier 2.2 (Fig. 1 and supplementary information; Manni et al. 2004). Area A separated the Chatham Islands (OCH) from the east coast of the South Island (MTB, TIM, GOB) and was identified first (contained the maximum pairwise Φ_{ST}) in 100% of the resampled matrices. Two other areas, B and C, were identified among the top five potential barriers in $\geq$ 98% of the replicate data sets. Area B separated the southwest coast of the North Island (IHM and AHU) from the northwest coast of the South Island (WST and PHD), while area C separated the southeast of the South Island (NPT) from the east coast of the South Island (TIM). Area D occupied the northeast corner of the North Island with

Table 3 Polymorphism data, Tajima's D, and Fu's F_s for concatenated mitochondrial fragments across proposed groups (Table 3)

Group	N	Number of polymorphic sites	Number of haplotypes (number of private haplotypes)	Haplotype diversity $h \pm$ SD	Nucleotide diversity $\pi \pm$ SD	Tajima's D (p value)	Fu's F_s (p value)
All	534	129	147 (124)	0.900 ± 0.008	0.004 ± 0.002	−2.293 (<0.001)*	−25.174 (<0.001)*
Mainland	521	126	144 (121)	0.899 ± 0.008	0.004 ± 0.002	−2.291 (<0.001)*	−25.148 (0.001)*
OCH	13	12	6 (3)	0.821 ± 0.082	0.005 ± 0.003	1.359 (0.929)	1.370 (0.769)
Split across cook strait							
North Island	261	103	105 (93)	0.943 ± 0.008	0.004 ± 0.002	−2.250 (<0.001)*	−25.369 (<0.001)*
South Island	260	56	51 (39)	0.832 ± 0.013	0.003 ± 0.002	−1.826 (0.006)	−25.798 (<0.001)*
Split across upwelling regions							
North Island + TCL + PHD	301	110	117 (110)	0.941 ± 0.008	0.004 ± 0.002	−2.263 (<0.001)*	−25.290 (<0.001)*
South Island—TCL–PHD	220	41	34 (27)	0.812 ± 0.014	0.003 ± 0.002	−1.515 (0.030)	−15.578 (0.001)*
Split according to Barrier 2.2 results							
AHU, IHM, GLN, SPB, DBL, OPT, CRW	*136*	*70*	*67 (53)*	*0.951 ± 0.010*	*0.004 ± 0.002*	*−2.025 (0.004)*	*−25.594 (<0.001)**
EAI	*24*	*19*	*10 (3)*	*0.877 ± 0.038*	*0.002 ± 0.001*	*−1.905 (0.014)*	*−3.005 (0.051)*
OLB, MHP, WAI, MAT, WLG, CBL, TCL, PHD, WST, CHN, JCH, DSD, CCB, STR, NPT, TIM, MTB, TSK, GOB	*361*	*88*	*88 (72)*	*0.865 ± 0.010*	*0.003 ± 0.002*	*−2.145 (<0.001)**	*−25.469 (<0.001)**

Results were generated in Arlequin 3.5 (Excoffier et al. 2005)

* Indicates significance after Bonferroni correction ($p = 0.0013$, determined across Table 3 and supplementary information Table 2)

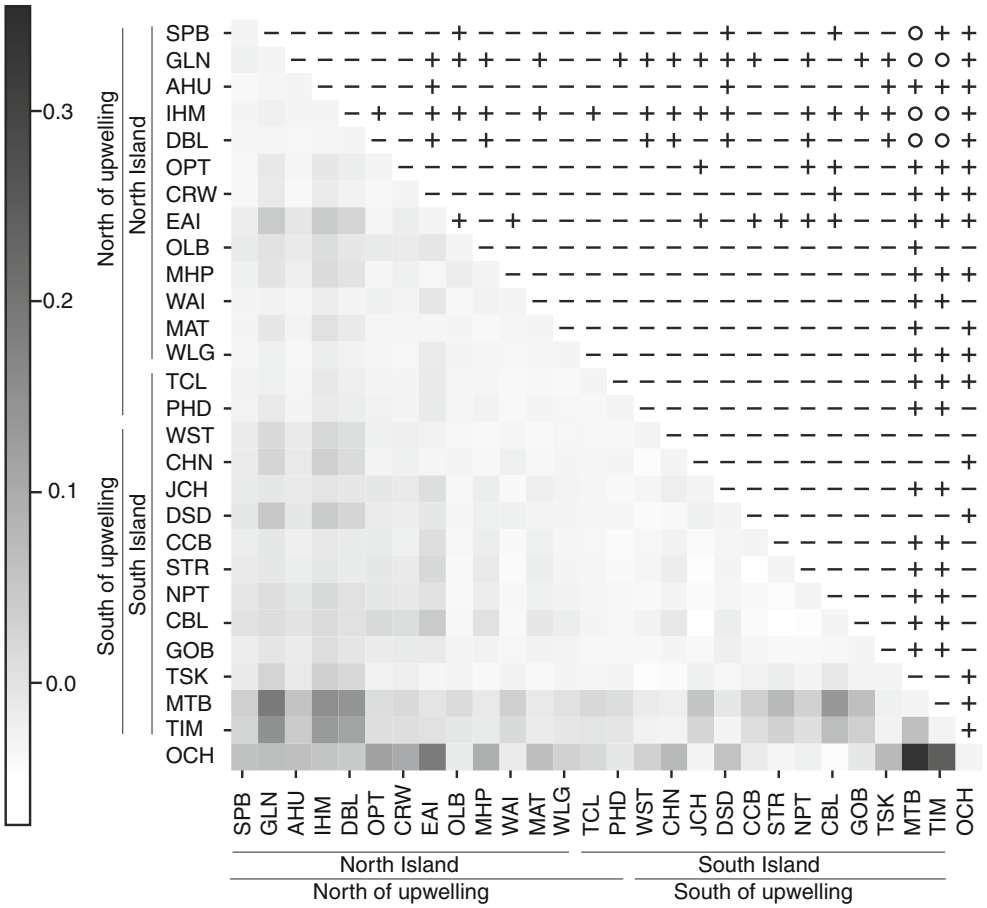

Fig. 3 Pairwise Φ_{ST} (below the diagonal) for 28 sampling locations around New Zealand. Above the diagonal, a + indicates $p < 0.05$ and an *open circle* (○) indicates significance after Bonferroni correction ($p = 0.00014$). Samples are arranged first according to island and then roughly north to south along the west coast and north to south along the east coast of each island

potential barriers occurring between EAI and OLB (supported with 99% of the resampled matrices), OLB and CRW (supported with 62% of the resampled matrices), and CRW and EAI (supported with 51% of the resampled matrices, supplementary information).

For comparison to groupings divided by Cook Strait, the sampling locations were partitioned into three groups (1. samples north of areas A and D, 2. samples south of areas A and D, 3. EAI), based on the areas of genetic differentiation predicted with Barrier 2.2 (Manni et al. 2004). Analyses using this grouping showed significant differentiation between groups (Φ_{CT}) and no significant differentiation within groups (Φ_{SC}, Table 2). Haplotype diversity was larger for the group of sampling locations north of areas A and D (Table 3), and common haplotypes 18 and 19 were absent from all sampling locations north of areas A and D. No isolation by distance was detected in either group.

Mismatch analyses of individuals grouped according to sampling locations could not reject models of demographic and range expansions. Modes were in the range of 0–4 and 7–9 pairwise differences, consistent with common haplotypes (9, 11, 18 and 19) being either 1–2 or 7–9 mutations apart (Fig. 2), and as a result, population parameters estimated from the mismatch distributions varied. For instance, coalescence time in mutational time units (τ) ranged from 0.313–9.852 for demographic expansions and 0.504–8.069 for spatial expansions (supplementary information). Although mismatch analyses suggested patterns of expansions, significance of Tajima's D and Fu's F_s varied according to sampling location (supplementary information). Tajima's D and Fu's F_s were negative and significant when all individuals were treated as a single group and for all North, South, and Chatham Island groupings (Table 3). Given that the mismatch analyses for sampling locations all supported models of expansions, and the large excess of recent mutations indicated by large negative Fu's F_s and the star-shaped region of the haplotype network (Fig. 2), these significant negative values could indicate population expansion instead of selection on mtDNA.

Discussion

Paua's relatively short larval duration (Tong et al. 1992) and passive larval stage (McShane 1992) suggested its population genetic structure would be vulnerable to features associated with population genetic structure identified in other New Zealand coastal marine invertebrates (Ross et al. 2009). Paua samples collected from around New Zealand had highly significant but modest genetic structure. All AMOVAs resulted in significant structure, suggesting that the Chatham Islands sample was different from the mainland samples and the northern samples were different from southern samples. Monmonier's maximum difference algorithm identified additional potential genetic breaks at the southeast of the South Island and the northeast of the North Island.

The Chatham Islands vs. North and South Islands

The separation of the Chatham Islands sample from the North and South Island samples was consistent with Smith and McVeagh's (2006) preliminary genetic study of paua. The differentiation between Chatham Islands and mainland paua was also consistent with the few intraspecific studies of New Zealand coastal marine invertebrates that have incorporated samples from the Chatham Islands (Smith et al. 1989; Clarke 2001; Goldstien et al. 2009). Differentiation between paua from the mainland and paua from the Chatham Islands probably resulted from isolation by distance.

The combination of moderate genetic structure and shared haplotypes supports a conclusion of very limited gene flow between the Chatham Islands and the mainland. Limited gene flow may occur via chance dispersals due to delayed metamorphosis. Roberts and Lapworth (2001) reported that paua larvae that underwent metamorphosis at 26 and 30 days could survive, but at reduced rates compared to larvae that underwent metamorphosis in under 10 days. This borders the lower limits of a 30–50 day period estimated for simulated passive planktonic larvae (or particles) traveling from the South Island to the Chatham Islands (in fact, the 10^{-4} percentile dispersal time was 27 days; Chiswell 2009).

North Island vs. South Island

The inclusion of more sampling locations, more individuals, and/or an additional 459 bp of mtCOI resulted in the detection of highly significant genetic structure among mainland samples that was not previously identified with mtDNA in Smith and McVeagh's (2006) preliminary genetic study. AMOVAs rejected the hypothesis of homogeneity around the Cook Strait region, but unlike other New Zealand invertebrates, such as *Sypharochiton pelliserpentis* (Veale 2007), *Cellana ornata* (Goldstien et al. 2006), *Patiriella regularis* (Waters and Roy 2004; Ayers and Waters 2005), and *Perna canaliculus* (Apte and Garder 2002; Star et al. 2003), the structuring did not necessarily correspond to known regions of upwelling as proposed by Veale (2007).

The lack of a strong partition in pairwise Φ_{ST} and the low level of divergence ($\Phi_{ST} = 0.039$) emphasized that paua do not have an obvious genetic structure, as compared to the level and pattern of genetic structures identified in *C. ornata* ($\Phi_{ST} = 0.829$, Goldstien et al. 2006) and *S. pelliserpentis* ($\Phi_{ST} = 0.45$, Veale 2007). Although Φ_{ST} values are not directly comparable, the level of differentiation in paua was more similar to species like *P. regularis* with a 9–10-week pelagic larval stage ($\Phi_{ST} = 0.072$, Waters and Roy 2004) or *P. canaliculus* with a greater than 4-week pelagic larval stage ($\Phi_{ST} = 0.162$, Apte and Gardner 2002). The lack of concordance in pattern and level of population differentiation among New Zealand coastal invertebrates suggest that the effects of potential barriers to gene flow in the Cook Strait are species-specific. Differences in larval behavior and life-history characteristics (Hedgecock1986; Bohonak 1999; Ross et al. 2009), ecology (Reid et al. 2006) and/or demography may limit how and when potential barriers of the Cook Strait region influence population genetic structure.

Additionally, a north–south split may not be an accurate description of paua genetic structure. At least three areas of reduced gene flow were evident around the mainland and only Area B corresponds with a portion of the Cook Strait region (Fig. 1). Area D, at the eastern promontory of the North Island, corresponds with the offshore divergence of the East Auckland Current and a series of offshore eddies that can impinge on the coastline to the south (Heath 1985, Chiswell and Roemmich 1998). Potentially, these features could sweep larvae offshore or entrain larvae beyond survival and, thereby, limit gene flow. Such a pattern of limited gene flow has been identified in two amphipods (*Paracorophium* spp., Stevens and Hogg 2004) and an anemone (*Actinia tenebrosa*, Veale 2007). Further, the presence of a barrier in this location is also supported by biogeographic data, with a strong biogeographic boundary observed in a range of taxa around East Cape (Powell 1961; Pawson 1961; Moore 1961; Francis 1996). Unlike Areas B and D, Area C does not correspond to an obvious hydrographic barrier, nor has it been identified in other organisms as a potential barrier to gene flow. The significant differentiation of samples MTB and TIM in this area from a large number of other samples may indicate the role

of other processes such as local retention of larvae in shaping the genetic structure of paua.

Note, Monmonier's (1973) maximum differentiation algorithm is considerably less accurate at identifying true population structure in instances of high levels of gene flow and/or low ratios of within to between group gene flow (Dupanloup et al. 2002), both or either of which may be the case with paua. Also although the correspondence of genetic breaks and contemporary hydrography has been emphasized, we cannot exclude other explanations for genetic structure such as past hydrography (e.g., Barber et al. 2006), the effects (i.e., genetic patchiness) of repeated variability of new recruits (Sainsbury 1982, Hedgecock 1994), and/or the potential non-neutrality (which could not be excluded using Tajima's D and Fu's F_s) mtDNA fragments (Meiklejohn et al. 2007). Testing concordance of the structure found here to that obtained with multiple neutral nuclear markers can help to address these alternatives.

Furthermore, issues such as small sample sizes, temporal variation in sampling, and the paua fishery may confound results. Small sample sizes may reflect statistical sampling errors in which the samples do not capture the true variation in a population (Holsinger and Weir 2009). Collecting a larger number of individuals from these locations would reduce these errors, and given the small pairwise Φ_{ST} values between sampling locations considerably larger samples (e.g., $N = 100$) would be needed (Kalinowski 2005). The paua were collected in just over a three-year period (January 2005–February 2008), so variation between samples may reflect temporal variation (e.g., due to variability in recruitment) rather than or in addition to spatial variation (e.g., Lee and Boulding 2007, 2009). Unfortunately, we do not have temporally varying samples from the same location to untangle temporal vs. spatial variation. Based on pairwise Φ_{ST} results, the effect of temporal variation seems small. The five locations with the earliest sample dates (GOB, DSD, CCB, NPT, MAT; sampled in January 2005) were not significantly different from the four locations (JCH, CHN, WAI, MHP) sampled over 2.5 years later. A clear temporal pattern could not be identified among significant comparisons that did not fit one of the north–south genetic splits examined in this paper. For example, the South Island sample MTB was significantly divergent from other South Island samples collected within a month (CCB, GOB, and NPT) to samples collected within 2.5 years (JCH), while it was not divergent from samples collected within 1 month (DSD and GOB) to samples collected within 2.75 years (CHN). The fact that the samples most likely contained a range of cohorts may dampen the effect of temporal variation resulting from variable recruitment (Hedgecock 1994). Finally, prolonged periods of intense fishing results in reduced genetic diversity and fishery-induced selection (Hauser et al. 2002; Allendorf et al. 2008). The amount of commercial fishing around New Zealand varies according to the estimated stock size of different regions and could potentially alter the genetic make-up of some samples. Teasing apart the influence of all these factors on the main pattern found here requires significant additional work that includes collecting a large number of samples from commercially fished, non-commercially fished, and marine preserves over a long time frame.

Noticeably, northern (lower latitude) samples had a larger number of private haplotypes and higher haplotype diversity; a pattern that has also been identified in the endemic cushion star (*P. regularis*, Ayers and Waters 2005). Such a pattern, combined with significant neutrality tests, suggests population expansion and mirrors a pattern indicative of climate-driven expansions observed in the northern hemisphere (Maggs et al. 2008). Although mismatch distributions support demographic and spatial expansion, they cannot clearly identify a common expansion time (supplementary information).

Similar to other abalone species (e.g., *H. cracherodii*, Gruenthal and Burton 2008; *H. rufescens*, Burton and Tegner 2000; *H. midae*, Evans et al. 2004; and *H. rubra*, Conod et al. 2002), paua mtDNA variation was characterized by high haplotype diversity resulting from a large number of rare haplotypes with few nucleotide differences (or low nucleotide diversity). Large levels of genetic variation can accumulate in populations that are ancient, occupy a diversity of niches, or have an increased mutation rate. Potentially, the large levels of diversity are indicative of large effective population sizes in abalone slowing the loss of genetic diversity due to genetic drift.

Conclusion

The mtDNA gene regions used in this study identified genetic differentiation among paua from around New Zealand. In general, the genetic splits between the Chatham Islands and the mainland and between northern and southern mainland samples are consistent with those identified in other coastal marine invertebrates (Ross et al. 2009), but the magnitude of differentiation in paua was lower. Although this may be a product of marker choice, paua could potentially have higher levels of gene flow or may have larger population sizes buffering against the effects of genetic drift. Further spatial and temporal sampling, as well as studies of adult and larval movement, is needed to elucidate processes occurring in the Cook Strait region, the northeast of the North Island, and the southeast of the South Island. Additional genetic structure among mainland samples may also exist (see Smith and McVeigh

2006), but will only be identified with more variable markers (Waples 1998, Kalinowski 2002).

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