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Method paper

Unravelling the effects of gene flow and selection in highly connected populations of the silver-lip pearl oyster (*Pinctada maxima*)

Maria G. Nayfa *, Kyall R. Zenger

Centre for Sustainable Tropical Fisheries and Aquaculture, College of Marine and Environmental Sciences, James Cook University, Townsville 4811, QLD, Australia

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ABSTRACT

Many marine organisms often display weak levels of population genetic structuring as a result of both environmental characteristics (*e.g.*, ocean currents) and life history traits (*e.g.*, widely dispersed planktonic larval stages) maintaining high levels of gene flow. This can lead to the assumption that these organisms can be managed as a single stock based on high levels of population connectivity. However, this neglects to account for other micro-evolutionary forces such as selection, which also shape these populations. This study utilizes 1130 genome-wide SNP loci to unravel the effects of gene flow and selection shaping three highly connected populations of the silver-lip pearl oyster (*Pinctada maxima*) in the ecologically and economically important Indo-Pacific region (Aru, Bali, and West Papua). Twenty-two loci under directional selection were identified amongst the populations, providing further supporting evidence of strong local adaptation (*i.e.*, G × E effects) among populations in this region. Global F_{st} values for directional outliers (0.348) were up to eight times greater than for neutral markers (0.043). Pairwise F_{st} comparisons between Aru and Bali revealed the largest directional differences (0.488), while Bali and West Papua had the least (0.062). Unrooted neighbour-joining (NJ) distance trees and genetic diversity indices of directional outliers revealed that individuals from Bali and West Papua had reduced allelic variation ($MAF_{avg} = 0.144$, $H_o = 0.238$ and $MAF_{avg} = 0.232$, $H_o = 0.369$, respectively) compared to Aru ($MAF_{avg} = 0.292$, $H_o = 0.412$). This indicates that directional selection is most likely acting upon genetic variation within the Bali and West Papua populations. NJ distance trees, discriminant analysis of principal components, and F_{st} analyses of directional outliers revealed two divergent groups ("Bali/West Papua"; "Aru") that had previously gone unrecognized. This study not only illustrates that relatively strong local adaptive forces are occurring despite high gene flow, but identifies the populations that are most likely experiencing selection. Additionally, this study highlights the need to understand all micro-evolutionary forces acting on populations when resolving stock structure.

1. Introduction

Identifying discrete management units (MUs) or stocks in marine ecosystems is sometimes more difficult than in terrestrial systems due to high levels of connectivity generated by ocean currents and life history traits (Gray, 2000; Kool et al., 2011). This high degree of connectivity can lead to the general assumption of population panmixia, or where all individuals are potential mates due to limited physical and ecological boundaries (Kritzer and Sale, 2004), that can be managed as a single meta-population (Kritzer and Sale, 2010). High connectivity is thought to negate selective pressures and reduce local adaptation through large amounts of gene flow (Lenormand, 2002). However, the effects of heterogeneous environments (*i.e.* marine systems) influencing selective pressures and local adaptation must also be considered. Recent genetic studies have shown that animal geographic proximity and dispersal patterns may be insufficient to accurately determine overall patterns of population genetic variation for many marine taxa (Toonen et al., 2011). One of the major shortcomings of these factors is that they neglect to account for local adaptation, or when genotype by environment (G × E) interactions result in populations displaying traits which are advantageous to their local environments (Nielsen et al., 2009).

Local adaptation can occur within populations that are highly connected if the effects of directional selection are stronger than the homogenizing effects of gene flow (Limborg et al., 2012). Under this model, genetic differentiation amongst populations is primarily due to selection acting upon specific genes within a population, and consequently those loci physically linked to them in strong linkage disequilibrium (*i.e.* hitchhiker effect; Smith and Haigh, 1974; Barton, 2000). In recent years, the identification of population genetic divergence has become easier to detect on a finer-scale due to advancements in the field of population genomics through improved accuracy, speed, and the reduced cost of genome-wide single nucleotide polymorphism (SNP) genotyping (Helyar et al., 2011).

* Corresponding author.
E-mail addresses: mnayfa12@gmail.com (M.G. Nayfa), kyall.zenger@jcu.edu.au (K.R. Zenger).

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Traditional genetic analyses mostly rely on neutral loci (*i.e.* those alleles which do not have an established advantageous nor disadvantageous effect on a species' fitness; Hedrick, 2001), while genomic analyses are typically also able to identify outlier loci (*i.e.* those loci which are influenced by selective processes and can affect the fitness of an organism; Butlin, 2010). Characterisation of outlier loci is becoming an important tool towards fully understanding all the micro-evolutionary forces that are at play within a population (Pujolar et al., 2014; Funk et al., 2012). Traditional genetic markers (*e.g.* allozymes, microsatellite loci, amplified fragment length polymorphisms, and mitochondrial DNA) are restricted by technological limitations which can only process a limited number of markers (Zarraonaindia et al., 2012). In contrast, genome-wide SNP genotyping uses information from thousands to millions of loci, thereby increasing its analytical power (Luikart et al., 2003; Frazer et al., 2007; Stranger et al., 2007). These SNP markers cover a larger portion of the genome and allow for the detection of fine-scale gene flow and selection signatures (Stapley et al., 2010). This enhancement in genetic resolution improves our understanding of population structure, and can ultimately be used to create better defined MUs and subsequently, more effective and tailored management regimes for marine species (Waples et al., 2008; Funk et al., 2012).

Improving marine management practices is particularly important in biodiversity "hotspots", those areas with exceptionally high concentrations of species richness (Myers et al., 2000; Allen, 2008; Renema et al., 2008). These "hotspots" provide a number of ecosystem services and functions, which are important for both marine life and human food security (Moberg and Folke, 1999). In order to preserve and maintain these ecosystem services and functions, it is essential that the biodiversity in these "hotspots" be conserved, as it plays a key role in both preserving and regulating ecosystem processes (Mace et al., 2012). Therefore, it is important that we understand population dynamics in these areas in order to improve upon current management regimes.

In particular, the Indo-Pacific region is one such "hotspot" and incorporates a geographic region known as the "Coral Triangle", a well-recognized global centre of marine biodiversity (Allen, 2008; Veron et al., 2009). One common, and commercially valuable organism found in this area is the silver-lip pearl oyster, *Pinctada maxima* (Shirai, 1994; Lind et al., 2007). This organism produces the Indo-Pacific's economically important and lucrative "white" South Sea pearls (Taylor and Strack, 2008). Sessile during its adult stage, *P. maxima is* a broadcast spawner that relies upon its extended planktonic larval stage (17–24 days) and oceanic currents to disperse throughout the Indo-Pacific (Rose and Baker, 1994). It is in this stage that populations are subjected to new immigrants and additions to the population (Treml et al., 2008; Selkoe and Toonen, 2011). However, the planktonic larvae are highly vulnerable to environmental conditions, leading to differential survival among different families (Lind et al., 2010). The potential for high gene flow observed in *P. maxima* and many other marine taxa, has resulted in much debate concerning the proper management and definition of marine MUs, or stocks, for these wild organisms (Gosling, 2015). Some require only genetic distinctiveness among populations, while others consider more biological differences amongst the organisms. However, the ability to fully resolve both is becoming necessary for stocks to be clearly discriminated. Currently, a stock for marine taxa with a sessile adult stage can be as large as an entire geographic region where gene flow is possible during planktonic juvenile stages to as small as a single settlement (*e.g.* bivalve bed; Waldman, 2005; Gosling, 2015). Understanding and incorporating knowledge of both gene flow and functional evolutionary change (i.e. local adaptation) of these and all marine taxa is vital for improving management regimes and efforts to enhance stock performance (Waples et al., 2008).

A study by Lind et al. (2007) using six polymorphic microsatellite genetic markers revealed very weak genetic structuring amongst individuals from Aru, Bali, and West Papua within the Coral Triangle region (average $F_{st} = 0.0036$), whereby all sampled individuals could be considered part of a single homogeneous population. However, for the silver-lip pearl oyster, genetic differentiation among populations may not be limited by the effects of gene flow alone. A recent G × E study by Kvingedal et al. (2010) observed variations in *P. maxima* grow-out potential (*i.e.* variations in growth rate) when families originating from the three aforementioned Indonesian regions were translocated from their native environments to new locales in Bali and Lombok. The authors detected significant G × E effects among these populations, with individuals from both Bali and West Papua growing significantly larger than those from Aru in both grow-out locations, indicating that local adaption may be at play. Other marine species such as, Atlantic cod (*Gadus morhua*; Nielsen et al., 2009) and Atlantic herring (*Clupea harengus*; Limborg et al., 2012), have demonstrated that local selection pressures can in fact negate the effects of high levels of gene flow and cause selection to occur at a limited number of loci, while all other loci in the organism's genome remain unaffected by selective forces. This selection can in turn lead to a reduction in offspring fitness through outbreeding of genetically mismatched individuals, *i.e.* individuals adapted to different local environments or conditions (Gharrett et al., 1999; Edmands and Timmerman, 2003; Edmands, 2007). Consequently, to fully understand how different micro-evolutionary forces are shaping *P. maxima* populations in this region, it is essential to perform robust population genomic analysis. This genomic information will not only assist in defining relevant commercial *P. maxima* stock MUs, but it will also serve as a model for other sessile, broadcast spawning marine invertebrates.

This study aims to unravel the effects of gene flow and selection currently shaping silver-lip pearl oyster (*P. maxima*) populations in the Indo-Pacific region. Genome-wide SNP loci were used to examine population diversity statistics and population differentiation at both neutral and outlier loci in order to understand the micro-evolutionary forces acting on these populations, their interactions, and the implications for management of *P. maxima* and similar marine taxa.

2. Methods

2.1. Data collection and SNP validation

In order to quantify the effects of gene flow and local adaption as previously demonstrated in *P. maxima* populations in the Indo-Pacific (Lind et al., 2007; Kvingedal et al., 2010), a total of 85 adult silver-lip pearl oysters from the same families as those individuals in the three previously evaluated wild populations in the Indo-pacific region were obtained: Aru (27 specimens; 6.43′S, 134.63′E); Bali (33 specimens; 8.32′S, 114.92′E); and West Papua (25 specimens; 1.13′N, 130.54′E; Fig. 1). High-quality genomic DNA was extracted from all individuals using a revised CTAB protocol, DNA samples were genotyped as per manufacturer's instructions using the Illumina 3K iSelect custom arrays at PathWest Medical Laboratories, Perth, Western Australia (Steemers and Gunderson, 2007), and genotypes were calculated using GenomeStudio V2011.1 by Illumina Inc. as detailed by Jones et al. (2013a). Additionally, genetic linkage maps were constructed as described by Jones et al. (2013b). In brief, SNPs were only utilised for this study if they amplified successfully, each locus had a call rate (*i.e.* the proportion of genotypes with non-missing data; Anderson et al., 2010) of >90%, returned distinct and unduplicated genotype calling clusters, globally conformed to Mendelian expectations, and were polymorphic across all three populations (Jones et al., 2013a). Additionally, only those SNPs with a minor allele frequency (MAF) >0.02 across populations and which conformed to Hardy–Weinberg Equilibrium (HWE) in at least one population were included. This stringent filtering process was to ensure that any identified outlier loci were a result of selective forces on populations and not genotyping or genomic artefacts. In total, 1130 SNPs met these criteria and were included in all subsequent analysis.

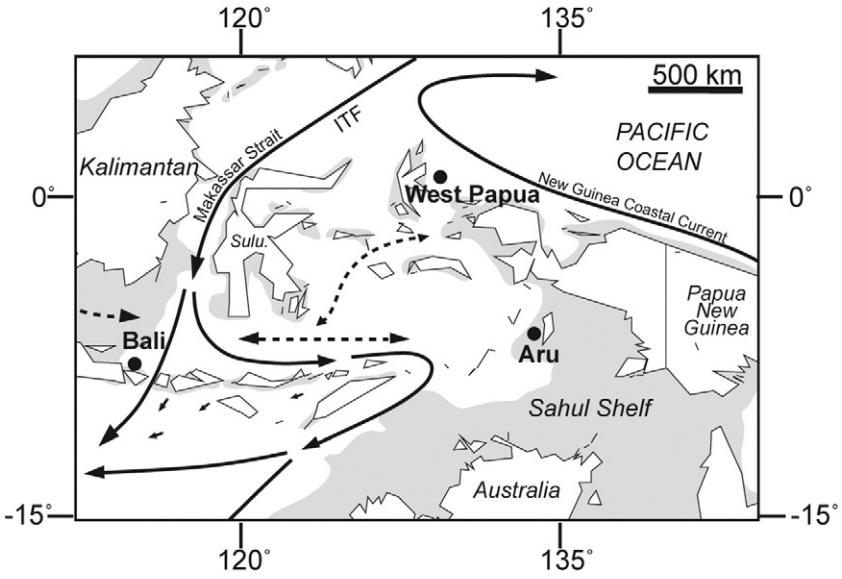

Fig. 1. Map displaying Indonesian sampling sites for *Pinctada maxima* where solid black arrows indicate major ocean currents and dotted black arrows indicate seasonally reversing currents.
Adapted from Lind et al. (2012).

2.2. Outlier analysis

Two independent methods were used to identify candidate loci under selection: *BayeScan 2.1* (Foll and Gaggiotti, 2008; Foll, 2012) and *LOSITAN* (Beaumont and Nichols, 1996; Antao et al., 2008). Only those candidate SNPs that were jointly identified by both programs were categorized as highly probable directional outliers. These two programs use two different statistical approaches (Beaumont and Nichols, 1996; Antao et al., 2008; Foll and Gaggiotti, 2008; Foll, 2012), and were used in conjunction with one another to confidentially identify outliers (Larmuseau et al., 2010; White et al., 2010; Kovach et al., 2012; Pujolar et al., 2014). Only *LOSITAN* identified balancing outliers.

BayeScan 2.1 analyses were based on 1:10 prior odds for the neutral model and included 20 pilot runs consisting of 5000 iterations each, followed by 100,000 iterations with a burn-in length of 50,000 iterations as suggested per the manual (Foll, 2012). Bayes factors (BF), which represent posterior odds (*i.e.* the ratio of posterior probabilities), were then calculated to determine whether a neutral or selection model is at play for each SNP. *BayeScan 2.1* utilizes Jeffreys' interpretation to determine which of these models the posterior odds favour (Foll, 2012). For this analysis, only those SNPs with the highest support from Jeffreys' interpretation (*i.e.* categorized as decisive with a p-value ≤ 0.01) for the selective model were identified as outlier SNPs. Positive alpha values were then used to distinguish SNPs under diversifying (*i.e.* directional) selection, while negative alpha values were used to indicate balancing or background selection (Foll, 2012).

LOSITAN analyses were run with 50,000 iterations, a 95% confidence interval, and a false discovery rate (FDR) of 0.20 as per recommendations for acceptable levels of false positives by Hayes (2013). The setting "Neutral" mean F_{st} was used in order to increase the reliability of the estimated initial mean F_{st} by removing potential non-neutral loci from this preliminary computation (Antao et al., 2008). Additionally, a "Force mean F_{st}" was used to approximate a desired mean F_{st} for the dataset by applying a bisection algorithm over repeated simulations. All other program options remained at their default settings.

Quantile–quantile plots (QQ-plots) with a 95% confidence interval for all and neutral markers were constructed using *GWASTools 3.1* (Gogarten et al., 2012) to confirm the normality of markers (Hayes, 2013; Dominik, 2013). If neutral markers conformed to the assumption of normality it was deemed that most, if not all, directional and balancing outliers had been detected jointly by *BayeScan2.1* and *LOSITAN*.

2.3. Population diversity statistics

Observed (H_o) and expected (H_e) levels of heterozygosity in addition to tests for departure from Hardy–Weinberg Equilibrium (HWE) for all loci within each population were conducted using a Markov Chain (MC) length of 1,000,000 and 100,000 dememorizations in *ARLEQUIN 3.5* (Excoffier and Lischer, 2010). The inbreeding coefficient (F_{is}) for each population was also calculated in *ARLEQUIN 3.5* using 16,000 permutations to compute a distance matrix based on the number of different alleles. Individual average multilocus heterozygosity (*MLH*) was computed for all individuals and populations according to Slate et al. (2004). Average minor allele frequency (MAF_{avg}) was calculated within each population as low frequency alleles are sensitive to a range of microevolutionary processes (Templeton, 2006). All diversity analyses were conducted across all major loci classes (*i.e.* all-inclusive, neutral, directional and balancing). Additionally, effective population sizes based on the linkage disequilibrium method (N_{eLD}) for all three populations were approximated using *NeEstimator V2* (Do et al., 2014).

2.4. Population differentiation

Pairwise and global F_{st} values were calculated for all-inclusive, neutral, directional and balancing in *ARLEQUIN 3.5* to determine the level of differentiation among populations (Excoffier and Lischer, 2010). In order to obtain an unbiased estimate of genetic divergence among *P. maxima* populations and individuals, a 1-proportion of shared alleles (1-PSA) distance matrix was generated between all individuals using the program *MICROSAT V1.4d* (Minch et al., 1995). The 1-PSA distance matrix was utilized due to its proven ability to accurately correlate genetic similarity with geographic location (Bowcock et al., 1994; Moalic et al., 2011) down to the subpopulation level (Estoup et al., 1995). Unrooted Neighbour-Joining (NJ) distance trees were then constructed using the generated 1-PSA distance matrix in the program *MEGA6* (Tamura et al., 2013) to determine between-group differentiation. This method was employed because it does not base calculations on *a priori* hypotheses (individual groupings), and allows genotypic

similarities and patterns to emerge naturally among individuals and populations (Zenger et al., 2007).

Moreover, to determine between-group differentiation discriminant analyses of principal components (DAPCs) were conducted for all locus categories (all-inclusive, neutral, directional and balancing) using the package *adegenet 1.4.2* in *R.3.1.0* (Jombart, 2008, 2013; Jombart and Ahmed, 2011; Jombart et al., 2010). Cross-validation was used to ascertain the optimal number of principal components to retain while Bayesian information criterion (BIC) scores were used to determine the number of genetic clusters within the data (Jombart, 2013).

2.5. Regions under selection

Loci were assigned to linkage positions previously established by either genetic linkage maps or LODE mapping scores created by Jones et al. (2013b). Manhattan plots were then created using the *R 3.1.0* package *qqman V 0.1.2* (Turner, 2014). These plots were used to detect genomic regions under selection, and to further confirm the significance of outliers.

3. Results

3.1. Outlier analysis

Twenty-two unique directional outliers were jointly identified by *BayeScan2.1* and *LOSITAN* through comprehensive and pairwise analyses of populations (Table 1). The number of directional outlier loci identified by pairwise comparisons revealed that Bali and West Papua populations have the least number of outlier SNPs with only one outlier identified in either program, and none jointly identified. Aru and Bali populations have the greatest number of outlier SNPs with 18 of the 22 directional outliers present. All outlier SNPs identified by *BayeScan2.1* exhibited positive alpha values, denoting that the program only detected SNPs putatively undergoing directional selection. Conversely, *LOSITAN* identified 76 unique balancing outliers which were equally distributed between pairwise comparisons of Aru and West Papua in addition to Aru and Bali. No balancing outliers were observed between Bali and West Papua.

QQ plots of all available SNPs revealed that the data violated the assumption of normality (Supplementary Material 1A). However, when both directional and balancing outliers were removed the remaining neutral SNP data fit the assumption of normality, with the majority of SNPs falling within the 95% confidence interval, validating that most putative outliers had been identified from the dataset (Supplementary Material 1B).

3.2. Population diversity statistics

Effective population size estimates (N_{eLD}) were determined to be infinite for all three populations, indicating large effective population sizes for all three populations. All loci were found to conform to Hardy–Weinberg Equilibrium following Bonferroni corrections (p-value ≥ 0.05). F_{is} values across all three populations as well as per individual population were not found to vary significantly from zero, ranging between −0.047 and 0.004 across all loci classes. All three populations showed comparable levels of heterozygosity and average minor allele frequency when evaluated across all-inclusive, neutral, and balancing loci (Table 2). Directional loci displayed the greatest discrepancy in allelic variation, with Bali displaying the lowest level (MAF_{avg} = 0.144, MLH = 0.216, H_o = 0.238), West Papua an intermediate level (MAF_{avg} = 0.232, MLH = 0.369, H_o = 0.369) and Aru the greatest level (MAF_{avg} = 0.292, MLH = 0.393, H_o = 0.412) of genetic diversity (Table 2).

Table 1
The directional and balancing outlier loci identified in *LOSITAN* at an FDR of 0.20 and categorized as "decisive" on the Jeffreys' Scale in *BayeScan 2.1* for directional outliers.

	Directional outliers			Balancing outliers
	LOSITAN	*BayeScan2.1*	Jointly identified	*LOSITAN*
All populations	16	34	14	1
Aru *vs*. Bali	19	38	18	33
Aru *vs*. West Papua	11	16	7	43
Bali *vs*. West Papua	1	1	0	0
Total unique outliers	27	45	**22**	**76**

Bold numbers were the outlier SNPs used throughout the entire study.

3.3. Population differentiation

Global F_{st} values for directional outliers (F_{st} = 0.348) were up to eight times greater than those for neutral loci (F_{st} = 0.043; Table 3). Pairwise comparisons of all-inclusive and neutral loci found that both categories were very similar in magnitude with F_{st} values between 0.022 and 0.065 (Table 3). Pairwise F_{st} values of directional outliers revealed that the greatest genetic divergence was between Aru and Bali (F_{st} = 0.488) followed by Aru and West Papua (F_{st} = 0.345), whereas Bali and West Papua were the most similar (F_{st} = 0.062). F_{st} values for balancing outliers revealed little to no genetic differences among the populations.

The NJ distance tree of neutral loci revealed weak but significant population genetic structure, indicated by the small branch distances at the internal nodes separating the populations. Within each population similar branch lengths of individuals indicated that all individuals are generally equally related to one another (Fig. 2a). There was no difference in tree topology and branch lengths when comparing neutral loci and all-inclusive loci NJ distance trees. This population divergence was most apparent when only directional outliers (n = 22) were evaluated (Fig. 2b). Directional outliers revealed that there is a distinct separation amongst individuals and populations, with Bali and Aru pairwise comparisons displaying the greatest degree of genetic divergence and population separation. Individuals from West Papua were notably more interspersed with individuals from Bali than Aru. This pattern is also reflected in the pairwise population F_{st} data (Table 3). Individuals from Bali and West Papua both exhibit shorter branch lengths (and general diversity indices; Table 2) than individuals from Aru, indicating a reduction of genetic variation at these loci and subsequently signifying that directional selection may be occurring in these populations. When balancing outliers were examined, all populations and individuals were equally related to one another and no population structuring could be discerned (Fig. 2c). All-inclusive loci revealed similar results to those displayed by neutral loci, with slightly more distinct population structuring observed.

Similar patterns were observed in the DAPCs, with BIC scores identifying three genetic clusters for the neutral locus category, two genetic clusters for directional loci, and one genetic cluster when only balancing loci were considered (Supplementary Material 3). An analysis of the first discriminant function for the directional locus category revealed that Bali and West Papua overlapped, whereas all three regions overlapped when balancing loci were examined. These results indicate that the majority of variation observed in these two locus categories is due solely to the first discriminant function.

3.4. Regions under selection

The Manhattan plot (Supplementary Material 2) revealed that as no single genomic region or gene(s) of major effect were observed (*i.e.* a strong selective sweep), the selective forces are most likely polygenic sweeps acting across different regions of the genome. Note that only 900 of the 1130 loci analysed in this study were used to create the subsequent Manhattan plots, as Jones et al. (2013b) were unable to map these particular loci. These unmapped loci included two outliers.

Table 2
Average minor allele frequency (MAF_{avg}), expected heterozygosity (H_e), observed heterozygosity (H_o), and average individual multilocus heterozygosity (±standard deviations when applicable) for all-inclusive, neutral, directional, and balancing loci.

		MAF_{avg}	H_e	H_o	MLH
All-inclusive loci	Aru	0.223 (±0.149)	0.306 (±0.165)	0.328 (±0.168)	0.309 (±0.010)
1130 SNPs	Bali	0.217 (±0.150)	0.300 (±0.166)	0.312 (±0.168)	0.311 (±0.017)
	West Papua	0.224 (±0.145)	0.312 (±0.159)	0.340 (±0.165)	0.303 (±0.032)
Neutral loci	Aru	0.221 (±0.149)	0.307 (±0.164)	0.329 (±0.165)	0.305 (±0.008)
1032 SNPs	Bali	0.219 (±0.151)	0.304 (±0.165)	0.315 (±0.168)	0.306 (±0.015)
	West Papua	0.224 (±0.146)	0.313 (±0.156)	0.315 (±0.165)	0.312 (±0.018)
Directional loci	Aru	0.292 (±0.147)	0.379 (±0.129)	0.412 (±0.130)	0.393 (±0.115)
22 SNPs	Bali	0.144 (±0.127)	0.241 (±0.155)	0.238 (±0.156)	0.216 (±0.090)
	West Papua	0.232 (±0.109)	0.369 (±0.123)	0.369 (±0.139)	0.369 (±0.194)
Balancing loci	Aru	0.226 (±0.138)	0.318 (±0.151)	0.315 (±0.165)	0.304 (±0.080)
76 SNPs	Bali	0.221 (±0.134)	0.314 (±0.145)	0.314 (±0.152)	0.326 (±0.041)
	West Papua	0.222 (±0.140)	0.313 (±0.158)	0.341 (±0.166)	0.312 (±0.032)

4. Discussion

4.1. Population differentiation and diversity

Analyses of all-inclusive and neutral loci found weak but significant genetic differences ($F_{st} \leq 0.065$; p-value ≤ 0.0002) between Aru, Bali, and West Papua populations. However, when only directional loci were considered, the level of genetic differences among populations ($F_{st} \leq 0.488$; p-value ≤ 0.0000) was significantly higher (~8 fold), with a reduction in genetic diversity observed in Bali, and to a lesser extent in West Papua (Table 2). This indicates that these two populations are most likely experiencing the effects of local directional selection for particular trait(s) more heavily than individuals in Aru. Additionally, NJ distance trees of directional outliers revealed that individuals from Bali and West Papua are freely interspersed with one another, while those in Aru are segregated from these two regions, suggesting that Bali and West Papua are most likely experiencing similar selective pressures and should likely be considered as a discrete stock management unit from those in Aru. These selective pressures may be initiating local adaptation in Bali and West Papua populations, resulting in individuals that might display an increase in fitness to these regions. These local adaptations are likely the driving factor behind the variations in grow-out potential observed in a G × E study conducted by Kvingedal et al. (2010) which utilized individuals from the same genetic stock (*i.e.* families) as those from this study. In this G × E study, families translocated from Aru exhibited slower growth-rates when moved to Bali than families translocated from Bali and West Papua, which displayed similarly fast growth-rates. This variation in family growth rates suggests that selective forces are most likely at play in one or more of the populations, rather than environmental conditions in Bali being universally more favourable for growth.

The increased genetic differentiation observed when only using directional outlier loci is mitigated by the large number of neutral and balancing loci, with population structuring becoming weak when all-inclusive loci were evaluated. This pattern is observed in both the DAPCs and NJ distance trees. NJ distance trees and genetic distance estimates using neutral and all-inclusive loci show that all three populations are exhibiting relatively equal levels of genetic divergence to one another. This suggests that while adaptation is occurring, it is only affecting allele frequencies in certain regions of the genome. The lack of strong genetic differentiation under neutral loci supports high levels of gene flow among all three populations. Additionally, 'infinite' estimates of N_{eLD} for all three populations in conjunction with prior knowledge of *P. maxima*'s life history – *i.e.* a long planktonic larval stage (Rose and Baker, 1994) – and strong regional ocean currents (Lind et al., 2007, 2012) also suggest high levels of gene flow amongst these regions. Nevertheless, the extent of how selection is influencing populations with such high gene flow still needs to be considered.

Table 3
Genetic distance (F_{st}) values for all-inclusive loci, neutral loci, balancing loci, and directional loci.

	All-inclusive loci	Neutral loci	Directional outliers	Balancing outliers
All populations	0.050*	0.043*	0.348*	0.009
Aru & Bali	0.065*	0.052*	0.488*	0.009*
Aru & West Papua	0.060*	0.053*	0.345*	−0.002
Bali & West Papua	0.022*	0.022*	0.062*	0.016*

* Indicates a significant p-value < 0.05.

4.2. Potential drivers of population differentiation & diversity

Many environmental factors can influence both the rate of gene flow and level of genetic diversity of marine populations. Strong ocean currents, such as the Indonesian Throughflow Current (Lind et al., 2007, 2012), and long planktonic larval phases for *P. maxima* (17–24 days; Rose and Baker, 1994) can drive high connectivity and gene flow among these populations. However, the directional outliers identified in this study suggest that selection is acting upon specific regions of the genome strongly enough to negate the homogenizing forces of high gene flow for these particular loci, while the remaining genomic regions show little evidence of differentiation. Several potential explanations may be considered for this, including life history characteristics and environmental heterogeneity among these three geographic regions.

One potential explanation is that the selective pressures at play affect specific life stages of *P. maxima* more strongly than others, as has been documented in other marine invertebrates (Addison and Hart, 2005). Though selective pressures have decreased the genetic diversity of outlier loci in individuals from both Bali and West Papua, they have not acted similarly upon the Aru population. Effectively, certain traits have become selected for and their associated alleles are found at higher frequencies in Bali and West Papua. This does not indicate that these alleles have been removed from Aru, but rather that they are found at a lower frequency. This suggests that while migrants can flow freely among these highly connected populations, some individuals are better genetically predisposed to settle in West Papua or Bali and develop into adults than other individuals. However, models examining dispersal patterns of *P. maxima* through local currents are needed in order to verify whether or not this is truly the case.

If selection is occurring prior to adulthood, then a form of pre-settlement or post-settlement but pre-reproductive selection may be occurring in which only those individuals who exhibit the desired trait(s) or genotypes can settle and survive to adulthood in Bali and West Papua. For broadcast spawners, survival during their early life history stages and the distance that they can travel *via* ocean currents is dependent upon the amount of energy that they inherit from their mother, *i.e.* the size of the egg (Vance, 1973; Nishimura and Hoshino, 2009). This

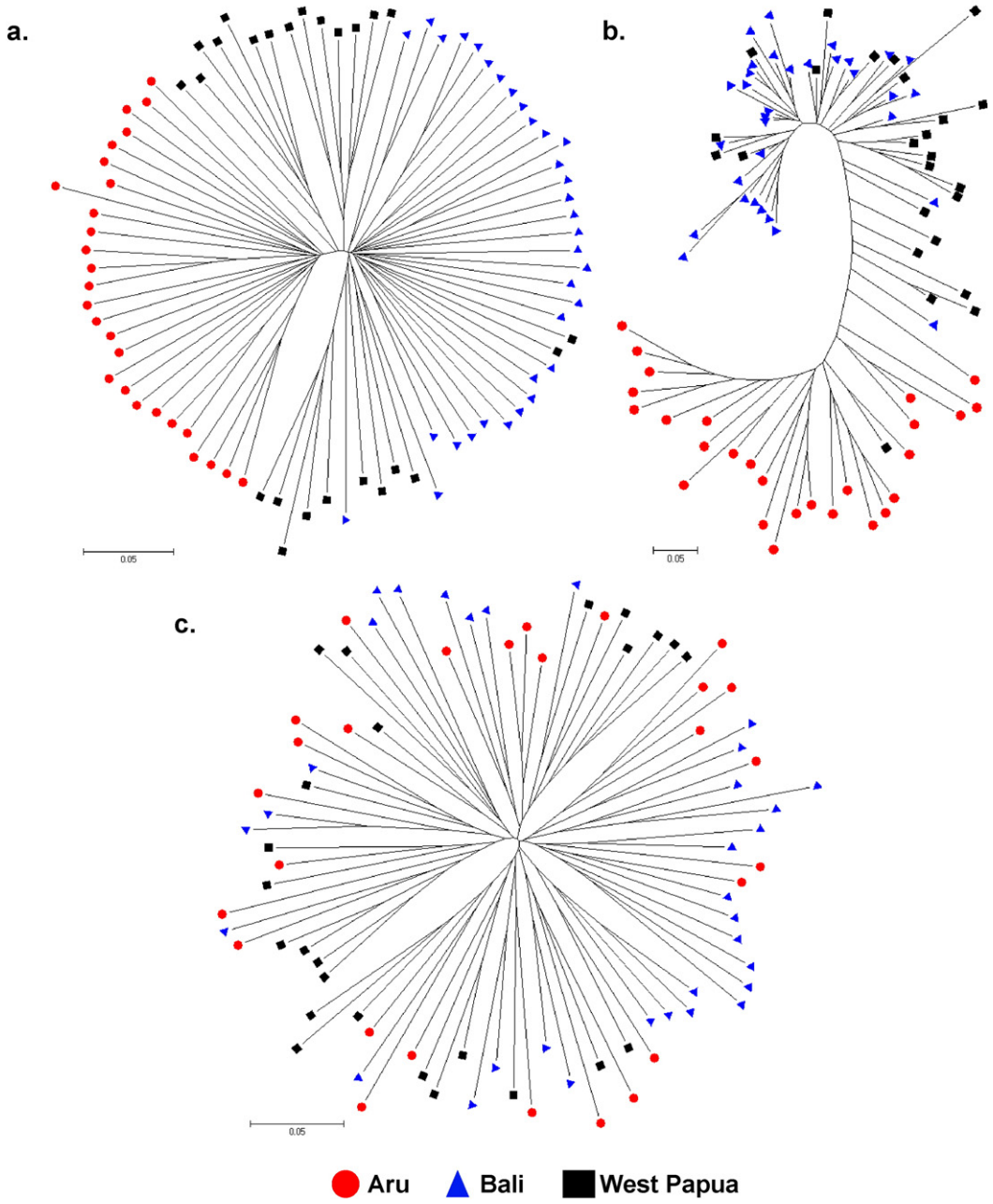

Fig. 2. Unrooted neighbour-joining trees that show the phylogenetic relationship among Aru, Bali, and West Papua using neutral (1032 SNPs; a), directional (22 SNPs; b), and balancing loci (76 SNPs; c) and where the scale bars indicate genetic distance among individuals.

results in substantial variations in family vulnerability, survivorship, and geographic range during these early life history stages. Additionally, anecdotal field observations indicate environmental differences in currents, water quality, and food availability among study sites, with Aru being the most divergent. Such environmental variations are known to affect the likelihood of an oyster recruit settling and surviving in any one location based upon whether or not they exhibit the necessary fitness traits (Saucedo et al., 2005). Despite these limitations, high levels of genetic variation in neutral loci, as observed by Lind et al. (2007) and confirmed by our study, amongst individuals in those regions most likely undergoing selection are still possible due to high levels of migrants (*e.g.* individuals from Aru and other regions) who still exhibit these favourable traits. This selective mortality of recruits has been established in other marine species such as *Neopomacentrus filamentasus*, a damselfish from Western Australia (Vigliola et al., 2007) and in the Caribbean octocoral, *Eunicea flexuosa* (Prada and Hellberg, 2014).

4.3. Implications for stock management of P. maxima

Genome-wide genomic data now allows for a more comprehensive understanding of all the micro-evolutionary processes shaping populations, resulting in improved stock assessments and management (Waples et al., 2008). The ability to resolve fine-scale population connectivity and signatures of selection is of exceptional importance to marine organisms, particularly broadcast spawners, which are highly connected through ocean currents and have the potential for high dispersal rates across heterogeneous habitats (Nielsen et al., 2009; Limborg et al., 2012). Using both neutral and adaptive loci, our data reveals two major genetically differentiated geographical groups or

commercial stocks (Bali/West Papua and Aru). The genetic differentiation identified among these stocks has previously been associated with variations in commercial fitness, i.e. significant disparities in growth rate and size of translocated individuals (Kvingedal et al., 2010). These variations in commercial fitness are key to improving commercial management practices for this species, as growth rate and oyster size at harvest are highly correlated with both pearl quality and production rates (Urban, 2000; Taylor and Strack, 2008). Since *P. maxima* are frequently translocated amongst locations for commercial purposes, it is important to have a clear understanding of the species functional stock structure in the region. Based on the current genomic data, Bali/West Papua should be managed as a separate commercial stock to individuals from Aru. However, further population genomic analyses should be performed across this species distribution, and in divergent habitats to gain additional insights into stock structure in the Indo-Pacific Region

5. Conclusion

Using genome-wide loci, this study has elucidated the combined effects of gene flow and selection acting upon populations of *P. maxima* from three neighbouring geographic regions in Indonesia. This study demonstrated that selection can occur despite high gene flow, highlighting the importance of understanding micro-evolutionary forces when evaluating population genetic structure. Reductions in branch lengths of NJ distance trees coupled with genetic diversity indices of directional outliers suggest that individuals from Bali, and to a lesser extent West Papua, are the most likely candidate populations experiencing selective pressures. Yet, as no testing on variations in environmental conditions or the seasonal strengths of currents and eddies was conducted, it is unclear what is driving selection. Reductions in genetic variation observed in directional outliers in addition to a previous ecological study demonstrating G × E interactions for growth rate, suggest that selection is differentially impacting these populations. Although there is weak but significant population genetic structure based on neutral loci, the strong signatures of selection observed in directional outliers should be taken into consideration when managing these populations, particularly for translocation or commercial grow-out operations in aquaculture. Accordingly, the Bali/West Papua region and Aru may need to be managed separately in these commercial environments. A wider-scale study is recommended in order to determine how micro-evolutionary forces are affecting the Indo-Pacific at a variety of spatial scales, and to more accurately inform management in this region. Nevertheless, this study establishes the importance of using genome-wide data to better understand the micro-evolutionary forces shaping populations, particularly for high-gene flow species such as, broadcast spawners.

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Maria G. Nayfa is a PhD candidate in Marine and Environmental Sciences at James Cook University, Queensland, Australia. Maria's research focuses on utilizing population genomic techniques to resolve population structuring, relatedness, connectivity, and evolutionary relationships within aquatic species. Her previous education includes a BS in Environmental Science from Duke University, NC, U.S.A. and a MSc in Marine Biology from James Cook University, Queensland, Australia.

Kyall R. Zenger is an Associate Professor of Sustainable Tropical Aquaculture at James Cook University in Queensland, Australia. He has over 18 years of experience in applied animal production, population ecology, biotechnology and quantitative/evolutionary genetics on both aquatic and terrestrial animals. His current work involves several large-scale research programs encompassing population genomics, phylogenomics, genome mapping and genetic evaluation of aquatic species including pearl oysters, marine shrimp, barramundi and tilapia. A/Prof. Zenger's experience has allowed him to bridge both applied and pure basic research areas, and his current teaching responsibilities encompass wildlife conservation and applied animal production at both undergraduate and postgraduate levels.