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Meso-scale genetic structure of the intertidal, crevice-dwelling, stalked barnacle *Ibla cumingi* (Crustacea: Cirripedia): an interplay of life history and local hydrographic conditions

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Variation in life history characteristics is believed to play an important role in dispersal and thus shaping the population and genetic structure of marine invertebrates. The genetic structure of Ibla cumingi*, a small intertidal stalked barnacle that broods lecithotrophic larvae, was evaluated using 145 random amplified polymorphic DNA markers on 100 individuals from five locations across Hong Kong waters. Shallow genetic structure was observed along open-coast shores, and there was no indication of isolation by geographical distance. A significant genetic divergence, however, was observed between samples inside and outside Tolo Harbour, a semi-enclosed, sheltered and estuarine bay located in the north-eastern quadrant of Hong Kong, indicating the presence of a genetic sub-structuring pattern. In addition, relatively lower genetic diversities were described for samples inside Tolo Harbour than those from open-coast shores. This could be associated with an increase in inbreeding events attributed to local settlement caused by larval retention. This study provides an insight into how the interaction of life history and local, enclosed, hydrographic conditions could result in a substantial genetic structuring of* I. cumingi *over a meso-scale geographical distance.*

Keywords: Crustacea, intertidal stalked barnacles, enclosed bay, genetic sub-structure, larval mode

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INTRODUCTION

The pelagic larval stage of marine invertebrates plays an important role in their dispersal (Palmer & Strathmann, 1981) and, hence, in maintaining gene flow and shaping population structure (Palumbi, 1994). Studies on the dispersal and connectivity between populations is essential for understanding local population dynamics and population genetic structures, and has important implications for marine resources management (Palumbi, 2003). Dispersal capability is believed to be a function of synergistic intrinsic factors including planktonic period, larval behaviour (Palumbi, 2003) and larval strategies (planktotrophy versus lecithotrophy) (Todd *et al.*, 1998). Abiotic factors, however, also either increase or impede larval flow, depending on the local physical environment. Examples of these factors include ocean currents (Palumbi, 1994), large- and small-scale hydrographic/topographic variabilities (Lessios *et al.*, 2003; Ayers & Waters, 2005), temperature (Borsa *et al.*, 1997), and anthropogenic influences (O'Foighil *et al.*, 1999).

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These mixed variables may result in population patterns ranging from low differentiation over a large geographical extent in species with short dispersal periods (Ignacio *et al.*, 2000; McCormack *et al.*, 2000) to high differentiation within a small area, despite a long planktonic larval life (Goldson *et al.*, 2001; Taylor & Hellberg, 2003; Kirkendale & Meyer, 2004; Rocha *et al.*, 2005). A more complex case, termed 'chaotic genetic patchiness', has been identified in marine organisms demonstrating large-scale genetic homogeneity but having fine-scale genetic heterogeneity (Johnson & Black, 1982; Watts *et al.*, 1990). Such genetic patchiness is believed to be associated with a 'sweepstakes'-pattern of reproductive success (Hellberg *et al.*, 2002; Hedgecock *et al.*, 2007), temporal variations in larval sources (Kordos & Burton, 1993) and selective mortalities at either pre- or post-settlement stages (Johnson & Black, 1984).

Barnacles are a diverse group of marine crustaceans that play significant roles in structuring benthic communities especially in the intertidal zone (Anderson, 1994). They have a bi-phased life cycle with sessile adults producing pelagic larvae, and such characters make them good models for studies of larval dispersal. *Ibla cumingi* (Darwin, 1852) (Ibliformes: Iblidae) is one of only a few stalked intertidal barnacles (Foster, 1987) that is widely distributed in the Indo-West Pacific region (Liu & Ren, 1985; Jones *et al.*,

2000) and occurs as a significant member of the rocky-shore crevice and fissure fauna in tropical regions including Hong Kong (Morton & Morton, 1983; Britton, 1990). The species has a small body (<7.0 mm capitulum width) due to phylogenetic constraints (Anderson, 1994) and individuals can therefore only carry a small brood. Fecundity is similarly low but the species is able to reproduce throughout the year in Hong Kong (Leung, 2003).

Unlike many other intertidal barnacles, which have planktotrophic larvae, *Ibla cumingi* produces lecithotrophic ones (Anderson, 1994). Although these seem able to feed (Høeg *et al.*, 2009), unlike other planktotrophic larvae, they can develop most larval stages (nauplii stage I to V) without recourse to food (Yan *et al.*, 2005). Energy resources are putatively reserved in significantly larger vitellogenic oocytes for their early larval stages, as compared with another planktotrophic stalked barnacle, *Capitulum mitella* Linnaeus, 1758 (Leung, 2003). The larval development period is abbreviated, as it only takes seven days to reach the final cyprid stage (Yan *et al.*, 2005).

Similar to other local rocky-shore sessile animals, *Ibla cumingi* experiences a range of hydrographic conditions in Hong Kong. Along the wave-exposed shores of the Hong Kong coastline, western waters are largely affected by the outflow from the Pearl River, especially during summer months when there is heavy rainfall, whereas southern and eastern waters are oceanic. North-eastern waters include Tolo Harbour, a large (~50 km^2), semi-estuarine, enclosed bay with poor flushing (Morton, 1982). Within enclosed marine environments, restrictions to larval dispersal can be common (Bilton *et al.*, 2002; Sköld *et al.*, 2003). It has also been reported that the effects of local hydrographic conditions on the structuring of a population's genetics would be more profound in species with abbreviated and lecithotrophic larvae than those with long-lived planktotrophic larvae (Goldson *et al.*, 2001). In enclosed bay areas, therefore, the possible restricted dispersal of abbreviated and lecithotrophic larvae may lead to local settlement in the vicinity of their parents and could result in an increased frequency of inbreeding events (Grosberg, 1987; Miller, 1998).

In the present study, using *Ibla cumingi* as a model species characterized by low fecundity with an abbreviated larval period, we aimed to evaluate its potential genetic variation in Hong Kong waters. We hypothesized that larval dispersal restriction would be substantial inside enclosed Tolo Harbour. Genetic differentiation was thus expected between samples from inside and outside the embayment. This study also aimed to provide: (i) baseline information on the virtually unknown genetic constitution of a local marine sessile invertebrate; and (ii) to reveal any patterned structure that may be attributable to hydrographic variables and about which information is still limited for the marine environment.

MATERIALS AND METHODS

Sample collections

A total of 100 individuals of *Ibla cumingi* were collected from intertidal rock crevices from five locations in Hong Kong during the period from March to May 2002. Two samples were collected from inside Tolo Harbour, that is, Ma Shi Chau (MSC) and Starfish Bay (SFB); and three samples from open-coast shores, that is, Cape d'Aguilar (CD, the south-eastern-most shore of Hong Kong Island), Lung Ha Wan (LHW, eastern waters of Hong Kong) and Butterfly Beach (BF, western waters of Hong Kong) (Figure 1). All the samples were kept alive in aquaria before DNA extraction.

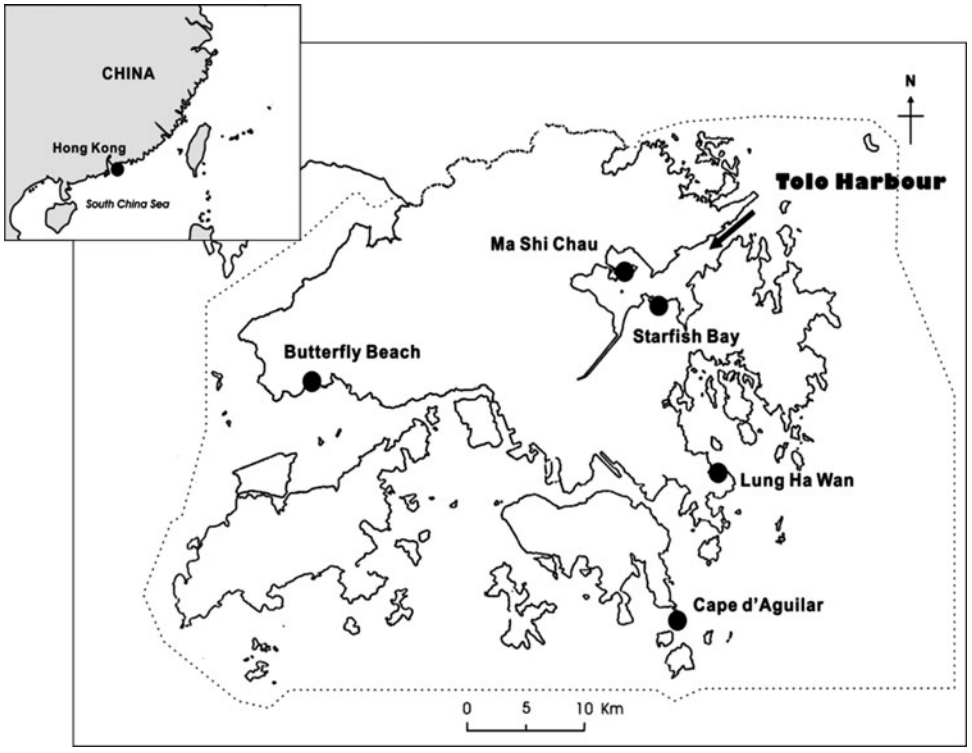

Fig. 1. A map showing the five sampling sites in Hong Kong and from where *Ibla cumingi* was obtained.

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DNA isolation and PCR-RAPD procedures

Approximately 50 mg of body tissues, including the stalk, adductor muscle and ovaries (but excluding the prosoma because the gut may contain parasites) of each *Ibla cumingi* individual were dissected out and washed with sterile distilled water. Tissues were cut into small pieces and transferred to 1.5 ml centrifuge tubes containing 500 µl of lysis buffer (100 mM Tris–HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2.0% hexadecyltrimethylammonium bromide (CTAB), and 0.2% 2-mercaptoethanol). Each tissue sample was incubated overnight at 65°C with 50 µl of proteinase K (1 mg ml^{-1}). After digestion, genomic DNA was extracted using the phenol–chloroform extraction method (Hoelzel & Green, 1992). The final DNA pellets were re-suspended in 40 µl of TE buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA, pH 8.0), and treated with 2 µl RNAse (1 mg ml^{-1}) for 2 hours. The DNA was quantified using a recording spectrophotometer (UV-160, Shimadzu Corporation) and stored at 4°C until required.

Twenty-two random primers (10 base-pairs) (Invitrogen™ Life Technologies Co.) were screened for a sub-sample of five individuals from each site in Hong Kong. Seven primers (Table 1) exhibiting highly reproducible and easily scored results, that is, several clear bands with a low level of background (Wilson *et al.*, 1997), were chosen for determination of population genetics.

RAPD-polymerase chain reaction (PCR) was performed in a 25 µl reaction mixture containing 20 ng of template DNA, 1U *Taq* DNA polymerase (Invitrogen™ Life Technologies Co.), 0.5 µM of each primer, 0.2 mM of each dNTPs (dATP, dCTP, dGTP and dTTP), 1.5 mM $MgCl_2$ and 1 × PCR buffer (200 mM Tris–HCl, pH 8.4, 500 mM KCl). Amplification was carried out in a PTC-100 Thermocycler (MJ Research) using an initial DNA denaturation step of five minutes at 94°C followed by 40 cycles: 1 minute denaturation at 94°C, 1 minute annealing at 37°C and 3 minutes extension at 72°C. This was followed by a 20-minute final extension period at 72°C. The RAPD reactions were conducted twice for each experimental sample to ensure the reproducibility of band profiles. The amplified products (12 µl) and 1.6 µl 100-bp DNA ladder (GeneRuler™) were electrophoresed with 1 × TAE buffer (40mM Tris–Acetate, 10 mM EDTA) on a 2% agarose gel stained with ethidium bromide. The gel was initially run at 130 V for 2 minutes and followed by a constant voltage of 70 V for ~2 hours. Gels were then visualized and photographed under UV light.

RAPD data analysis

For each primer, the RAPD band profile of each sample was scored for either the presence (1) or absence (0) of bands of a specific size. To minimize possible reproducibility problems, positive bands were scored only if they were produced consistently in the two separate amplifications. For each individual, the scored banding profiles of all primers were merged. By grouping the scoring of each individual, a 1/0 data matrix (100 individuals × 145 loci) was created for all individuals. The allele frequencies at each band were estimated using a Bayesian method implemented in ALFP-SURV 1.0 software (Vekemans, 2002). The genetic diversity within sample was determined using portion of polymorphic loci (PLP) and the unbiased expected heterozygosity (H_j, Nei's gene diversity; Nei, 1973). Pairwise distance matrices were estimated using Nei's distance (Nei, 1972) with the correction of Lynch & Milligan (1994). The computation of Nei's distance and 1000 bootstrapping were undertaken using AFLP-SURV 1.0 software.

Table 1. Sequences, percentage of GC contents and number of bands scored for the seven chosen RAPD primers.

RAPD primer code	% GC	Sequence (5′ to 3′)	No. of bands scored
B-05	70	CCAATCGAGG	18
B-08	70	GGTCCACCGA	17
B-11	70	GTCCACTCGG	19
B-12	60	GTCGTACAGC	26
B-14	60	GGCTATCTGC	18
B-19	70	AGGTCCCTCG	19
B-21	60	GGGCTAGAAG	28

Assessing the admixture pattern using clustering, assignment and PCO analyses

The admixture pattern between samples obtained from Hong Kong sites was further revealed using the assignment test of the Bayesian clustering and assignment software STRUCTURE v2.2 (Pritchard *et al.*, 2000; Falush *et al.*, 2003). The analysis was performed for the 100 individuals with no population information included and under a model of admixture assumption. Number of genetic clusters (K) from 1 to 10 with 20 repetitions for each K was tested (10,000 burn-in and 10,000 Markov chain Monte Carlo replicates for each run). Selection of K from the output data was achieved using an *ad hoc* statistic ΔK (Evanno *et al.*, 2005). The average proportion of membership (admixture coefficient, q) of all the samples will be assessed using the inferred clusters according to the selected K value.

Multivariate analysis, the principal co-ordinate (PCO) analysis implemented in PRIMER 6.1.12, was also employed to discriminate the admixture pattern of the five samples based on the allele frequencies for all loci.

Analysis of genetic differentiation

Analysis of molecular variance (AMOVA) was performed using ARLEQUIN version 3.01 software (Excoffier *et al.*, 2005) to examine hierarchical population structure by pooling the samples into two different groupings, that is: (i), inside (IT: SFB and MSC); and (ii) outside Tolo Harbour (OT: CD, BF and LHW). The analysis was tested for statistical significance against a distribution of 16,000 permutations to guarantee $<1\%$ difference with the exact probability in 99% of cases (Guo & Thompson, 1992). Isolation by distance (IBD) between samples was evaluated using the Mantel test as implemented in GENETIX v.4.05 (10,000 permutations) by correlating linearized F_{ST} (i.e. F_{ST} $(1\text{-}F_{ST})^{-1}$) to hydrographical distance measured as the minimum along-shore, between site, distances.

RESULTS

Genetic diversity

Of the seven primers used, a total of 145 loci of specific sizes were recorded among the five Hong Kong samples of *Ibla*

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Table 2. Sampling locations, sample sizes (N) and genetic diversity of the five samples.

Sample	N	P loc	PLP	H_j	SE (H_j)
Starfish Bay	20	131	90.3	0.349	0.012
Ma Shi Chau	20	132	91.0	0.353	0.011
Cape d'Aguilar	20	140	96.6	0.386	0.010
Lung Ha Wan	20	141	97.2	0.383	0.010
Butterfly Beach	20	137	94.5	0.370	0.010

N, number of specimens; P loc, number of polymorphic loci at the 5% level; PLP, proportion of polymorphic loci at the 5% level expressed as a percentage; H_j, expected heterozygosity under Hardy–Weinberg genotypic proportions (synonymous with Nei's gene diversity; SE (H_j), standard error of H_j).

cumingi (100 individuals) with 139 loci (95.9%) being polymorphic, and the number of loci per primer varied between 17 and 28 (Table 1). Genetic diversity in terms of the proportion of polymorphic loci (PLP) and H_j is shown in Table 2. Genetic diversity ranged from 90.3% to 97.2% for PLP, and ranged from 0.349 to 0.386 for H_j. Sites from eastern waters generally had a relatively higher genetic diversity (CD: PLP = 96.6%, H_j = 0.386; LHW: PLP = 97.2%, H_j = 0.383). Generally lower genetic diversities were reported upon for both sites inside Tolo Harbour (SFB: PLP = 90.3%, H_j = 0.349; MSC: PLP = 91.0%, H_j = 0.353).

Assessing the admixture pattern using clustering, assignment and PCO analyses

Based on the admixture analysis, a high ΔK at $K = 2$ was found (Figure 2). That is, the presence of two clusters in every individual best interpreted the RAPD data observed. Under the admixture model of $K = 2$, the proportion of membership (q, the admixture coefficient) of all individuals was further evaluated in the clustering analysis (Figure 3).

The results showed how well the five samples could be assigned to the two clusters. An individual with a q value that was >0.8 was considered as being strongly assigned to either cluster 1 (Outside Tolo Harbour) or cluster 2 (Inside Tolo Harbour). Almost all individuals from BF, LHW and CD were strongly assigned to the Outside Tolo Harbour cluster (N = 59/60, 98.3%). Likewise, most of the individuals from MSC and SFB were assigned to the Inside Tolo Harbour cluster (N = 33/40, 82.5%). Three individuals from MSC and four individuals from SFB were not well assigned ($q < 0.8$) to the Inside Tolo Harbour cluster, and one individual from SFB was assigned to the Outside Tolo Harbour cluster.

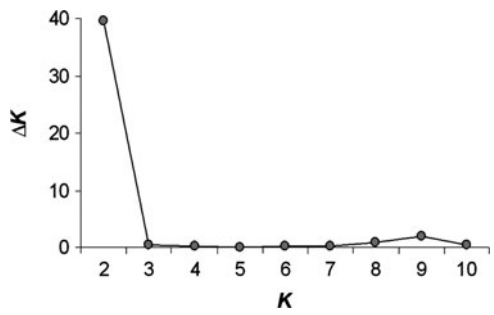

Fig. 2. *Ibla cumingi*. The rate of change in posterior probability (ΔK) over the number of clusters (K). A maximal at $K = 2$ indicates two clusters that best explain the RAPD data.

PCO analysis also agreed well with the assignment analysis (Figure 4). Most of the individuals from MSC and SFB were grouped together to form a cluster and those from CD, LHW and BF formed another cluster.

Analysis of genetic differentiation

The five Hong Kong samples were also analysed using AMOVA by sorting into two groups, i.e. (i) Inside Tolo (sites: MSC and SFB) and (ii) Outside Tolo (sites: CD, LHW and BF). AMOVA revealed significant genetic differentiation between the two groups (Φ_{CT} = 0.041, 4.13%, $P < 0.0001$; Table 3). Pairwise Φ_{ST} supported the cluster and AMOVA observations (Table 4). The pairwise Φ_{ST} did not, however, agree well with geographical proximity, and the Mantel test exhibited no significant correlation between F_{ST} and hydrographic distance (r = 0.421, $P > 0.05$) indicating no IBD in the five samples. The results showed highly significant pairwise Φ_{ST} estimates between all pairs, except for three pairs, i.e. SFB versus MSC, BF versus LHW and BF versus CD.

DISCUSSION

This study has identified a meso-scale genetic structuring of the intertidal stalked barnacle *Ibla cumingi* in Hong Kong waters. Samples obtained from across the open waters of Hong Kong (CD, LHW and BF) showed a generally panmictic genetic structure. Conversely, significant genetic divergence was detected between samples from the open waters and those from Tolo Harbour (SFB and MSC). Similarly, a higher genetic diversity was reported for the open-coast samples when compared with those from inside Tolo Harbour.

Genetic structure and gene flow in marine organisms is generally correlated with larval dispersal ability (Palumbi, 1994), and marine species with dispersive larvae show less genetic structuring over large distances while those with limited dispersal abilities demonstrate structuring over a finer spatial scale (Hedgecock, 1986; Bohonak, 1999). In the present study, the lack of genetic structure among the open-coast sites across Hong Kong waters, together with the non-significant result of isolation by distance, suggests that the life history of *Ibla cumingi*, a marine sessile invertebrate with an abbreviated larval period and low fecundity, does not result in population sub-structuring.

Shallow genetic structuring has been reported recently for Hong Kong species with long life spans and planktotrophic modes of larval development, such as corals (Ng & Morton, 2003), mantis shrimps (Lui *et al.*, 2010) and the limpet *Cellana grata* (Gould, 1859) (Ng *et al.*, 2010). Similar to these studies, the lecithotrophic and abbreviated larval development of *Ibla cumingi* does not seem to pose a significant effect on general population connectivity in Hong Kong waters, with samples showing weak genetic structuring from western, southern and eastern open-coast shores. These findings also agree with other studies, which have suggested that larval duration (planktonic or abbreviated) and dispersal mechanism (active or passive, and exported or retained) may have little effect on among-population connectivity (Teske *et al.*, 2007).

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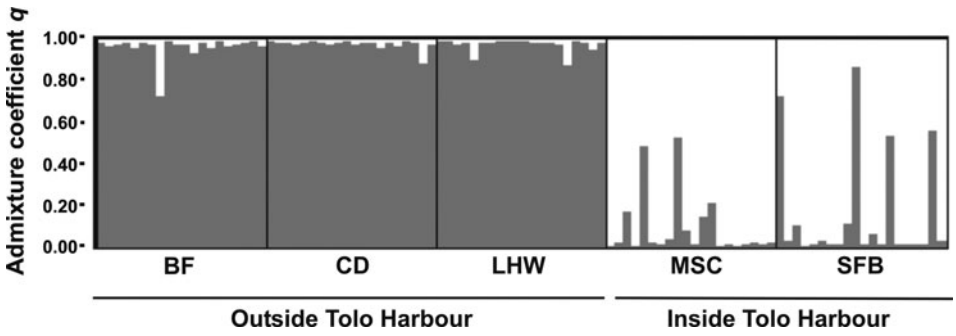

Fig. 3. *Ibla cumingi*. Bar plot of the results obtained from Structure using $K = 2$. Each individual is represented by a vertical line partitioned into two colour segments with length proportional to the admixture coefficients of the two clusters. For abbreviations see text.

The life history of *Ibla cumingi*, in combination with local topographical variations, such as large enclosed systems, as in Tolo Harbour, could result in significant genetic divergence between samples at a local meso-scale, as reported in the present study. In addition to genetic divergence, genetic diversities were different for *I. cumingi* between samples obtained from inside and outside Tolo Harbour. From the assignment analysis, seven out of 40 individuals from sites inside Tolo Harbour were not assigned unambiguously while assignment failure was recorded in only one of 60 individuals from outside Tolo Harbour. This observation implies a directional gene flow from sites outside to inside Tolo Harbour, but not vice versa. Tolo Harbour is a semi-enclosed, elongate, shallow, estuarine environment with weak tidal flushing (Lee *et al.*, 2003). Few population genetic studies have been conducted upon marine taxa in this enclosed bay. One recent study of the stomatopod, *Oratosquilla oratoria* (de Haan, 1844), across Hong Kong waters reported that a site close to the outer channel of Tolo Harbour showed a different haplotype composition to another open-water site only ~30 km away (Lui *et al.*, 2010). Another study on picoeukaryote diversity revealed less distinct seasonal variations in species composition inside Tolo Harbour than the open-waters outside, again relating to the highly enclosed nature of this harbour (Cheung *et al.*, 2008).

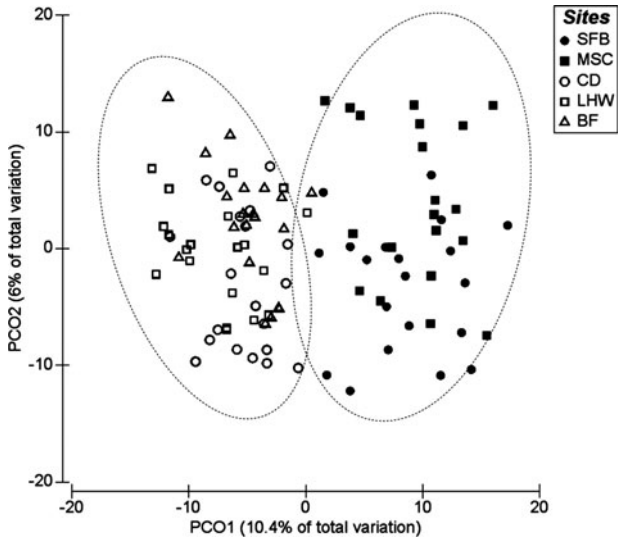

Fig. 4. *Ibla cumingi*. Principal co-ordinate (PCO) analysis based on allele frequencies of 145 RAPD loci for 100 individuals from the 5 samples. The first and second principal co-ordinates represent 10.4% and 6.0% of the total variation. The MSC and SFB are inside Tolo Harbour (symbols in black), and CD, LHW and BF are outside Tolo Harbour (symbols in white). For abbreviations see text.

Studies from elsewhere have reported that enclosed marine environments like estuaries and fjordic seascapes could lead to gene-flow restriction, and that genetic structuring may be enhanced by such hydrographic conditions that act on species with low dispersal abilities (Bilton *et al.*, 2002; Watts & Johnson, 2004). Other studies have also revealed significant meso-scale genetic differentiation in species with dispersive planktotrophic larvae associated with enclosed hydrographic regimes (Sköld *et al.*, 2003). There is no other similar population genetic study on intertidal stalked barnacles with lecithotrophic larvae. However, a recent study on an intertidal stalked barnacle *Pollicipes pollicipes* with planktotrophic larvae also demonstrated meso-scale genetic differentiation and suggesting such differences may relate to the local hydrographic patterns (Quinteiro *et al.*, 2007).

Ibla cumingi broods lecithotrophic larvae (Anderson, 1994; Yan *et al.*, 2005), which do not engage in active swimming (Høeg *et al.*, 2009). Such characters could provide constraints to larval dispersal distances within a low flushing embayment. In addition, the expected abbreviated larval life may limit settlement duration and pre-metamorphic larvae may mainly recruit back into their parental area inside Tolo Harbour. This hypothesis is supported by a demographic analysis that recruitment by *I. cumingi* inside Tolo Harbour was sustained but small and with no obvious pulse over the course of the year as there is in more open-coast situations (Leung, 2003).

Taken together, it is suggested that the enclosed environment of Tolo Harbour could have a significant effect on larval retention by *Ibla cumingi* causing genetic divergence between areas inside and outside it. The relatively low genetic diversity observed in the Tolo Harbour samples may

Table 3. *Ibla cumingi*. Analysis of molecular variance (AMOVA) among the five Hong Kong samples. Samples were grouped into two regions, that is (1) Inside Tolo Harbour: SFB and MSC and (2) OutsideTolo Harbour: CD, LHW and BF.

Variance component	df	Variance	% total	Φ-statistics
Between groups (Inside-Tolo versus Outside-Tolo)	1	1.12	4.44	$\Phi_{CT} = 0.044^*$
Among samples within group	3	0.44	1.66	$\Phi_{SC} = 0.017^*$
Within samples	95	25.16	93.90	$\Phi_{ST} = 0.061^*$

*, indicates $P < 0.001$.

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Table 4. *Ibla cumingi*. A matrix of pairwise approximate geographical distance (km) (above diagonal) and Φ_{ST} (below diagonal) based on the five Hong Kong samples (Starfish Bay, Ma Shi Chau, Cape d'Aguilar, Lung Ha Wan and Butterfly Beach).

Sample	SFB	MSC	LHW	CD	BF
Starfish Bay	–	3.5	40	50	85
Ma Shi Chau	0.0177	–	40	50	85
Lung Ha Wan	0.0698*	0.0685*	–	14	38
Cape d'Aguilar	0.0551*	0.0664*	0.0233*	–	55
Butterfly Beach	0.0523*	0.0551*	0.0121	0.0154	–

*, indicates $P < 0.001$.

also indicate inbreeding. It has been shown from elsewhere that recruitment of offspring into parental habitats may increase inbreeding leading to a reduction in diversity (Grosberg, 1987; Miller, 1998).

In addition, some recent studies have proposed that the spatial genetic variations seen in a species, which should, in theory, demonstrate high gene flow among populations, may relate to a 'sweepstakes' pattern of reproduction (Hellberg *et al.*, 2002; Hedgecock *et al.*, 2007). That is, the high variance in reproductive success may lead to a small effective population size resulting in strong genetic drift relative to gene flow. The sweepstakes hypothesis is usually applied to marine species that exhibit free-spawning and high fecundity (Hellberg *et al.*, 2002). *Ibla cumingi* is able to reproduce year-round, but has a relatively low fecundity as compared with other intertidal barnacles (Leung, 2003; Yan *et al.*, 2006). During the peak reproductive period in early summer (March to May), Tolo Harbour samples may comprise >80% of brooding individuals. Recruitments performances were, however, low and no obvious seasonal pulse was detected (Leung, 2003). It has further been reported that hypoxic events attributable to eutrophication have caused summer mortalities of benthos and declines in species abundance and diversity in the Tolo Harbour area (Wu, 1982), and may reduce the settlement of new recruits of some benthic species (Ganmanee *et al.*, 2004). Such hypoxic events may also impact upon *I. cumingi*, accounting for the observed variation in reproductive success, and this too may be explained by the sweepstakes concept.

In the present study, the three samples of *Ibla cumingi* obtained from outside Tolo Harbour were not genetically different between eastern and western waters. Eastern waters are oceanic throughout the year while the western waters of Hong Kong are affected much more by the freshwater output from the Pearl River, especially during the summer rainy season (May to September), when surface salinities can be as low as 1–2‰ (Morton & Morton, 1983). Low salinity is believed to affect the larval recruitment of some intertidal barnacles in Hong Kong's western waters (Chan *et al.*, 2001). Moreover, the reproductive success of the mantis shrimp, *Oratosquilla oratoria*, was suggested to be negatively affected by such low salinity incursions (Lui, 2005), and may be associated with the species' patchy genetic structure across Hong Kong waters (Lui *et al.*, 2010). In contrast, *I. cumingi* is widely distributed on estuarine rocky shores throughout Hong Kong waters (Morton & Morton, 1983) and seems to be less impacted by low salinities. The genetic contiguity of *I. cumingi* between eastern and western sites indicate, therefore, that salinity is probably not a selection force for the larval dispersal phase and beyond of this small barnacle.

From across Hong Kong's open-coastal waters, a relatively high genetic diversity has herein been reported for the Cape d'Aguilar (CD) sample of *Ibla cumingi*. Cape d'Aguilar has been a designated marine reserve since 1995 for its diverse coastal flora and fauna and geological features (Morton & Harper, 1995). No previous study has examined the connectivity of marine species between Hong Kong's marine protected areas and other local shores. Although *I. cumingi* is of no commercial importance, the present study is the first report on genetic diversity and population connectivity in a species with low fecundity and an abbreviated larval period, between local shores and a marine protected area. Goals of marine reserves are to preserve biodiversity and increase fisheries yields (Botsford *et al.*, 2003). A network of interconnected reserves is necessary to span the range of any particular marine species (Allison *et al.*, 2003; Buonaccorsi *et al.*, 2005). It is, therefore, essential to explain the population connectivity of various marine taxa in order to further our understanding of marine resource management. This study of *I. cumingi* is a first step towards this understanding in Hong Kong.

In conclusion, this study provides evidence for possible meso-scale gene flow restriction in *Ibla cumingi*, which exhibits a low fecundity and abbreviated larval period. Genetic structuring of this species probably results from the combined biotic and abiotic characteristics of low larval dispersal potential and the semi-enclosed estuarine environment of Tolo Harbour within the framework of the broader hydrographic environment of Hong Kong and along the coastline of southern China.

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