



ELSEVIER

Journal of Experimental Marine Biology and Ecology 325 (2005) 46–54

**Journal of
EXPERIMENTAL
MARINE BIOLOGY
AND ECOLOGY**

www.elsevier.com/locate/jembe

Fine-grained spatial genetic structure in the bivalve *Gemma gemma* from Maine and Virginia (USA), as revealed by Inter-Simple Sequence Repeat markers

Marco Casu^{a,*}, Ferruccio Maltagliati^b, Piero Cossu^a, Tiziana Lai^a,
Marco Curini Galletti^a, Alberto Castelli^b, John A. Commito^c

^aDipartimento di Zoologia e Antropologia Biologica, Università di Sassari, Via F. Muroli 25, Sassari 07100, Italy

^bDipartimento di Scienze dell'Uomo e dell'Ambiente, Università di Pisa, Via A. Volta 6, Pisa 56126, Italy

^cEnvironmental Studies Department, Gettysburg College, Gettysburg, PA 17325, USA

Received 20 January 2005; received in revised form 15 March 2005; accepted 15 April 2005

Abstract

Gemma gemma is a small ovoviviparous bivalve distributed in shallow sand flats along the North American Atlantic and Gulf of Mexico coasts. Genetic variation in *G. gemma* was analysed by means of Inter-Simple Sequence Repeats (ISSRs) at the following levels: (i) between localities (Maine and Virginia), (ii) among 10-m-diameter patches within localities, and (iii) within patches. Thirty individuals/patch and three patches/locality were analysed. Individuals were genotyped for 67 ISSR polymorphic loci from five primers. The portion of the genetic variation found between localities (2%) was small compared to that found either among patches within localities (37%) or within patches (61%). ISSRs in *G. gemma* allowed the detection of significant differentiation at individual and patch levels. By contrast, a low degree of genetic variability was found between localities. The small-scale genetic heterogeneity does not follow a simple, consistent pattern. Our results contrast with the generally accepted rule that aplanic species are locally homogeneous and globally heterogeneous and teleplanic species are the inverse.

© 2005 Published by Elsevier B.V.

Keywords: Dispersal; *Gemma gemma*; Gene flow; Genetic variation; ISSRs; Spatial structure

1. Introduction

One of the aims of population geneticists is to define accurately the spatial extent of populations. In some instances population boundaries correspond to clear topographic constraints, but many marine invertebrates are wide-ranging species with virtually

* Corresponding author. Tel.: +39 79 22 86 62; fax: +39 79 22 86 65.

E-mail address: marcasu@uniss.it (M. Casu).

continuous distributions. As a consequence, it is difficult to identify or even hypothesise a priori population boundaries. In this context, a species' dispersal capability assumes relevance because dispersal can be perceived not only as a way of colonising or recolonising new areas, but also as a means of exchanging individuals and genes between geographically separated (sub-)populations.

It is generally assumed that species lacking pelagic larval stages (aplantic) and those with short larval life-spans (anchiplantic) have a very limited potential for dispersal, whereas the opposite is true for species with medium or long larval life-spans (actaeplantic and teleplantic) (Havenhand, 1995). Accordingly, many studies have reported considerable genetic divergence among populations of aplantic and anchiplantic species, and little genetic divergence between distant population of actaeplantic and teleplantic species (e.g., Gooch et al., 1972; Palumbi and Kessing, 1991). However, exceptions to this rule are provided by a number of studies on benthic invertebrates (e.g., Johannesson, 1988).

In this paper we report the analysis of genetic variation in the amethyst gem clam, *Gemma gemma* (Totten, 1834) (Eulamellibranchia, Veneridae), at different spatial scales. *G. gemma* is a small ovoviviparous bivalve (max. shell length \cong 5 mm) widely distributed along the North American coast from Nova Scotia to Texas, where the species can be a dominant member of the infauna in intertidal and shallow subtidal sandy areas (Bradley and Cooke, 1959; Sellmer, 1967; Schneider and Mann, 1994). Furthermore, its presence in five Californian bays has been recorded as a result of human-mediated introductions (Carlton, 1992). Unlike most marine bivalves, *G. gemma* does not have free-swimming dispersing larval stages. It broods its young and releases them as benthic juveniles (Sellmer, 1967). Since there is little evidence for active horizontal movement through the sediment (J. Belt and J. Commito, unpublished data), dispersal is accomplished through passive transport (Commito et al., 1995). These authors found that individuals of *G. gemma* can be easily transported in the bedload, and there is little indication that movement is size- or age-selective (Commito et al., 1995). This clam is therefore a particularly appropriate organism for the study of population genetic structure across different spatial scales.

Genetic analysis employing highly variable genetic markers can provide important information about population structure and gene flow, especially when coupled with recently introduced powerful statistical approaches, such as Bayesian statistics (e.g., Shoemaker et al., 1999; Vekemans, 2002; Bertorelle et al., 2004). Indeed, DNA techniques have been proven valuable tools in gathering useful genetic information from natural populations (Avice, 1994). However, despite the increasing use of PCR-based genetic markers, their application to the study of marine invertebrates generally lags behind that in investigations of terrestrial organisms.

In this study we used Inter-Simple Sequence Repeat (ISSR) markers, also known as Random Amplified Microsatellites (RAMS) (Zietckiewicz et al., 1994). This technique is based on the amplification of the regions between closely spaced, inversely oriented microsatellites (or Simple Sequence Repeats, SSRs), by means of a single primer composed of a short microsatellite sequence (typically 18–20 base pairs) with one to three degenerate nucleotides anchored at the 3' or 5' end. Such amplifications do not require genome sequence information and lead to multilocus and highly polymorphic banding patterns (Zietckiewicz et al., 1994). While ISSRs have been used by plant biologists for a variety of applications (e.g., see Wolfe and Liston, 1998), only recently have they been used in vertebrates (Kostia et al., 2000; Borner and Branchard, 2001; Haig et al., 2003; Hassan et al., 2003) and terrestrial invertebrates (Abbot, 2001; Luque et al., 2002; Chatterjee and Mohandas, 2003; Chatterjee et al., 2003).

We conducted the analysis of *G. gemma* population genetic structure using ISSRs in samples from two Atlantic coast localities in North America in order to understand how *G. gemma* varies genetically across different spatial scales.

2. Materials and methods

2.1. Samples and DNA extraction

A total of 180 individuals of *G. gemma* were collected at Tom's Cove, Assateague Island, Virginia, USA (T, 37°53'N; 75°20'W) in March 2002, and North Carrying Place Cove, Lubec, Maine, USA (L,

44°51'N; 66°59'W) in July 2002. At each locality, we sampled within three 10-m-diameter patches approximately 100 m from each other. Thirty individuals per patch were analysed. Samples were labelled and preserved in absolute ethanol until genetic analyses were conducted. Genomic DNA was extracted from entire individuals using QIAGEN® DNeasy Tissue kit (QIAGEN Inc., Valencia, California) according to the manufacturer's instructions. We analysed only individuals with shell lengths from 2.5 to 3.0 mm to minimise the presence of individuals belonging to different age classes. Once extracted, DNA was stored in solution at 4 °C until ISSR-PCR amplifications.

2.2. ISSRs

The ISSR-PCR products result in dominant, diallelic Mendelian markers when divergence in SSR sites or chromosomal structural rearrangement occurs (Wolfe and Liston, 1998; Wolfe et al., 1998). Each band corresponds to a DNA sequence delimited by two inverted microsatellites. ISSRs are generated by protocols very similar to those of RAPDs (Random Amplified Polymorphic DNA), except that ISSR primer sequences are planned for microsatellite regions. Moreover, ISSRs are quick and easy to handle, and they have reproducibility because the longer lengths and higher annealing temperatures of their primers decrease the amount of template primer mismatch artefacts typical of RAPDs.

Thirteen sequences among the 18 ISSR primers we assayed were found on the web (<http://www.biosci.ohio-state.edu/~awolfe/ISSR/protocols.ISSR.html>), whereas the remaining five sequences were designed directly by the authors. Primers were provided by Proligo® Primers and Probes, Proligo France SAS. Primers contained different di- and tri-nucleotide repeat motifs to screen different parts of the genome (Table 1). They were initially tested on four individuals of *G. gemma* from both localities in order to find repeats producing a suitable number of variable bands. The preliminary screening allowed the identification of five informative and reliable primers (Table 1). The PCR reaction mixture of 25 µl volume contained 0.5 units of Taq DNA Polymerase (Pharmacia®), 1× reaction buffer (Pharmacia®), 3 mM MgCl₂, 0.2 µM primer, 200 µM of each dNTP (Roche®), and up to 30 ng of genomic DNA. PCR

Table 1

Gemma gemma: primer names and sequences used in the ISSR analysis, number of polymorphic bands per primer and range of molecular weight in base pairs (bp) amplified by PCR-ISSR for 180 individuals

Primer	Sequence (5'–3')	No. of polymorphic bands	Size range of polymorphic bands (bp)
UBC 809	(AG) ₈ G	14	100–3000
UBC 811	(GA) ₈ C	15	100–2700
UBC 827	(AC) ₈ G	13	200–2600
SAS 1	(GTG) ₄ C	11	200–2600
SAS 3	(GAG) ₄ C	14	100–3000

amplification was performed in a MJ PTC-100 Thermal Cycler (MJ research®) programmed for 1 cycle of 3 min at 94 °C, 45 cycles of 40 s at 94 °C, 45 s at 50 °C (for primers UBC 809, UBC 811 and UBC 827) or 55 °C (for primers SAS 1 and SAS3), and 1 min and 40 s at 72 °C to complete partial amplification. At the end a post-treatment for 5 min at 72 °C and a final cooling at 4 °C were performed. For each primer, negative controls and replicates were included in the amplifications to verify repeatability of results.

2.3. Electrophoresis and analysis of amplification products

The PCR products were analysed by electrophoresis using a 1.5% agarose gel in 1× TAE buffer (0.04 M Tris–acetate and 0.001 M EDTA). Gels were run at 60 V for 2 h and stained by soaking gel in a 1 µl/10 ml ethidium bromide solution for 15 min. ISSR banding patterns on gels were visualized using a photo-UV transilluminator system and recorded by digital photography. We obtained bands corresponding to dimensions of amplified fragments of approximately 100 bp to 3000 bp (Table 1). One hundred base pair ladders (DNA Molecular Weight Marker XIV, Roche®) were run for reference with each primer.

2.4. Statistical analyses of ISSR data

Since ISSR markers are interpreted as dominant diallelic markers, the dominant allele determines the presence of the band, namely *AA* and *Aa* individuals have the (1) phenotype, whereas *aa* individuals have the (0) phenotype. Absence of a band is interpreted as primer divergence, loss of a locus through deletions of

SSR site, or chromosomal rearrangement (Wolfe and Liston, 1998). The program AFLP-SURV (Veekmans, 2002) was used to obtain allelic frequencies at each marker locus in each sample and to estimate genetic diversity within and between samples. We carried out the analysis assuming Hardy–Weinberg equilibrium ($F_{IS}=0$). Furthermore, we assumed low ($F_{IS}=0.05$), moderate ($F_{IS}=0.10$) and high ($F_{IS}=0.25$) disequilibria, because heterozygote deficiency is very common in bivalves (reviewed in Zouros and Foltz, 1984; Gaffney et al., 1990). Based on the estimates of allele frequency, we used the Lynch and Milligan (1994) approach, which employs the average expected heterozygosity of the presumptive loci, or Nei's (1978) gene diversity, as measure of genetic diversity. We calculated heterozygosity (H) and Wright's fixation index (F_{ST}) using the Bayesian method with non-uniform prior distribution of allele frequencies (Zhivotovsky, 1999). The Bayesian method is supposed to give more accurate results with respect to other approaches, such as the square root method (Zhivotovsky, 1999). It provides the frequency of the null allele at each locus, extracted from the sample size and the number of individuals in the sample that lack the ISSR fragment. This approach estimates the distribution of allele frequencies based on the variation over loci of the frequencies of ISSR fragments in the sample. Genetic differentiation among samples and localities was tested using a permutation procedure with 10,000 pseudoreplicates. The null hypothesis is that there is no genetic differentiation among the groups.

Hierarchical relationships were estimated by analysis of molecular variance (AMOVA). Total genetic variation was partitioned into (i) between localities, (ii) among patches within localities, and (iii) within patches using AMOVAPREP (Miller, 1998) and WINAMOVA (Excoffier et al., 1992). Significance levels were calculated using randomisation tests. A null distribution was obtained by allocating each individual to randomly chosen populations and the variance components estimated from 10,000 permutations. This procedure eliminated the normality assumption required for the analysis of variance but which is inappropriate for molecular data (Excoffier et al., 1992). For Φ_{ST} (within patches), the variance component was tested by randomisations across all

patches; for Φ_{SC} (among patches), it was assumed that the localities are real but patches are not, so that randomisations occurred within localities; and for Φ_{CT} (among localities), it was assumed that the patches were real and the localities were artificial, so that randomisations of populations were made across localities. All statistical analyses were performed applying the method outlined by Lynch and Milligan (1994).

UPGMA consensus dendrograms of Nei's (1978) genetic distances between the six patches were constructed with the program TFPGA (Miller, 1997) on the basis of the four matrices obtained from the Bayesian analyses with four different prior distributions ($F_{IS}=0, 0.05, 0.10$ and 0.25). Nodes of the dendrogram were tested using bootstrapping with 10,000 replicates.

3. Results

The numbers and sizes of bands resolved per primer ranged from 11 to 15 and from 100 bp to 3000 bp, respectively (Table 1). We detected a total of 62 bands in the sample from Virginia and 58 in the sample from Maine, with nine and five locality-private bands, respectively (Table 2). The frequencies of the dominant alleles are reported in Table 3.

With the unrealistic hypothesis of Hardy–Weinberg equilibrium at the two localities, the values of heterozygosities were $H=0.184 \pm 0.018$ in the sample from Virginia, and $H=0.169 \pm 0.018$ in the sample from Maine (Table 4). With the assumption of increasing disequilibrium due to deficit of heterozygote individuals, the values of heterozygosity in-

Table 2
Gemma gemma: summary of ISSR products per patch and locality

Locality	Patch	Sample size	No. of bands	No. of unique bands
Virginia	A	30	46	2
	B	30	34	2
	C	30	36	1
	A+B+C	90	62	9
Maine	A	30	44	3
	B	30	36	–
	C	30	41	–
	A+B+C	90	58	5

Table 3

Gemma gemma: frequencies of dominant alleles estimated using Lynch and Milligan's (1994) method at all loci in all samples

		Primer UBC809													
bp	100	150	200	300	400	450	500	900	1300	1500	2500	2600	2800	3000	
Virginia-A	0.42	0.79	0.45	0.09	0.2	0.24	0	0.09	0.02	0	0.14	0	0	0	
Virginia-B	0	0.16	0.34	0.09	0	0.22	0.36	0.02	0	0	0	0	0	0	
Virginia-C	0	0.29	0.42	0.09	0	0.18	0.34	0	0	0	0	0	0	0	
Maine-A	0	0.12	0.24	0.24	0	0.39	0.03	0.51	0.03	0.03	0	0.02	0.03	0.02	
Maine-B	0.22	0.51	0.07	0.12	0.03	0.51	0.14	0.07	0	0	0.03	0.02	0	0	
Maine-C	0	0.05	0.22	0.39	0.07	0.14	0.05	0.48	0.14	0	0.05	0.07	0	0	

		Primer UBC811													
bp	100	120	150	200	300	400	450	500	900	1000	1100	1400	1500	2600	2700
Virginia-A	0	0.02	0.51	0.07	0.03	0	0.05	0.09	0.07	0.16	0.03	0.02	0.05	0.05	0
Virginia-B	0.05	0.02	0.51	0.02	0.22	0.12	0	0.16	0.02	0.02	0	0.05	0	0	0
Virginia-C	0	0.05	0.55	0	0.31	0.05	0.09	0.07	0.09	0	0	0.03	0.07	0	0
Maine-A	0	0	0	0.09	0.27	0.05	0	0.1	0.02	0.09	0	0	0.1	0.03	0.05
Maine-B	0	0	0	0.02	0	0	0	0.02	0	0.03	0	0	0	0.14	0.07
Maine-C	0.67	0.07	0.67	0.05	0	0	0	0.05	0	0.03	0.09	0	0.02	0	0.09

		Primer UBC827											
bp	150	200	220	300	450	500	600	700	1000	1100	1500	2600	2640
Virginia-A	0.73	0.45	0	0.09	0	0	0.51	0.02	0.58	0.03	0	0	0.51
Virginia-B	0.55	0.48	0.05	0	0	0.02	0.45	0	0	0.34	0	0	0
Virginia-C	0	0.62	0	0	0.18	0.24	0	0.05	0.02	0	0.39	0.36	0.03
Maine-A	0.39	0.34	0.07	0.07	0	0.09	0.03	0.24	0	0.62	0	0.12	0
Maine-B	0.39	0.45	0	0.05	0.05	0.02	0	0.34	0.05	0.48	0	0.03	0
Maine-C	0	0	1	0.16	0.73	0.05	0.09	0.1	0.05	0.45	0	0.18	0

		Primer SAS1									
bp	150	200	300	400	500	900	1000	1500	2500	2700	2900
Virginia-A	0	0.02	0.45	0.07	0.73	0	0.29	0.03	0.02	0	0
Virginia-B	0.36	0	0	0.79	0.79	0	0	0.31	0	0	0
Virginia-C	0	0	0.1	0	0	0.62	0	1	0.36	0.02	0.1
Maine-A	0.2	0	0.02	0.55	0.36	0.1	0	0	0.03	0.03	0
Maine-B	0	0	0.14	0	0.51	0	0.12	0	0.1	0.02	0
Maine-C	0.31	0	0.09	0	0.58	0	0.18	0	0	0	0

		Primer SAS3												
bp	100	150	200	250	300	450	500	600	1000	1100	1500	1600	2700	3000
Virginia-A	0.03	0.07	0.05	0.16	0.09	0.67	0.07	0.39	0.36	0.02	0	0.05	0	0
Virginia-B	0	0	0	0	0.02	0.03	0.48	0.18	0.67	0.24	0.07	0.18	0.05	0.27
Virginia-C	0	0	0.16	0.02	0.54	0.22	0.2	0.03	0	0.14	0	0	0.36	0
Maine-A	0	0.34	0.07	0.09	0.45	0.22	0	0.07	0.14	0	0	0.1	0.16	0
Maine-B	0	0	0.34	0.12	0.1	0.62	0	0.51	0.39	0	0	0.09	0.34	0
Maine-C	0	0	0.36	0.02	0.55	0.39	0.03	0.16	0.12	0.02	0	0.2	0	0

Approximate band sizes are in bold.

creased slightly (Table 4). A summary of the values of H calculated for each patch at the two localities is reported in Table 4.

The greatest portion of the genetic variation was found within patches (61.0%), and the variance among patches within localities accounted for most

Table 4

Gemma gemma: estimates of genetic diversity obtained by patch (suffix pat) and pooling patches within the two localities (suffix loc), applying the Lynch and Milligan (1994) approach to ISSR data

Locality	Patch	Deficit of heterozygotes	H_{pat}	H_{loc}	F_{STpat}	F_{STloc}
Virginia	A	Absent ($F_{IS}=0$)	0.170 ± 0.022	0.184 ± 0.018	$0.249 \pm 0.038^*$	$0.080 \pm 0.039^*$
	B		0.151 ± 0.023			
	C		0.152 ± 0.022			
Maine	A		0.159 ± 0.020	0.169 ± 0.018		
	B		0.143 ± 0.022			
	C		0.157 ± 0.021			
Virginia	A	Low ($F_{IS}=0.05$)	0.169 ± 0.023	0.186 ± 0.018	$0.263 \pm 0.039^*$	$0.083 \pm 0.041^*$
	B		0.149 ± 0.023			
	C		0.150 ± 0.023			
Maine	A		0.158 ± 0.021	0.171 ± 0.019		
	B		0.142 ± 0.023			
	C		0.155 ± 0.021			
Virginia	A	Moderate ($F_{IS}=0.10$)	0.170 ± 0.023	0.190 ± 0.019	$0.279 \pm 0.046^*$	$0.085 \pm 0.040^*$
	B		0.150 ± 0.023			
	C		0.151 ± 0.023			
Maine	A		0.160 ± 0.021	0.174 ± 0.019		
	B		0.144 ± 0.024			
	C		0.156 ± 0.021			
Virginia	A	High ($F_{IS}=0.25$)	0.172 ± 0.022	0.200 ± 0.019	$0.291 \pm 0.041^*$	$0.094 \pm 0.039^*$
	B		0.153 ± 0.024			
	C		0.155 ± 0.023			
Maine	A		0.167 ± 0.022	0.183 ± 0.019		
	B		0.148 ± 0.023			
	C		0.160 ± 0.021			

Bayesian with non-uniform prior distribution method (see Materials and methods) was applied to calculate heterozygosity (H) and Wright's fixation index (F_{ST}).

* $p < 0.001$ by permutation tests with 10,000 pseudoreplicates.

of the remaining molecular variance (36.7%). The amount of variance found between localities was substantially lower (2.3%) (Table 5). The Φ -statistics revealed significant molecular differentiation within patches and among patches within localities ($\Phi_{ST} = 0.390$ and $\Phi_{SC} = 0.376$, both $p < 0.001$), but not among localities ($\Phi_{CT} = 0.023$, $p = 0.196$) (Table 5), indicating that genetic heterogeneity was present at small spatial scales.

Assuming Hardy–Weinberg equilibrium and the three degrees of disequilibrium considered, F_{ST} values showed no evidence of genetic differentiation between localities (Table 4). In contrast, F_{ST} values estimated among patches were substantially higher (Table 4). All the F_{ST} values were significantly greater than zero by probability tests ($p < 0.001$).

For each assumed F_{IS} value, we constructed a dendrogram based on Nei's (1978) genetic distances

Table 5

Gemma gemma: results of hierarchical analysis of molecular variance (AMOVA) derived from the cluster analysis computed from the distance matrix constructed using the formula of Excoffier et al. (1992)

Source of variation	df	MS	Variance component	Percentage of variance	Φ -statistics	p
Between Maine and Virginia	1	132.217	0.22098765	2.29	$\Phi_{CT} = 0.023$	0.1958
Among patches within localities	4	112.328	3.54773946	36.71	$\Phi_{SC} = 0.376$	<0.0001
Within patches	174	5.896	5.89559387	61.00	$\Phi_{ST} = 0.390$	<0.0001

p -values, calculated from a random permutation test (10,000 replicates), and Φ -statistics represent the probability of obtaining by chance alone a more extreme variance than the observed values (Excoffier et al., 1992).

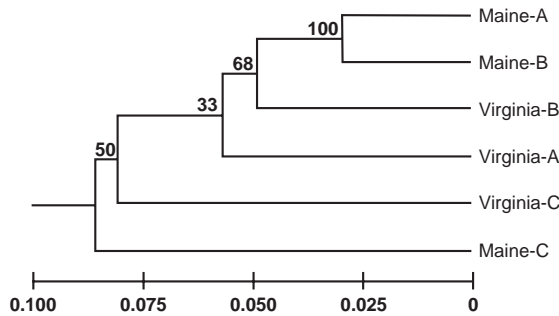


Fig. 1. *Gemma gemma*. UPGMA consensus dendrogram of Nei's (1978) genetic distances between patches. Bootstrap values were obtained after 10,000 replicates.

between patches. Genetic distances were greater with increasing F_{IS} values, but the topologies of the four dendrograms were identical (data not shown), hence we reported only the dendrogram obtained with $F_{IS}=0.10$. The dendrogram showed inconsistency in geographic separation among patches (Fig. 1). Bootstrap support was low to moderate, with the exception of the high value relative to the node linking patches A and B in Maine (Fig. 1).

4. Discussion

Our work represents the first application of ISSRs to a marine invertebrate. Technical results of the present study provide evidence for the reliability and usefulness of ISSR markers, especially in defining fine-scale population genetic substructuring. A result that stands out from the present study was the considerable small-scale genetic heterogeneity in *G. gemma* at both within- and among-patch levels. By contrast, little additional large-scale genetic variation was detected. Nine private bands in samples from Virginia and five in those from Maine (Tables 2 and 3) were not sufficient to significantly differentiate the two localities because, with a few exceptions, dominant alleles had very low frequencies (Table 3). The pooling of patches with high within-patch and between-patch genetic heterogeneity decreased the observed fixation indices (F_{ST} and Φ_{CT}) between Maine and Virginia. The pooling of a set of highly divergent samples at each locale may account for the absence of significance of these two parameters. In fact, long distance gene flow should produce a decrease in the number of private bands in both

Maine and Virginia and not an increase in private bands, as observed. Consistent with our results obtained at the larger spatial scale, a high degree of genetic homogeneity was found in a preliminary study employing sequences of the mitochondrial DNA Cytochrome Oxidase I gene in samples from seven sites from Maine to Virginia (K. Holland, J. Commito, A. Dickerson and T. Losch, unpublished data). These investigators observed very low levels of genetic differentiation, with only 0.3–0.5% nucleotide sequence variation between sites.

Strathmann and Strathmann (1982) argued that brooding is a life-history strategy of small-size benthic species because as adults they can be easily dispersed and thus gain little advantage from planktonic larvae. This strategy is adopted because planktonic larvae suffer possible disadvantages. They include dispersal away from favourable habitat, mismatches between larval and adult physiological tolerances, greater susceptibility to environmental stresses, predation, and various costs that may be associated with metamorphosing in response to specific chemical cues and postponing metamorphosis in the absence of those cues (Pechenik, 1999). Furthermore, it has been hypothesised that dispersal among populations of *Abra tenuis*, a small-size aplanic bivalve ecologically similar to *G. gemma*, may occur by means of transport by water currents and transport by waterfowl (Holmes et al., 2004). Therefore, a degree of medium-to-large scale gene flow cannot be a priori excluded in *G. gemma*.

The sensitivity of the ISSRs to detect genetic diversity among *G. gemma* individuals accounts for the absence of identical genotypes across all individuals analysed. The hypervariability of the markers allowed the detection of fine-grained spatial genetic structure, with high within- and among-patch genetic heterogeneity. Patches were differentiated quite clearly from each other, but this degree of differentiation was not consistent with their close geographic proximity. Similar structures were referred to as "chaotic genetic patchiness" in the limpet *Siphonaria jeaneae* (Johnson and Black, 1982; 1984) and in the bivalve *Spisula ovalis* (David et al., 1997). Our results suggest that, locally, the genetic structure of *G. gemma* can be influenced by the occurrence of a mosaic constituted by groups of sibs. However, the presence of a number of diverse genotypes within the patches suggests that

passive dispersal plays a role at local spatial scale. But the simple pattern described is further complicated because there are other factors that may have caused the observed fine-scale genetic heterogeneity, such as temporal variation in the abundance, distribution and paternal genetic component of newly released juveniles (Botton, 1984; Weinberg, 1985, 1989; Commito et al., 1995). Mortality of juveniles could also affect the genetic patchiness, as heavy local mortality could result from particular conditions of weather, tides or predation. Furthermore, patterns of heterogeneity resulting from localised recruitment are ephemeral. Each genetic patch will fade as old cohorts die and later batches of new females, some of which will immigrate from different sites, produce new mosaics of genotypes.

The present study opens the way to further research that should (i) focus on the fine-scale genetic structure in locations with different rates of passive dispersal resulting from dissimilar hydrodynamic regimes; (ii) consider sampling in different periods of the same year in order to assess the local fine-scale temporal dynamics; and (iii) enlarge the study area in order to give insight on the long-distance gene flow (if any) and assess the possible presence of major biogeographic discontinuities with genetic breaks or the presence of large-scale isolation by distance.

5. Conclusion

Results of the present study are in contrast with the generalisation that aplanic and anchiplanic species, in genetic terms, have locally homogeneous but globally heterogeneous populations, whilst actaeplanic and teleplanic species have locally heterogeneous but globally homogenous populations (for examples see Gooch et al., 1972; Gaines et al., 1974; Palumbi and Kessing, 1991; Hunt, 1993; Huang et al., 2000; Holmes et al., 2004). In addition, our results cannot reject the hypothesis that, if body size is relatively small, passive transport of adult benthic aplanic and anchiplanic invertebrates of low mobility may be a means of long-distance dispersal (Strathmann and Strathmann, 1982; Johannesson, 1988). From a population genetic perspective, passive dispersers without free-swimming larvae may be characterised by large-scale genetic patterns similar to those of most of species with medium- to long-lived planktonic larvae.

Acknowledgments

We thank many members of the Commito family for assistance in the field. We also wish to thank Giorgio Binelli and two anonymous reviewers, whose comments greatly improved the quality of the manuscript. This project was funded in part by a Gettysburg College Research and Professional Development Grant to JAC and University of Pisa Research Grant to AC. [SS]

References

- Abbot, P., 2001. Individual and population variation in invertebrates revealed by Inter-simple Sequence Repeats (ISSRs). *J. Insect Sci.* (<http://www.insectscience.org/1.8>).
- Avise, J.C., 1994. *Molecular Markers, Natural History and Evolution*. Chapman and Hall, New York.
- Bertorelle, G., Bruford, M., Chemini, C., Vernesi, C., Hauffe, H.C., 2004. New, flexible Bayesian approaches to revolutionize conservation genetics. *Conserv. Biol.* 18, 584.
- Bornet, B., Branchard, M., 2001. Nonanchored Inter Simple Sequence Repeat (ISSR) markers; reproducible and specific tools for genome fingerprinting. *Plant Mol. Biol. Rep.* 16, 139–146.
- Botton, M.L., 1984. Spatial distribution of three species of bivalves on an intertidal flat: the interaction of life-history strategy with predation and disturbance. *Veliger* 26, 282–287.
- Bradley, W.H., Cooke, P., 1959. Living and ancient populations of the clam *Gemma gemma* in a Maine coast tidal flat. *Fish. Bull.* 137, 305–334.
- Carlton, J.T., 1992. Introduced marine and estuarine molluscs of North America: an end-of-the-20th-century perspective. *J. Shellfish Res.* 11, 489–505.
- Chatterjee, S.N., Mohandas, T.P., 2003. Identification of ISSR markers associated with productivity traits in silkworm, *Bombyx mori* L. *Genome* 46, 438–447.
- Chatterjee, S.N., Mohandas, T.P., Taraphdar, T., 2003. Molecular characterization of the gene pool of *Exorista sorbillans* (Diptera: Tachinidae) a parasitoid of silkworm, *Bombyx mori*, in India. *Eur. J. Entomol.* 100, 195–200.
- Commito, J.A., Currier, C.A., Kane, L.R., Reinsel, K.A., Ulm, I.M., 1995. Dispersal dynamics of the bivalve *Gemma gemma* in a patchy environment. *Ecol. Monogr.* 65, 1–20.
- David, P., Perdieu, M.-A., Pernot, A.-F., Jarne, P., 1997. Fine-grained spatial and temporal population genetic structure in the marine bivalve *Spisula ovalis*. *Evolution* 51, 1318–1322.
- Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA data. *Genetics* 131, 479–491.
- Gaffney, P.M., Scott, T.M., Kohen, R.K., Diehl, W.J., 1990. Interrelationships of heterozygosity, growth rate and heterozygote deficiency in the coot clam, *Mulinia lateralis*. *Genetics* 124, 687–699.

- Gaines, M.S., Caldwell, J., Vivas, A.M., 1974. Genetic variation in the mangrove periwinkle, *Littorina angulifera*. Mar. Biol. 27, 327–332.
- Gooch, J.L., Smith, B.S., Knupp, D., 1972. Regional survey of gene frequencies in the mud snail *Nassarius obsoletus*. Biol. Bull. 142, 36–48.
- Haig, S.M., Mace, T.R., Mullins, D., 2003. Parentage and relatedness in polyandrous comb-crested jacanas using ISSR. J. Heredity 94, 302–309.
- Hassan, M., Harmelin-Vivien, M., Bonhomme, F., 2003. Lessepsian invasion without bottleneck: example of two rabbitfish species (*Siganus rivulatus* and *Siganus luridus*). J. Exp. Mar. Biol. Ecol. 291, 219–232.
- Havenhand, J.N., 1995. Evolutionary ecology of larval types. In: McEdward, L.R. (Ed.), Ecology of Marine Invertebrate Larvae. CRC Press, New York, pp. 79–122.
- Holmes, S.B., Dekker, R., Williams, I.D., 2004. Population dynamics and genetic differentiation in the bivalve mollusc *Abra tenuis*: aplanic dispersal. Mar. Ecol. Prog. Ser. 268, 131–140.
- Huang, B.X., Peakall, R., Hanna, P.J., 2000. Analysis of genetic structure of blacklip abalone (*Haliotis rubra*) populations using RAPD, minisatellite and microsatellite markers. Mar. Biol. 136, 207–216.
- Hunt, A., 1993. Effects of contrasting patterns of larval dispersal on the genetic connectedness of local populations of two intertidal starfish, *Patriella calcar* and *P. exigua*. Mar. Ecol. Prog. Ser. 92, 179–186.
- Kostia, S., Ruohoen-Letho, M., Vainola, R., Varvio, S.L., 2000. Phylogenetic information in inter-SINE and inter-SSR fingerprints of the Arctiodactyla and evolution of the Bov-tA SINE. Heredity 84, 37–45.
- Johannesson, K., 1988. The paradox of Rockall: why is a brooding gastropod (*Littorina saxatilis*) more widespread than one having a larval dispersal stage (*L. littorea*)? Mar. Biol. 99, 507–513.
- Johnson, M.S., Black, R., 1982. Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. Mar. Biol. 70, 157–164.
- Johnson, M.S., Black, R., 1984. Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution 38, 1371–1383.
- Luque, C., Legal, L., Staudter, H., Gers, C., Wink, M., 2002. ISSR (Inter Simple Sequence Repeats) as genetic markers in Noctuids (Lepidoptera). Hereditas 136, 251–253.
- Lynch, M., Milligan, B.G., 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3, 91–99.
- Miller, M.P., 1997. Tools for population genetic analyses (TFPGA 1.3): a windows program for the analysis allozyme and molecular population genetic data. Computer software distributed by the author at <http://bioweb.usu.edu/mpmbio/index.htm>.
- Miller, M.P., 1998. AMOVA-PREP 1.01: a program for the preparation of the AMOVA input files from dominant-marker raw data. Computer software distributed by the author at <http://bioweb.usu.edu/mpmbio/index.htm>.
- Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.
- Palumbi, S.R., Kessing, B., 1991. Population biology of trans-arctic exchange: mtDNA sequence similarity between Pacific and Atlantic sea urchins. Evolution 45, 1790–1805.
- Pechenik, J.A., 1999. On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Mar. Ecol. Prog. Ser. 177, 269–297.
- Schneider, F.I., Mann, K.H., 1994. Rapid recovery of fauna following simulated ice rafting in Nova Scotian seagrass bed. Mar. Ecol. Prog. Ser. 78, 57–70.
- Sellmer, G.P., 1967. Functional morphology and ecological life history of the gem clam, *Gemma gemma* (Eulamellibranchia: Veneridae). Malacologia 5, 137–233.
- Shoemaker, J.S., Painter, I.S., Weir, B.S., 1999. Bayesian statistics in genetics. Trends Genet. 15, 354–358.
- Strathmann, R.R., Strathmann, M.F., 1982. The relationship between adult size and brooding in marine invertebrates. Am. Nat. 119, 91–101.
- Vekemans, X., 2002. AFLP-SURV version 1.0. Distributed by the Author at <http://www.ulb.ac.be/sciences/lagev/aflp-surv.html>. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.
- Weinberg, J.R., 1985. Factors regulating population dynamics of the marine bivalve *Gemma gemma*: intraspecific competition and salinity. Mar. Biol. 86, 173–182.
- Weinberg, J.R., 1989. Predicting population abundance and age structure: testing theory with field data. Mar. Ecol. Prog. Ser. 53, 59–64.
- Wolfe, A.D., Liston, A., 1998. Contributions of PCR-based methods to plant systematics and evolutionary biology. In: Soltis, D.E., Soltis, P.S., Doyle, J.J. (Eds.), Plant Molecular Systematics, vol. II. Chapman and Hall, New York, pp. 43–86.
- Wolfe, A.D., Xiang, Q.-Y., Kephart, S.R., 1998. Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable inter simple sequence markers. Mol. Ecol. 7, 1107–1125.
- Zhivotovsky, L.A., 1999. Estimating population structure in diploids with multilocus dominant DNA markers. Mol. Ecol. 8, 907–913.
- Zietkiewicz, E., Rafalsky, A., Labuda, D., 1994. Genome fingerprinting by Inter-Simple Sequence Repeat (ISSR)-anchored polymerase chain reaction amplification. Genomics 20, 176–183.
- Zouros, E., Foltz, D.W., 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. Malacologia 25, 583–591.