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Genetic and morphological variation in four populations of the surf clam *Donax serra* (Röding) from southern African sandy beaches

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Abstract

The surf clam *Donax serra* (Bivalvia, Donacidae) dominates sandy-beach communities of two southern African biogeographical regions, a cold (Benguela current) and warm province (Agulhas current). Morphometric and behavioural differences led to a controversial discussion of whether or not populations from the two provinces belong to the same species. Shell size measurements confirmed morphological differences: clams from the cold province were significantly rounder, flatter and less wedge-shaped than clams from the warm province. In this study a genetic approach was used to relate phenotypic differences to genetic variability of four populations of *D. serra* separated by up to 2500 km of shoreline. Genetic analysis of 22 protein-coding loci was carried out by starch-gel electrophoresis. Populations studied are conspecific (genetic distances range from 0.003 to 0.044) and possess genetic variation (alleles per locus: 1.73–1.91; mean heterozygosity: 18–22%; percentage polymorphism: 45.5–59.1%) in the range of most other marine bivalves, which allows for potential adaptation to environmental changes. Wright's fixation indices show little to moderate genetic divergence among the subpopulations relative to the limiting amount under complete fixation (F_{ST} = 0.016–0.089), moderate divergence of individuals relative to the total population (F_{IS} = 0.265–0.452), and comparably high divergence of individuals relative to the compound population (F_{IT} = 0.300–0.473). The effective number of individuals exchanged between populations in each generation is high enough (1.44–8.65) to counteract genetic drift. We propose that the observed differences represent phenotypic plasticity enabling this species to inhabit

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different biogeographic regions. Gene flow, balanced selective pressure and evolutionary inertia are discussed as explanations for similarities of the two outlying populations. The substantial subdivision of the two Namibian populations indicates a potential biotic barrier and requires separate studies of the population dynamics.
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Keywords: Allozyme electrophoresis; Biogeography; *Donax serra*; Evolutionary inertia; Morphology; Phenotypic plasticity; Population genetics; Southern Africa

1. Introduction

The surf clam *Donax serra* (Röding, 1798) is the largest-sized member of the genus Donacidae and inhabits the intertidal and upper subtidal zone of extended and exposed sandy beaches along the Namibian and South African coast. Biomass reaches 8500 g shell free dry mass per metre shoreline. Clams are exploited for bait and food and might be a valuable aquaculture species with their unique and delicate taste showing rapid growth and high densities (Donn, 1987; McLachlan, 1996). Based on an economic evaluation Sims-Castley and Hosking (2003) calculated a possible price range between US$6.50–60.00/kg for export markets. Morphological studies on Donacidae indicated high interspecific (Ansell, 1985; Nelson et al., 1993; McLachlan et al., 1995) and intraspecific (Wade, 1967; Nelson et al., 1993) variation. Regarding *D. serra* populations from the West and the South-east Coast, it is not clear whether all clams belong to the same species ('population' in this paper refers to all animals inhabiting the geographic location without any implication of reproductive connections). Shell morphology differs significantly (Donn, 1990a; Soares et al., 1998) and colour differences of soft tissue are common between coasts (Donn and Els, 1990). Additional behavioural dissimilarity was found between adults and juveniles inhabiting different beach zones (Donn, 1990b; Soares et al., 1998). These differences may indicate that the populations belong to different (sub)species or stocks. They have been associated with habitat differences (Donn, 1990a) and directional selection with microevolutionary changes maintained by geographical isolation (Soares et al., 1998). However, significant differences in sperm morphology (van der Horst et al., 1986) and growth rates (Schoeman, 1997) were not detected. Further, culturing of larvae from crosses was successful (H. van der Horst, personal communication fide Donn, 1990a) supporting the hypothesis that populations are closely related.

Genetic analysis may not only clarify the phylogenetic relationships, but also provides an estimate to analyse intraspecific larval dispersal between regions and the dependence of recruitment on local stocks. Levinton and Suchanek (1978) postulate that high gene flow occurs in species with planktonic larvae resulting in high levels of genetic variation provided that the populations import reconfigured genes. If populations belong to the same species, if large amounts of variation had occurred in the founder population, and if the recent populations are not isolated, we expect the northern population to be genetically more connected due to larval import by the

northerly surface-flow of the Benguela current. Conversely, we expect South-east Coast populations to be most divergent due to restricted genetic input. Our study examines biometric parameters of populations of *D. serra* along the southern African coastline to confirm morphological differences. In order to relate these variations to genetic determination we analysed enzymatic proteins with starch-gel electrophoresis in order to: (1) compare genotypic variation within the populations with expectations for a sexually reproducing species mating randomly under free recombination of genes; (2) find partition allelic variation among the different populations in order to infer the levels and patterns of gene flow; and (3) determine if there is an association between allelic and morphological variation, possibly linked to environmental parameters.

2. Material and methods

2.1. Study areas and sampling

Four exposed sandy beaches of two different biogeographic provinces, two Namibian (Langstrand, 22°47′S, 14°33′E; Meob Bay, 24°38′S, 14°43′E) and two South African beaches (Bloubergstrand, 33°51′S, 18°09′E; Maitlands in St Frances Bay 34°6′S, 25°13′E) were selected (Fig. 1). For detailed descriptions of the habitats see McLachlan (1986) (Langstrand, I), Holtzhausen (1999) (Meob Bay, II), Soares et al. (1996) (Bloubergstrand, III) and Schoeman (1997) (Maitlands, IV).

Due to coastal upwelling the water temperature of the Benguela current is 13 °C

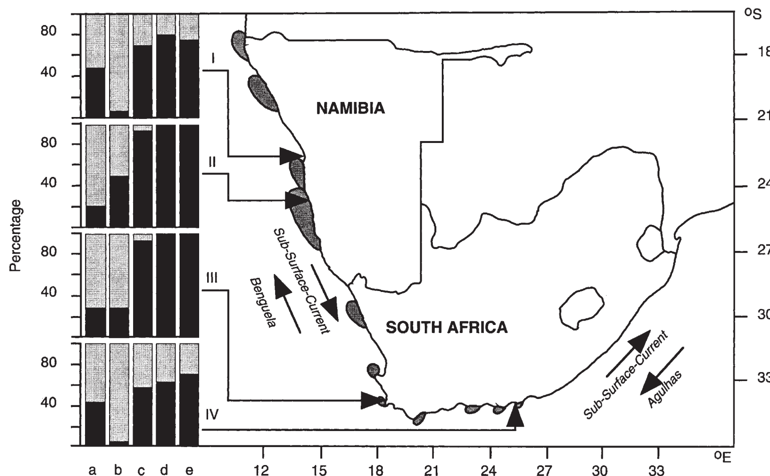

Fig. 1. Study sites along the southern African coast: (I) Langstrand; (II) Meob Bay; (III) Bloubergstrand; and (IV) Maitlands. Principal upwelling cells (stippled areas) in the Benguela and Agulhas and the subsurface-currents are included (Shannon and Nelson, 1996). Allozyme variation is shown on the left side (a, EST-2; b, PEP-B2; c, PEP-C2; d, PGM-1; e, PGM-2), bars indicate percentage of A (black) and B (grey) allele. Note the related pattern of the two outlying in contrast to the two intermediate populations.

on average (Walker et al., 1984). Sea surface temperature (SST) for Site I decreases to about 12 °C in winter and rises to 23 °C in summer with seasonal means at Walvis Bay of 16 °C (summer/autumn) and 14 °C (winter/spring) (Shannon, 1985). The SST at Site II is annually 13.5 °C ranging between 10 and 19.5 °C (summer/autumn: 15 °C, winter/spring: 12 °C) (C. Bartholomae, unpublished data). Site III is exposed to even lower temperatures with an annual mean of 13 °C (8–14 °C in summer and 11–17 °C in winter) (Walker et al., 1984). Site I and III are in a high-energy intermediate morphodynamic state (Soares et al., 1996; Schoeman, 1997; Laudien et al., 2001), whereas Site II is in a reflective state with waves breaking almost directly in the intertidal zone (J. Laudien, personal observation). All three are, however, open ocean beaches exposed to continuous wave action and subject to subequal semidiurnal tides with a maximum tide range of about 2 m (springs average 1.4 m, neaps 0.7 m) (McLachlan, 1986).

Site IV (Maitlands) is located at the South-east Coast and is influenced by the south-eastwards flowing Agulhas current. At the Agulhas Bank it is deflected southwards (Brown and Jarman, 1978). The mean annual SST is 22 °C (15–17 °C in winter, 26 °C in summer) (Ansell and McLachlan, 1980). Sporadically this region may, however, experience coldwater upwelling during strong eastern winds in summer (Goschen and Schumann, 1995) albeit with a much lesser frequency than at the West Coast (Shannon, 1985). The beach is in a high-energy intermediate to dissipative morphodynamic state (McLachlan, 1990; Soares et al., 1998).

Sampling was done during spring low water in March 1999. A total of 32 adult *D. serra* were collected randomly within a 50 m stretch at each sample site. Surf clams were transported to the laboratory alive. Holotypes are deposited in the Research Institute and Natural History Museum Senckenberg, Frankfurt, Germany (SMF 323605/1) and the National Museum of Namibia, Windhoek, Namibia (SMN 77104).

2.2. Morphometric and analytic methods

Four morphological variables were measured according to Soares et al. (1998): shell length (anterior–posterior), height (ventro–dorsal) and width (left–right) to 0.01 cm with vernier callipers. Additional wet mass (including shell) was recorded to the nearest 0.01 g. Thereafter clams were frozen at −25 °C and extracts were prepared by homogenisation of the body (except stomachs in order to exclude interference from nutrition) in an equal volume of distilled water.

Allozymes were analysed by starch-gel electrophoresis (12% potato starch, Sigma Chemicals Co.), using the electrophoretic procedures, method of interpretation of gel banding patterns and locus nomenclature in van der Bank et al. (1992). Loci were numbered from the anodal end of the gel. Gels were stained for allozymes listed in Table 1. Aspartate transaminase (ATA, 2.6.1.1), alcohol dehydrogenase (ADH, 1.1.1.1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1.2.1.12), glycerol-3-phosphate dehydrogenase (G3PDH, 1.1.1.6), hexokinase (HK, 2.7.1.1), L-iditol dehydrogenase (IDDH, 1.1.1.14), L-lactate dehydrogenase (LDH, 1.1.1.27), malate dehydrogenase (MDH, 1.1.1.37), 6-phosphogluconate dehydrogenate (PGDH,

Table 1
Locus abbreviations, enzyme commission numbers (EC no.) and buffers providing the best results

Protein	Locus	EC no.	Buffer
Adenylate kinase	AK-1	1.1.1.1	TC–EDTA
Creatine kinase	CK-1[a]	2.7.3.2	MF
Esterase	EST-1, -2	1.2.1.12	MF
Glucose-6-phosphate isomerase	GPI-1	5.3.1.9	RW
Isocitrate dehydrogenase	IDH-1	1.1.1.27	TC
NADP-dependent malate dehydrogenase	MDHP-1	1.1.1.38	MF
Mannose-6-phosphate isomerase	MPI-1[a], -2	5.3.1.8	MF
Peptidase		3.4.-.-	
Substrate			
Glycyl-L-leucine (GL)	PEP-A1[a]		MF
L-Leucylglycyl-glycine (LGG)	PEP-B1, -B2		MF
Leucyl-DL-alanyl (LA)	PEP-C1, -C2		MF
L-phenylalanyl-L-proline (PHP)	PEP-D1		MF
L-leucine-β-naphthylamide (CAP/LAP)	PEP-E1	3.4.11.1	LiOH
L-leucyl-tyrosine (LT)	PEP-S1		MF
Phosphoglycomutase	PGM-1, -2	5.4.2.2	RW
General protein	PROT-1[a]		RW
Superoxide dismutase	SOD-1[a], -2[a]	1.15.1.1	LiOH

See Section 2 for abbreviations of buffers.
[a] Monoallelic loci.

1.1.1.44), AK-2 and IDH-2 were also stained for, but showed insufficient activity for interpretation.

The following buffers were used to separate the enzymes: (1) MF—a continuous Tris, boric acid and EDTA buffer system (pH 8.6) (Markert and Faulhaber, 1965); (2) RW—a discontinuous Tris, citric acid (gel pH 8.7), lithium hydroxide and boric acid (electrode pH 8.0) buffer (Ridgway et al., 1970); (3) TC—a continuous Tris and citric acid buffer system (pH 6.9) (Whitt, 1970); (4) LiOH—a discontinuous lithium hydroxide, boric acid (gel pH 8.4), additionally Tris and citric acid monohydrate (electrode pH 8.3) buffer (Selander et al., 1971); and (5) TC–EDTA—a continuous Tris and EDTA buffer (pH 9.6) (Harris and Hopkinson, 1976) (Table 1).

2.3. Statistics

Electrophoretic results were analysed using BIOSYS-I (Swofford and Selander, 1981). Descriptive statistics were calculated for each population including percentage polymorphic loci (P), mean number of alleles per locus (A) and average heterozygosity (H) according to Nei (1978). To assess statistical significance of allele class deviations from expected numbers of heterozygotes and homozygotes and χ^2 analyses according to Levene (1949) (adapted for small samples) were done. Results were adjusted for the number of tests performed with the Bonferroni technique (Lessios, 1992). For polymorphic loci, allele classes among populations were compared using contingency χ^2 analysis. Allele frequency differences were integrated across loci by calculating genetic distances for all pairs of populations (Nei, 1978; Wright, 1978) using BIOSYS-I. Different fixation indices were used to analyse genetic divergence between populations (Wright, 1978), where F_{IT} and F_{IS} are the fixation indices of individuals relative to the total population and its subpopulation respectively, and F_{ST} measures the amount of divergence among subpopulations relative to the limiting amount under complete fixation. To apply F-statistics the four populations were considered as subsamples of a hypothetical compound population resulting from the sum of all analysed specimens. F_{IS} values were tested according to the method of Waples (1987):

$$\chi^2 = F_{IS}^2 \cdot N \cdot (k-1); \quad \mathrm{DF} = k \cdot \frac{(k-1)}{2} \tag{1}$$

where N is the total number of individuals and k is the number of alleles at the locus. Significant differences (5% level) of F_{IT} values were tested according to a bootsrapping method implemented in the program ‘Genetic Data Analysis’ (Lewis and Zaykin, 2001).

F_{ST} values were tested according to Workman and Niswander (1970):

$$\chi^2 = 2NF_{ST}(k-1); \quad \mathrm{DF} = (k-1)(s-1) \tag{2}$$

where N is the total number of specimens, k the number of alleles per locus, and s is the number of populations. For an essentially one-dimensional array of sampling sites such as described in this study Wright’s (1969) ‘island model’ may be used to express approximate equilibrium levels of gene flow (expressed as the effective num-

ber of migrants per generation, N_{EM}) within a structured population. Pairwise N_{EM} values were estimated from F_{ST} according to Takahata (1983):

$$N_{EM} = \frac{(1/F_{ST})-1}{4\alpha} \quad (3)$$

where N_E is the effective population size, M the number of migrants per generation, $\alpha = [n/(n-1)]^2$ and n is the total number of populations. The genetic distances, D (standard; Nei, 1972) and D_{78} (unbiased and adapted for small sample sizes; Nei, 1978) were calculated between populations.

3. Results

3.1. Morphometrical comparisons between populations of *D. serra*

Morphological comparisons between width vs. height (*W*/*H*) and height vs. length (*H*/*L*) revealed a significant difference in shell shape (Kruskal–Wallis, *W*/*H*: $p < 0.001$, DF = 3; *H*/*L*: $p < 0.001$, DF = 3). West Coast clams were rounder, flatter and less wedge-shaped than South-east Coast clams. Fig. 2 presents *W*/*H* plotted against *H*/*L*; population means of *W*/*H* and *H*/*L* (filled symbols) are (I) 0.68 and 0.55, (II) 0.67 and 0.54, (III) 0.68 and 0.54, (IV) 0.63 and 0.59, respectively.

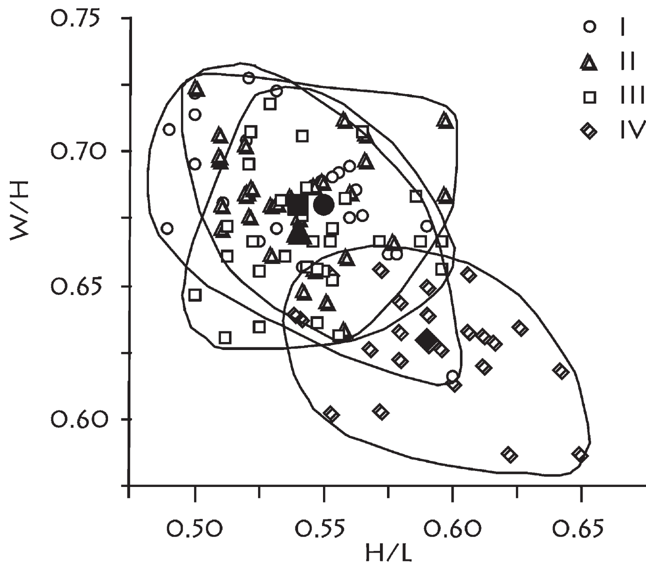

Fig. 2. Shell shape variability of four *D. serra* populations from the West Coast (I–III) and the South-east Coast (IV) of southern Africa. Width to height ratios (*W*/*H*) are plotted against height to length ratios (*H*/*L*), means of populations are given as filled symbols.

3.2. Allozyme variation

Twenty-two protein-coding loci provided interpretable results. Locus abbreviations, enzyme commission numbers, buffers used giving the most satisfactory results and monoallelic loci are presented in Table 1. Products of all protein-coding loci migrated anodally. Significant (χ^2, $p < 0.05$) deviations of allele distributions occurred between populations I and II at seven (53.8%) loci (EST-2, PEP-B2, PEP-C1, -2, PEP-E1, PGM-1, -2); between populations I and III at five (31.25%) loci (PEP-B2, PEP-C1, -2, PGM-1, -2); between populations I and IV at five (33.3%) loci (MPI-2, PEP-C1, PEP-E1, PEP-S1, PGM-1); between populations II and III at two loci (15.4%) (PEP-B2, PEP-E1); between populations II and IV at eight (57.1%) loci (EST-2, MPI-2, PEP-B2, PEP-C1, -2, PEP-S1, PGM-1, -2); and between populations III and IV also at eight (57.1%) loci (MPI-2, PEP-B2, PEP-C1, -2, PEP-E1, PEP-S1, PGM-1, -2). Only the two intermediate populations did not deviate significantly when all loci were taken into consideration. Table 2 presents the genotypes, coefficients of heterozygote deficiency (negative values) or excess, degrees of freedom, the individual heterozygosity values (h) and sample sizes at all polyallelic loci. The h values ranged from 0.031 to 0.728. Significant ($p < 0.05$) genotypical deviations from Hardy–Weinberg proportions are indicated in Table 2. Allele classes at MPI-2 deviated in all populations, PEP-B2 and -E1 in I and III, EST-2, PEP-S1 and PGM-2 in I and IV, PEP-C1 in I, II and IV, PEP-C2 and PGM-1 only in I, PEP-B1 in I, II and III and PEP-D1 only in III.

Within populations genotypic frequencies at five of the polymorphic loci (31.25%) (AK-1, EST-1, GPI-1, IDH-1, MDHP-1) closely approximated Hardy–Weinberg expectations, while the other polymorphic loci showed deficiencies of heterozygotes (Table 2). Between populations, significant ($p < 0.05$) deviations of allele distributions occurred at nine (56.25%) of the 16 polyallelic loci, namely EST-2, MPI-2, PEP-B2, -C1, -C2, -E1, -S1, PGM-1 and -2. Rare alleles for the AK-1 locus occurred in population III (C alleles) and population IV (A alleles) where the other two populations were fixed for B alleles. Rare B alleles were found in I and II at EST-1 where the other two populations were monoallelic. In contrast, rare B alleles were found at the MDHP-1 locus for III and IV, whereas the two Namibian populations were monomorphic. GPI-1 was fixed only in III and IDH-1 was only polyallelic in I. Three alleles were detected at I and IV, and two alleles in the other two populations at MPI-2. The outlying populations were fixed at PEP-D^b, whereas III had two and II three alleles. Populations II and III were fixed for PGM-1 and -2, whereas II and III were polyallelic. The mean number of alleles per locus (A) 1.91 ± 0.21 (I), 1.73 ± 0.21 (II), 1.77 ± 0.21 (III) and 1.86 ± 0.19 (IV) was not significantly different among populations (Kruskal–Wallis test, $n = 22$, $H = 0.784$, $p > 0.05$). The mean heterozygosity per locus (H), 0.223 ± 0.057 (I), 0.182 ± 0.056 (II), 0.198 ± 0.057 (III) and 0.211 ± 0.052 (IV) was also not significantly different (Kruskal–Wallis test, $n = 22$, $H = 0.526$, $p > 0.05$). The percentage of polyallelic loci (P) (0.95 criterion) was 45.5 (I), 36.4 (II), 40.9 (III) and 45.5% (IV), respectively.

Table 2
Genotypes, heterozygote deficiency (indicated with −) or excess from Hardy-Weinberg proportions (*d*), chi-square (χ^2) values, degrees of freedom (DF) and individual heterozygosities (*h*) for all polyallelic loci and sample size (*N*)

Locus	Population	Genotypes										*d*	χ^2	DF	*h*	*N*
		AA	AB	AC	AD	BB	BC	BD	CC	CD	DD					
AK-1	I					24										24
	II					16										16
	III					15	1					0	0	1	0.061	16
	IV		1			31						0	0	1	0.031	32
EST-1	I	29	2									0.017	0.017	1	0.062	31
	II	30	2									0.016	0.016	1	0.061	32
	III	32														32
	IV	29														29
EST-2	I	7	7			10						−0.420	4.419*	1	0.492	24
	II	1	10			19						0.024	0.019	1	0.320	30
	III	2	8			11						−0.089	0.178	1	0.408	21
	IV	6	6			9						−0.431	4.096	1	0.490	21
GPI-1	I	31	1									0	0	1	0.031	32
	II	30	2									0.016	0.016	1	0.061	32
	III	32														32
	IV	30	2									0.016	0.016	1	0.061	32
IDH-1	I	31	1									0	0	1	0.031	32
	II	32														32
	III	32														32
	IV	32														32
MDHP-1	I	7														7
	II	13														13
	III	8	2									0.056	0.059	1	0.180	10
	IV	5	1									0	0	1	0.153	6
MPI-2	I	5	7	1		19						−0.423	8.578*	3	0.426	32
	II	7	5			19						−0.627	12.733*	1	0.425	31
	III	6	5			13						−0.554	7.752*	1	0.457	24
	IV		4			24			1			−0.287	57.145*	3	0.190	29

(*continued on next page*)

Table 2 (*continued*)

Locus	Population	Genotypes										d	χ^2	DF	h	N
		AA	AB	AC	AD	BB	BC	BD	CC	CD	DD					
PEP-B1	I	7	5	3		7	3		5			−0.454	13.387*	3	0.660	30
	II	7	2	5		7	6		3			−0.357	10.508	3	0.663	30
	III	4		3		9	11		4			−0.288	16.978*	3	0.624	31
	IV	5	3	4		7	9		2			−0.195	6.756	3	0.652	30
PEP-B2	I	2				30						−1.000	42.034*	1	0.117	32
	II	8	12			8						−0.158	0.726	1	0.500	28
	III	5	7			17						−0.428	5.574*	1	0.414	29
	IV		3			29						0.033	0.051	1	0.089	32
PEP-C1	I	6	1	2	1	4	1		9	1	3	−0.704	46.981*	6	0.712	28
	II	1	1			3	2	3	5	4	6	−0.434	25.434*	6	0.692	25
	III	2	1	1	2	1	1	3	6	4	5	−0.358	12.019	6	0.705	26
	IV					3	1		17	1	4	−0.840	42.279*	3	0.473	26
PEP-C2	I	10	1			4						−0.847	11.852*	1	0.420	15
	II	24	3									0.039	0.061	1	0.105	27
	III	28	4									0.050	1.105	1	0.117	32
	IV	5	16			2						0.385	3.563	1	0.491	23
PEP-D1	I					32										32
	II					32										32
	III	1				31						−1.000	63.016	1	0.061	32
	IV	32														32
PEP-E1	I	1	2	6		3	4		10	1	1	−0.256	22.061*	6	0.613	28
	II			1		3	10	1	8	6	3	−0.110	5.786	6	0.622	32
	III	4		3	2	5	3	2	7	2	2	−0.460	21.837*	6	0.728	30
	IV			2		1	10	3	9	2	4	−0.144	11.978	6	0.630	31
PEP-S1	I	6	1	3		9	4		9			−0.625	27.422*	3	0.656	32
	II	1		4		4	13		9			−0.032	4.692	3	0.557	31
	III	4	1	1		6	8		12			−0.489	24.691*	3	0.602	32
	IV		1			14	3		9			−0.708	16.752*	3	0.497	27

Table 2 (*continued*)

Locus	Population	Genotypes										d	χ^2	DF	h	N
		AA	AB	AC	AD	BB	BC	BD	CC	CD	DD					
PGM-1	I	23	2			5						−0.795	20.428*	1	0.320	30
	II	32														32
	III	32														32
	IV	14	12			6						−0.213	1.498	1	0.469	32
PGM-2	I	15	3			4						−0.645	9.899*	1	0.375	22
	II	17														17
	III	16														16
	IV	21	3			8						−0.779	20.306*	1	0.417	32

See Section 2 for population designations.*Significant deviation of allele classes from expected Hardy-Weinberg proportions, $p < 0.05$, adjusted by Bonferroni technique.

3.3. Genetic divergence

The genetic distances (D) and (D_{78}) are presented in Table 3. A genetic distance of 0.030 (D) was computed between I and II. The two intermediate populations (II and III) have the smallest genetic distance of 0.008 (D) and 0.003 (D_{78}), whereas highest values were obtained between sites II and IV with 0.049 (D) and 0.044 (D_{78}). The calculated fixation indices (Wright, 1965, 1978) also describe genetic divergence between populations: the F_{IS} statistic indicates the degree of allelic fixation in individuals relative to the subpopulation and averaged 0.362 (Table 3). Pair-wise pooling revealed the following values: 0.402 (I + II), 0.452 (I + III), 0.426 (I + IV), 0.289 (II + III), 0.265 (II + IV) and 0.323 (III + IV). These values are moderate since a value of 1 shows fixation of alleles due to inbreeding and a value close to 0 indicates random mating (Nei, 1986). The F_{IT} statistic is also moderate (0.409) and indicates the amount of inbreeding in the population due to population subdivision. Pair-wise comparisons yielded 0.435 (I + II), 0.473 (I + III), 0.442 (I + IV), 0.300 (II + III), 0.331 (II + IV) and 0.372 (III + IV). F_{ST} (Table 3) measures the amount of differentiation among subpopulations relative to the limiting amount under complete fixation and provides a mean measure of the affiliation of individuals within the population (Swofford and Selander, 1981). The average weighted F_{ST} values from

Table 3
Genetic comparison between four populations of *D. serra*

Population	Parameter	II	III	IV
I	D	0.030	0.020	0.016
	D_{78}	0.025	0.015	0.010
	F_{IT}	0.435	0.473	0.442
	F_{IS}	0.402	0.452	0.426
	F_{ST}	0.055	0.037	0.028
	N_{EM}	2.416	3.660	4.882
II	D	–	0.008	0.049
	D_{78}	–	0.003	0.044
	F_{IT}	–	0.300	0.331
	F_{IS}	–	0.289	0.265
	F_{ST}	–	0.016	0.089
	N_{EM}	–	8.648	1.439
III	D		–	0.041
	D_{78}		–	0.036
	F_{IT}		–	0.372
	F_{IS}		–	0.323
	F_{ST}		–	0.073
	N_{EM}		–	1.786

D, genetic distance according to Nei (1972); D_{78}, genetic distance according to Nei (1978), genetic differentiation according to Wright (1965, 1978), indicating the mean weighted F_{IT}, F_{IS} and F_{ST} statistics values. Additionally the effective number of individuals exchanged between populations in each generation (N_{EM}) using formula 3 (Takahata, 1983) is included (see Section 2 for population designations).

pair-wise comparisons revealed the following values: 0.055 (I + II), 0.037 (I + III), 0.028 (I + IV), 0.016 (II + III), 0.089 (II + IV) and 0.073 (III + IV).

According to Wright's (1969) island model the N_{EM} values in the present study are high enough to counteract genetic drift and ranged between 1.44 (II and IV) and 8.65 (II and III) (Table 3). The genetic distances indicate that population I is closer related to the geographically furthest population IV than to the closer populations II and III. The two intermediate populations are closest related (Table 3).

4. Discussion

Variability of morphological and behavioural characteristics of many species are believed to be determined by changes in the physical (Grant, 1991; Soares et al., 1999) and/or biological (Levitan, 1988) environment. The phenotypic characteristics may be determined exclusively by the genotype (Rothwell, 1993), or by the interaction between genes and the environment (Pigliucci, 1996; Soares et al., 1999). Selection pressure acting upon a diverse, genetically determined phenotype will trigger evolutionary changes while environmentally determined plasticity may buffer them (Grant, 1991). Therefore, it has been controversially discussed whether different phenotypes of the surf clam *D. serra*, inhabiting two biogeographic provinces along the southern African coast, belong to the same species (Donn, 1990a; Soares et al., 1998).

4.1. Morphological variation

Results of our morphological analyses supported significant differences in shell shape between both coasts (Donn, 1990a; Soares et al., 1996). Soares et al. (1998) reported these differences already in juvenile stages. Since smaller and less wedge shaped clams burrow faster than thick shelled ones (Trueman et al., 1966), the authors proposed that the different shell morphologies are the result of directional selection: more wedge shaped, elongate clams would be selected for in the intertidal (South-east Coast) because they burrow faster and swash-ride more efficiently and thus, are less exposed to predators, whereas flat, disc shaped shells are selected for on the West Coast due to increased stability in the subtidal habitats (Donn, 1990b). If the observed low differences in allozymes between Maitlands (IV) and the most northerly located population would mirror differences of the total genomes, it would be unlikely that significantly different shell shape is genetically determined and therefore environmental factors would be likely to cause the different morphologies resulting from phenotypic plasticity. Transformation experiments of marked spat could verify this hypothesis.

4.2. Genetic variation

Within populations, *D. serra* showed moderate to high levels of genetic variation: *A*, *H* and *P* values were in the range commonly found in other marine bivalves

Table 4
Average number of alleles per locus (*A*), mean heterozygosity (*H*) and percent polymorphism (*P*) for different freshwater (F) and marine (M) bivalves

Species	*A*	*H* (%)	*P* (%)	Reference
Aulacomya ater regia (M)	2.31	22–29	–	Blot et al. (1987)
Bathymodiolus spp. (M)	1.27–2.09	–	9–36	Moraga et al. (1994)
Coelatura kunenensis (F)	1.31–1.55	–	28.6	van der Bank (1995)
D. deltoides (M)	3–4			Murray-Jones and Ayre (1997)
D. serra (M)	1.73–1.91	18–22	45.5–59.1	This study
Mytilus edulis (M)	–	9.5	30	Ahmad et al. (1977)
		13–23	–	Grant and Cherry (1985)
M. galloprovincialis (M)	–	18–30	–	Grant and Cherry (1985)
M. desolationis (M)	2.02	23–27	–	Blot et al. (1987)
Ostrea edulis (M)	1.88	8.9	18.2–40.9	Saavedra et al. (1993)
Quadrula quadrula (F)	2.06	20–27	50–70	Berg et al. (1998)
Venus antiqua (M)	1.88	8.8	38.5–69.2	Gallardo et al. (1998)

(Table 4). Significant heterozygote deficiency relative to Hardy-Weinberg expectations was found for all analysed populations (confirmed by a moderate mean F_{IS} value: 0.362). Results from other bivalves range between 50 and 67%, whereas for population I it is 77%, the other three populations are less deficient (Table 5). The deficiencies could be expected ideal since Hardy-Weinberg proportions do not occur in nature (Altukhov, 1981) and significant heterozygote differences are common in bivalve species (Gaffney et al., 1990; Johnson and Joll, 1993; Gallardo et al., 1998). Singh and Green (1984) proposed four possible explanations for such deficiencies: inbreeding, the Wahlund (1928) effect, the presence of null alleles and selection. Population size of *D. serra*, the dispersal via planktonic larvae (Birkett and Cook, 1987) and the fact that only specific loci are affected in the analysed populations, provide sufficient evidence to discount inbreeding or the Wahlund effect, resulting

Table 5
Percentage of deficiencies related to Hardy-Weinberg expectations of four freshwater (F) and one marine (M) clam species

Species	Percentage of deficiency related to Hardy-Weinberg expectations (%)	Reference
Quadrula quadrula (F)	67	Berg et al. (1998)
European anadontines (F)	64	Nagel et al. (1996)
Utterbackia imbecillis (F)	65	Hoeh et al. (1998)
Utterbackia sp. (F)	50	Hoeh et al. (1998)
D. serra (M)	Population I, 77; II, 20; III, 45; IV, 31	This study

from the inclusion of genetically distinct groups into a single population sample (Gaffney et al., 1990). Nevertheless, different timing of gametocyte release between genotypes throughout the six months spawning period (Laudien et al., 2001) may play a significant role for the deficiency (Smith, 1987), which is supported by relatively high F_{IT} values. In addition, spatially or temporally separated cohorts may mimic a Wahlund effect within the population. Null alleles were not detected. Additional factors like non-random mating, self-sorting crossings and mutations are unlikely to be involved since they usually affect all loci.

Five loci (EST-2, PEP-B2, PEP-C2, PGM-1, PGM-2) show clinal variation from sites II and III to the two sites I and IV, respectively (Fig. 1). While populations II and III inhabit the cold bases of upwelling cells, periods of wind-relaxation (mid to late summer) result at beach I in a stratification (Shannon and Nelson, 1996) and rapid temperature increase (23 °C, J. Laudien, unpublished). Therefore Langstrand (I) is only 3 °C colder than Maitlands (IV), and the maximal SST may be associated with genetic variability since specific activities of enzymes or additional digestive isozymes may be induced by temperature (Lombard and Grant, 1986). The repeated trend in the parameters *A*, *H* and *P* appear to be related to temperature. Further the *D* values are also highest, when a cold and a warm site are paired. This is in agreement with Gartner-Kepkay et al. (1980), who also concluded that environmental factors can explain deficiencies and variation for mussels.

Although the analysed populations of *D. serra* show no specific fixed allele differences, the genetic divergence (D_{78} = 0.003–0.044) (Table 3) is in the range for conspecific populations (0–0.05; Ferguson, 1980) and compares well with *Mytilus galloprovincialis* (D = 0.032 ± 0.001) (Quesada et al., 1995) and *Macoma baltica* (D = 0.025–0.029) (Meehan, 1985). The present values are also much lower than those of sympatric *Donax variabilis* and *Donax parvula* populations (Nelson et al., 1993) and also between *Mytilus* species (D_{78}= 0.16–0.28 based on 16–23 loci) (Skibinski et al., 1980; Grant and Cherry, 1985; Väinölä and Hvilson, 1991). The observed low genetic divergence of populations of *D. serra* is supported by the relatively low *F*-values of divergence among subpopulations (F_{ST} = 0.016–0.089). In essence, 1.6–8.9% of the allozyme diversity was explained by variation among samples, and therefore 91.1–98.4% within local populations. Values of genetic variation reported for other bivalve molluscs with meroplanktonic larvae cover a wide range (*Donax deltoides*, F_{ST} = 0.009 for 1200 km, Murray-Jones and Ayre, 1997; *Ostrea edulis*, F_{ST} = 0.062, Saavedra et al., 1993; *Pinctada maxima*, F_{ST} = 0.104 over 3400 km, Johnson and Joll, 1993). High similarities have been related to habitats with current systems (e.g. *Chlamys opercularis*, Macleod et al., 1985; *Choromytilus meridionalis*, Lombard and Grant, 1986; *D. deltoides*, Murray-Jones and Ayre, 1997), evolutionary inertia (i.e. not diverged genetically through evolutionary time, Soares et al., 1999) and a long meroplanktonic time period of widespread bivalve populations (Varvio et al., 1988). Our study reflects a low state of population separation indicating that larval life of *D. serra* is long enough to allow dispersal. The relatively low genetic differences between populations observed in the present study are in line with results from the black mussel *C. meridionalis* in the Cape Province (Lombard and Grant, 1986) and the sand-whelk *Bullia digitalis* (Grant and Da Silva-Tatley 1997) and can

be explained: (1) as the result of extensive gene flow between populations; (2) due to balancing selective pressure acting upon populations; and/or (3) evolutionary inertia.

4.2.1. Gene flow

N_{EM} values ranged between 1.44 and 8.65, and therefore indicate substantial gene flow as a global value of $N_{EM} > 1$ prevents genetic drift/divergence (Slatkin, 1987). The present data compare well to estimates for populations of *Mytilus edulis* (N_{EM} = 8.4, Slatkin, 1985; Quesada et al., 1995), whereas much higher levels ($N_{EM} >$ 23) have been reported for *D. deltoides* separated by up to 1200 km distance (Murray-Jones and Ayre, 1997). Larvae of *D. serra* from the southern West Coast beaches may be transported equator wards with the Benguela current and thus contribute to populations located further north. This hypothesis was supported as population I shows higher genetic variability than II or III. We hypothesise that the back transport of larvae may occur via the subsurface current (Gordon et al., 1995), which flows conspicuous pole-wards throughout the year (Shannon and Nelson, 1996) (Fig. 1). However, hydrographic patterns are unlikely to explain similarities between the two outlying populations. Recruits would be closely related to the bypassed populations (II and III) and larvae would have to stay meroplanktic over a very long time period as the Agulhas current leaves the shelf at the southern termination of the Agulhas Bank and eddies move commonly westwards (Shannon and Nelson, 1996). Only an analogous trend for the outlying populations in contrast to the intermittent beaches could explain their low genetic distance with both getting recruits from beaches mainly located against the surface currents while the counter-currents may back-transport some larvae. However, the intrapopulational divergence is much higher at IV than at the other beaches and therefore gene import at IV is much lower. Grouping of the two outlying populations is therefore unlikely to be related to hydrography.

There is a possibility of gene exchange by human interference, which could explain the lower genetic distance of I and IV as transfer of *D. serra* has happened at some beaches of the eastern Cape and at Cape Cross (J. Laudien, personal communication with local fishermen, nature conservationists and staff of the Namibian Ministry of Fisheries and Marine Resources).

4.2.2. Balancing selective pressure

Balanced selection related to SST is much more likely to explain similarities between sites I and IV. Karl and Avise (1992) also suggested selection to explain the uniformity of their genetic analyses. Allozymes may have effects on overall fitness and growth rates (Koehn and Gaffney, 1984; Singh and Green, 1984) and therefore selection for the most beneficial enzyme could be the cause for heterogeneity of the allozyme frequencies. This is supported by results from the study of Gartner-Kepkay et al. (1980), who found indications in *M. edulis* that variable environmental parameters are mirrored in populations with variable genomes and thus reflect a functional aspect of adaptability. In *D. serra* several loci are affected differently, thus we postulate that there is a different sensitivity of the enzyme systems to various environmental selective forces. Analysed clams show allelic diversity

particularly at the peptidase (PEP) locus. Variations at the cytosol aminopeptidase (PEP-E, formerly CAP or LAP) locus, are in agreement with findings for *M. edulis* and *Geukensia demissa* (Young et al., 1979; Garthwaite, 1986). Different PEP-E genotypes provide differences in growth rate and tissue weight (Koehn and Gaffney, 1984; Garthwaite, 1989) and therefore this could be a useful locus for selective breeding and aquaculture. As a digestive enzyme PEP may be subject to the availability of certain food compounds. Thus, varying nutrition between sites may contribute, although not significantly (McMillan, 1993), to genetic distances.

4.2.3. Evolutionary inertia

Sandy-beach organisms are generalists (Brown and McLachlan, 1990) and a co-adapted genome supporting high plasticity allows them to withstand and rapidly adapt to the changes of the dynamic environment. Kreitman and Akashi (1995) assume that strong selection pressure for phenotypic plasticity may override stochastic processes, such as random genetic drift and mutation, which promote divergence of populations. If different phenotypes are fit for different environmental conditions (Sultan, 1995), populations can theoretically survive long geological periods without the necessity for genetic changes. This may also explain the genetic similarity of populations of *D. serra*, in line with explanations for other sandy-beach molluscs (Soares et al., 1999).

4.3. Genetic isolation

For species with planktonic larvae, direct determination of the subdivision of populations is virtually impossible and genetic comparison provides an indirect but powerful tool. Our results show a substantial geographic separation of the two Namibian populations. Several studies indicated that the intense upwelling cell at Lüderitz (Shannon, 1989) (Fig. 1) could cause a biological discontinuity (van der Bank and Holtzhausen, 1998–1999), initiated near 24°30′S in the vicinity of Meob Bay (Agenbag and Shannon, 1988). This effective biotic barrier could be an expanded cold water filament generated in the Lüderitz upwelling cell, a deflection of the poleward under-current located in the vicinity of Meob Bay (cf. Monteiro, 1999) or the combination of changes in circulation, turbulence and stratification (Agenbag and Shannon, 1988). The present genetic analysis resembles the picture observed for other marine bivalves separated by biogeographic borders (Saavedra et al., 1993; Sanjuan et al., 1994; Quesada et al., 1995) and supports the hypothesis that environmental forces may be the major causal elements determining genetic differentiation. We emphasise that the observed genetic differences between populations could also reflect historical patterns of gene flow such as large-scale climatic changes, which occurred in the Atlantic over the Pleistocene (Thunell and Belyea, 1982) and could have initiated the gene divergence among neighbouring populations (Endler, 1977). Therefore, population dynamics for stocks should be analysed separately. If these parameters are not similar, proper management of future commercial *D. serra* harvests must be adjusted respectively.

5. Conclusions

This preliminary genetic study on *D. serra* reveals that populations are conspecific and possess genetic variation in the range of most other marine bivalves that potentially allows for adaptation to environmental changes. Our results reveal a discrepancy between morphological differences and genetic similarity. Therefore, it is likely that different shell shapes are resulting from phenotypic plasticity caused by environmental factors. The allozymes described show a good basis for further studies on genetic distribution patterns and enzyme polymorphism. Within-area and additional population sampling is required to substantiate the present results. Further confirmation of the strength of larval connections may involve surveying genetic variation in newly settled larvae and juveniles in particular. The substantial subdivision of the two Namibian populations requires separate analyses of population dynamics.

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