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W. S. Grant · F. M. da Silva-Tatley

Lack of genetically-subdivided population structure in *Bullia digitalis*, a southern African marine gastropod with lecithotrophic development

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Abstract Larval shell morphology in fossil and present-day gastropods is often used to infer modes of larval development and levels of dispersal. Dispersal ability influences not only genetic population structure, but also is thought to influence a species' geographical range and evolutionary duration. We tested these predictions in *Bullia digitalis*, a sandy-beach whelk, by examining genetic variability at 33 protein-coding loci in nine samples ($N = 739$) taken in 1984 to 1985 at localities extending over about three-quarters of the geographical range of this species in southern Africa. Females of this species deposit eggs into benthic or brooded capsules in which larvae develop through the trochophore and veliger stages to emerge as crawling juveniles. Scanning electron micrographs confirmed a protoconch morphology typical for gastropods with lecithotrophic larval development. Contrary to expectations, subpopulations of *B. digitalis* had high levels of variability ($H = 0.102$) and lacked a genetically-fragmented structure ($\theta = 0.013$). The lack of a genetically-subdivided population structure would not have been correctly inferred, if this species were known only from well-preserved fossil shells. Indirect estimates of migration between populations based on θ and the island model of migration, which assumes drift-mutation equilibrium, ranged between 19 and 23 individuals per generation. Either an undescribed mechanism of dispersal facilitates gene flow between populations, or the geographical range of this species has recently expanded to produce the appearance of high levels of gene flow. Gene-frequency distributions showed that relative to four other species of *Bullia*, populations of *B. digitalis* were in mutation-drift disequilibrium, with a significant excess of low-frequency alleles that is consistent with a recent rapid expansion from a small population. Also contrary to expectations, this species has a large geographical range (2 400 km) and an apparently long evolutionary history extending 5 to 20 million years, as estimated from an allozyme phylogeny with four other species of *Bullia*.

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W.S. Grant (✉)[1] · F.M. da Silva-Tatley[2]
Marine Biology Research Institute, University of Cape Town, Rondebosch 7700, Republic of South Africa

Present address:
[1]Conservation Biology Program, Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, Washington 98112, USA

[2]Department of Medical Biochemistry, Medical School, University of Cape Town, Observatory 7925, Republic of South Africa

Introduction

Biological features of extinct species have been important for inferring mechanisms of speciation. For example, exoskeletal morphology of fossil invertebrates has been used to infer modes of larval development (Shuto 1974; Emlet 1989), paleoecologies (Bromley 1975; Vermeij 1977), genetic variability (Palmer 1986), and phylogenetic relationships (Runnegar and Pojeta 1974). Many of these inferences, combined with observations of present-day species, have been used to build evolutionary models of speciation (Hansen 1980; Jablonski 1986). Among these inferences is the attempt to estimate gene flow between subpopulations of extinct species, since gene flow is central to most models of evolutionary change (Wright 1951) and speciation (Mayr 1963). Gene flow is thought to have a cohesive effect on subpopulations, preventing them from diverging from one another through random genetic drift and natural selection (Scheltema 1971, 1978; Crisp 1978).

In marine gastropods, the structure of the protoconch, the minute, pre-metamorphic larval shell at the tip of the spire of the adult shell, has been used to infer mode of larval development in extinct species known only from fossil remains (Shuto 1974; Hansen 1980; Jablonski 1986), and in extant species such as deep-sea gastropods, whose development is otherwise unknown. Species with planktotrophic larvae tend to have

elongated, sculptured protoconchs, and species with lecithotrophic larvae tend to have globose, unornamented protoconchs (Powell 1942; Thorson 1950). Numerous studies indicate that present-day marine gastropods with planktotrophic larvae show more genetic uniformity among populations (Gooch et al. 1972; Johnson and Black 1984) than do species with development in benthic capsules (Janson 1987; Grant and Utter 1988; Day 1990). The apparent reason is that the passive movement of free-swimming larvae in ocean currents and subsequent recruitment into distant populations promotes gene flow among populations.

These observations have been generalized and used as assumptions to formulate biogeographical and speciation models for gastropods. Several authors have argued that over evolutionary time, species with long-lived planktotrophic larvae more readily invade suitable, but unoccupied, habitats (Crisp 1978; Scheltema 1978; Templeton 1980; Valentine and Jablonski 1983; Jablonski 1986). As a result, these species are predicted to have large geographical ranges (but see Johannesson 1988) and are thought to be less likely to speciate because of the homogenizing effect of gene flow (Hansen 1980; Jablonski 1986). Lineages of such species are also thought to resist extinction better than species with genetically-fragmented subpopulations, and hence to have longer species' durations. In contrast, lineages of gastropods with lecithotrophic development are predicted to have greater genetic fragmentation among populations because of reduced dispersal and gene flow. Reduced dispersal leads to restricted geographical ranges and to shorter species' duration because of greater rates of speciation and extinction.

In this study, we examined the genetic population structure of the sandy-beach whelk *Bullia digitalis*, which inhabits dissipative sandy beaches exposed to medium to strong wave action from Namibia to the Transkei, South Africa (Fig. 1) (Brown and McLachlan 1990). Only a few sandy beaches along the coast of southern Africa are suitable for this species' burrowing and swash-riding to search for food. Brown (1971) found that *B. digitalis* cannot successfully burrow in sand with grain sizes >3.2 mm. Juveniles are sensitive to sand particle-size; one having a shell 0.5 cm in length, for instance, cannot burrow into sand with grains >1.4 mm. Variability in wave action also influences the distribution of *B. digitalis*, which is most abundant on beaches with moderate wave action and absent from both very calm beaches and beaches with heavy surf. The greatest impediment to dispersal along the coast is this species' mode of development. Females may mate with several males in late spring and summer (November to January), and numerous capsules, each containing ≥150 eggs, are deposited 4 to 12 cm in the sand or held on the ventral surface of the foot by females (Brown 1971; da Silva and Brown 1985), behavior that is rare in gastropods. The relative survivals of individuals in free and brooded capsules, however, is not known. Crawling juveniles emerge from the capsules after a few weeks (Brown 1971, 1982).

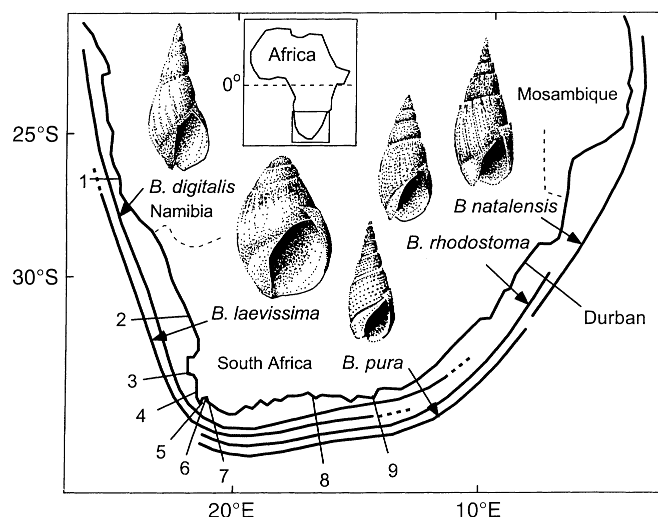

Fig. 1 *Bullia* spp. Lines represent geographical distributions (*1–9* sample locations of *B. digitalis* numbered as in Tables 1 and 3)

Although this whelk has lecithotrophic benthic development, it appears to contradict the expectation of a small geographical range thought to be a consequence of benthic development. The goal of the study was to examine in detail the genetic population structure of this marine gastropod to search for genetic characteristics that may resolve this apparent contradiction. If other species of *Bullia* are short-lived because of high rates of speciation and extinction, as predicted for species with lecithotrophic, non-planktonic development, we might find such genetic signatures as reduced levels of gene diversity, drift-mutation disequilibria, and short branches on a shallow phylogeny. We also examined electrophoretic variability of enzymes in four additional species of *Bullia* in southern Africa and estimated a phylogenetic tree for the five species.

Materials and methods

Bullia digitalis

Scanning electron micrographs of three field-collected juveniles, ~4 mm in length, were made at the Electron Microscope Unit, University of Cape Town, South Africa. Shells were vacuum-dried, mounted on stubs, coated with gold-palladium, and examined at 50 to 100× magnification with a Cambridge S-200 scanning electron microscope.

Samples of *Bullia digitalis* ($N = 739$) were collected at low tide along 20 to 50 m of beach at each of nine localities in southern Africa (Table 1) after baiting the swash zone with crushed mussels or fish offal. Whelks were transported in damp cloth, or in buckets, and kept live in recirculated sea water at 15 °C until electrophoresis (within 2 wk). Tissues were mascerated in equal volumes of 0.1 *M* Tris-HCl, pH 8.0, and centrifuged at 1000 ×*g* for 10 min. Soluble proteins were separated by electrophoresis in 12% starch gels (Sigma Chemical Co., St Louis, Missouri, USA) for 3 to 5 h, depending upon buffer system. We used three buffer systems: LiOH-borate-tris-citrate (pH 8.5), tris-citrate (pH 6.9), and tris-borate-EDTA (pH 8.7) (Buffers I, II and IV of Grant and Leslie 1993, respectively).

Table 1 *Bullia* spp. Sample localities in southern Africa, dates and sample sizes. Sample numbers correspond to Locations 1 to 9 in Fig. 1; species abbreviations are used in Table 6 and Fig. 6

Sample, location	Date	(N)
B. digitalis (*dig*)		
Namibia		
1: Luderitz	Sep. 1986	(85)
South Africa		
2: Lambert's Bay	Oct. 1984	(87)
3: Yzerfontein	Aug. 1984	(100)
4: Ou Skip	June 1984	(99)
5: Muizenberg	July 1984	(100)
6: Mnandi	Aug. 1984	(49)
7: Strand	Aug. 1984	(81)
8: Sedgefield	Jan. 1985	(44)
9: Maitland	Jan. 1985	(94)
Ou Skip (resampling)	Aug. 1986	(50)
B. laevissima (*lae*)		
Muizenberg	Aug. 1986	(26)
B. natalensis (*nat*)		
Durban	Nov. 1986	(30)
B. pura (*pur*)		
Muizenberg	Aug. 1986	(35)
B. rhodostoma (*rho*)		
Strand	Aug. 1986	(51)

Gene expressions of isozymes in foot muscle, mantle, digestive gland, brain and gonad were examined in 99 whelks from Ou Skip beach to establish the best buffer–tissue combinations for surveying genetic variability. The gene products of 33 putative loci (Table 2) were identified with the histochemical protocols of Murphy et al. (1996). A total of 19 loci were monomorphic and were not examined in the remaining samples.

Enzymes encoded by *Ark*, *Mpi*, and *Opdh* had two-banded heterozygous phenotypes typical of monomeric enzymes. The primary bands of *Opdh* phenotypes were accompanied by as many as four satellite bands with intensities decreasing anodally. Proteins encoded by *Est-1*, *Gpi-1*, *Idh-1*, *Idh-2*, *Mpi*, *Pgl-1* and *Pgdh* had 3-banded heterozygous phenotypes typical of dimeric enzymes. Allozymes encoded by *Ppp* showed broad-banded and 3-banded heterozygous phenotypes, depending upon the mobilities of alleles. Heterozygotes of *Iddh-1* and *Ldh-1* had five bands typical of tetramric enzymes. Locus and allelic nomenclature followed Murphy et al. (1996). When several bands appeared on a gel, the corresponding loci were numbered beginning from the cathodal end of a gel. Alleles were designated as the mobility relative to the most common allele, which was designated 100. Alleles encoding proteins migrating cathodally from the origin were prefixed with a minus sign.

We compared genotypic proportions with Hardy–Weinberg proportions with the goodness-of-fit *G*-test (Sokal and Rohlf 1981; Lessios 1992). *F*-statistics for 19 independent alleles at six polymorphic loci were estimated with Eqs. (1) to (4) of Weir and Cockerham (1984). The variable, $f\ (=F_{IS})$, is the weighted average of Wright's inbreeding coefficient "*F*" over polymorphic loci at each locality, and was tested for deviation from zero with $\chi^2 = f^2N$ ($df = 1$), where N is the average sample size (Li and Horvitz 1953). $F\ (=F_{IT})$, inbreeding in individuals relative to the total population, was tested with student's $t = F\sqrt{N_T}$ and $df =$ infinity, where N_T is the total number of individuals (Brown 1970). $\theta\ (=F_{ST})$, the standardized variance of allelic frequencies among samples, was tested for departure from zero with $\chi^2 = 2N_T\theta$ and $df = s-1$, where s is the number of subpopulations (Workman and Niswander 1970). Geographical structure was further analyzed by nesting gene frequencies into three physically-distinct coastal regions: (1) Namibia, (2) the west coast, and (3) the south coast of South Africa, and by testing for gene-frequency shifts amongst these groups with the *G*-test of independence (Sokal and Rohlf 1981). We used sequential Bonferroni adjustments of rejection probabilities to account for increased Type I error resulting from repeated tests of the same hypothesis with six polymorphic loci (Rice 1989). The same nested geographic model was used to partition the total gene diversity into its geographical components (Chakraborty 1980). Sample gene diversities were estimated following Nei (1978), and standard errors were calculated by jackknifing over loci.

Two indirect methods based on the island model of migration were used to estimate the number of migrants between populations per generation. First, the number of migrants, N_em, was estimated with

$$\theta = 1/(4N_em + 1) \ ,$$

where N_e = subpopulation size and m = rate of immigration into each subpopulation from a central pool of migrants. Second, N_em was also estimated with the private-alleles method of Slatkin (1985). If gene flow in *Bullia digitalis* more closely follows a linear stepping-stone model of dispersal instead of dispersal from a central pool of migrants as assumed in the island model, these estimates of N_em greatly underestimate gene flow between neighboring populations.

The association between geographical distance and genetic differences between pairs of samples was tested with Mantel's (1967) matrix correlation coefficient, r, (NTSYS, Exeter Software, New York) with an approximate student's t statistic with $df =$ infinity. We also used Moran's I (Sokal and Oden 1978; Cliff and Ord 1981) to test for spatial autocorrelation in 19 alleles with standard normal significance testing, following Jumars et al. (1977) and Sokal and Oden (1978). We used Gabriel connections (Gabriel and Sokal 1969) and geographical distance (km) between samples to detect isolation by distance on large geographic scales, and the inverse of geographic distance (1/km) between samples to detect isolation by distance on small scales.

Bullia spp.

Although 11 species of *Bullia* occur in southern Africa (Kilburn and Rippey 1982), we were able to collect only four additional species accessible at low tide. In 1986, *B. laevissima* was collected from extreme low-intertidal sandy beaches, and *B. pura* and *B. rhodostoma* from mid- to high-intertidal sandy beaches, all in False Bay, South Africa (Fig. 1; Location 5). *B. natalensis* was collected near Durban, Natal, by R. Kilburn (Natal Museum). For comparisons, we resampled *B. digitalis* from Ou Skip and examined a set of 32 loci that overlapped those used in the population study (Table 2). Protein electrophoretic protocols were the same as those for *B. digitalis*.

For each species, expected unbiased gene diversities (Nei 1978) were calculated for each locus from allelic frequencies, then averaged. Significant differences in the average gene diversity between species was tested with Student's t-tests of average arcsine-transformed single-locus heterozygosities (Archie 1985). Nei's unbiased genetic distance, D, (Nei 1978; Hillis 1984) between taxa was calculated from gene frequencies for 32 loci. Majority-rule consensus trees were made from 500 bootstrapped UPGMA (unweighted pair-group method using arithmetic averages) (Sneath and Sokal 1973) and 500 bootstrapped Fitch–Margoliash (Fitch and Margoliash 1967) trees (PHYLIP Version 3.5C; J. Felsenstein, Department of Genetics, University of Washington, Seattle, Washington 98195, USA). A cladogram was constructed from ordered character states at each locus with the minimum-alleles turnover model (Mickevich and Mitter 1983) and with PAUP (phylogenetic analysis using parsimony) (Swofford 1985) to produce a minimal-length tree with Wagner parsimony.

Table 2 *Bullia* spp. Enzymes, locus abbreviations, and tissues–buffer combinations giving best results [*DG* digestive gland; *F* foot; *M* mantle; *Buffer I* LiOH-borate-tris-citrate (pH 8.5), *II* tris-citrate (pH 6.9), *IV* tris-borate-EDTA (pH 8.7); *Sample 1 B. digitalis* (Ou Skip sample only), *2 B. digitalis* (Samples 1 to 9), *3* species comparison]

Enzyme (EC No.)	Locus abbreviation	Tissue	Buffer	Samples examined
Alkyline phosphatase (3.1.3.1)	*Alp*	DG	IV	3
Adenosine deaminase (3.5.4.4)	*Ada*	DG	IV	1
Adenylate kinase (2.7.1.20)	*Ak*	F	II	2,3
Aldolase (4.1.2.7)	*Ald-1*	F	IV	1
Aldehyde oxidase (1.2.3.1)	*Ao*	DG	I	1,3
Arginine kinase (2.7.3.3)	*Ark*	F	IV	2,3
Aspartate amino transferase (2.6.1.1)	*Aat-2*	F	I	2,3
Esterase (3.1.1.1)	*Est-1*	M	IV	2,3
	Est-2	M	IV	2,3
	Est-3	M	IV	2,3
Fumerate hydratase (4.2.1.2)	*Fumh*	M	IV	1,3
Glucose-6-phosphate dehydrogenase (1.1.1.49)	*G6pdh*	DG	I	1,3
Glucose-6-phosphate isomerase (5.3.1.9)	*Gpi-1*	F	I	2,3
Glyceraldehyde-phosphate dehydrogenase (1.2.1.12)	*Gapdh*	F	II	2,3
Glycerol-3-phosphate dehydrogenase (1.1.1.8)	*G3pdh-1*	F	II	2,3
	G3pdh-2	F	II	2,3
Hexokinase (2.7.1.1)	*Hk*	F	IV	1
Iditol dehydrogenase (1.1.1.14)	*Iddh-1*	DG	I	2,3
Isocitrate dehydrogenase (1.1.1.42)	*Idh-1*	DG	II	2,3
	Idh-2	F	II	2,3
Lactate dehydrogenase (1.1.1.27)	*Ldh-1*	F	I	2
	Ldh-2	DG	I	2,3
Leucine amino peptidase (3.4.11.-)	*Lap-1*	DG	II	2,3
Malate dehydrogenase (1.1.1.37)	*Mdh-1*	F, DG	II	2,3
	Mdh-2	F	II	2,3
Mannose-phosphate isomerase (5.3.1.8)	*Mpi*	F, M	IV	2,3
Octopine dehydrogenase (1.5.1.11)	*Opdh*	M	I	2,3
Peptidase (3.4.11.-)				
Substrate: Glycyl-leucine	*Pgl-1*	M, DG	II	2,3
	Pgl-2	M, DG	II	2,3
Leucyl-glycyl-glycine	*Plgg-1*	DG, M	II	1,3
Phenylalanyl-proline	*Ppp*	M, DG	II	2,3
Phosphoglucomutase (5.4.2.2)	*Pgm-1*	M	I	3
Phosphogluconate dehydrogenase (1.1.1.44)	*Pgdh*	F	II	2,3
Superoxide dismutase (1.15.1.1)	*Sod-1*	DG	I	2,3
	Sod-2	M	I	3
Xanthine dehydrogenase (1.2.3.2)	*Xdh*	DG	IV	1,3

Results

Variability in *Bullia digitalis*

Scanning electron micrographs of protoconchs of three field-collected juveniles of ~4 mm long confirmed previous descriptions with light microscopy (da Silva and Brown 1985). The protoconch had ~2.5 volutions, a large nucleus, a blunt globose apex, and no sculpturing or ridging (Fig. 2). This morphology is typical of species with lecithotrophic, nonplanktonic larvae (Shuto 1974).

Gene frequencies for the 14 polymorphic loci appear in Table 3. No deviations from Hardy–Weinberg proportions were detected for any of the loci in any of the samples, and no systematic heterozygote deficits or excesses appeared in the samples; f, the average within-population inbreeding coefficient over loci, was 0.001 and was not significant (Table 4). F, inbreeding relative to the total population, averaged 0.014 over loci and was also not significant.

Gene-frequency shifts among samples were small, but significant for *Ark* ($P < 0.001$), *Est*-1 ($P < 0.001$), *Idh-1* ($P < 0.05$), *Mpi* ($P < 0.05$), *Opdh* ($P < 0.05$), *Pgl-1* ($P < 0.05$), and *Ppp* ($P < 0.001$) (after sequential Bonferroni adjustment of rejection probabilities). Most of this heterogeneity was due to frequency differences among samples from Lambert's Bay to False Bay (Localities 2 to 7) over a distance of ~400 km. However, after pooling genotypic frequencies into a single sample ($N = 739$), a significant departure from Hardy–Weinberg proportions that would suggest Wahlund's effect failed to appear for any locus. A component analysis of the total gene diversity in *Bullia digitalis* indicated that on average 98.2% of the total variability was contained within samples, 0.24% was due to differences among three samples collected over 30 km in False Bay (Localities 5 to 7), 0.93% was due to differences over a scale of ~400 km, and 0.61% was due to differences on a regional scale of 2200 km. The average θ for 19 alleles at nine loci was 0.013, and did not differ significantly from zero (Table 4).

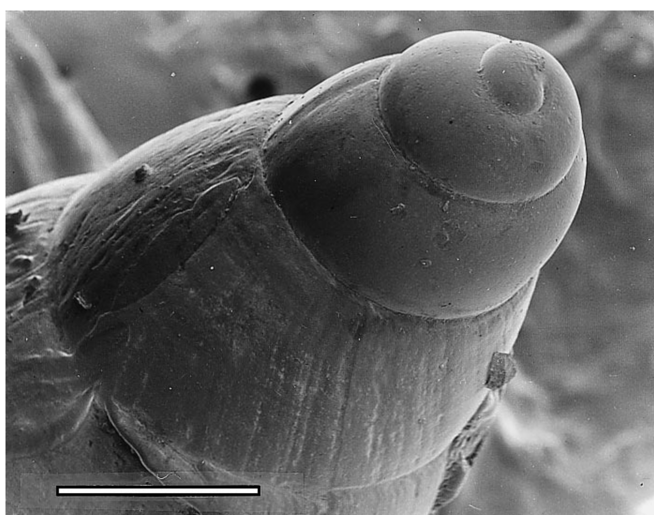

Fig. 2 *Bullia digitalis*. Electron micrograph of protoconch (Scale bar = 0.5 mm)

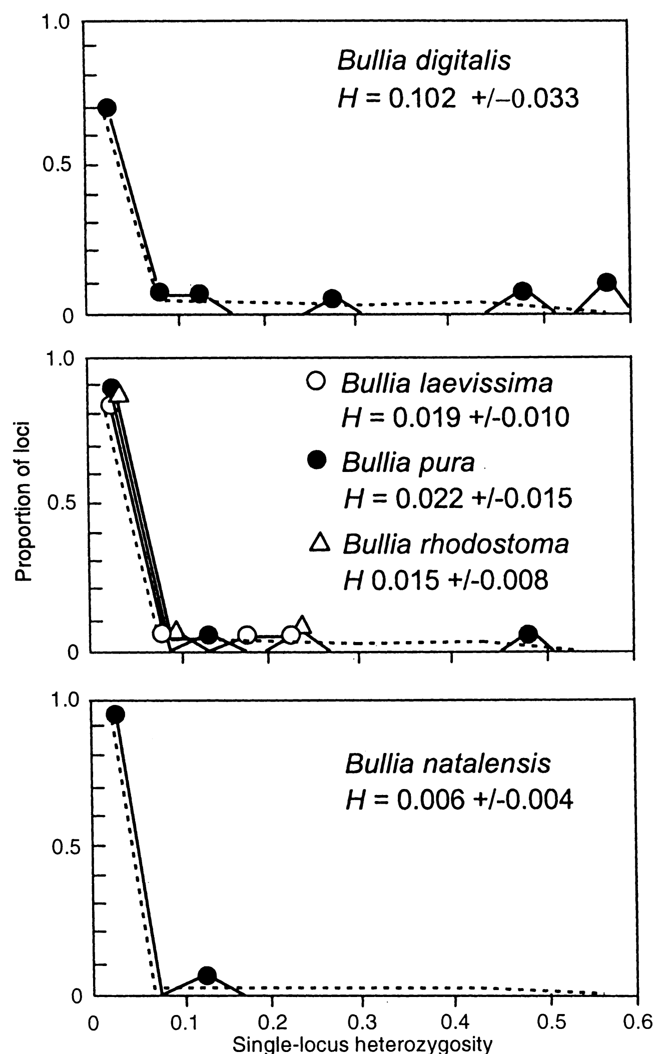

Fig. 3 *Bullia* spp. Distributions of single-locus heterozygosity (H) in five species

We also tested for geographical structure with Mantel's statistic (Table 5) and spatial autocorrelation. Significant correlations between genetic differences and geographic distances between samples should appear if gene flow is largely restricted to nearby populations, as might be expected in a whelk with direct benthic development. None of the 19 alleles showed a significant correlation with distance ($P > 0.05$ after sequential Bonferroni adjustment), with the inverse of the shore-line distance between samples, or with Gabriel connections. Nei's D served as a multilocus measure of differences between populations and was compared with the long-shore distances between samples. D between samples ranged from 0.0000 to 0.0019 and averaged 0.00086. Mantel's correlation between genetic distances and geographical distances between samples was $r = 0.134$, but was not significant (Student's $t = 0.592$, $P > 0.05$).

Two indirect methods of estimating the number of genetically-effective migrants between populations indicated high levels of gene flow between populations. An estimate of N_em made with the average θ over loci and the island model of migration was about 19 immigrants per population each generation. The private-alleles method of Slatkin (1985) yielded an estimate of 23 immigrants per generation. These results are consistent with high levels of gene flow, or alternatively with a recent radiation from a single panmictic population.

Variability among species of *Bullia*

Gene diversities ranged from 0.006 (SE = 0.004) in *Bullia natalensis* to 0.022 (SE = 0.015) in *B. pura*, except for *B. digitalis* with a gene diversity of 0.102 (SE = 0.033) that was significantly greater than those for the four other species (Student's *t*-test; $P < 0.05$). The distribution of unbiased, single-locus gene diversities, h, closely followed the L-shaped distribution (Fig. 3) expected in the infinite-alleles model for populations in equilibrium with mutation and random drift (Fuerst et al. 1977). Comparisons of observed with expected gene diversities, however, have low probabilities of detecting disequilibria, because low-frequency alleles contribute little to heterozygosity.

We therefore compared the observed frequency distributions with expected distributions for populations in drift-mutation equilibrium in the infinite alleles model (Watterson 1984). A significant excess of low-frequency alleles appeared in *Bullia digitalis* from the second collection at Ou Skip (Fig. 4A; Kolmogorov–Smirnov test, $P < 0.05$). We then pooled gene frequencies for the nine samples of *B. digitalis* and included the 19 monomorphic loci examined initially in the first Ou Skip sample. This provided a large sample with considerable power for detecting disequilibria. An even greater excess of rare alleles appeared in this pooled sample (Fig. 4B; $P < 0.01$). Significant departures from expected drift-

Table 3 *Bullia* spp. *Ada*, *Ald*, *Ao*, *Gapdh*, *Ldh-1* and *Ldh-2* were assumed to be fixed for the same allele, respectively, in all samples of *B. digitalis*; *Ao*, *Gapdh* and *Ldh-2* were fixed for the same allele, respectively, among *Bullia* spp. (*Localities 1 to 9* as in Fig. 1 and Table 1; *locus abbreviations* as in Table 2)

Locus, allele	*Bullia digitalis* locality									*Bullia* spp.				
	1	2	3	4	5	6	7	8	9	*dig*	*lae*	*nat*	*pur*	*rho*
Alp														
220	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02	–	–	–	–
200										–	–	–	0.01	–
190										–	–	–	0.06	–
160										0.04	–	–	0.93	–
150										–	1.00	–	–	–
100										0.38	–	–	–	–
0										–	–	–	–	1.00
−50										–	–	1.00	–	–
−100										0.56	–	–	–	–
Ak														
150	–	–	–	–	–	–	–	–	–	–	1.00	–	1.00	1.00
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	1.00	–	–
Ark														
125	–	0.01	0.01	0.01	0.07	0.06	0.09	–	–	–	–	–	–	–
100	1.00	0.99	0.99	0.99	0.93	0.94	0.91	1.00	1.00	1.00	–	–	–	–
97	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–
80	–	–	–	–	–	–	–	–	–	–	–	–	–	1.00
75	–	–	–	–	–	–	–	–	–	–	–	–	1.00	–
Aat-2														
120	ND	ND	ND	–	ND	ND	ND	ND	ND	–	–	1.00	–	1.00
100				1.00						1.00	–	–	1.00	–
85				–						–	1.00	–	–	–
Est-1														
200	–	–	–	–	–	–	–	–	–	–	–	–	0.99	–
170	–	–	–	–	–	–	–	–	–	0.01	–	–	–	–
135	–	–	–	–	–	–	–	–	–	–	1.00	–	–	–
125	0.01	0.01	0.01	–	–	–	–	–	–	0.01	–	–	0.01	–
115	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–
110	0.03	0.04	0.02	0.08	0.02	0.03	0.02	–	0.02	0.09	–	–	–	–
107	0.06	0.06	–	0.01	0.05	0.01	–	–	–	0.05	–	–	–	–
105	0.01	0.05	0.10	0.11	0.08	0.11	0.06	0.10	0.05	0.01	–	–	–	–
104	–	–	0.01	–	–	–	–	–	–	–	–	–	–	–
102	0.11	0.12	0.15	0.10	0.16	0.11	0.15	0.14	0.13	0.14	–	–	–	–
100	0.74	0.63	0.61	0.61	0.60	0.63	0.65	0.73	0.70	0.66	–	–	–	–
97	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–
95	0.01	0.01	0.01	0.02	0.01	–	0.01	–	–	–	–	–	–	–
90	0.01	0.01	0.02	0.01	0.01	0.02	0.05	–	0.03	–	–	–	–	1.00
85	–	0.06	0.01	0.05	0.03	0.01	0.01	0.03	0.07	–	–	–	–	–
80	–	–	0.05	0.01	0.03	0.05	0.04	–	–	–	–	–	–	–
75	–	0.01	0.01	–	–	0.01	–	–	–	–	–	–	–	–
65	0.02	–	–	–	–	0.01	0.01	–	–	–	–	–	–	–
50	–	0.01	0.01	–	0.01	0.01	–	–	–	–	–	–	–	–
Est-2														
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–	1.00	1.00
95	–	–	–	–	–	–	–	–	–	–	1.00	1.00	–	–
Est-3														
110	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–
105	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–
100	1.00	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	–
92	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–
85	–	–	–	–	–	–	–	–	–	–	–	–	–	1.00
Fumh														
130	ND	ND	ND	–	ND	ND	ND	ND	ND	–	–	–	1.00	–
120				–						–	1.00	–	–	0.98
110				–						–	–	1.00	–	0.02
100				1.00						1.00	–	–	–	–

Table 3 (continued)

Locus, allele	*Bullia digitalis* locality									*Bullia* spp.				
	1	2	3	4	5	6	7	8	9	*dig*	*lae*	*nat*	*pur*	*rho*
G6pdh														
100	ND	ND	ND	1.00	ND	ND	ND	ND	ND	1.00	1.00	1.00	–	1.00
95				–						–	–	–	1.00	–
Gpi-1														
500	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01
410	–	–	–	–	–	–	–	–	–	–	–	–	–	0.99
400	–	–	–	–	–	–	–	–	–	–	–	–	1.00	–
380	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–
220	0.01	–	0.01	–	0.01	–	–	–	–	–	–	–	–	–
180	–	–	–	–	0.01	–	0.01	–	–	–	–	–	–	–
100	0.99	0.99	0.99	0.98	0.98	1.00	0.97	0.99	0.99	1.00	–	–	–	–
50	–	–	–	–	–	–	–	0.01	–	–	–	–	–	–
10	–	–	–	0.02	–	–	–	–	0.01	–	–	–	–	–
−80	–	0.01	–	–	–	–	0.01	–	–	–	–	–	1.00	–
−120	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–
G3pdh-1														
100	1.00	1.00	1.00	1.00	1.00	100	1.00	1.00	1.00	1.00	1.00	1.00	–	1.00
20	–	–	–	–	–	–	–	–	–	–	–	–	1.00	–
G3pdh-2														
400	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–
120	–	–	–	–	–	–	–	–	–	–	1.00	–	–	–
110	–	–	–	–	–	–	–	–	–	–	–	–	–	1.00
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–	–
80	–	–	–	–	–	–	–	–	–	–	–	–	1.00	–
Iddh-1														
300	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–
250	–	–	–	–	–	–	–	–	–	–	–	–	0.01	–
200	–	–	–	–	0.01	–	–	–	–	–	–	–	0.99	–
180	–	–	–	0.01	0.02	–	0.01	–	0.02	–	–	–	–	1.00
100	0.99	1.00	1.00	0.99	0.97	1.00	0.99	0.99	0.97	1.00	1.00	–	–	–
10	0.01	–	–	–	–	–	–	–	0.01	–	–	–	–	–
−50	–	–	–	–	–	–	–	0.01	–	–	–	–	–	–
Idh-1														
145	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03
135	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–
125	–	0.01	–	–	0.01	0.03	0.02	–	0.01	–	–	–	–	0.97
110	0.02	0.01	–	–	–	0.01	0.02	–	0.02	–	–	–	1.0	–
105	–	–	–	–	–	–	–	–	–	–	0.98	–	–	–
103	–	–	–	–	–	–	–	–	–	–	–	0.07	–	–
100	0.91	0.97	0.97	0.96	0.92	0.92	0.92	0.97	0.93	0.96	–	–	–	–
95	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–
90	–	–	–	–	–	–	–	–	–	–	–	0.93	–	–
60	0.07	0.01	0.03	0.03	0.05	0.03	0.04	0.03	0.04	0.04	–	–	–	–
50	–	–	–	0.01	0.01	0.01	–	–	–	–	–	–	–	–
Idh-2														
130	–	–	–	–	–	–	–	–	0.01	–	–	–	–	–
125	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–
115	0.02	–	–	0.01	0.01	0.02	0.01	–	0.02	–	–	–	–	–
100	0.98	1.00	1.00	0.99	0.99	0.98	0.97	1.00	0.97	1.00	1.00	–	–	–
95	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–
85	–	–	–	–	–	–	0.01	–	–	–	–	–	–	1.00
75	–	–	–	–	–	–	–	–	–	–	–	–	1.00	–
Lap-1														
300	–	–	–	–	–	–	–	–	–	–	–	–	–	1.00
250	–	–	–	–	–	–	–	–	–	–	1.00	–	–	–
150	–	–	–	–	–	–	–	–	–	–	–	–	1.00	–
125	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–	–
Mdh-1														
200	0.01	–	–	–	0.01	–	–	0.01	0.01	–	–	–	–	–
150	–	–	–	–	–	–	–	–	0.01	–	–	–	–	–
105	–	–	–	–	–	–	–	–	–	–	1.00	–	–	–

(continued overleaf)

Table 3 (continued)

Locus, allele	*Bullia digitalis* locality									*Bullia* spp.				
	1	2	3	4	5	6	7	8	9	*dig*	*lae*	*nat*	*pur*	*rho*
100	0.98	1.00	1.00	1.00	0.99	1.00	1.00	0.99	0.98	1.00	–	–	–	1.00
90	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–
30	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–
0	–	–	–	–	–	–	–	–	–	–	–	–	1.00	–
Mdh-2														
200	–	–	–	–	–	–	–	–	–	–	–	–	0.36	–
120	0.01	–	–	–	–	–	0.02	–	–	–	–	–	–	–
100	0.99	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	–	0.02	0.64	–
90	–	–	–	–	–	–	–	–	–	–	1.00	–	–	–
60	–	–	–	–	–	–	–	–	–	–	–	–	–	1.00
30	–	–	–	–	–	–	–	–	–	–	–	0.98	–	–
Mpi														
108	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–
105	0.01	0.04	0.06	0.08	0.02	–	0.01	0.01	0.02	–	–	–	–	–
102	0.01	0.01	–	–	–	–	–	–	–	–	–	–	–	–
100	0.96	0.95	0.91	0.92	0.96	0.97	0.98	0.99	0.97	0.99	–	–	–	–
95	0.02	–	–	–	0.01	0.01	–	–	–	0.01	–	–	–	–
90	–	–	–	–	–	0.02	0.01	–	0.01	–	–	–	–	–
80	–	–	0.01	–	0.01	–	–	–	–	–	1.00	–	–	0.04
75	–	–	–	–	–	–	–	–	–	–	–	–	1.00	–
70	–	–	–	–	–	–	–	–	–	–	–	–	–	0.96
50	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–
Opdh														
200	–	0.03	0.01	0.02	0.01	0.01	–	0.01	–	–	–	–	–	–
110	–	0.01	–	–	–	–	–	–	–	–	–	–	–	–
100	1.00	0.95	0.96	0.96	0.99	0.99	1.00	0.99	1.00	1.00	–	–	–	–
95	–	0.01	0.02	0.01	–	–	–	–	–	–	1.00	–	–	–
90	–	–	0.01	–	–	–	–	–	–	–	–	1.00	–	–
80	–	–	–	–	–	–	–	–	–	–	–	–	–	0.98
30	–	–	–	0.01	–	–	–	–	–	–	–	–	1.00	–
10	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02
Pgl-1														
170	0.04	0.02	0.03	0.09	0.08	0.05	0.03	0.01	0.02	0.01	–	–	–	–
160	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–
155	–	–	–	–	–	–	–	–	–	0.01	–	–	–	1.00
150	–	–	0.13	–	–	0.09	0.04	–	–	0.01	–	–	–	–
145	0.43	0.39	0.25	0.37	0.34	0.18	0.22	0.24	0.37	0.27	–	–	1.00	–
125	0.02	–	0.02	–	–	0.02	0.04	–	–	–	–	–	–	–
120	–	–	–	–	–	–	–	–	–	–	0.96	–	–	–
100	0.50	0.56	0.54	0.52	0.57	0.64	0.65	0.74	0.60	0.70	–	–	–	–
95	0.01	0.01	0.01	–	–	–	–	–	–	–	–	1.00	–	–
80	–	–	0.01	–	–	–	–	–	–	–	–	–	–	–
75	–	0.02	0.01	0.02	0.01	0.02	0.02	0.01	0.01	–	–	–	–	–
Pgl-2														
105	–	–	–	–	–	–	–	–	–	–	1.00	–	1.00	–
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–	1.00
90	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–
Plgg-1														
135	ND	ND	ND	–	ND	ND	ND	ND	ND	–	–	1.00	–	–
130				–						–	–	–	–	1.00
120				–						0.08	–	–	1.00	–
100				1.00						0.92	1.00	–	–	–
Ppp														
100	0.89	0.91	0.87	0.74	0.89	0.91	0.86	0.87	0.89	0.99	0.04	1.00	–	–
107	0.02	0.02	0.06	0.15	0.03	0.03	0.03	0.01	–	0.01	–	–	–	–
95	–	–	–	–	–	0.01	–	–	–	–	–	–	0.99	–
115	0.07	0.05	0.03	0.10	0.06	0.05	0.07	0.11	0.09	–	0.96	–	–	1.00
122	–	–	–	0.01	–	–	–	–	–	–	–	–	–	–
90	0.02	0.02	0.03	–	0.01	–	0.01	0.01	0.02	–	–	–	–	–
75	–	–	0.01	–	0.01	–	0.03	–	–	–	–	–	–	–
60	–	–	–	–	–	–	–	–	–	–	–	–	0.01	–
Pgm-1														
115	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	–	1.00	–	–

Table 3 (continued)

Locus, allele	*Bullia digitalis* locality									*Bullia* spp.				
	1	2	3	4	5	6	7	8	9	*dig*	*lae*	*nat*	*pur*	*rho*
107										0.04	–	–	–	–
100										0.64	–	–	1.00	0.87
97										0.04	0.86	–	–	–
95										–	–	–	–	0.13
90										0.28	–	–	–	–
87										–	0.14	–	–	–
Pgdh														
100	0.85	0.83	0.73	0.78	0.81	0.77	0.76	0.85	0.82	0.85	–	–	–	–
110	–	–	–	–	–	–	–	–	–	–	–	1.00	0.01	1.00
30	0.14	0.16	0.27	0.22	0.15	0.23	0.22	0.14	0.17	0.13	–	–	–	–
−20	0.01	0.01	–	–	–	–	0.01	0.01	0.01	–	–	–	–	–
60	–	–	–	–	0.02	–	–	–	–	–	–	–	0.99	–
15	–	–	–	–	0.01	–	0.01	–	–	–	1.00	–	–	–
120	–	–	–	–	0.01	–	–	–	–	0.02	–	–	–	–
Sod-1														
250	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–
−90	–	–	–	–	–	–	–	–	–	–	–	–	1.00	–
−250	–	–	–	–	–	–	–	–	–	–	–	–	–	1.00
Sod-2														
115	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.40	–	–	–	–
110										–	–	–	1.00	–
100										0.60	–	–	–	–
95										–	–	1.00	–	–
85										–	–	–	–	1.00
80										–	0.90	–	–	–
70										–	0.10	–	–	–
Xdh														
115	ND	ND	ND	–	ND	ND	ND	ND	ND	–	–	0.02	–	–
110				–						–	–	0.98	–	–
105				–						–	–	–	1.00	–
103				–						0.06	–	–	–	–
100				1.00						0.94	–	–	–	1.00
95				–						–	1.00	–	–	–

mutation distribution did not occur in the four other species of *Bullia* (Fig. 5).

A phylogenetic analysis of gene frequencies with different methods gave conflicting trees. A total of 149 alleles was detected among the five species of *Bullia*; 116 of these were autapomorphs (occurring in only one taxon), 30 were sympliesiomorphs (shared derived alleles), and 3 were common to all taxa. *D* between species ranged from 1.07 between *Bullia digitalis* and *B. rhodostoma* to 2.07 between *B. natalensis* and *B. pura* (Table 6). One important feature of the phenetic and cladistic trees, was that each method of tree construction produced a different tree topology (Fig. 6). The consensus UPGMA bootstrap tree showed support $>50\%$ only for the *B. natalensis*–*B. pura* group. The consensus Fitch–Margoliash bootstrap tree, on the other hand, showed support $>50\%$ for two groupings: *B. laevissima*–*B. natalensis* and *B. pura*–*B. digitalis*. The PAUP cladogram had yet a different topology. A consistency index of 0.75 for this tree indicated a moderate number of homoplasies in the tree (1.0 = no homoplasies). Another important feature of these trees is that the branches leading to the various taxa were very long. A considerable number of homoplasies have apparently accumulated since these species diverged from one another, so that their phylogenetic relationships are not resolved by these allozyme data.

Discussion

Genetic population structure in *Bullia digitalis*

One goal of this study was to examine the relationship between genetic population structure and protoconch morphology in *Bullia digitalis*. Species with planktotrophic pelagic development are thought to have a large potential for gene flow that leads to genetic homogeneity among populations, but the degree of homogeneity depends on the extent to which gene flow is actually realized (Gooch et al. 1972; Berger 1973; Johnson and Black 1984). Shortened pelagic existence, temperature or salinity barriers to passive dispersal in ocean currents, and failure to recruit into distant populations may reduce

Table 4 *Bullia digitalis*. Average gene frequency (*P*), unbiased *F* statistics, θ (correlation of genes between individuals within a subpopulation relative to total population); *f* (average inbreeding over subpopulations), and *F* (inbreeding in individuals relative to the total population) for nine populations estimated from 16 independent alleles encoded by nine loci (Weir and Cockerham 1984) (*Locus abbreviations* as in Table 2)

Locus, allele	*P*	θ	*f*	*F*
Ark				
100	0.972	0.0392	0.0154	0.0247
Est-1				
110	0.031	0.0107	0.0565	0.0666
105	0.072	0.0109	0.0397	0.0501
102	0.129	0.0005	0.0422	0.0427
100	0.646	0.0078	−0.0192	−0.0112
85	0.030	0.0139	0.0519	0.0651
av		0.0072	0.0153	0.0223
Gpi-1				
100	0.988	−0.0007	0.0082	0.0089
Idh-1				
100	0.949	0.0085	0.0548	0.0628
60	0.035	0.0048	0.0871	0.0914
av		0.0069	0.0680	0.0745
Mpi				
105	0.029	0.0188	0.0490	0.0668
100	0.958	0.0108	0.0108	0.0214
av		0.0141	0.0265	0.0402
Opdh				
100	0.984	0.0164	0.0459	0.0615
Pgl-1				
170	0.042	0.0157	0.0131	0.0286
145	0.320	0.0243	−0.0112	0.0134
100	0.585	0.0115	−0.0544	−0.0423
av		0.0171	−0.0299	0.0123
Ppp				
107	0.044	0.0464	−0.575	−0.0085
100	0.867	0.0245	0.0410	0.0646
av		0.0286	−0.0022	0.0264
Pgdh				
100	0.801	0.0087	0.0027	0.0114
30	0.190	0.0109	0.0130	0.0237
av		0.0092	0.0028	0.0136
Average over loci		0.0133	0.0100	0.0232

gene flow and lead to genetic differentiation among populations (Burton 1983; Schaeffer et al. 1985; Hedgecock 1986). Nevertheless, many gastropods with pelagic larvae tend to show genetic homogeneity over large portions of their ranges. On the other hand, gastropods with lecithotrophic benthic development have low potentials for gene flow among populations and tend to have genetically-fragmented population structures (Berger 1973; Snyder and Gooch 1973; Janson and Ward 1984; Janson 1987; Grant and Utter 1988; Day 1990; Dempster 1995). Since *B. digitalis* has a protoconch morphology typical of species with lecithotrophic development and has encapsulated juveniles with apparently benthic development (da Silva and Brown 1985), the finding of genetic homogeneity among populations is unexpected.

It is difficult to explain why *Bullia digitalis* failed to show strongly subdivided genetic structure over three quarter of its geographic range. Although pelagic dispersal occurs in some gastropods with lecithotrophic development (Hadfield and Strathmann 1990), gene flow in *B. digitalis* appears to be limited to juvenile or adult migration, aided by wave action along sandy beaches, which along the southwest coast are largely confined to a few sheltered bays. Adult migration between sandy beaches is unlikely in the intertidal zone; although adults are known to extend the foot to capture the energy of waves in the swash zone of sandy beaches (Odendaal et al. 1992), whelks floating on the surface or in midwater have not been reported in near-shore plankton surveys. Unfortunately little is known about the behavior of *B. digitalis* in deeper outer coastal waters because of their inaccessibility.

The high degree of genetic homogeneity points to passive dispersal in currents of one or more life-history stages. Mature female *Bullia digitalis* breed once a year and produce ~30 000 eggs (da Silva and Brown 1985) encapsulated in cases typically measuring 2 × 1.2 cm and having short attachment threads at each end (Brown 1982). Egg cases are usually buried, unattached, 4 to

Table 5 *Bullia digitalis*. Mantel's (1967) correlations, *r*, and Student's *t*-test values for significance of *r* for gene-frequency shifts and geographic distance; degrees of freedom equal infinity; sequential Bonferroni adjustment of rejection probabilities was used (*Locus abbreviations* as in Table 2)

Locus, allele	Geographic distance		Inverse of distance	
	r	*t*	*r*	*t*
Ark				
100	−0.173	0.778	−0.142	0.753
Est-1				
110	−0.064	0.216	−0.144	0.650
105	0.414	2.028	−0.121	0.664
102	−0.121	0.821	0.285	1.762
100	0.495	2.347	−0.281	1.523
90	−0.094	0.336	0.190	0.887
85	0.282	1.369	−0.288	1.579
Gpi-1				
100	−0.412	1.585	0.453	2.209
Idh-1				
100	0.043	0.333	−0.266	1.702
Mpi				
100	−0.070	0.297	−0.284	1.460
Opdh				
100	0.001	0.005	−0.140	0.673
Pgl-1				
170	−0.236	1.017	0.047	0.243
145	0.092	0.584	−0.008	0.048
100	0.275	1.045	−0.201	0.972
Ppp				
100	−0.291	0.939	−0.025	0.110
Pgdh				
100	−0.036	0.185	−0.173	0.967
Nei's *D*	0.134	0.592	−0.329	1.725

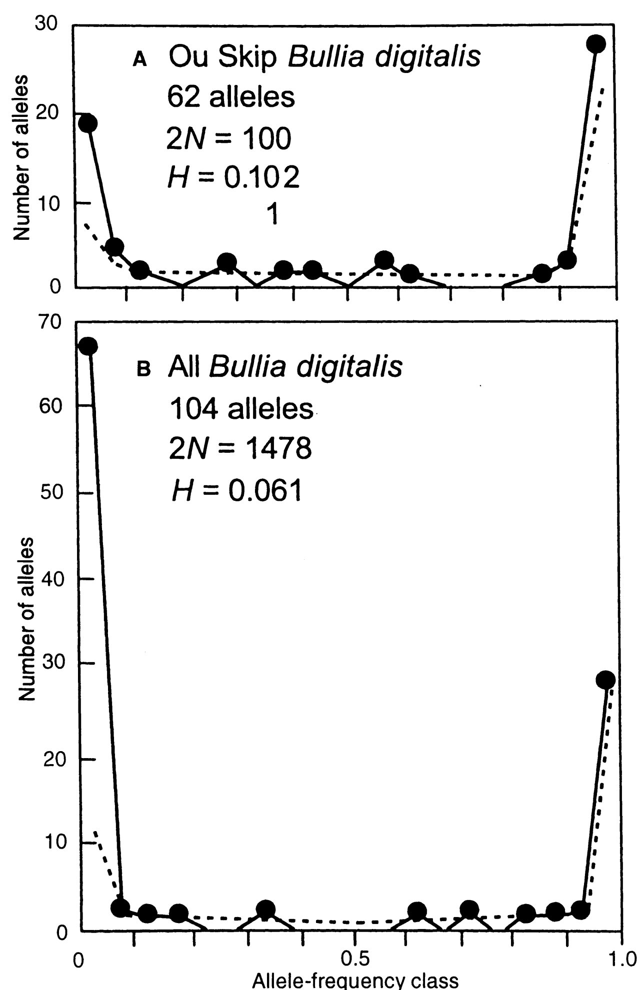

Fig. 4 *Bullia digitalis*. Expected (*dashed line*) and observed (●) distributions of gene frequencies. **A** Sample from Ou Skip, South Africa; **B** pooled for 9 samples

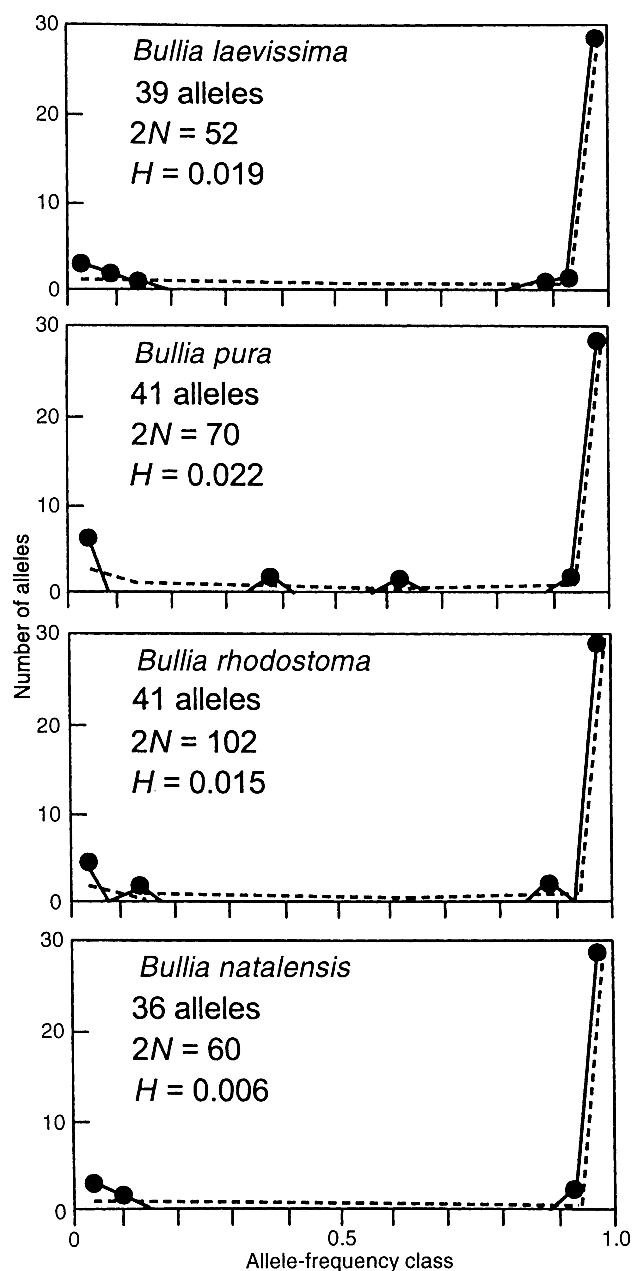

Fig. 5 *Bullia* spp. Expected (*dashed line*) and observed (●) distributions of gene frequencies

12 cm into sand below low water of spring tides (Brown 1982). In the laboratory, newly-emerged juveniles are moved easily by small currents, whereas large individuals are not (AC Brown, Department of Zoology, University of Cape Town, personal communication). Martel and Chia (1991) showed that, for some gastropods, passive dispersal of small juveniles in strong surf alongshore was common, and suggested that such dispersal may be important in maintaining local populations regardless of the mode of development. Surf-zone movements of juveniles have been observed in some gastropods (Newell 1964; Anderson 1971), but are not considered to be important for long-distance dispersal (Highsmith 1985). Nevertheless, frequent current-mediated dispersal over short distances can bring about apparent genetic homogeneity over large areas. The southwesterly Agulhas Current along the south coast and the fast-moving Benguela Current on the west coast (Shannon 1985) provide mesoscale currents and eddies that potentially retain and transport *B. digitalis* juveniles.

Circumstantial evidence suggests that dispersal by rafting on flotsam or macroalgae be may important in some gastropods. For example, *Littorina saxatilis* has benthic brooded development, but has a larger geographic range than *L. littorea*, a species with planktotrophic larval development (Johannesson 1988). In a study of invertebrate populations on suspended kelp holdfasts, some individuals survived for several months, and rafting may contribute to long-distance movement between some areas (Edgar 1987). Females of *Bullia digitalis*, however, do not attach egg capsules to substrata that could be carried by currents, and this species occurs on sandy beaches devoid of macroalgae.

Table 6 *Bullia* spp. Genetic distance (Nei 1978; Hillis 1984) between species (*Species abbreviations* as in Table 1)

	dig	*lae*	*nat*	*pur*	*rho*
dig					
lae	1.15				
nat	1.52	1.51			
pur	1.23	1.86	2.07		
rho	1.07	1.38	1.52	1.67	

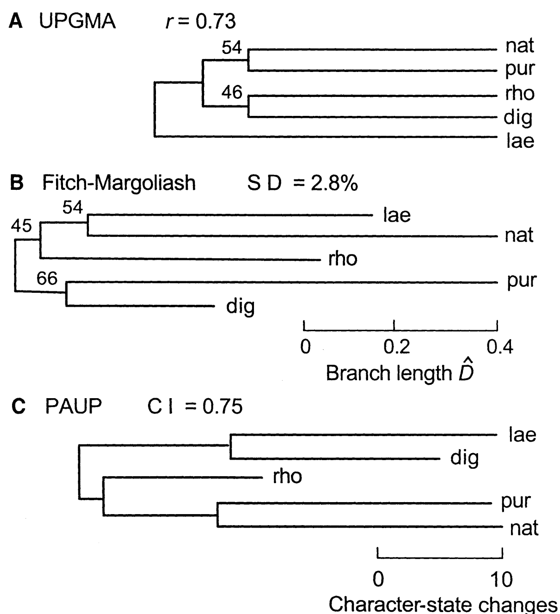

Fig. 6 *Bullia* spp. **A** UPGMA tree of genetic distance (Nei 1978) based on 32 allozyme encoding loci (*numbers at nodes* percentage bootstrap support for nodes); **B** Fitch–Margoliash tree of Nei's genetic distances (*numbers at nodes* percentage bootstrap support for nodes); **C** PAUP (Swofford 1985) tree of ordered character-state changes (minimum alleles turnover model) with Wagner parsimony (*Species' abbreviations* as in Table 1; *CI* consistency index

In our inferences of gene flow between subpopulations of *Bullia digitalis*, we assumed that the geographical distributions of allozyme variability were principally influenced by gene flow between populations and by random genetic drift. The possibility that natural selection may be producing the genetic homogeneity observed in this study must also be considered (Koehn et al. 1980; Karl and Avise 1992). Selection would complicate inferences about population genetic structure from gene frequencies. However, sampling across a strong environmental gradient around Cape Point should have enhanced the probability of detecting the effects of natural selection if it were an important influence on gene frequencies. For example, strong allozyme-frequency shifts were detected in populations of the brown mussel *Perna perna* at the faunal boundary at Cape Point (Grant et al. 1992). Populations of this mussel were otherwise genetically homogeneous over several hundred kilometers on the south coast of South Africa. The lack of genetic heterogeneity in samples of *B. digitalis* from the Cape region is consistent with a selectively-neutral interpretation of allozyme-frequency variability.

Two genetic characteristics of *Bullia digitalis*, a high level of genetic diversity and an excess of low-frequency alleles, give conflicting indications of population history. *B. digitalis* had significantly higher levels of gene diversity than four other intertidal species of *Bullia*, which have similar modes of development (Brown 1982). This implies that, relative to other species of *Bullia*, *B. digitalis* has had a longer history of large populations in which mutant alleles have drifted to moderate frequencies. The low levels of genetic diversity (*H* 0.006 to 0.022) in the other intertidal species of *Bullia* may indicate that they have a history of small population sizes, that they have experienced reductions in population size (Nei et al. 1975), or that gene diversity has been eroded by a metapopulation structure of local extinctions and recolonization (Gilpin 1991).

On the other hand, a significant excess of low-frequency alleles in *Bullia digitalis* relative to populations in drift-mutation equilibrium with the same level of gene diveristy (Watterson 1984) implies that populations of this species have expanded rapidly from a small population and have accumulated new mutations that have not yet moved to intermediate frequencies by random drift (Maruyama and Fuerst 1984). This may also explain the absence of correlation between genetic and geographical distances between samples. Small genetic distances between sites may reflect similarity from recent common ancestry rather than similarity from gene flow, which is overestimated by assuming drift-migration equilibrium. If this hypothesis is correct, an analysis of mitochondrial DNA variability may show a nucleotide sequence mismatch distribution characteristic of rapidly expanding populations (Rogers and Harpending 1992).

Species longevity and geographic range in *Bullia digitalis*

One expectation for gastropods with direct development is that pelagic development contributes to large geographical ranges because a large supply of colonists is available to invade unoccupied, but suitable habitats (Mileikovsky 1971; Crisp 1978; Jablonski 1986; Scheltema 1986). The geographical range of *Bullia digitalis* extends from the east coast of South Africa to central Namibia, a distance of ~2400 km. This range is similar to the median range of 1860 km for fossil gastropods with planktotrophic development reported by Jablonski. The median range for species with nonplanktotrophic larval development was only 380 km (Jablonski 1986).

Theory predicts that species with lecithotrophic benthic (direct) development are more likely to speciate because reduced levels of gene flow between populations allow greater levels of genetic divergence between populations than do high levels of gene flow in species with planktotrophic pelagic development (Hansen 1980; Jablonski 1986). Another prediction is that species with

direct development are more likely to become extinct because of the reduced ability to colonize new and vacated habitats (Hansen 1980; Jablonski 1986). Thus, the terminal branches in a phylogeny of related species with direct development should be relatively short. The trees we found in *Bullia* had several homoplasies and long branches, which we take to imply ancient speciations and long evolutionary durations for the five species. The magnitudes of genetic distance between species is at the upper limit of the useful range for measuring genetic distances, and these estimates probably underestimate genetic distance because of the inability to detect multiple amino acid replacements with protein electrophoresis (Nei 1978).

Although temporal scaling of protein genetic distances is controversial (Ayala 1986; Scherer 1990), even approximate values are useful for estimating species longevity in *Bullia* spp. Branch lengths may be scaled with time by correlating cladogenesis with a dated geologic event or fossil chronology. However, calibrating the allozyme clock for *Bullia* spp. is difficult because of the absence of an outgroup taxon in southern Africa to test assumptions of rate uniformity along the branches and because of the lack of dated geologic events associated with cladogenesis in this group. Temporal scaling may be possible by using calibrations from gastropods that dispersed through the Bering Strait after it opened a connection between the Atlantic and Pacific Oceans 3.0 to 3.5 million years ago (see reviews by Grant 1987; Cunningham and Collins 1994). Nei's genetic distance between two clades of *Littorina* that dispersed into the Atlantic from the Pacific Ocean averages 0.75 (Zaslavskya et al. 1992), and this gives about 4.7 million years for $D = 1.0$. Temporal calibrations for other marine invertebrates and for marine fishes generally range between 5 and 18 million years for $D = 1.0$ (Bermingham and Lessios 1993; Grant 1987). This range of calibrations gives times since divergence between species of *Bullia* of 5 to 20 million years, and is much longer than a median of about 2 million years estimated for nonplanktotrophic, Late Cretaceous fossil gastropods (Jablonski 1986) and a median of 1.0 million years for nonplanktotrophic Early Cenozoic gastropods (Hansen 1980).

If *Bullia digitalis* were known only from a few well-preserved fossil shells, biologists would be able to infer correctly from its globose, unornamented protoconch that it had lecithotrophic development, but would not be able, within the current theoretical framework, to infer the lack of genetic subdivision among its populations, or its deep evolutionary history. The results of this study suggest that either populations of *B. digitalis* have expanded recently on a large geographical scale, or that some yet unidentified mechanism of dispersal exists in this species. The presence of lecithotrophic larvae in this species may not preclude dispersal through egg-case or juvenile rafting. In any case, the population genetic structure of this species greatly weakens any generalities about modes of larval development and population genetic structure in gastropods.

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