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Morphological and genetic adaptation to a lagoon environment: a case study in the bryozoan genus *Alcyonidium*

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Abstract The Fleet (southern England) is a stable (ca. 5,000 years) coastal saline lagoon that supports a population of *Alcyonidium* resembling the common coastal epiphyte, *Alcyonidium gelatinosum* (L.). A combination of morphological, reproductive, and ecological characters was used to compare lagoonal and non-lagoonal proximate populations. Comparisons revealed a difference in the timing of spawning, considered to be related to the temporally restricted availability of viable substrata within the lagoonal basin. Allochronous spawning and spatial separation together suggest that the lagoonal taxon is reproductively isolated. The two populations were further compared with seven other coastal populations of *Alcyonidium* using randomly amplified polymorphic DNA (RAPD) analysis. The results confirm the individuality of the lagoonal taxon but also a close relationship with three *A. gelatinosum* populations. We present and consider four hypotheses that may account for the presence of this genetically distinct taxon: (1) diversification within the Fleet; (2) colonisation from another lagoon; (3) a southern lagoonal species at its northern limit; and (4) introduction by shipping or other anthropogenically mediated dispersal mechanism. Significant diversification on the time scale involved has been demonstrated for isolated freshwater environments and, therefore, is feasible within a saline lagoon. Hypothesis 1 and, to a lesser extent, hypothesis 2 are consistent with the recognition of individual lagoons as 'biogeographic' islands of importance for their unique or characteristic biodiversity. The study also represents the first example of concordant morphological, reproductive, and genetic diversification in a marine bryozoan.

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Introduction

Saline lagoons often form behind sedimentary barriers associated with microtidal coasts. Seawater and biological exchange occur through one or more inlets or by percolation. A typical lagoonal basin is an isolated, low energy ecosystem characterised by extreme shelter from wave action, an attenuated tidal range, weak currents and poor flushing, and a prevalence of shallow sublittoral mud beds often colonised by seagrasses (Barnes 1980).

Lagoons are well recognised as being unique in terms of their biodiversity. A range of characteristic invertebrate morphospecies is known to be common to many saline lagoons in north-west European waters (Barnes 1980, 1988, 1989, 1994; Bamber et al. 1992), some of the species being closely related to estuarine or open coastal counterparts. For example, the lagoonal cockle *Cerastoderma glaucum* (Bruguière) and estuarine cockle *Cerastoderma edule* (L.) are only differentiable according to relatively minor morphological, biological, and ecological characteristics; the species co-exist in intermediate environments and can hybridise (Barnes 1980). The existence of characteristic lagoonal morphospecies suggests that physical isolation and low energy regimes may exert strong selective pressures on marine colonisers, fostering the evolution of differentiated taxa. Rapid genetic differentiation has recently been demonstrated for populations of fish isolated within post-glacial freshwater lakes (McPhail 1993; Duvernell and Turner 1998; Foster et al. 1998). Coastal saline lagoons formed behind sedimentary barriers are also post-glacial entities, typically no more than 10,000 years old. It is, therefore, reasonable to hypothesise that rapid genetic differentiation may also occur within saline lagoons.

We used a combination of morphological, reproductive, and ecological characters, in conjunction with randomly amplified polymorphic DNA (RAPD) analy-

sis to compare lagoonal and non-lagoonal populations of the marine bryozoan genus *Alcyonidium* (Lamouroux) as found within and outside the Fleet Lagoon (southern England). This genus is particularly suitable for study because of its tendency to produce cryptic species (Thorpe et al. 1978a, b), partly attributable to the limited dispersal potential of the ephemeral lecithotrophic larvae characteristic of most British species within the genus. Here, we examine the hypothesis that relatively stable saline lagoons may foster the evolution of unique morphological and genetic variants.

The RAPD technique has previously been used to investigate population genetic structure within clonal marine organisms, including cnidarians (Levitan and Grosberg 1993; Grosberg et al. 1997), freshwater bryozoans (Okamura et al. 1993; Hatton-Ellis et al. 1998) and ascidians (Bishop et al. 1996). Although the RAPD technique is not always favoured for technical reasons (Haymer 1994; Reiseberg 1996), recent work has established its value for differentiating taxa, provided that certain assumptions are fulfilled and appropriate control experiments are performed (Haymer 1994; Reiseberg 1996; Perez et al. 1998). The RAPD technique is particularly powerful when used in combination with other characters (Reiseberg 1996).

Materials and methods

Ecological studies

The Fleet is a 13-km-long saline lagoon formed between a shingle barrier (Chesil Bank) and the mainland coast of southern England (Fig. 1; 50°40.5′N, 2°34.3′E). The lagoonal basin exhibits classically sheltered conditions with weak tidal circulation and poor flushing (Robinson et al. 1983). The waters of the lagoonal basin are mainly brackish although fully saline conditions can prevail along most of the basin during dry periods (Whittaker 1978). The shallow subtidal bottom (< 1 m) is dominated by organic muds supporting seagrass meadows (two species of *Zostera*, two of *Ruppia*) (Whittaker 1978). A species of *Alcyonidium* colonising mollusc shells within the lagoonal basin was first mentioned by Seaward (1978) and identified only as a member of the *A. mytili/polyoum* 'complex' (Seaward 1981). Recent field surveys have revealed this population to be concentrated within the central section of the lagoon basin (P.E.J. Dyrynda, personal observation). In contrast, *A. gelatinosum* (L.) is absent from the lagoonal basin but occurs intertidally on *Fucus serratus* L. at the lagoon mouth (Fig. 1). Lagoonal *Alcyonidium* was collected by sweep netting at Langton Hive Point (Fig. 1) in winter, and by hand gathering by snorkellers whilst vegetation was dense in summer. Thirty colonies of the lagoonal *Alcyonidium* were collected at 4- to 6-week intervals between January 1995 and January 1997 (31 January 1995, 6 April 1995, 1 May 1995, 9 June 1995, 15 July 1995, 10 August 1995, 7 September 1995, 8 October 1995, 12 November 1995, 28 March 1996, 4 May 1996, 14 January 1997). Fronds of *F. serratus* bearing *A. gelatinosum* were hand collected at 'Small Mouth' (Fig. 1) at low tide. Samples were preserved in 70% ethanol. Additional colonies were maintained live for observation, morphometric analysis, and photography. Seasonal monitoring of vegetation cover and invertebrate populations within the central lagoon was undertaken over the same period (Fig. 2).

Colonies of *A. gelatinosum* were also collected from the Menai Strait (53°13.4′N, 4°10.2′W) and Camel Estuary (50°32.6′N, 4°55.3′W). Five populations representing two other littoral *Alcyonidium* species were sampled as follows: *A. mytili* (Dalyell) Longnidry (56°3.0′N, 3°49.0′W), Falls of Lora (56°25′N, 5°24′W), Cleddau Bridge (51°42.3′N, 4°55.8′W); *A. reticulum* Ryland and Porter (2000), Porlock (51°12′N, 3°27′W), Watwick (51°42.0′N, 5°09.3′W).

Laboratory studies

Morphometric analyses

Morphometric analysis of live colonies was undertaken using image analysis [Wild M420 Makroscop, video camera, and PC running

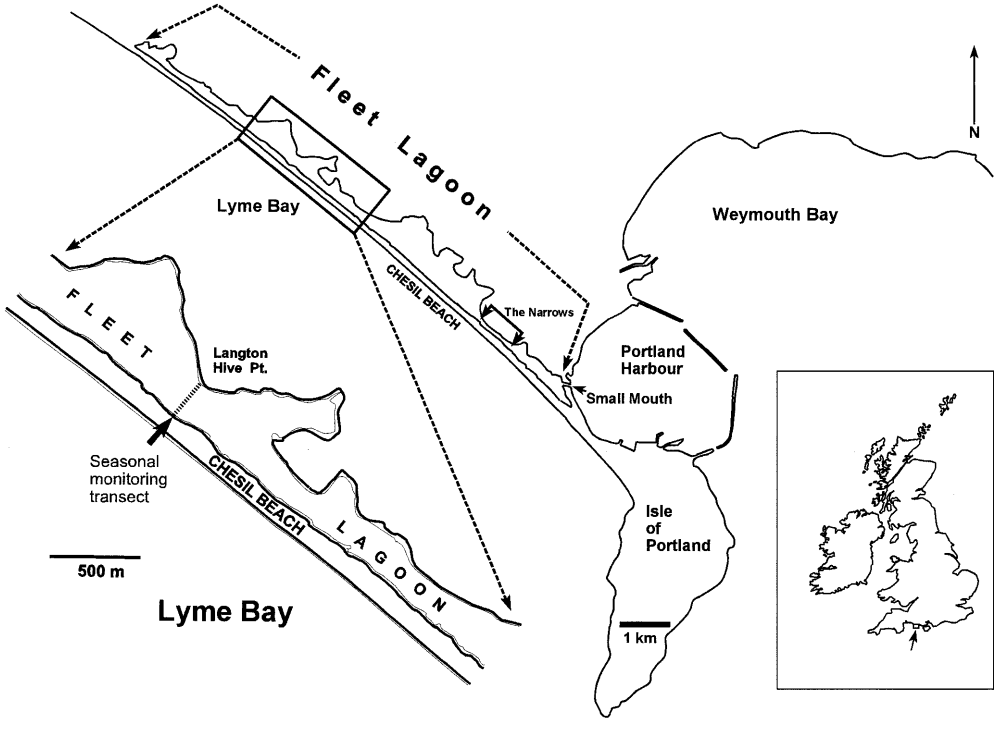

Fig. 1 *Alcyonidium gelatinosum*. Map of study area

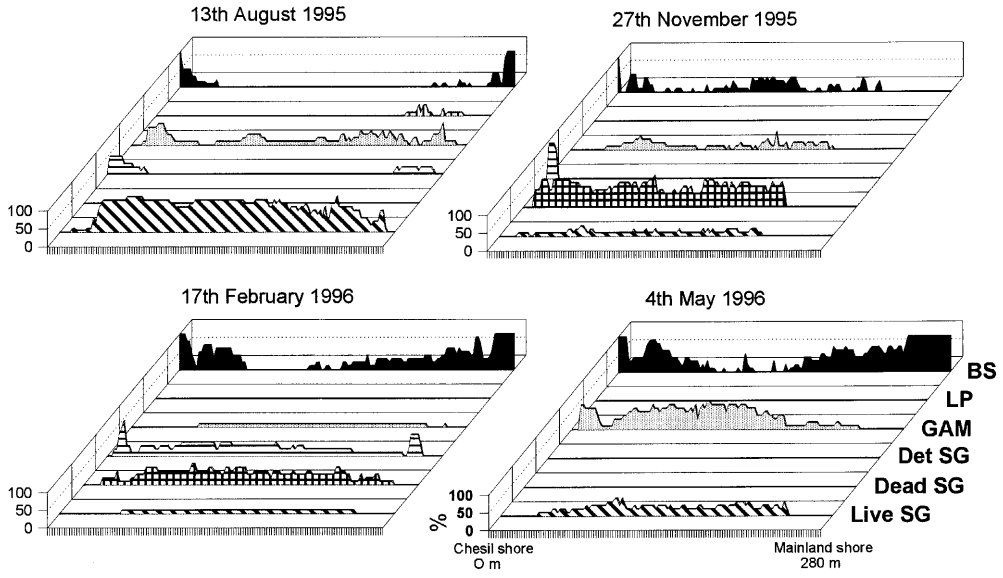

Fig. 2 *Alcyonidium gelatinosum*. Seasonal variation in vegetation cover across the Fleet Lagoon at Langton Hive Point (selected months shown). Seagrasses dominate the profile in summer alongside waning quantities of green algae. Seagrass leaves die in autumn but mainly remain intact until mid-winter. Fragmentation of seagrass detritus in winter is accompanied by mobilisation of sediments and invertebrates and the exposure of shell material. Green algal growth progresses ahead of seagrass growth in spring. *Live SG* Live seagrass; *Dead SG* dead but intact seagrass leaves; *Det SG* detached seagrass detritus; *GAM* green algal mats; *LP* stonewort *Lamprothamnion papulosum*; *BS* bare substratum

MeasurementTV (Updegraff 1990)]. Zooid length and width were measured ($n = 250$) and subjected to regression, product-moment correlation, and analysis of covariance (ANCOVA). Tentacle number distributions ($n = 150$) were compared using the Kolmogorov–Smirnov two-sample test (Sokal and Rohlf 1997). The presence of zooids containing testes or developing oocytes/embryos was noted and the diameters of the latter measured and compared using ANCOVA. Colour was defined for embryos within intact colonies and excised embryos using the Munsell System (Ryland 1958). Reproductive data were compiled for both populations over a 2-year period to determine the seasonal timing of reproduction.

Molecular analyses

Preserved colonies were prepared for DNA extraction by evaporating off the ethanol. Samples of individual colonies were macerated in 1.5-ml micro-centrifuge tubes to which 20 µl Proteinase K (10% solution), 50 µl Tris-HCl (pH 8.0), 25 µl SDS (25% solution), and 500 µl TNE buffer (100 m*M* Tris, 10 m*M* EDTA, 1.0 *M* NaCl, pH 8.0) had been added. The tubes were incubated at 37°C for 12 h. Samples were extracted twice with 600 µl phenol:chloroform (pH 7.5) followed by a single extraction with 600 µl chloroform:isoamyl alcohol (24:1). The supernatant was decanted, and DNA precipitated with 1 ml of 95% ethanol, at –20°C. Samples were centrifuged at 13,000 rpm (5 min) and the pellets washed twice with 70% ethanol, rotated for 1 h, dried at 37°C (15 min) and re-suspended in 100 µl of TE buffer (10 m*M* Tris, 1 m*M* EDTA). DNA concentrations were quantified in a Pharmacia Genequant and adjusted to 20 ng/µl with ultra-pure water.

PCR amplification of samples

Each reaction mixture contained 20 ng of sample DNA, 100 µ*M* dATP, dCTP, dGTP, and dTTP (Promega), 2.5 m*M* $MgCl_2$, 0.4 µ*M* primer (Operon Technologies), and 2.5 µl 10X PCR buffer (100 m*M* Tris HCl, 15 m*M* $MgCl_2$, 500 m*M* KCl, pH 8.0) supplied with *Taq* DNA polymerase (Boehringer Mannheim). For each reaction, 1.0 unit of *Taq* polymerase was used, in a final reaction volume of 25 µl overlaid with mineral oil (Sigma, UK). Mixtures were placed in a Hybaid Omnigene Thermocycler with the program 94.0°C (2 min), 36.0°C (1 min), 72.0°C (2 min) for 1 cycle, then 94.0°C (30 s), 36.0°C (1 min), 72.0°C (2 min) for 44 cycles. Amplification products were electrophoresed for 3.5 h in 6% polyacrylamide gels with 1X TBE buffer (0.089 *M* Tris borate, 0.002 *M* EDTA), followed by silver staining (Tegelstrom 1987). Silver-stained polyacrylamide gels were preferred to agarose gels because they allowed greater resolution of bands and thus helped to eliminate noise in a RAPD data set by reducing the chance of misidentification of homologous bands between samples (Rieseberg 1996). Gels were run with a Marker VI DNA ladder (Boehringer Mannheim).

Twenty 10-base-pair oligonucleotide primers (Operon Technologies) were tested, six of which gave clear reproducible results with polymorphic marker bands. Reproducibility of amplification products was established by (1) running controls without template DNA to test for contamination; (2) repeating PCR reactions three times; and (3) extracting different parts of the same colony in separate extractions and subsequently performing independent repeated PCR amplifications. A single brand and batch number of *Taq* was employed for all samples. DNA concentration was a critical factor in the clarity of RAPD profiles and the optimal concentration was found to be 20 ng of DNA for each 25-µl reaction.

Data analysis

The program RAPDdist (Black 1995) was used to calculate genetic distances based on similarity coefficients (distance $= 1 - S$), where $S = 2n_{ab}/n_a + n_b$; n_{ab} is the number of bands common to individuals a and b; n_a and n_b are the bands unique to individuals a and b, respectively (Nei and Li 1985). Cluster analysis was performed using the neighbour component of Phylip 3.5 C (Felsenstein 1993). Dendrograms were produced using Treeview (Page 1998). Frequencies of RAPD loci were calculated from the presence/absence of data (Lynch and Milligan 1994). This method estimates conventional population parameters with co-dominant markers. It assumes that (1) each locus has two alleles corresponding to either the presence or absence of an amplifiable site, and (2) the genotype frequencies at the loci are in Hardy–Weinberg equilibrium. Gene frequencies were calculated according to Lynch and Milligan (1994):

$$q' = \sqrt{x}\left(\frac{1 - \mathrm{Var}\ x}{8x}\right)^{-1}$$

where q is the frequency of the null allele, x is the proportion of n sampled individuals that do not exhibit the marker, and Var x is the variance of x. From frequency data, genetic distance D was calculated using the method of Nei (1972) in the RAPDdist package (Black 1995). A phenotypic analysis was also used where presence and absence data were used to calculate distance using Manhattan's distance. Bootstrapping was subsequently used to assess the robustness of the genetic distance trees.

Results

Morphology and reproduction of *Alcyonidium*

Correlation coefficients were calculated for zooid length against zooid width measurements using data from the two localities ($n = 250$ for each population). The product-moment correlation gave an r value of 0.85 ($P < 0.0001$) at Langton Hive Point indicating a good positive correlation between zooid length and width; in comparison, at Small Mouth the r value was 0.52 ($P < 0.0001$), indicating a more variable relationship between zooid length and width at this location (see Fig. 3). The high r value for the Langton Hive Point population indicates a greater regularity of zooid shape. Using ANCOVA, the measurements were also significantly different between the two populations ($P = 0.0015$, $df = 499$).

Tentacle number data showed no significant difference in frequency distribution between five colonies of *A. gelatinosum* at Small Mouth ($n = 190$). In contrast, there was a significant difference ($P < 0.02$) in 26.7% of the 15 pairwise comparisons (after Bonferroni adjustments) between six colonies at Langton Hive Point ($n = 224$). In a Kolmogorov–Smirnov two-sample test comparing the distribution of tentacle number at the two sites, there was a significant difference ($P < 0.001$) in the tentacle number distributions (range 17–21, mode 19 for *A. gelatinosum*; range 16–19, mode 18 for lagoonal *Alcyonidium*) (Fig. 4).

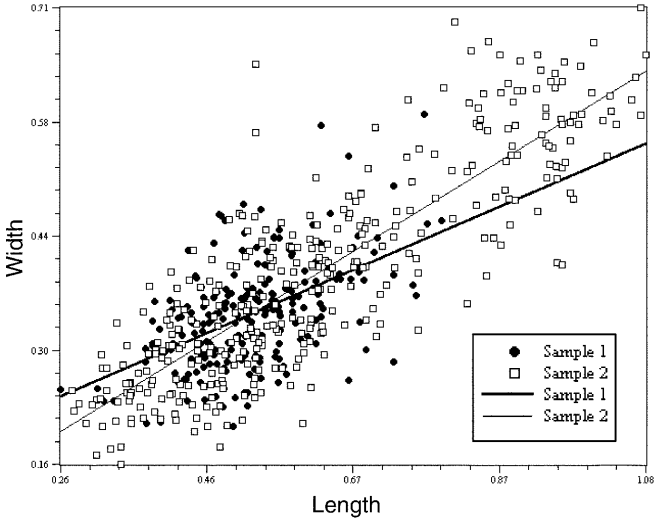

Fig. 3 *Alcyonidium gelatinosum*. Zooid length and width data comparison between Langton Hive Point (*sample 1*) and Small Mouth (*sample 2*) populations

Embryos collected from colonies at Langton Hive Point were larger in diameter (range 0.14–0.18×015–0.20 mm) than those collected from colonies at Small Mouth (range 0.11–0.18×0.14–0.19 mm). Embryos from colonies at Langton Hive Point exhibited colour intensities of 7.5YR 8/6 inside the colony, and 10.0 YR 8/4 when excised (Munsell classification). Embryos within colonies from Small Mouth had a colour of 7.5YR 8/4, and excised embryos were designated 10.0YR 7/2. Embryo colour has been a useful character in distinguishing other *Alcyonidium* species (Ryland and Porter 2000).

Detailed observations of the reproductive condition of the two populations were collected over a 2-year period (Fig. 5). *A. gelatinosum* spawns in September/October, whereas the lagoonal *Alcyonidium* spawns during January/February.

Molecular data

Following tests for consistent amplification and reproducibility of profiles over several amplifications, RAPD profiles were analysed for presence and absence of bands at specific loci, from which genetic distances were calculated (Table 1) and plotted as a consensus neighbour-joining tree after 100 bootstraps were performed (see Fig. 6). The three trees generated were identical in topology; therefore only one of them is shown here. The Nei's D tree in Fig. 6 shows three major clades. Clade A indicates two populations of *A. reticulum* grouping together, clade B shows three populations of *A. mytili* grouping together, and clade C shows three populations of *A. gelatinosum* and the lagoon population (Langton Hive Point) clustering together. Bootstrapping showed good support for clades A and B, but clade C is less well supported. With Nei's D the support is 55% and with the phenotypic analysis it is 77%. This suggests that the true relationship between the *A. gelatinosum* and Fleet Lagoon populations is poorly resolved in this type of genetic analysis. The results of the genetic analysis do, however, show that the Langton Hive population is more closely related to *A. gelatinosum* than to either *A. mytili* or *A. reticulum*. Additional genetic study may reveal further insights as to the specific status of the lagoon taxon.

Spatial distributions of *Alcyonidium* populations within the Fleet

Recent field surveys suggest that this species is confined to the central lagoonal basin (P.E.J. Dyrynda, unpublished; current study), where it is most prevalent along the landwards third of the sublittoral lagoonal

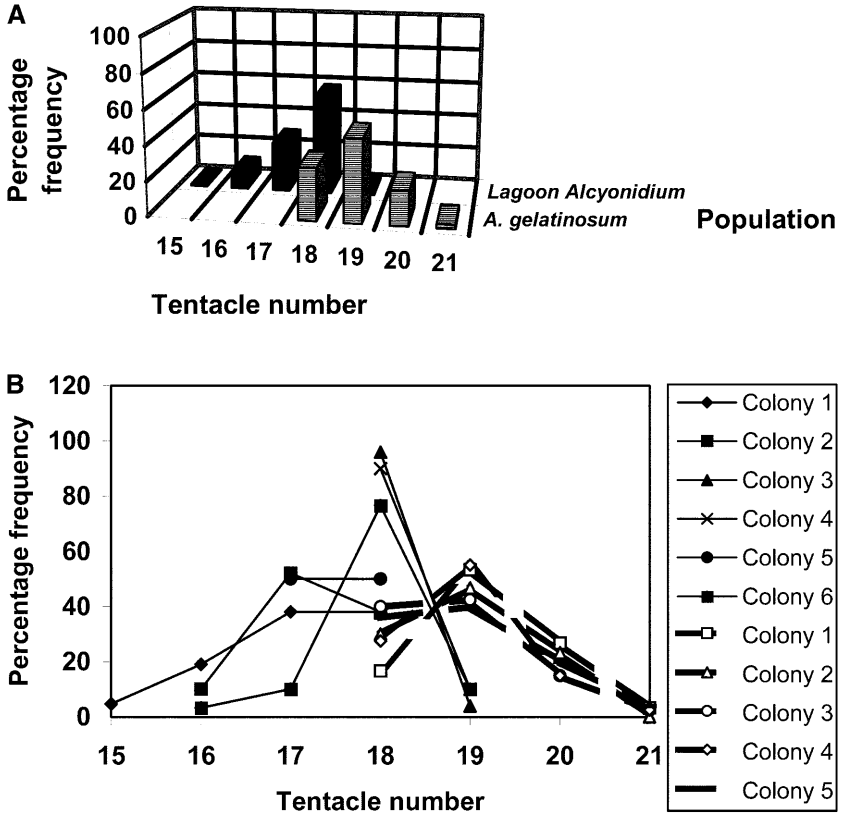

Fig. 4 *Alcyonidium gelatinosum*. **A** Comparison of tentacle number distribution between Small Mouth (*A. gelatinosum*) and Langton Hive Point (Lagoon *Alcyonidium*) sites. **B** Comparison of tentacle number distribution for six colonies of *Alcyonidium* at Small Mouth (*grey*) and five colonies at Langton Hive Point (*black*)

bottom at Langton Hive Point (Figs. 1, 2). Well-developed colonies were found upon several substrata including the shells of live molluscs, seagrass rhizomes, and large cobbles (Fig. 7). In contrast, *A. gelatinosum* is confined to the outer lagoon and occurs in abundance upon fronds of the littoral alga *Fucus serratus* within the current-scoured entrance channel at Small Mouth. A small number of colonies occurred further upstream within the 'Narrows' tidal rapids (Fig. 1), growing on *F. vesiculosus*.

Temporal variations in the occurrence of *Alcyonidium* in the Fleet

Established colonies of the lagoonal *Alcyonidium* are conspicuous in late winter/early spring, at which time the sublittoral lagoon bed is characterised by minimal vegetation cover with tracts of bare mud featuring emergent seagrass rhizomes, the shells of live and dead lagoon cockles, gastropod molluscs, and occasional stones and cobbles (Fig. 2). Colonies become much less conspicuous once rapid growth of green algae and seagrasses commences, usually in late April. Green algae dominate the epibenthic cover in May/June followed by seagrasses in July/August (Fig. 2). Significant quantities of fine sediment accrete within the developing seagrass stands, progressively smothering the veneer of dead shells and other hard substrata exposed earlier in the year. Searches undertaken during summer months revealed substantial numbers of *Alcyonidium* colonies surviving upon the shells of live gastropods grazing the seagrass leaves and upon live cockles inhabiting the understorey. Small numbers survive upon the proximal sections of growing seagrass leaves. In autumn, the stands of seagrass leaves die and turn brown. A substantial proportion, however, persists intact through to mid-winter, after which the leaves quickly disintegrate, triggering the mobilisation of associated sediments and benthic invertebrates. The removal of surface fine sediment reveals, once more, the underlying layer of mollusc shells, stones, and seagrass rhizomes. Over-wintered *Alcyonidium* colonies are conspicuous upon the shells of live cockles that maintain their position relative to the sediment surface, and upon gastropods that have gravitated from the disintegrated vegetation canopy to congregate upon cobbles and cockle shells (Fig. 7).

Discussion

Morphological, physiological, ecological, and reproductive evidence clearly indicates that the *Alcyonidium* populations at Small Mouth and Langton Hive Point comprise differentiable entities. Although of similar external appearance, significant differences exist in zooid size, tentacle number, embryo colour, reproductive season, and substratum preferences. Molecular data enable the two to be differentiated through genetic similarity indices. The amount of differentiation

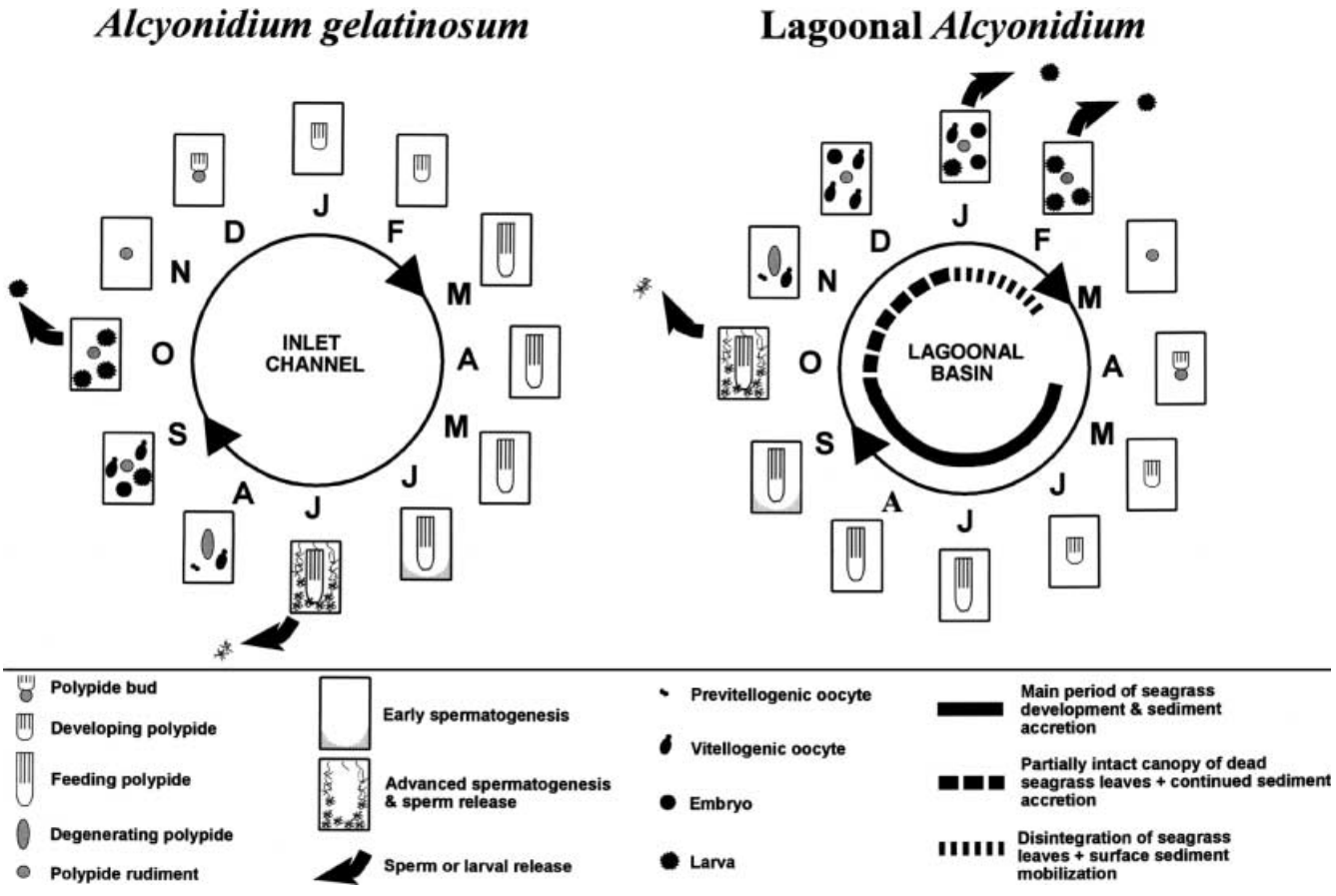

Fig. 5 *Alcyonidium gelatinosum*. Diagram of reproductive seasonality of the two *Alcyonidium* populations studied

between the lagoon population and *A. gelatinosum* from Small Mouth and other localities is less than that between *A. gelatinosum* and two other common encrusting *Alcyonidium* species, that is, *A. mytili* and *A. reticulum*. Thus, there is a substantial degree of morphological, ecological, and reproductive differentiation but a lesser amount of corresponding genetic variation, at least as shown using RAPD, between the coastal and lagoonal *Alcyonidium* populations. Integrity of the lagoonal population appears to be maintained by differences in the availability of viable substrata, and spatial and temporal barriers to gene flow, therefore causing reproductive isolation.

Viable substrata are defined here as those persisting for the time interval between settlement and spawning. The timing of recruitment of the lagoonal population (January–February) is synchronised to the seasonal availability of viable substrata (Fig. 2). The availability of hard substrata on the lagoon bottom is greatest in late winter when vegetation cover is minimal. Large cobbles and the shells of live gastropods and cockles are viable in that they remain silt free throughout the year, whereas dead shells and small stones become smothered by silt

Table 1 *Alcyonidium gelatinosum*. Genetic distance values for populations of *A. gelatinosum* (Small Mouth, Menai Strait, and Camel Estuary), *A. mytili* (Falls of Lora, Longniddry, and Cleddau Bridge), *A. reticulum* (Watwick and Porlock), and Langton Hive Point. The 1–S interpopulation values are shown below the diagonal, 1–S intrapopulation values on the diagonal (bold) and Nei's *D* values above the diagonal

Population	Small Mouth $n=29$	Menai Strait $n=17$	Camel Estuary $n=22$	Falls of Lora $n=25$	Longniddry $n=28$	Cleddau Bridge $n=24$	Watwick $n=29$	Porlock $n=27$	Langton Hive Point $n=29$
Small Mouth	**0.3214**	0.0471	0.0350	–	–	–	–	–	–
Menai Strait	0.3343	**0.3471**	0.0370	–	–	–	–	–	–
Camel Estuary	0.3226	0.3359	**0.3247**	–	–	–	–	–	–
Falls of Lora	–	–	–	**0.5924**	0.0143	0.0247	–	–	–
Longniddry	–	–	–	0.0760	**0.5925**	0.0184	–	–	–
Cleddau Bridge	–	–	–	0.1026	0.0996	**0.6236**	–	–	–
Watwick	–	–	–	–	–	–	**0.3042**	0.0746	–
Porlock	–	–	–	–	–	–	0.2913	**0.2785**	–
Langton Hive Point	–	–	–	–	–	–	–	–	**0.3191**

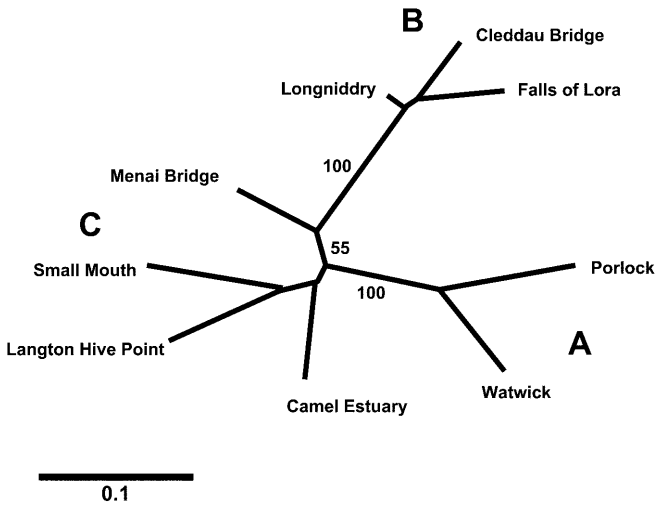

Fig. 6 *Alcyonidium gelatinosum*. Genetic distance tree for Nei's *D* values after 100 bootstrap replications, for three populations of *A. gelatinosum* (from Small Mouth, Menai Bridge, and Camel Estuary), three of *A. mytili* (Longniddry, Falls of Lora, and Cleddau Bridge), two populations of *A. reticulum* (Porlock and Watwick), and the *Alcyonidium* lagoon population (Langton Hive Point, Fleet Lagoon)

from spring to autumn, and seagrass leaves and ephemeral algae only survive for part of the year. *A. gelatinosum* at Small Mouth spawns September–October, as do other populations in southern Britain (de Putron and Ryland 1998). Its preferred substratum, *F. serratus*, typically survives intact and silt free for more than a year and is, therefore, viable. Spawned larvae of *A. gelatinosum* entering the lagoon basin on spring flood tides are unlikely to recruit in the absence of *F. serratus* (or *F. vesiculosus* L.).

Contrasting substratum preferences, varying degrees of substratum specificity, and different spawning seasons are characteristic of many bryozoan genera including *Alcyonidium* (Hayward 1985). Substratum specificity and reproductive season, or more specifically, the synchrony of spawning to particular environmental conditions, are defining characteristics for many bryozoan species. Since they often differ between species within a single genus it is likely that changes in larval substratum selection behaviour and spawning season represent adaptive steps attainable relatively quickly. Based on data presented herein, there are four hypotheses to account for the existence of this distinct population of *Alcyonidium*.

Hypothesis 1: origination within the Fleet

In geological terms, bar-built saline lagoons are ephemeral (Barnes 1980). The Fleet is estimated to be about 5,000 years old (Carr and Blackley 1974), relatively long lived by lagoon standards (Barnes 1980). The enclosing barrier may have formed further offshore than its present location (Carr and Blackley 1974). A wider, more hydraulically energetic and less isolated protolagoonal basin may have supported *F. serratus* bearing *A. gelatinosum*. The lagoonal basin *Alcyonidium* may have differentiated phenotypically and genetically in response to gradually changing selective pressures associated with increasing isolation, declining hydraulic energy, and the changing availability of viable substrata accompanying the landwards migration of the barrier. Alternatively, selective pressures may have acted upon *A. gelatinosum* larvae introduced into the lagoon in its present form.

Reproductive isolation of the lagoonal population from coastal *A. gelatinosum* may have arisen as a result of a shift in spawning season and increasing spatial separation during the evolution of the lagoon. The two populations are currently separated by a distance of 7 km, which may, alone, be adequate to ensure reproductive isolation. Dilution and the 6-h tidal phase lag separating the two localities (Robinson et al. 1983) are likely to prevent the exchange of spermatozoa, although the rafting of fertile colonies upon drift vegetation could conceivably facilitate gene flow if gametogenesis were synchronous in the two populations (which it is not). Drifting masses of algal and seagrass vegetation are characteristic of the lagoon (P.E.J. Dyrynda, personal observation).

Previous evidence of genetic differentiation within saline lagoons is limited. Planes et al. (1998) reported genetic differentiation in coral reef fish, which may be a consequence of isolation through partitioning of water bodies within the New Caledonia lagoon. Kott (1995) described a new ascidian morphospecies unique to a single Pacific coral atoll lagoon and inferred speciation through isolation.

Hypothesis 2: origination within another lagoon and subsequent natural colonisation of the Fleet

The structural and ecological characteristics and concomitant selective pressures associated with the Fleet are comparable to those of many bar-built lagoons around the world (Barnes 1980, 1989). It is feasible that the lagoonal *Alcyonidium* first diverged from *A. gelatinosum* or an as yet unknown ancestor in another lagoon. Lagoonal species are known to spread from one geographically remote lagoon to another, although the mechanisms involved are often poorly understood (Barnes 1980). Algal rafting enables the dispersal of bryozoans and other taxa producing larvae of limited dispersal capacity between remote localities (Watts et al. 1998). Sara et al. (1993) investigated four sympatric species of the sponge *Tethya* inhabiting an isolated atoll lagoon. Although all four were found to be genetically isolated, their origins were hypothesised to represent immigration and local adaptation rather than in situ speciation.

The successful introduction of a taxon to a remote lagoon represents a founder event. In the absence of

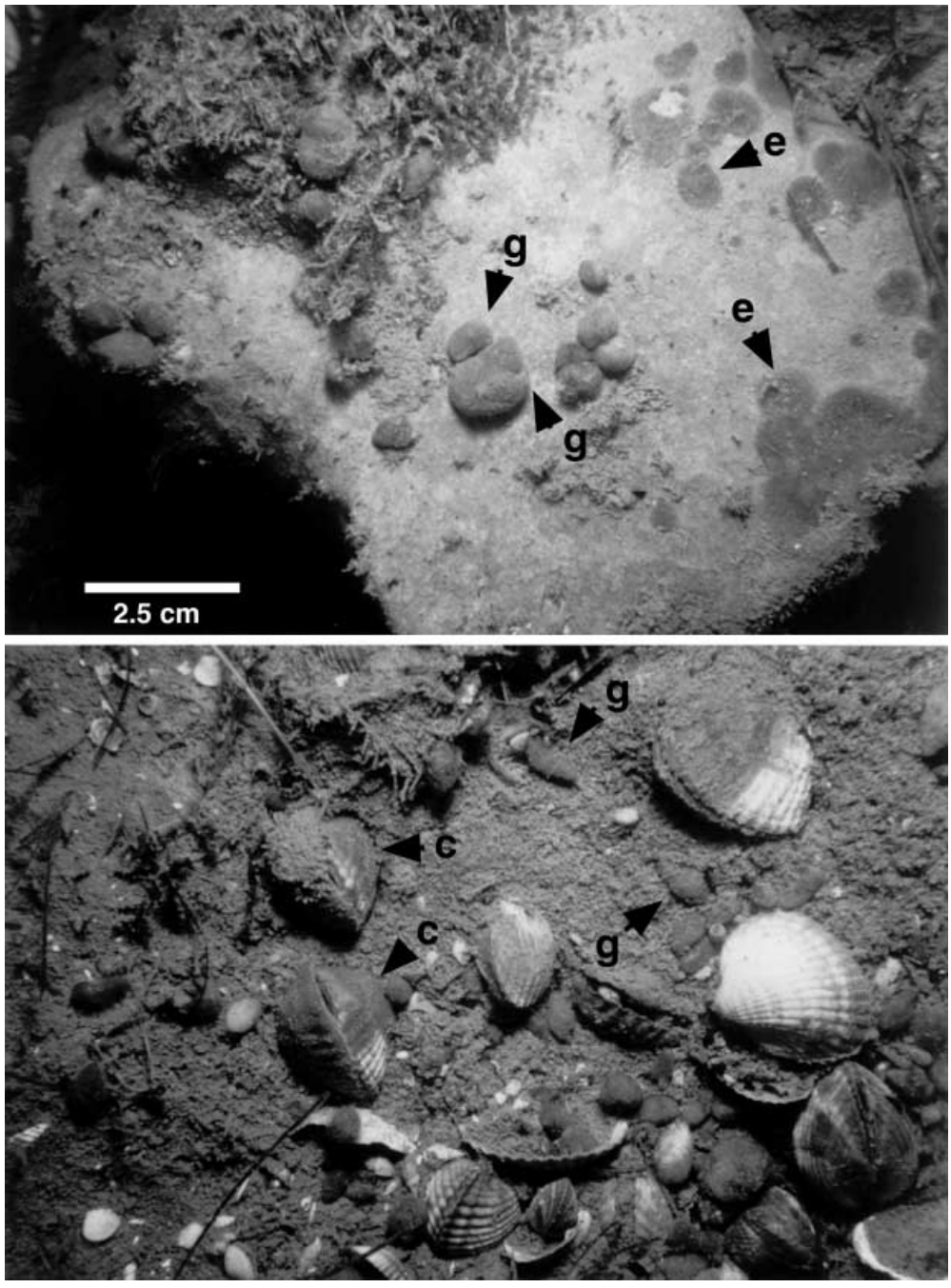

Fig. 7 *Alcyonidium gelatinosum*. Live colonies of *Alcyonidium* inhabiting the bottom of the lagoon adjacent to Langton Hive Point (April 1996). *Top* A cobble supports encrusting colonies (*e*) and live individuals of the gastropod mollusc *Littorina saxatilis* (*g*), the shells of which are almost entirely overgrown by the bryozoan. *Bottom* The mud bed features half-buried live lagoon cockles (*c*); the emergent parts of the valves bear encrusting colonies of *Alcyonidium*. The shells of small live gastropods (*g*) inhabiting the surface of the mud (*Hydrobia ventrosa* and/or *Rissoa membranacea*) are, with the exception of the opercular orifice, colonised by *Alcyonidium*

significant gene flow, differentiation would be anticipated, particularly if the population size remained small. If the lagoonal *Alcyonidium* did originate elsewhere, it may differ significantly from its source population. It would also be expected to occur in other British lagoons. Over recent years, the faunas of many United Kingdom lagoons have been surveyed (Bamber et al. 1992), and, to our knowledge, the Fleet is the only locality where this *Alcyonidium* has been recorded. Poole Harbour, a bar-built estuarine basin approximately 50 km (by sea) to the east of the Fleet contains lagoon-like subsystems. Intertidal brackish water pools at a location within the inner recesses of the harbour support a population of *Alcyonidium* encrusting small stones. This population was evaluated during the course of the current investigation. According to morphological criteria this taxon corresponds with *A. reticulum* and not the Fleet taxon. Poole Harbour supports both intertidal and shallow subtidal populations of the cockle *Cerastoderma edule* and of *C. edule*/*glaucum* hybrids (Boyden and Russell 1972). Live individuals and dead shells have been encountered during previous benthic surveys of Poole Harbour (P.E.J. Dyrynda, personal observation) and on no occasion were they found to be colonised by *Alcyonidium*. Within United Kingdom waters, other species of *Alcyonidium* are known to colonise live

mollusc shells. *A. mytili* grows upon *Mytilus edulis* L. (Cadman and Ryland 1996), and a hitherto undescribed encrusting *Alcyonidium* occurs on intertidal *Littorina obtusata* (L.) at several locations in Scotland (J.S. Porter, personal observation). Atypically, *A. gelatinosum* has been recorded growing on *M. galloprovincialis* Lamarck at Padstow, Cornwall (J.S. Ryland and J.S. Porter, personal observation). Further afield, *A. nodosum* O'Donoghue and de Watteville is epizoic on the prosobranch *Burnupena papyracea* (Bruguière) off the south and western Cape, South Africa (Ryland 2001). Ongoing surveys of *Alcyonidium* populations within United Kingdom waters, to date involving more than 70 sites representing various coastal ecosystems, have failed to find a comparable taxon (J.S. Porter and J.S. Ryland, personal observation).

Hypothesis 3: the lagoonal taxon is a southern species at its northern limit

It is possible that the lagoonal population could represent a relatively warm-water taxon at the northern limit of its distribution in southern England, in which case the Fleet Lagoon may provide a warm-water refuge. The Fleet is known to contain a range of southern species, most numerous within the tide-scoured inlet channel between Small Mouth and the Narrows (Dyrynda and Farnham 1985) (Fig. 1). If this hypothesis were correct, other records of this taxon would be expected for localities further south on the European Atlantic coast. None are apparent to date.

Hypothesis 4: introduced by shipping or other anthropogenically mediated dispersal mechanism

It is possible that this *Alcyonidium* is non-native. Many exotic taxa have been translocated from one global region to another as fouling organisms on ships (Carlton and Hodder 1995). A remote overseas source is a possibility in view of the proximity of the Fleet to the docks within Portland Harbour (Fig. 1). According to this hypothesis other populations would be expected to occur as fouling organisms within nearby docks or marinas. None, however, has been found in these habitats to date (P.E.J. Dyrynda, personal observation).

The genetic evidence suggests that this distinct *Alcyonidium* population is most likely to have originated within the Fleet. Although differentiable and apparently reproductively isolated from non-lagoonal populations of *A. gelatinosum*, its closest known relative, it is not clear whether the lagoonal *Alcyonidium* represents a true species or an intra-specific variant.

Barnes (1980, 1994) considered the evolution of lagoonal specialists and pointed out that of the species known to be characteristic of lagoons in north-west Europe, some also occur in other tideless saline environments. He hypothesised that such species may have evolved within a primordial tideless sea such as the Tethys. This is consistent with the generally accepted hypothesis that speciation normally takes at least 10^5–10^6 years. Taylor (1988), however, noted that the evolution of brooded, ephemeral larvae among cheilostomate bryozoans in the mid-Cretaceous (Albian) era was followed by a period of rapid species radiation. Stratigraphic studies of the bryozoan genera *Metrarabdotos* and *Stylopoma* (reviewed by McKinney and Jackson 1989; Jackson and Cheetham 1999) showed that evolution within these genera was characterised by long intervals of stasis punctuated by rapidly splitting lineages. Recent molecular research suggests that individual speciation events may be much more frequent than the fossil record implies. In Lake Malawi, more than 500 endemic species of cichlid fish are believed to have evolved, by micro-allopatric speciation, over a period of 700,000 years (Knight et al. 1999). The three-spined stickleback provides evidence of the rapid evolution of distinct variants and species within post-glacial freshwater lakes formed over the past 22,000 years (McPhail 1993; Foster et al. 1998). Populations of the Death Valley pupfish are a classic example of rapid allopatric divergence since the Pleistocene (which ended 11,000 years BP). Several pupfish populations show distinct differences in morphology, physiology, and behaviour, which are supported by evidence from molecular studies (Duvernell and Turner 1998). The time frame involved approaches the 5,000 years estimated for the life of the Fleet Lagoon.

We suggest that bar-built lagoons constitute relatively transient biogeographic islands (MacArthur and Wilson 1967) that typically form during periods of rising sea levels. Selective pressures imposed within stable lagoons facilitate progressive genetic biodiversification of enclosed biota that may ultimately lead to speciation. The life of saline lagoons of this type is curtailed by migration of the enclosing barrier, transition to a freshwater environment, or falling sea levels (Barnes 1980). Under such circumstances, genetic variants will only survive and may only progress to distinct species if they have colonised other habitats. Since many lagoons around the world have already been severely degraded by anthropogenic activities (Kalicharran and Diab 1993; Bettinetti et al. 1996) it is likely that much of the associated biodiversity has already been lost. The need to investigate genetic biodiversity within saline lagoons that are relatively undisturbed is, therefore, pressing.

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