Algal genotype and photoacclimatory responses of the symbiotic alga *Symbiodinium* in natural populations of the sea anemone *Anemonia viridis*

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SUMMARY

As an approach to investigate the impact of solar radiation on an alga–invertebrate symbiosis, the genetic variation and photosynthetic responses of the dinoflagellate algal symbiosis in an intertidal and a subtidal population of the sea anemone *Anemonia viridis* were explored. Allozyme analysis of the anemones indicated that the two populations were genetically very similar, with a Nei's index of genetic identity (*I*) of 0.998. The algae in all animals examined were identified as *Symbiodinium* of clade *a* by PCR-RFLP analysis of the small subunit ribosomal RNA gene. The symbiosis in the two populations did not differ significantly in algal population density, chlorophyll *a* content per algal cell or any photosynthetic parameter obtained from studies of the relationship between photosynthesis and irradiance. We conclude that there is not necessarily genetic variation or photosynthetic plasticity of the symbiotic algae in *Anemonia viridis* inhabiting environments characterized by the different solar irradiances of the subtidal and intertidal habitats.

1. INTRODUCTION

Many marine Cnidaria, including corals and sea anemones, harbour symbiotic dinoflagellate algae, *Symbiodinium* (also known as 'zooxanthellae'), from which they derive photosynthetic carbon (Muscattine 1990). Solar radiation is a major determinant of algal photosynthesis and, consequently, it influences both the amount of algal photosynthate translocated to the animal tissues and its contribution to the carbon requirements of the animal. For example, Muscattine *et al.* (1984) calculated that algal photosynthesis is sufficient to fuel the entire respiratory carbon requirement of sun-acclimated colonies of the coral *Stylophora pistillata*, but just 47–59% of the requirement of shade-acclimated colonies; and Davies (1991) found that algal photosynthesis can meet the carbon requirements of several shallow-water coral species on sunny, but not overcast, days.

The occurrence of many alga–invertebrate symbioses over wide ranges of water depth (e.g. Goreau 1959; Done 1983; Shick 1991) has been attributed to phenotypic plasticity in both the modes of animal nutrition and photosynthetic responses of the symbiosis (Falkowski 1990; Gattuso *et al.* 1993). The latter includes variation in both the density and chlorophyll content of the algae, and also their photosynthetic performance, as characterized by the photosynthesis–irradiance

(P:I) relationship of the algae. Two recent publications, however, suggest that the phenotypic plasticity of the photosynthetic systems in *Symbiodinium* may have been overestimated. Iglesias-Prieto & Trench (1994) demonstrated that the photoacclimatory responses of the algal cells to irradiance varied between *Symbiodinium* taxa in laboratory culture; and Rowan & Knowlton (1995) found that the dominant genotype of *Symbiodinium* in the coral species complex *Montastraea annularis* varied in a regular fashion with depth at Panama. Taken together, these studies raise the possibility that irradiance could be an important determinant of the distribution of *Symbiodinium*, with different algal genotypes occurring predominantly in the light environment to which its photosynthetic characteristics are well-suited.

The impact of irradiance on the distribution of *Symbiodinium* in symbiosis may be complicated by other factors. In particular, those cnidarian species in which *Symbiodinium* is transmitted vertically have a very restricted, and, possibly, no opportunity to form an association with any alga apart from that derived from its parent; while the identity of *Symbiodinium* in many species (including the coral *Montastraea annularis*, described above) that acquire their complement of algae from the environment depends on poorly understood recognition processes that are not necessarily correlated with the photosynthetic characteristics of the algae. These potentially confounding effects are particularly important if, as with the *M. annularis* complex

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(above), the symbioses in different animal species are compared.

The study reported here was designed to explore directly the photosynthetic characteristics of *Symbiodinium*, by a parallel analysis of algal genotype and P:I relationships in natural populations inhabiting environments characterized by different levels of solar radiation. The algal genotypes were assessed by restriction fragment length polymorphisms (RFLP) of the ssRNA gene, a method increasingly used to distinguish dinoflagellate symbionts (e.g. Rowan & Knowlton 1995; Gast & Caron 1996; Billingham *et al.* 1997). We chose the temperate sea anemone *Anemonia viridis*, which contains algae with the ultrastructural features of *Symbiodinium*. The taxonomy of *A. viridis* is not fully understood (Manuel 1988), and it was important for the interpretation of this study to establish whether the intertidal and subtidal populations were conspecific. This was done using allozymes, a technique of proven value in the identification of morphologically indistinguishable, but reproductively isolated species of sea anemones and other Cnidaria (e.g. Solé-Cava & Thorpe 1992; Knowlton *et al.* 1992).

2. MATERIALS AND METHODS

(a) Collection and sampling of anemones

Anemones were collected from non-overlapping subtidal and intertidal populations of *Anemonia viridis* (= *Anemonia sulcata*) at Lamorna Cove, Cornwall, UK in January–May 1995. The subtidal anemones were located at the base of kelp beds at 5–10 m depth, and the intertidal anemones were in mid-shore rock pools, at 1–3 m depth at high tide. The scalar irradiances, as measured with a LiCor 193-SB Spherical Quantum Sensor, were 30–90 and 300–1700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR) at the subtidal and intertidal sites, respectively, varying with weather conditions and season (J. C. Bythell, unpublished data). Eleven anemones from each population were used for photosynthesis experiments, and 50 were used for genetic analysis.

The photosynthetic responses were determined over two days after collection. Prior to analysis, the animals were maintained in the shade under natural illumination, with a maximum of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR. Regression analysis of each of the measured photosynthetic parameters with time from collection indicated that this treatment did not significantly influence the photosynthetic response of either the intertidal or subtidal anemones ($p > 0.05$) (J. C. Bythell, unpublished data). Following the P:I analysis, each anemone was frozen and stored in liquid nitrogen until its dry weight, chlorophyll content, and algal population density were determined.

For the genetic analysis of the anemones, seven samples of three tentacles were excised from each anemone, and homogenized in 0.5 ml ice-cold ZIB buffer of Rowan & Powers (1991a) in a hand-held glass homogenizer, until the animal tissue was disrupted but the algal cells were still intact. The homogenate was centrifuged at 12,000 g for 10 min, the supernatant was used immediately for allozyme analysis of the anemones, while the pellet was stored at -80°C for algal DNA analysis.

(b) The biomass of anemones, their algal population densities, and chlorophyll content

Each anemone was cut through the central disc into three equal parts, with wet weights used to obtain exact proportions.

One-third was dried to constant weight at 60°C for determination of dry weight; one-third was homogenized and used for quantification of algae in a haemocytometer slide; and chlorophyll was extracted from the final third in cold 90% acetone, with determination of chlorophylls a and c_2 by spectrophotometry (Jeffrey & Humphrey 1975).

(c) Allozyme analysis of anemones

The allozyme analysis used Titan III cellulose acetate plates (Helena Laboratories, Beaumont, Texas, USA), with runs of 15–30 min at 200 V, following the protocol of Searle (1985) and the histochemical staining procedure of Harris & Hopkinson (1976). The enzymes that were consistently resolvable were as follows: hexokinase (HK, EC 2.7.1.1), leucyl-valine peptidase (LVP, EC 3.4.11.13), glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1), glucose phosphate isomerase (GPI, EC 5.3.1.9), phosphoglucosyltransferase (PGM, EC 5.4.2.2), malate dehydrogenase (MDH, EC 1.1.1.37) and mannose phosphate isomerase (MPI, EC 5.3.1.8). The buffers were 40 mM of Tris-10 mM citrate (pH 7.6) for HK and LVP, 25 mM of Tris-190 mM glycine (pH 8.5) for GPI, PGM, MDH and MPI; and 40 mM of phosphate (pH 6.3) for GOT. For every plate, the position of isozymes of the anemones was scored relative to a standard, comprising a sample from kidney (HK, GOT, GPI, PGM, MPI) or liver (LVP, MDH) of the inbred mouse strain C57BL/6J.

Allelic and genotypic frequencies were calculated for the two populations, and the genotypic diversity within each population (Hunter 1993) and differentiation between populations were assessed (Nei 1972, 1987; Rogers 1972).

(d) RFLP analysis of algae

DNA was extracted from the algal samples (obtained as described above) by the method of Rowan & Powers (1991a). The PCR amplifications used 0.02 μg of template DNA per μl of reaction volume, with 1.5 mM of MgCl_2 , 0.1 mM each of dNTPs, 0.05 units of Taq DNA polymerase (Promega) per μl of final volume and 0.2 μM each of two primers. The primers were either the 'universal' primers, ss5 and ss3, or 'zooxanthellae-specific' primers, ssz5 and ssz3, of Rowan & Powers (1991a), for the ssRNA gene. The amplification conditions were 28 cycles of 45 s at 94°C , 45 s at 56°C , 2 min at 72°C , with a further 8 min at 72°C in the final cycle, to ensure complete extension. All amplifications included a sample lacking template DNA as the negative 'PCR-control'.

The amplified DNA was digested with *TaqI*, *DpnII* or *SacII* (New England BioLabs) by the procedure of Gardes & Bruns (1995). For sequential digests, DNA from the first digestion reaction was precipitated with one-ninth volumes of 5 M sodium acetate and one volume of isopropanol, washed twice in 70% ethanol and resuspended in distilled water. Half of each sample was digested with the second restriction enzyme, and the remainder was incubated in parallel, without the enzyme, as a control. The products of PCR amplification and digestion with restriction enzymes were subjected to electrophoresis in 2% agarose gels, and stained with ethidium bromide for detection of DNA under 320 nm UV transillumination. The molecular weight markers were P-9577 (Sigma Chemical Co., Poole, UK).

(e) Photosynthesis–irradiance relationships

Each anemone was incubated in a stirred 0.36 l respirometer cell submerged in natural sea water in a thermostatic water bath at $17 \pm 1^\circ\text{C}$, and the dissolved oxygen flux was

determined from changes in the oxygen concentration, which was recorded with a polarographic sensor (YSI 5739). Irradiance was provided by Siemens HR metal halide lamps, raised or lowered on a pulley system to provide stepwise ascending and descending irradiances between 64 and $1960 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR, measured with LiCor 193-SB Spherical Quantum Sensor. The rate of oxygen consumption or production was measured for 10 min at each irradiance, and the P:I response curve for each anemone was modelled from the hyperbolic tangent function: $P_{\text{net}} = P_{\text{gmax}} (\tanh(I/I_k) + R)$, where P_{net} is net productivity, P_{gmax} is the asymptotic maximum gross productivity, I is irradiance, I_k is the irradiance at which the initial slope intersects the asymptote (saturation irradiance), and R is the dark respiration rate (Falkowski *et al.* 1990). The units for these parameters are provided in table 2. The model was assessed using iterative nonlinear regression analysis. Two further parameters derived from the model were I_c , the irradiance at zero net oxygen flux (compensation irradiance), and a , the initial slope of the curve (photosynthetic efficiency). The fitted parameters were tested for homogeneity of variances (F_{max} test), and normality (Shapiro–Wilk test), and analysed by repeated measures ANOVA (ascending versus descending irradiances) with one grouping factor (intertidal and subtidal).

3. RESULTS

(a) *Allozyme variation in A. viridis*

Four of the eight enzymes tested, GOT, MDH, PGM and GPI, showed mobility variation that could reasonably be attributed to multiple alleles at single genetic loci. The two most common alleles at each locus were the same in the intertidal and subtidal populations of *A. viridis* (table 1a), and the two populations were genetically similar, with Nei's mean genetic identity (I) of 0.998 and Roger's genetic distance (D) of 0.024. They probably did not, however, comprise a single panmictic unit because (i) two alleles (*Pgm*-C and *Gpi*-C) were unique to the subtidal population (table 1a); and (ii) the allele frequencies of *Mdh* differed significantly between the two populations $\chi^2_1 = 5.7$, $0.01 < p < 0.05$; test of Nei (1987).

A total of 17 multilocus genotypes were scored (table 1b), with genotype no. 1 (*Got*-AA, *Mdh*-AA, *Pgm*-AB, *Gpi*-AB) being the most common in both populations. *Pgm* and *Gpi* showed significant heterozygote excess ($p < 0.001$) over the expectation for the Hardy–Weinberg equilibrium in both populations (intertidal, *Pgm* $\chi^2_1 = 22.2$, *Gpi* $\chi^2_1 = 26.2$; subtidal, *Pgm* $\chi^2_1 = 16.4$, *Gpi* $\chi^2_1 = 22.2$). Overall, the genotypic diversity was low. Hunter's index of genotypic diversity (D^*) was 0.24 and 0.22, for the intertidal and subtidal populations, respectively.

(b) *Molecular typing of the symbiotic algae*

The greater part of the molecular analysis of algae was conducted on the amplification products obtained with the primers ssz5 and ssz3. For DNA samples of the algae from every animal from both the intertidal and subtidal populations, a single PCR product of length 1.6 kb was obtained (lane 9 in figure 1a); and the principal products of restriction digestion with *Dpn* II were 0.85 kb and 0.58 kb (lane 2 in figure 2a), and with *Taq* I were 0.70 kb and 0.60 kb (lane 5 in figure

Table 1. *Allozyme analysis of subtidal and intertidal populations of Anemonia viridis*

(a) allele frequencies

enzyme system/ allele	intertidal population	subtidal population
GOT A	0.94	0.96
B	0.06	0.04
MDH A	0.92	0.99
B	0.08	0.01
PGM A	0.60	0.63
B	0.40	0.36
C	0	0.01
GPI A	0.58	0.52
B	0.42	0.41
C	0	0.07

(b) multilocus genotypes

multilocus genotype	genotype				frequency of <i>A. viridis</i>	
	<i>Got</i>	<i>Mdh</i>	<i>Pgm</i>	<i>Gpi</i>	intertidal population ($n = 50$)	subtidal po- pulation ($n = 50$)
1	AA	AA	AB	AB	30	24
2	AA	AA	AA	AB	4	9
3	AA	AA	AB	AA	5	4
4	AB	AA	AB	AB	1	2
5	AB	AA	AA	AB	1	2
6	AA	AA	AA	AA	1	1
7	AB	AB	AA	AB	2	0
8	AB	AB	AB	AB	1	0
9	AB	AB	AB	AA	1	0
10	AA	AB	AA	AB	1	0
11	AA	AB	AB	AB	2	0
12	AA	AB	AA	AA	1	0
13	AA	AB	AB	AA	0	1
14	AA	AA	AB	AC	0	2
15	AA	AA	AB	BC	0	3
16	AA	AA	AA	BC	0	1
17	AA	AA	AC	AC	0	1

1a). This pattern of restriction fragments is diagnostic of *Symbiodinium* clade *a* (Rowan & Powers 1991b; Rowan & Knowlton 1995).

To construct a restriction map for the ssRNA gene of the algae, the products of digestion with either *Taq* I or *Dpn* II were digested with *Sac* II, which has a single restriction site at nucleotide no. 570 of the gene (Sadler *et al.* 1992). The smaller band of *Taq* I digestion, and larger band of the digestion by *Dpn* II were cut by *Sac* II (figure 1a, lanes 3 and 6, respectively), and the sizes of the fragments generated were used to construct the restriction map in figure 1b.

DNA samples of algae from ten each of the intertidal and subtidal animals were amplified with the primers ss5 and ss3. For every sample, a single 1.8 kb product was generated. The products of digestion with *Taq* I were 0.81 and 0.60 kb, and of digestion with *Dpn* II were 0.97 and 0.58 kb, these values being consistent with the data obtained above (see figure 1b).

Table 2. *Photosynthetic parameters derived from P:I curves of intertidal and subtidal Anemonia viridis*(Values of mean $\pm$ 95% confidence limits are shown ($n = 11$).)

	photosynthetic parameter				
	P_{gmax} mg O ₂ h ⁻¹	I_k $\mu\text{mol m}^{-2} \text{s}^{-1}$	I_c $\mu\text{mol m}^{-2} \text{s}^{-1}$	$\frac{a}{\text{mg O}_2 \text{ h}^{-1}}$ $\mu\text{mol m}^{-2} \text{s}^{-1}$	R mg O ₂ h ⁻¹
per g dry weight					
intertidal	3.1 $\pm$ 0.31	288 $\pm$ 51	70 $\pm$ 9	0.012 $\pm$ 0.0026	- 0.8 $\pm$ 0.09
subtidal	3.2 $\pm$ 0.62	349 $\pm$ 56	84 $\pm$ 13	0.009 $\pm$ 0.0016	- 0.7 $\pm$ 0.12
per 10 ⁹ algal cells					
intertidal	4.6 $\pm$ 1.08	308 $\pm$ 80	72 $\pm$ 17	0.014 $\pm$ 0.0038	- 1.1 $\pm$ 0.32
subtidal	6.2 $\pm$ 1.84	474 $\pm$ 142	107 $\pm$ 32	0.016 $\pm$ 0.0029	- 1.4 $\pm$ 0.47
per mg chlorophyll <i>a</i>					
intertidal	4.6 $\pm$ 0.98	282 $\pm$ 54	68 $\pm$ 8	0.016 $\pm$ 0.0029	- 1.1 $\pm$ 0.23
subtidal	5.1 $\pm$ 1.04	338 $\pm$ 82	83 $\pm$ 15	0.015 $\pm$ 0.0038	- 1.3 $\pm$ 0.36

Figure 1. Restriction analysis of ssRNA gene of *Symbiodinium* from *Anemonia viridis*. (a) Restriction pattern of PCR product obtained with the primers ssz5 and ssz3 of Rowan & Powers (1991a). Lane 1: molecular weight markers; lane 2: *DpnII*; lane 3: *DpnII*-*SacII*; lane 4: *DpnII*-*SacII* control; lane 5: *TaqI*; lane 6: *TaqI*-*SacII*; lane 7: *TaqI*-*SacII* control; lane 8: PCR control; lane 9: undigested PCR product. The controls (lanes 4, 7 and 8) are described in §2. (b) Map of restriction sites of *DpnII* ($\downarrow$) and *TaqI* ($\uparrow$). The nucleotide number is relative to the ssRNA gene of *Symbiodinium* (GenBank accession number M88521). The length of PCR products obtained with the primers ss5 & ss3 and ssz5 & ssz3, respectively, is indicated.

(c) *The photoacclimatory responses of the zooxanthellae*

The anemones from the intertidal habitat bore $6.8 \pm 1.24 \times 10^8$ algae g⁻¹ dry weight; and this did not

differ significantly from the density of $5.6 \pm 1.31 \times 10^8$ algae g⁻¹ dry weight of anemones in the subtidal region ($t_{22} = 1.48$, $p > 0.05$). Also, the chlorophyll content of the algae did not differ between the two sites. The algae contained 1.1 ± 0.43 and 1.2 ± 0.37 pg chlorophyll *a* cell⁻¹ in the intertidal and subtidal anemones, respectively ($t_{22} = 0.6$, $p > 0.05$).

The P:I curves obtained for intertidal and subtidal anemones were used to obtain the principal photosynthetic parameters, given in table 2. None of these parameters varied significantly between the intertidal and subtidal anemones, when expressed on the basis of total dry weight or algal chlorophyll *a* content; and, when expressed in terms of algal cell number, just one of the five parameters, I_k , was significantly lower in the intertidal animals than subtidal animals ($t_{22} = 2.7$, $0.01 < p < 0.05$). There was evidence for photoinhibition following exposure to high irradiances, with a significantly reduced P_{gmax} (repeated measures ANOVA, $F_{1,20} = 53$, $p < 0.001$), but this did not vary between the intertidal and the subtidal groups ($F_{1,20} = 0.25$, $p > 0.05$). The lack of significant repeated-measures effects on photosynthetic efficiency (*a*) or saturating irradiance (I_k) (at $p > 0.05$) suggests that photoinhibition was a transient effect, possibly a result of adaptive down-regulation of production rather than an indication of photosystem damage.

4. DISCUSSION

The core purpose of this study was to investigate the relationship between algal genotype and the photosynthetic responses of an alga-invertebrate symbiosis. The experimental data concern just one system, *Anemonia viridis*, and interpretation of the results depends on accurate distinction between those features of *A. viridis* that are specific to this system and those that are general to algal symbioses in animals. This issue is addressed in the first part of the discussion, and it is followed by a consideration of the photosynthetic responses of the symbiotic algae.

(a) The algal symbiosis in *A. viridis*

A. viridis is an unusual species for it harbours both symbiotic algae and occurs at high latitudes. Algal symbioses are common in shallow-water benthic Cnidaria, including corals and sea anemones in the tropics and subtropics, but their contribution to the benthic fauna declines progressively with increasing latitude. For example, just three of *ca.* 80 species of sea anemones reported around UK shores have symbiotic algae, and of these only *A. viridis* is common.

Despite the exceptional ecological circumstances of *A. viridis*, several aspects of its symbiosis are comparable to associations at lower latitudes. In particular, this study provides a definitive molecular demonstration that the symbiotic algae are *Symbiodinium*, and that they can be assigned to one of the three main clades, clade *a*, as identified by Rowan & Powers (1991b). Clade *a* has previously been identified in symbioses with Cnidaria in the Indo-Pacific and Caribbean. It exhibits very low levels of molecular variation, at least in the ssRNA gene (Rowan & Powers 1991b; R. Rowan, personal communication). Also, there is evidence that the symbiosis in *A. viridis* is functionally equivalent to associations at low latitudes. As in other symbioses involving *Symbiodinium*, the algae release a substantial proportion of the photosynthetically fixed carbon, which contributes to much or all of the respiratory carbon requirements of the animal (Tytler & Davies 1984; Stambler & Dubinsky 1987; Davy *et al.* 1996).

The intertidal and subtidal populations of *A. viridis* studied here can be interpreted reasonably as conspecific on the basis of their similarity of allozyme characteristics, as reflected in the high value of Nei's mean genetic identity, *I*. This system is, therefore, well-suited to the study of genotypic and phenotypic variation in the symbiosis between the two populations (see §1). The prevalence of a single multilocus genotype (no. 1 in table 1b) in both populations can be attributed, at least in part, to the high incidence of asexual reproduction in *A. viridis* (Manuel 1988). *A. viridis* populations at other locations in the UK are not dominated by this multilocus genotype (Z. Billinghamurst, J. B. Searle & A. E. Douglas, unpublished data), suggesting that geographically local (and probably recent) clonal expansion is an important factor shaping the population structure of *A. viridis* at Lamorna Cove.

We conclude from these considerations that the results on the photosynthetic response of the association in *A. viridis* are of general relevance to alga-invertebrate symbioses, including those at low latitudes.

(b) On the uniformity of the symbiosis in intertidal and subtidal populations of *A. viridis*

This study revealed no strong evidence for variation in either the genotype or the photoacclimatory responses of the symbiotic algae between natural populations of *A. viridis* living in different solar radiation environments.

The reasons for the apparent lack of genetic variation in *Symbiodinium* studied here cannot be established

until the algae in other *A. viridis* populations are studied. However, one important factor may be the vertical transmission of the algae during asexual and sexual reproduction of *A. viridis* (J. C. Bythell, unpublished observations). This would impose uniformity on the algal partner (Douglas 1995), unless there is (i) regular exchange of algae between the established symbiosis and the surrounding water and (ii) a diversity of *Symbiodinium* available in the water column. (It is not known if either process occurs.)

Recent studies on the genetic diversity of *Symbiodinium* in scleractinian corals at Bermuda suggest that their uniformity in *A. viridis* may not be exceptional. Each of most Bermudian coral species bore a single algal RFLP haplotype, independent of both depth (4–30 m) and whether the algae were transmitted vertically or acquired from the water column (Billinghurst *et al.* 1996; Billinghamurst 1997). Taken together, these studies suggest that the partitioning of *Symbiodinium* by depth and irradiance, which occurs in *Montastraea annularis* at Panama (Rowan & Knowlton 1995), may not be general, but particular to certain geographic locations and host species.

The lack of a statistically significant difference between the intertidal and subtidal populations of *A. viridis* in algal population density, chlorophyll content and photosynthetic parameters, contrasts sharply with most published studies on this subject, which describe clear-cut effects of irradiance or depth on *Symbiodinium* symbioses (reviewed in Falkowski *et al.* (1990) and Shick (1991)). Of particular relevance to this study, Davy *et al.* (1996) obtained differences in the P:I responses between geographically distant intertidal and subtidal populations of *A. viridis*, although, as the authors commented, the contribution of phenotypic and genetic differences between the algae to this effect was unknown. We consider it likely that the broad uniformity of the photosynthetic responses of the intertidal and subtidal symbioses in *A. viridis* studied here can be attributed largely to the lack of genetic differences between the symbiotic algae in the two situations. This interpretation is consistent with the finding that the photosynthetic plasticity of individual genotypes of *Symbiodinium* is lower than is generally appreciated (Iglesias-Prieto & Trench 1994).

The chief conclusion of this study, therefore, is that neither genetic variation nor photosynthetic plasticity of the alga can be assumed to contribute to the persistence of natural populations of alga-invertebrate symbioses living in widely different solar radiation environments.

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