Hydrobiologia (2012) 687:85–99
DOI 10.1007/s10750-011-0884-0

SPONGE RESEARCH DEVELOPMENTS

Relationship between genetic, chemical, and bacterial diversity in the Atlanto-Mediterranean bath sponge *Spongia lamella*

Charlotte Noyer · Mikel A. Becerro

Received: 30 March 2011 / Accepted: 6 September 2011 / Published online: 11 October 2011

Abstract Does diversity beget diversity? Diversity includes a diversity of concepts because it is linked to variability in and of life and can be applied to multiple levels. The connections between multiple levels of diversity are poorly understood. Here, we investigated the relationships between genetic, bacterial, and chemical diversity of the endangered Atlanto-Mediterranean sponge *Spongia lamella*. These levels of diversity are intrinsically related to sponge evolution and could have strong conservation implications. We used microsatellite markers, denaturing gel gradient electrophoresis and quantitative polymerase chain reaction, and high performance liquid chromatography to quantify genetic, bacterial, and chemical diversity of nine sponge populations. We then used correlations to test whether these diversity levels covaried. We found that sponge populations differed significantly in genetic, bacterial, and chemical diversity. We also found a strong geographic pattern of increasing genetic, bacterial, and chemical dissimilarity with increasing geographic distance between populations. However, we failed to detect significant correlations between the three levels of diversity investigated in our study. Our results suggest that diversity fails to beget diversity within a single species and indicates that a diversity of factors regulates a diversity of diversities, which highlights the complex nature of the mechanisms behind diversity.

Keywords Biodiversity · Correlations · Heterozygosity–fitness correlations · Bacterial diversity · Chemical diversity · Porifera · *Spongia agaricina* · *Spongia lamella*

Guest editors: M. Maldonado, X. Turon, M. A. Becerro & M. J. Uriz / Ancient animals, new challenges: developments in sponge research

C. Noyer
Department of Marine Ecology, Centro de Estudios Avanzados de Blanes (CEAB-CSIC), Acceso Cala St. Francesc 14, Blanes, 17300 Girona, Spain

M. A. Becerro (✉)
Natural Products and Agrobiology Institute (IPNA-CSIC), Avda Astrofísico Francisco Sánchez 3, 38206 La Laguna (Tenerife), Spain
e-mail: m.becerro@csic.es

Introduction

Tremendous efforts are underway to assess the world's biodiversity. The definition of "biodiversity" (or biological diversity) is a challenge in itself partly because of the complex, dynamic, and multifaceted concept of diversity (Gaston & Spicer, 2004). Biodiversity can thus go beyond the widespread species diversity notion to include the habitats, communities, and ecosystems where species live (so called ecosystem diversity), the genes and molecules found within a single species (genetic diversity), and the relationships between them (Feral, 2002). Whether this diversity of diversities is correlated and diversity begets diversity

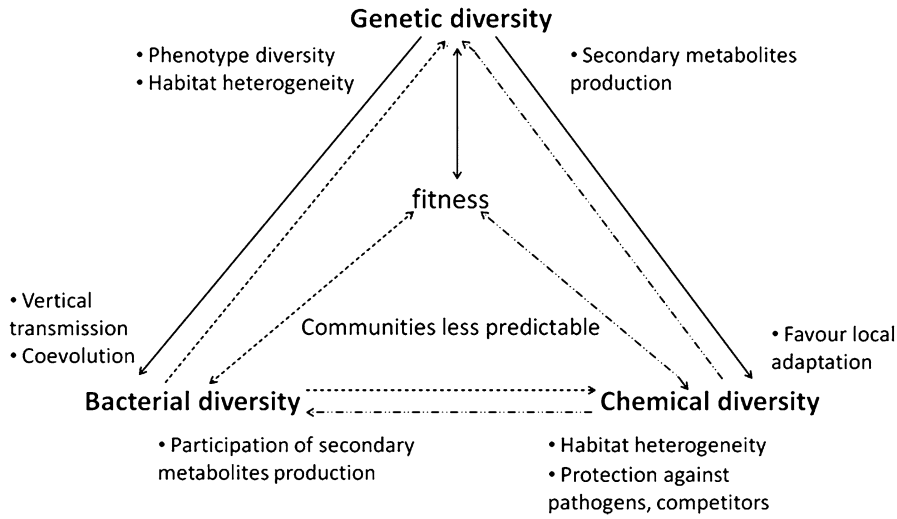

Fig. 1 Potential connections between genetic, bacterial, and chemical diversity

is an important challenge that could shed light on the mechanisms that generate and maintain biodiversity (Whittaker, 1972). Exploring how species diversity affects ecosystem functions has received certain attention from the scientific community (Cardinale et al., 2002; Lamosova et al., 2010). Other studies have aimed at the relationship between species and community diversity, or between species and genetic diversity (Vellend & Geber, 2005; Odat et al., 2010). However, linking diversities is a complex topic that remains controversial and observation-scale dependent (Yue et al., 2005).

According to Whittaker (1972), two main hypotheses explain how diversity in communities could influence diversity in other levels (Whittaker, 1972; Palmer & Maurer, 1997). The first is "diversity begets diversity" and three mechanisms support this suggestion (Palmer & Maurer, 1997). The first mechanism refers to the varying capacity of species to influence the environment, which generates a small-scale heterogeneity that could in turn create opportunities for new species to settle down (Palmer & Maurer, 1997). A second mechanism suggests that diverse communities are less predictable, making competitive exclusion less likely (Palmer & Maurer, 1997). Finally, "diversity breeding diversity" occurs for dependent communities (symbionts, commensals, pathogens…) that are likely to be diverse if their host communities also are diverse (Palmer & Maurer, 1997).

Counteracting the "diversity begets diversity" hypothesis, the niche saturation or "niche limitation" hypothesis assumes biological limits to species number in a community. As niches are limited, the likelihood of successful establishment for a species decreases as the number of species increases (Palmer & Maurer, 1997). Understanding the processes that drive diversity appears to be a challenging and problematic topic with many implications.

In our study, we aimed to unravel whether diversity begets diversity at a species level. We used genetic, bacterial, and chemical diversities that are intrinsically related to sponge evolution to investigate whether or not the data supports the diversity begets diversity hypothesis (Fig. 1). Sponges offer unique opportunities to further understand the multiple levels of diversity and their relationships, yet they have contributed minimally to this field (Becerro, 2008). Sponges are important components of marine sessile communities in a wide range of aquatic ecosystems (Diaz & Rutzler, 2001; Bell, 2008). Sponges host a large diversity of symbiotic microorganisms such as algae, archaea, and bacteria (Lee et al., 2001; Hentschel et al., 2006; Taylor et al., 2007). Sponges are also a major source of natural products, which can present biotechnological interests (Sipkema et al., 2005). Beyond losing species diversity, the loss of sponge species could result in the loss of bacterial and chemical diversities, whose biotechnological applications we are steadily increasing (Arrieta et al., 2010). Thus, these levels of diversity appeared intrinsically related to sponge evolution and could have strong implications for conservation. For this reason, we

tested whether levels of genetic, bacterial, or chemical diversity were associated with or remained independent of each other.

To investigate the relationship between genetic, bacterial, and chemical diversity we sampled nine populations of the endangered Mediterranean bath sponge *Spongia lamella*. *Spongia lamella* may be better known as the Mediterranean bath sponge *Spongia agaricina*. However, *S. agaricina* first description was performed on a now missing specimen from the Indian Sea (Pronzato & Manconi, 2008). Sharing a similar morphology and shape with the Mediterranean sponge (a massive gray color with inner white tissue sponge with a vase or dish-like shape), the name *S. agaricina* was generalized and used for the Mediterranean specimens. Recent evidence showed significant differences between the Philippine and Mediterranean specimens (Castritsi-Catharios et al., 2007; Pronzato & Manconi, 2008), which should be referred to as *S. agaricina* Pallas 1766 and *S. lamella* Schultze 1879, respectively (Pronzato & Manconi, 2008). *Spongia lamella* has a Mediterranean distribution, although occasionally reported in the Atlantic coastal waters of the Iberian Peninsula (Lopes & Boury-Esnault, 1981, J. Xavier, pers. comm.). Natural populations of commercial sponges including *S. lamella* decreased drastically in the Mediterranean as a consequence of overfishing, habitat degradation, and spread of diseases (Gaino & Pronzato, 1989, 1992; Pronzato, 1999) and are now registered in the Annex III of the Berne and Barcelona Conventions (Templado et al., 2004). Genetic studies have shown strong genetic differentiation between populations of this species probably linked to the life cycle (brooding sponge with low dispersal capabilities, slow growing animal) and overexploitation (Noyer et al., 2009; Noyer, 2010). The tissues of *S. lamella* contain abundant bacteria (Bertrand & Vacelet, 1971) dominated by *Chloroflexi* and *Acidobacteria* communities (Noyer, 2010, Noyer et al., 2010) and a great array of structurally diverse secondary metabolites with terpenes as mayor compounds (Cimino et al., 1975; Aiello et al., 1988; Fontana et al., 1996; Rueda et al., 1998; Noyer et al., 2011). Understanding the genetic, chemical, and bacterial diversity of this species can therefore be critical for the conservation and survival of this species. We investigated three of the four components of diversity: (i) the number of entities (richness), (ii) their abundances (evenness), and (iii) their dissimilarities (Magurran, 2004, Sala & Knowlton, 2006). The fourth component of diversity refers to the functional roles of the entities (Magurran, 2004; Sala & Knowlton, 2006) and was left out of this preliminary study because it requires experimental manipulations that will benefit from our observational approach. To quantify the three components of diversity, we used multi-locus heterozygosity (MLH), mean d^2, expected heterozygosity, and gene diversity for genetic data and the Shannon index on bacterial and chemical data, which were used to explore the potential connections between genetic, bacterial, and chemical diversity.

Materials and methods

Sample collection

Sampling was conducting by scuba diving at nine Atlanto-Mediterranean locations representing five regions: South France, Catalonia, Baleares, Gibraltar, and Portugal (Fig. 2). We collected sponges at three locations around Marseille in December 2006 at Plane (Pla), Pharillon (Far), and La Ciotat (Cio). In Catalonia, we sampled *S. lamella* at Cap de Creus (Cre) in summer 2006, Els Bullents (Bul) in summer 2008, and Arenys (Are) in winter 2006. Populations of Cabrera (Cab) in the Baleares Island and Ceuta (Ceu) in Gibraltar were sampled in winter 2005. Population of Portugal was sampled in the Berlengas archipelago in summer 2005 and 2007. For each specimen, a fraction on one edge of the sponge was cut underwater to minimize damage, placed into plastic bag containing sea water, and stored in cool box until further processing (usually 1–2 h after sampling). Sponge tissues were then manually cleaned of foreign tissues, soaked in a series of absolute ethanol baths to prevent ethanol dilution and degradation/contamination of sponge tissues, and conserved in absolute ethanol at −20°C until processed. Samples for population genetic analysis were extracted as in Noyer et al. (2009). For the quantitative analysis of bacterial communities we used DNAeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions, except for a 5 min incubation time before elution in a total volume of 75 ll supplied buffer (Noyer et al., 2010). In parallel, some of the specimens from Marseille (3 populations), Catalonia (3 populations) were also collected for

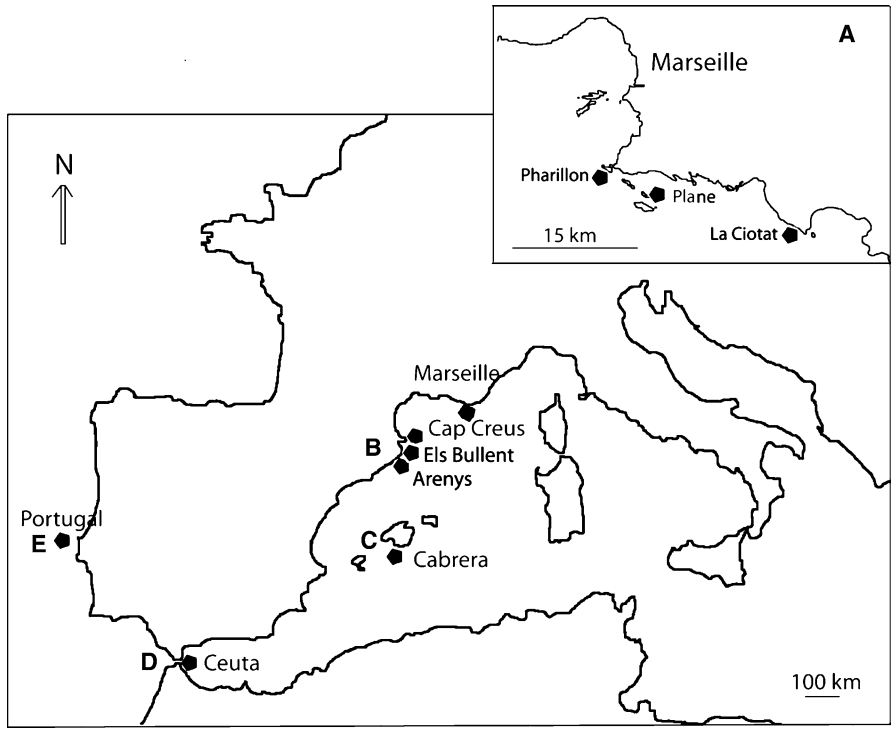

Fig. 2 Sampling sites for *Spongia lamella* in the western Mediterranean and Atlantic Iberian coast

chemical analyses, and were placed in a freezer at −20°C. An additional sample was also obtained from Ceuta. All of those specimens were then freeze-dried and stored in a −20°C freezer until chemical extraction (Noyer, 2010; Noyer et al., 2011).

Data analysis

We used three approaches to test multiple correlations: at individual level, at population level, and at inter-population level (i.e., population dissimilarities). Individuals were typed at seven microsatellite loci, genotyping protocols are described in Noyer et al. (2009, 2010). For each individual, we measured (1) MLH calculated as the proportion of loci that were heterozygous corrected for non-scored loci (Lesbarreres et al., 2007; Chapman et al., 2009; Pujolar et al., 2009) and (2) mean d^2 measured by $LD = \log$ (mean $d^2 + 1$) according to Da Silva et al. (2009). Mean d^2 provides a measure of the genetic distance between parental gamete genomes. Individuals with alleles at a given locus that differ the most in the number of repeat units are presumed to have higher levels of outbreeding and the parental alleles have more ancestral coalescence times (Da Silva et al., 2009; Hansson, 2010). At the population level, we used (1) expected heterozygosities (H_e) as a value for genetic diversity and (2) gene diversity (Noyer, 2010). At the interpopulation level, we used F_{ST} between pairwise populations as an index of genetic dissimilarities between sponge populations (Noyer, 2010).

Shannon index was used to assess bacterial and chemical diversity since (i) it is one of the most frequently used and (ii) it encompasses both richness (number) and evenness (equitability). This index is a measure of information entropy, it measures uncertainty (Sarkar, 2006), and is maximal when components are evenly distributed. Bacterial diversity was estimated by denaturing gel gradient electrophoresis (DGGE) and quantitative PCR (qPCR) analyses. We used the universal bacterial primers BAC358F (5′-CCT ACG GGA GGC AGC AG-3′) and BAC907RM (5′-CCG TCA ATT CMT TTG AGT TT-3′) to amplify fragments approximately 560 bp. We used a 40–75% vertical denaturant gradient (100% denaturant agent is 7 M urea and 40% deionized formamide) on 6% polyacrylamide gel in 1× TAE to separate bacterial bands, i.e., sequences (Muyzer & Smalla, 1998). We analyzed images of the gels using the Gels plot lanes tool of ImageJ software 1.38X (Wayne Rasband, National Institutes of Health, USA) according to Noyer (2010) and references therein. To perform

quantitative analyses using qPCR, we used five specific primer pairs designed in Noyer et al. (2010), to amplify and quantify specifically bacterial clades using a Stratagene Mx3005P QPCR system and 2× Brilliant SYBR® Green QPCR Master Mix (Stratagene) (Noyer, 2010). For each individual screened, we used Shannon diversity index from the DIVERSE procedure available in PRIMER 6 (Clarke & Warwick, 2001). Bacterial diversity was assessed by bacterial richness as the number of bands on the DGGE gels in an individual sample, and evenness through the relative intensity (quantity) of the bands on the gels (Casamayor et al., 2000). Individual Shannon index was also used to estimate diversity of the bacterial clades quantified by qPCR (*Chloroflexi* clade 1 and clade 2; *Acidobacteria* clade 1 and clade 2, and *Actinobacteria*). For each population, we averaged the individual indices within their original populations and we obtained the average index of bacterial diversity per sponge population. For the interpopulation comparisons, we used pairwise dissimilarities obtained from the SIMPER procedure available in PRIMER v6, both for the DGGE banding patterns and for the quantitative proportions of bacterial clades amplified.

Chemical diversity was estimated at the same individual, population and interpopulation levels. Secondary metabolites were extracted by dichloromethane/methanol (1:1) solution and extracts were analyzed by high performance liquid chromatography (HPLC) to characterize chemical profiles (Noyer, 2010; Noyer et al., 2011). In agreement with previous studies on *S. agaricina* secondary metabolites, chemical profiles showed two major compounds, the nitenin being the major compound, and a variety of minor metabolites belonging to the product class "terpenes" (Aiello et al., 1988; Rueda et al., 1998). For each individual, we used Shannon indices obtained from the DIVERSE procedure (available in PRIMER v6) from the chemical SPE–ELSD–HPLC profiles (Noyer et al., 2011). Thus, chemical diversity was assessed by chemical richness as the number of compounds in an individual sample, and chemical evenness through the abundance or quantity of this compound in the sample. For each population, we averaged individual Shannon indices within their original populations. For the interpopulation comparisons, we used pairwise dissimilarities from the SIMPER procedure (available in PRIMER v6). Pairwise dissimilarities were performed on 10 out of the 22 compounds that presented significant variations between sponge populations (Noyer, 2010; Noyer et al., 2011).

Multiples correlations were globally performed first between individual measures. However, only subsamples of each sponge populations used for the genetic analyses were used for bacterial and chemical analyses. Thus, only individuals that have triple genetic, bacterial, and chemical data were used in the individual-level analysis. As 10 individuals per populations were used for the qPCR analysis, we also investigated more closely the relationship between genetic and bacterial diversity at the individual-level per population. At the population level, multiple correlations were carried between the different measures of diversity averaged per population. At the interpopulation level, we visualized population dissimilarities with hitmaps using JColorGrid software (Joachimiak et al., 2006). We performed correlations between three matrixes to investigate whether there are relationships between genetic, bacterial, and chemical dissimilarities. The analyses of correlations were run separately for bacterial diversity using DGGE data or qPCR data to test one measure of genetic, bacterial, and chemical dissimilarities.

Results

Individual-level correlations

Multiple correlations of individuals that were typed for genetic, bacterial, and chemical analyses are summarized on Fig. 3. We observed negative correlations for both genetic measures, MLH and mean d^2 with chemical diversity, however, those correlations were not significant ($P = 0.325$ for MLH, and $P = 0.175$ for mean d^2 after Bonferroni corrections). We did not detect any correlations between chemical diversity and bacterial diversity using both DGGE and qPCR Shannon indices, or between genetic diversity (MLH and mean d^2) and bacterial diversity (DGGE and qPCR). The only significant correlation was obtained between the two measures of genetic diversity ($P = 0.015$, after Bonferroni corrections, Fig. 3).

As more individuals were scored for qPCR, we also investigated more deeply relationships between bacterial and genetic diversity at the individual-level per population (only individuals with paired genetic and

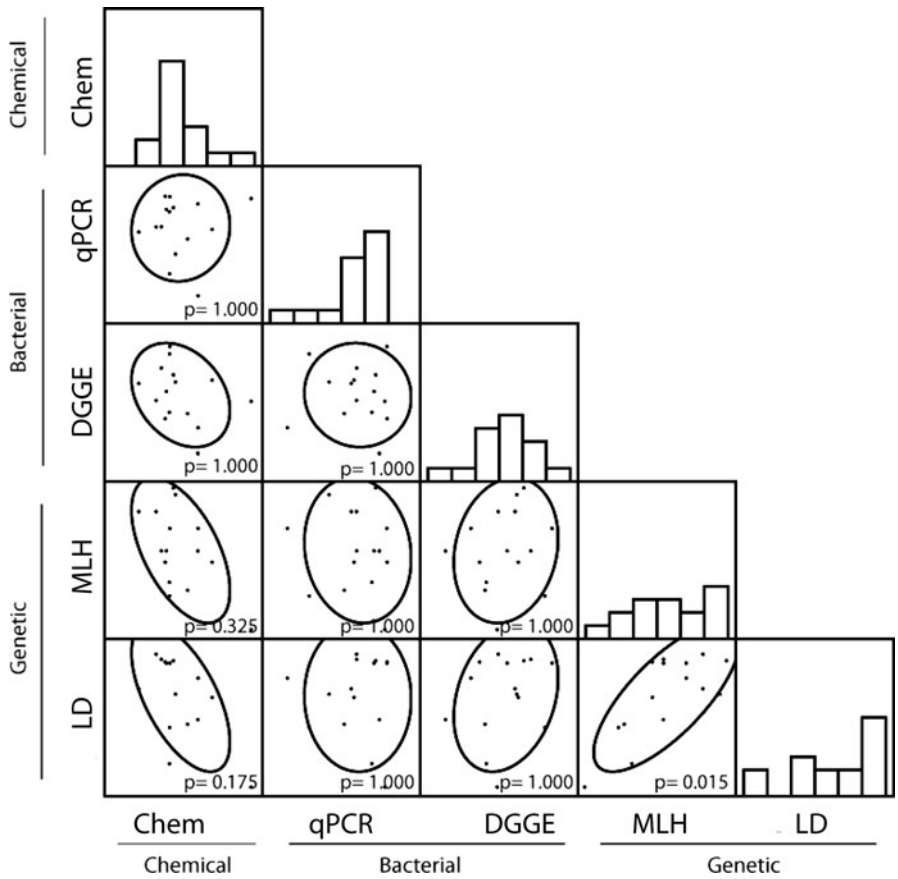

Fig. 3 Multiple individual-level correlations between chemical, bacterial, and genetic diversity. Chemical diversity was estimated by the Shannon index on the SPE–ELSD–HPLC profiles, qPCR diversity was estimated by Shannon index on proportions of bacterial clades amplified, DGGE diversity was estimated by Shannon index on the relative intensity of DGGE banding patterns, and genetic diversity was estimated on MLH (multi-locus heterozygosity) and *LD* [log (mean $d^2 + 1$)]. *P* values are Bonferroni corrected

qPCR data were included, Fig. 4). We did not find significant relationships between bacterial and genetic (both using MLH and mean d^2) diversities in any population. Thus, for genetic and qPCR bacterial diversities, we obtained the same consistent results when individual analyses were performed both globally (Fig. 3) or by populations (Fig. 4).

Population-level correlations

At the population level, when all indices were averaged by sponge population, we obtained similar results to those at the individual level (Figs. 3, 5). We did not detect significant correlation between chemical and bacterial (DGGE and qPCR), chemical and genetic (H_e and gene diversity), or bacterial (DGGE and qPCR) and genetic diversity (H_e and gene diversity). As at the individual level, we found significant correlations between He and gene diversity ($P = 0.012$, after Bonferroni corrections). However, we also detected a negative and significant correlation between the two methods used to assess bacterial diversity, mean qPCR and mean DGGE Shannon indices ($P = 0.018$, after Bonferroni corrections).

Population dissimilarities

Genetic, bacterial, and chemical dissimilarities showed the same pattern of higher dissimilarities for more distant sponge populations (Noyer, 2010; Noyer et al., 2011). Pairwise populations including Ceuta and Portugal presented the highest dissimilarities (Fig. 6). When we looked at correlations between population pairwise dissimilarities for the three levels, we obtained a positive and significant correlation between sponge genetic and chemical dissimilarities (Table 1). However, increasing genetic differences between sponge populations were not linked to increasing bacterial dissimilarities ($P = 1.000$ for both DGGE and qPCR data after Bonferroni corrections, Table 1). Increasing chemical dissimilarities was not linked to an increase of DGGE differences ($P = 1.000$). However, we observed a negative correlation between chemical dissimilarities and qPCR dissimilarities

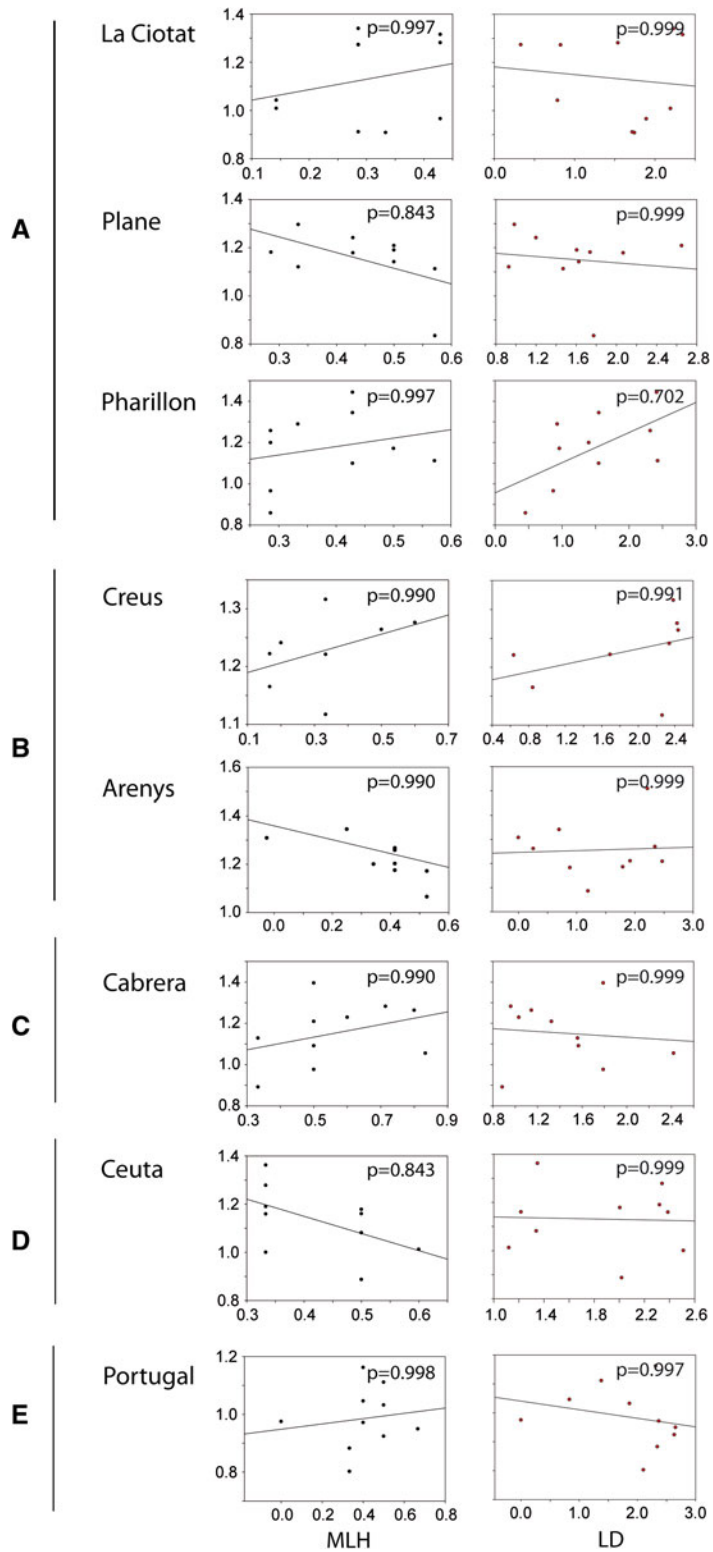

Fig. 4 Relationships between genetic diversity: MLH (*left*), *LD* (*right*), and diversity of bacterial clades amplified by qPCR, per sponge population. *P* values are Bonferroni corrected

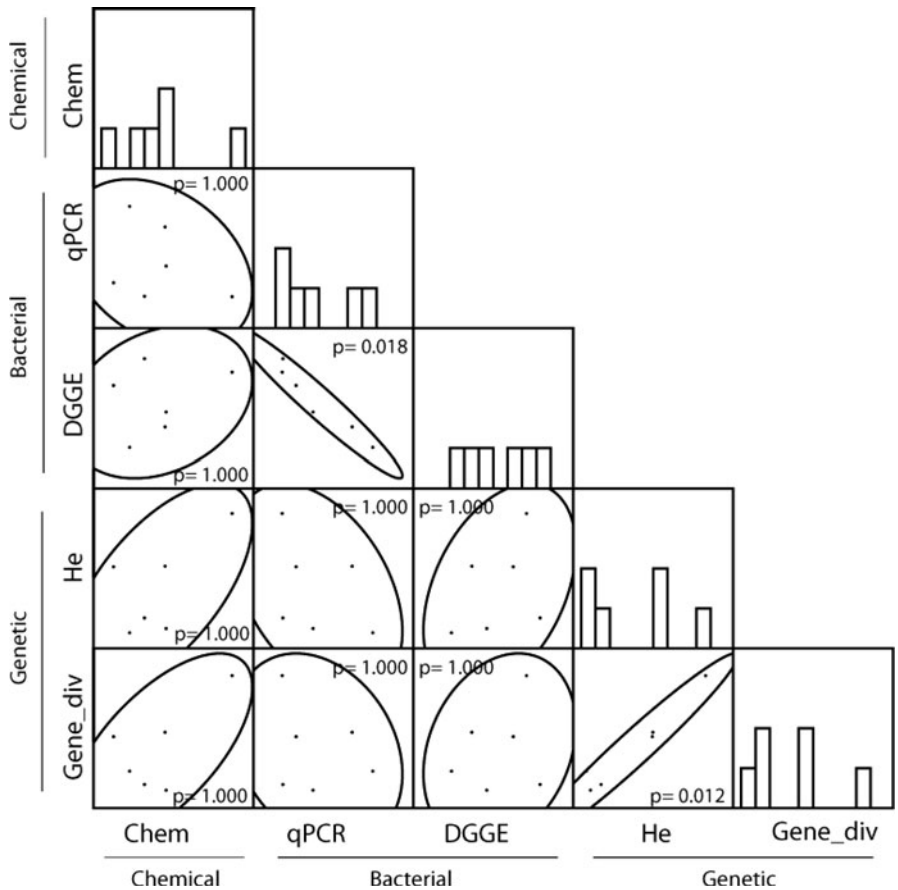

Fig. 5 Multiple population-level correlations between chemical, bacterial, and genetic diversity. Chemical and bacterial diversity were estimated as for Fig. 3, averaged per sponge population (except for Portugal and Cabrera populations, which did not contained chemical data and for Els Bullents, which did not contain bacterial data). Genetic diversity was estimated by expected heterozygosity under Hardy–Weinberg equilibrium and gene diversity among individuals. *P* values are Bonferroni corrected

($R = -0.587$, uncorrected $P = 0.021$). Increasing differences in bacterial clades amplified by qPCR were linked to a decrease of chemical dissimilarities between sponge populations, although this relationship turned not significant after Bonferroni corrections ($P = 0.064$).

Discussion

Does diversity beget diversity in *Spongia lamella*?

We used genetic, bacterial, and chemical diversities to investigate whether diversity begets diversity within the endangered sponge *S. lamella* (Fig. 1). We failed to detect multiple correlations between the three levels of diversity using two distinct methods to assess bacterial diversity and two measures of genetic diversity. Results were consistent both at the individual and population levels. The only significant and positive correlations were between the two measures of genetic diversity at individual level: heterozygosity (MLH) which is a measure of inbreeding, and mean d^2; and at population level: expected heterozygosity (H_e) and gene diversity. Paired measures of genetic diversity were highly correlated, which is in agreement with Chapman et al. (2009). At the population level, we also obtained a negative and significant correlation between the two methods of bacterial diversity. As Shannon index considers both richness and evenness, and as the same number of clades (richness) was amplified by qPCR for all sponge individuals, diversity of bacterial clades relied only on the evenness. However, each DGGE band refers theoretically to a bacterial sequence (Muyzer & Smalla, 1998). Thus, a bacterial clade could be represented by various sequences on the DGGE gels. Many sequences of the same clades at different positions on the DGGE gels increase number of DGGE bands and DGGE bacterial diversity. However, increasing proportions of a single clade would decrease bacterial clade evenness, and also decrease qPCR diversity. The two

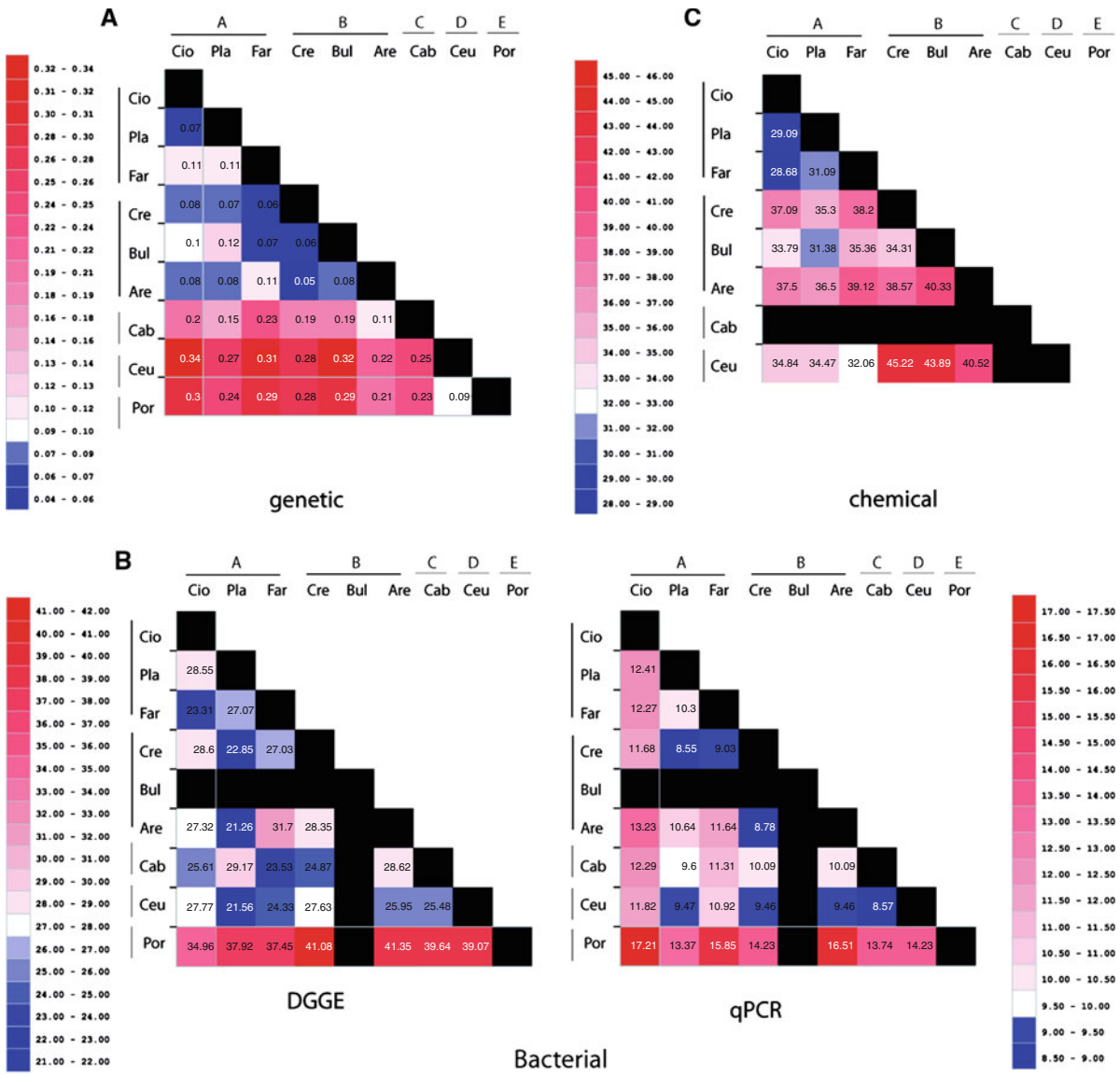

Fig. 6 Hit map of sponge population dissimilarities for **A** genetic differences; **B** bacterial (DGGE and qPCR) dissimilarities; and **C** chemical dissimilarities

measures of bacterial diversity are complementary rather than redundant as they focus on different phylogenetic levels, sequences, and clades.

In previous studies, we observed positive and significant relationships between geographical and genetic, bacterial, and chemical dissimilarities (Noyer, 2010). Among the multiple correlations between population dissimilarities we tested for those three levels, only chemical and genetic dissimilarities were significantly correlated. Increasing geographical distances between pairwise populations increased genetic differences by isolation by distance and, increasing genetic differences enhanced chemical dissimilarities. Though genetic and chemical diversity were not correlated per se, a certain connection seemed to exist. Production of secondary metabolites may have a genetic basis. Thus, increasing population genetic divergence through isolation and/or local selection might increase chemical dissimilarities between populations that are likely to be locally

Table 1 Correlations between genetic, bacterial, and chemical dissimilarities

	Genetic	Bacteria		Chemistry
	$F_{ST}/(1 - F_{ST})$	DGGE	qPCR	Chem.
Genetic				
$F_{ST}/(1 - F_{ST})$	–	−0.154	−0.059	**0.634**
Bacteria				
DGGE	$P = 1.000$	–		−0.013
qPCR	$P = 1.000$		–	−0.587
Chemistry				
Chem.	$P = 0.033$	$P = 1.000$	$P = 0.064$	–

Bonferroni corrected P values are given below the diagonal; Pearson correlation coefficients above the diagonal

adapted. Alternatively under specific ecological factors, some secondary metabolites only could present an advantage and increase sponge fitness. Then, differential production of secondary metabolites could also indirectly promote and strengthen genetic dissimilarities between populations through the selection of these genotypes. Finally, increasing genetic differences did not have any influence on bacterial dissimilarities as calculated either with DGGE and qPCR data. DGGE pairwise dissimilarities did not have any influence on chemical dissimilarities. Results suggested that all these factors may vary geographically, and highlight the complex nature of the mechanisms behind the variability in life.

The lack of significant correlations in this study could also reflect limitations in the ability of our data or analyses to detect them. Measures of genetic diversity, and its relevance to explain correlations (especially with fitness), remains a controversial and complex topic (Coltman & Slate, 2003; Balloux et al., 2004; Chapman et al., 2009; Da Silva et al., 2009). In this study, we used MLH and mean d^2 at the individual level. Various measures are generally used in heterozygosities–fitness correlations (HFCs). MLH has been the index most frequently used and has generally displayed strong size effect (Chapman et al., 2009; Pujolar et al., 2009).

Microsatellite variability is widely used to infer levels of genetic diversity in natural populations. However, criticisms have challenged the use of these markers to explain genome-wide variability (Vali et al., 2008). Nonetheless, microsatellites provide significantly stronger predictions of genome-wide-heterozygosity than do SNPs (Ljungqvist et al., 2010). In sponges, adequate genetic markers are limited and sequencing could be problematic. The mitochondrial COI gene, widely and extensively used, appears poorly polymorphic in Porifera (Duran et al., 2004a). Alternatively, microsatellites are highly polymorphic and have proven their usefulness in sponge studies (Duran et al., 2004b; Noyer et al., 2009; Blanquer and Uriz, 2010). Thus, microsatellite markers still remain the marker of choice for evaluating genetic diversity and assessing HFCs.

Alternative methods could have been applied to compare bacterial community and study bacterial diversity. Construction of bacterial clone libraries is a method widely used and gives indications of the identities/phylogeny and relative abundances of bacteria present in the sponge populations (Taylor et al., 2007 and reference herein). However, it is generally performed on few specimens of various species. First, it would appear time and money consuming when comparing nine populations from the same sponge species; second, clone libraries should be done at the population level which hinders analyses at the individual level. For these reasons, we choose the DGGE banding patterns to have a rapid and general insight of the bacterial community at the individual level and qPCR to compare quantitatively the main bacterial clades present.

One limitation of the Shannon index is the necessity to be compared across equivalent sampling designs (Clarke & Warwick, 2001). Here, we applied the same experimental design and method on each sample to sample the same "chemical" and "bacterial" diversity within each sponge specimen. Measure of bacterial diversity using qPCR was different. As we used the same set of primers for each sponge specimen, diversity values reflect only evenness (richness being equal to all samples, i.e., five bacterial clades). However, we chose to keep Shannon index to compare the same index between the different levels of chemical and bacterial diversity. Other diversity indices could be used too, but Simpson or Pielou's evenness indices lead to the same conclusions (data not shown).

Finally, to obtain a full picture of *S. lamella* system and get an insight on the functional role that sponge populations play in the associated organisms, it could

have been interesting to assess sponge habitat forming (diversity of sponge dwellers). Sponges also host a variety of other benthic invertebrates, such as scyphozoan, barnacles, decapodes, polychaetes, or amphipods among others (Rützler, 1976; Voultsiadou-Koukoura et al., 1987; Uriz et al., 1992; Ilan et al., 1999; author's unpublished data) and behave as efficient ecological niches.

Putative connections between genetic diversity, bacterial diversity, and chemical diversity

Our data failed to support the hypotheses that could explain potential links between genetic and bacterial diversity in *S. lamella* (Fig. 1). Increasing genetic diversity in a sponge population could increase diversity of host phenotypes for microorganisms to adapt. This would create habitat microheterogeneity in the host population and a greater possibility for various bacteria to use the resources. In their study based on the gastrointestinal (GI) tract, Zoetendal et al. (2001) found a positive and significant correlation between the similarity indices of bacterial communities and the genetic relatedness of their hosts, suggesting that the host genotype has a significant effect on the bacterial composition (Zoetendal et al., 2001). Another explanation relies on HFC and suggests that genetic diversity could be positively correlated with fitness. Populations with higher individual fitness would be less prone to local extinction promoting population survival especially for small inbreeding populations (Brook et al., 2002; Frankham, 2005). Survival of host populations would lead to the survival and evolution (coevolution) of associated symbionts. Increasing host genetic diversity through an increase of host fitness could thus promote bacterial diversity. An alternative relation could also occur if different bacteria would impart different functions that contribute to sponge metabolism (Lee et al., 2001; Taylor et al., 2007). Then, bacteria diversity would be linked to higher sponge fitness and potentially favor sponge populations. However, if only a few strains of microorganisms serve the sponge, increase diversity of microorganisms would be a burden because of higher competition between microorganism strains. Finally, relationship between genetic and bacterial dissimilarities could exist, since vertical transmission (Usher et al., 2001; Ereskovsky et al., 2005; de Caralt et al., 2007; Schmitt et al., 2007, 2008; Sharp et al., 2007; Noyer, 2010) and coevolution (Erpenbeck et al., 2002) occur. Thus, genetically different sponge populations could have differed more in their associated symbionts.

Whether genetic diversity controls or is controlled by chemical diversity is still an unresolved issue. Spatial and temporal variations in secondary metabolites could affect trophic and competitive interactions, increasing the chemical patchiness of the environment and promoting biodiversity at both genetic and species levels (Hay & Fenical, 1996). Alternatively, sponge genetic diversity could promote chemical diversity if secondary metabolites are produced by the sponge and the production has a heritable basis. Secondary metabolites present multiple ecological roles such as anti-predation, anti-fouling, mediation of spatial competition, facilitation for reproduction or protection against UV (Becerro et al., 1997; Mcclintock & Baker, 2001) and can be critical for species survival. Production of a cocktail of chemical compounds could confer advantages to a sponge individual that faces various threats, e.g., predators, competitors, pathogens, or environmental factors. If so, chemical diversity would increase sponge fitness. However, if only a limited number of chemical compounds are advantageous, increasing diversity of chemical compounds with ecologically irrelevant metabolites may dilute this benefit by allocating less energy to the production of the specific relevant compound. Thus, sponge populations with a low diversity of ecologically relevant compounds would be at an advantage.

If sponge-associated microorganisms produce even partially some of the secondary metabolites used by the host, the relationship between bacterial and chemical diversity could present direct effects. The production of some bioactive compounds has been ascribed to bacterial symbionts (Unson & Faulkner, 1993; Oclarit et al., 1994; Unson et al., 1994; Bewley et al., 1996). Also, symbiotic microorganisms could provide precursors or enzymes, being just one of the multiple cell compartments involved in the production of natural products (Kreuter et al., 1992; Ebel et al., 1997; Siegl & Hentschel, 2010; Sacristan-Soriano et al., 2011). We could therefore foresee a positive relationship between bacterial and chemical diversities. Alternatively, if a limited number of advantageous chemical compounds are produced by few strains of bacteria, increasing bacterial diversity may

increase competition between these different strains inhabiting sponge tissue, and could limit this advantage. Considering that bacteria do not influence production of secondary metabolites; secondary metabolites could potentially protect sponges from pathogens by preventing them to overrun the sponge tissues. This could have positive effect on both sponge fitness and symbiotic bacterial communities. Nevertheless, many factors may operate in these relationships, and the explanations represent basic interpretations that are not exhaustive. Hence, caution is required when attempting to understand mechanisms that could link those levels of diversity.

Measuring sponge fitness may have been interesting data to collect in order to unravel underlying ecological processes. Sponge fitness, defined as the capability to survive and reproduce, could be impacted by these three components (cf. above). Sponge populations with greater fitness are more likely to survive and adapt to environmental changes. Survival of various populations at different locations would promote maintenance of genetic diversity, of bacterial communities associated to the sponges and chemical diversity. Thus, besides the question "does diversity begets diversity," another fundamental question arises: does diversity at different levels favor the evolutionary potential of species?

Conclusion

Previous work performed on this species revealed that genetic, bacterial, and chemical diversity varied between sponge populations and presented significant geographical patterns (Noyer, 2010). However, we failed to show that those levels of diversity are related in *S. lamella* despite the various methods and measures used in this study. Measuring and understanding biological diversity is an important task with direct and indirect implications for conservation issues. However, assessing biodiversity remain very complex as there is no single all-embracing measure (Gaston & Spicer, 2004) and under different scales of observation, different indices of diversity might lead to different conclusions (Yue et al., 2003, 2005). Nevertheless, these three levels are intrinsically related to the sponge evolution and connections may still occur between the three components. However, in this model (target species, sampling design, technical, and statistical analyses), our data failed to support the diversity begets diversity hypothesis. This suggests that multiple factors can regulate the genetic, bacterial, and chemical diversity in *S. lamella*. In our study, all factors varied with geography as we found a clear pattern of changes in genetic, chemical, and bacterial diversity associated with geographical distance. These factors could represent multiple biotic and abiotic differences between populations, highlighting the complex nature of the mechanisms behind biodiversity.

Acknowledgments We thank Emilio Casamayor, Alastair Hamilton, and Olivier Thomas for technical support. We also thank Thierry Perez for the various samples in the area of Marseille, Joanna Xavier for the sponge specimens from Portugal, and Josep Carreras Carbonel, Andrea Blanquer, and Xavier Turon for their help during field sampling in Spain. Research funded by grants from the Agence Nationale de la Recherche (ECIMAR), from the Spanish Ministry of Science and Technology SOLID (CTM2010-17755) and Benthomics (CTM2010-22218-C02-01) and the BIOCAPITAL project (MRTN-CT-2004-512301) of the European Union. This is a contribution of the Consolidated Research Group "Grupo de Ecología Bentónica," SGR2009-655.

References

Aiello, A., P. Ciminiello, E. Fattorusso & S. Magno, 1988. 3-Beta, 5-alpha-dihydroxy-6-beta-methoxycholest-7-enes from the marine sponge *Spongia agaricina*. Journal of Natural Products 51: 999–1002.

Arrieta, J. M., S. Arnaud-Haond & C. M. Duarte, 2010. What lies underneath: conserving the oceans' genetic resources. Proceedings of the Natural Academy of Sciences 107: 18318–18324.

Balloux, F., W. Amos & T. Coulson, 2004. Does heterozygosity estimate inbreeding in real populations? Molecular Ecology 13: 3021–3031.

Becerro, M. A., 2008. Quantitative trends in sponge ecology research. Marine Ecology 29: 167–177.

Becerro, M. A., X. Turon & M. J. Uriz, 1997. Multiple functions for secondary metabolites in encrusting marine invertebrates. Journal of Chemical Ecology 23: 1527–1547.

Bell, J. J., 2008. The functional roles of marine sponges. Estuarine, Coastal and Shelf Science 79: 341–353.

Bertrand, J. C. & J. Vacelet, 1971. Association between Corneal and Bacterial Sponges. Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences Serie D 273: 638–641.

Bewley, C. A., N. D. Holland & J. D. Faulkner, 1996. Two classes of metabolites from *Theonella swinhoei* are localized in distinct populations of bacterial symbionts. Experientia 52: 716–722.

Blanquer, A. & M. J. Uriz, 2010. Population genetics at three spatial scales of a rare sponge living in fragmenteed habitats. BMC Evolutionary Biology 10: 13.

Brook, B. W., D. W. Tonkyn, J. J. Q'Grady, & R. Frankham, 2002. Contribution of inbreeding to extinction risk in threatened species. Conservation Ecology 6: 16.

Cardinale, B. J., M. A. Palmer & S. L. Collins, 2002. Species diversity enhances ecosystem functioning through interspecific facilitation. Nature 415: 426–429.

Casamayor, E. O., H. Schafer, L. Baneras, C. Pedros-Alio & G. Muyzer, 2000. Identification of and spatio-temporal differences between microbial assemblages from two neighboring sulfurous lakes: comparison by microscopy and denaturing gradient gel electrophoresis. Applied and Environmental Microbiology 66: 499–508.

Castritsi-Catharios, J., M. Magli & J. Vacelet, 2007. Evaluation of the quality of two commercial sponges by tensile strength measurement. Journal of the Marine Biological Association of the United Kingdom 87: 1765–1771.

Chapman, J. R., S. Nakagawa, D. W. Coltman, J. Slate & B. C. Sheldon, 2009. A quantitative review of heterozygosity–fitness correlations in animal populations. Molecular Ecology 18: 2746–2765.

Cimino, G., S. de Stefano, L. Minale & G. Sodano, 1975. Chemical patterns and classification of the Demospongiae. Comparative Biochemistry and Physiology 50B: 279–285.

Clarke, K. R. & R. M. Warwick, 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. 5-Primer-E Ltd, Plymouth Marine Laboratory, UK.

Coltman, D. W. & J. Slate, 2003. Microsatellite measures of inbreeding: a meta-analysis. Evolution 57: 971–983.

Da Silva, A., et al., 2009. Heterozygosity–fitness correlations revealed by neutral and candidate gene markers in roe deer from a long-term study. Evolution 63: 403–417.

De Caralt, S., M. J. Uriz, A. V. Ereskovsky & R. H. Wijffels, 2007. Embryo development of Corticium candelabrum (Demospongiae: Homosclerophorida). Invertebrate Biology 126: 211–219.

Diaz, M. C. & K. Rutzler, 2001. Sponges: an essential component of Caribbean coral reefs. Bulletin of Marine Science 69: 535–546.

Duran, S., M. Pascual, A. Estoup & X. Turon, 2004a. Strong population structure in the marine sponge Crambe crambe (Poecilosclerida) as revealed by microsatellite markers. Molecular Ecology 13: 511–522.

Duran, S., M. Pascual & X. Turon, 2004b. Low levels of genetic variation in mtDNA sequences over the western Mediterranean and Atlantic range of the sponge Crambe crambe (Poecilosclerida). Marine Biology 144: 31–35.

Ebel, R., M. Brenzinger, A. Kunze, H. J. Gross & P. Proksch, 1997. Wound activation of protoxins in marine sponge *Aplysina aerophoba*. Journal of Chemical Ecology 23: 1451–1462.

Ereskovsky, A. V., E. Gonobobleva & A. Vishnyakov, 2005. Morphological evidence for vertical transmission of symbiotic bacteria in the viviparous sponge Halisarca dujardini Johnston (Porifera, Demospongiae, Halisarcida). Marine Biology 146: 869–875.

Erpenbeck, D., J. A. J. Breeuwer, H. C. Van Der Velde & R. W. M. Van Soest, 2002. Unravelling host and symbiont phylogenies of halichondrid sponges (Demospongiae, Porifera) using a mitochondrial marker. Marine Biology 141: 377–386.

Feral, J. P., 2002. How useful are the genetic markers in attempts to understand and manage marine biodiversity? Journal of Experimental Marine Biology and Ecology 268: 121–145.

Fontana, A., L. Albarella, G. Scognamiglio, M. J. Uriz & G. Cimino, 1996. Structural and stereochemical studies of C-21 terpenoids from Mediterranean Spongiidae sponges. Journal of Natural Products 59: 869–872.

Frankham, R., 2005. Genetics and extinction. Biological Conservation 126: 131–140.

Gaino, E. & R. Pronzato, 1989. Ultrastructural evidence of bacterial damage to *Spongia officinalis* fibers (Porifera, Demospongiae). Diseases of Aquatic Organisms 6: 67–74.

Gaino, E. & R. Pronzato, 1992. Disease and overfishing drive commercial sponges to the brink of extinction in the Mediterranean basin. Bollettino del Museo Istituto di Biologia Università di Genova 56-57: 209–224.

Gaston, K. J. & J. I. Spicer, 2004. Biodiversity: An introduction. Blackwell Science, Oxford. 191 p.

Hansson, B., 2010. The use (or misuse) of microsatellite allelic distances in the context of inbreeding and conservation genetics. Molecular Ecology 19: 1082–1090.

Hay, M. E. & W. Fenical, 1996. Chemical ecology and marine biodiversity: insights and products from the Sea. Oceanography 9: 10–20.

Hentschel, U., K. M. Usher & M. W. Taylor, 2006. Marine sponges as microbial fermenters. FEMS Microbiology Ecology 55: 167–177.

Ilan, M., Y. Loya, G. A. Kolbasov & I. Brickner, 1999. Sponge-inhabiting barnacles on Red Sea coral reefs. Marine Biology 133: 709–716.

Joachimiak, M. P., J. L. Weisman & B. C. H. May, 2006. JColorGrid: Software for the visualization of biological measurements. BMC Bioinformatics 7: 225.

Kreuter, M. H., A. Robitzki, S. Chang, R. Steffen, M. Michaelis, Z. Kljajic, M. Bachmann, H. C. Schröder & W. E. G. Müller, 1992. Production of the cytostatic agent aeroplysinin by the sponge *Verongia aerophoba* in in vitro culture. Comparative Biochemistry and Physiology Part C-Pharmacology, Toxicology and Endocrinology 101: 183–187.

Lamosova, T., J. Dolezal, V. Lanta & J. Leps, 2010. Spatial pattern affects diversity–productivity relationships in experimental meadow communities. Acta Oecologica-International Journal of Ecology 36: 325–332.

Lee, Y. K., J. H. Lee & H. K. Lee, 2001. Microbial symbiosis in marine sponges. Journal of Microbiology 39: 254–264.

Lesbarreres, D., D. S. Schmeller, C. R. Primmer & J. Merila, 2007. Genetic variability predicts common frog (Rana temporaria) size at metamorphosis in the wild. Heredity 99: 41–46.

Ljungqvist, M., M. Akesson & B. Hansson, 2010. Do microsatellites reflect genome-wide genetic diversity in natural populations? A comment on Vali et al. (2008). Molecular Ecology 19: 851–855.

Lopes, M. T. & N. Boury-Esnault, 1981. Contribution à la connaissance des éponges cornées de la côte de l'Arrábida et de l'Algarve. Arquivos do Museu Bocage, A, I 6: 95–110.

Magurran, A. E., 2004. Measuring Biological Diversity. Blackwell Science Ltd., Oxford.
Mcclintock, J. B. & B. J. Baker, 2001. Marine Chemical Ecology. CRC Press, Boca Raton.
Muyzer, G. & K. Smalla, 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 73: 127–141.
Noyer, C., 2010. Relationship between genetic, bacterial and chemical diversity of the Mediterranean sponge *Spongia agaricina* (*S. lamella* Schultze). PhD thesis, University of Barcelona: 278 pp.
Noyer, C., G. Agell, M. Pascual & M. A. Becerro, 2009. Isolation and characterization of microsatellite loci from the endangered Mediterranean sponge Spongia agaricina (Demospongiae: Dictyoceratida). Conservation Genetics 10: 1895–1898.
Noyer, C., A. Hamilton, O. Sacristán-Soriano & M. A. Becerro, 2010. Quantitative comparison of bacterial communities in two Mediterranean sponges. Symbiosis 51: 239–243.
Noyer, C., O. P. Thomas & M. A. Becerro, 2011. Patterns of chemical diversity in the mediterranean sponge Spongia lamella. PLoS ONE 6(6): e20844. doi:10.1371/journal.pone.0020844.
Oclarit, J. M., H. Okada, S. Ohta, K. Kaminura, Y. Yamaoka, T. Iisuka, S. Miyashiro & S. Ikegami, 1994. Anti-bacillus substance in the marine sponge, *Hyatella* species, produced by an associated *Vibrio* species bacterium. Microbios 78: 7–16.
Odat, N., F. H. Hellwig, G. Jetschke & M. Fischer, 2010. On the relationship between plant species diversity and genetic diversity of Plantago lanceolata (Plantaginaceae) within and between grassland communities. Journal of Plant Ecology-UK 3: 41–48.
Palmer, M. W. & T. A. Maurer, 1997. Does diversity beget diversity? A case study of crops and weeds. Journal of Vegetation Science 8: 235–240.
Pronzato, R., 1999. Sponge-fishing, disease and farming in the Mediterranean Sea. Aquatic Conservation-Marine and Freshwater Ecosystems 9: 485–493.
Pronzato, R. & R. Manconi, 2008. Mediterranean commercial sponges: over 5000 years of natural history and cultural heritage. Marine Ecology-An Evolutionary Perspective 29: 146–166.
Pujolar, J. M., D. Bevacqua, F. Capoccioni, E. Ciccotti, G. A. De Leo & L. Zane, 2009. Genetic variability is unrelated to growth and parasite infestation in natural populations of the European eel (Anguilla anguilla). Molecular Ecology 18: 4604–4616.
Rueda, A., E. Zubia, M. J. Ortega, J. L. Carballo & J. Salva, 1998. New metabolites from the sponge *Spongia agaricina*. Journal of Natural Products 61: 258–261.
Rützler, K., 1976. Ecology of Tunisian commercial sponges. Tethys 7: 249–264.
Sacristan-Soriano, O., B. Banaigs, E. O. Casamayor & M. A. Becerro, 2011. Exploring the links between natural products and bacterial assemblages in the sponge *Aplysina aerophoba*. Applied and Environmental Microbiology 77: 862–870.
Sala, E. & N. Knowlton, 2006. Global marine biodiversity trends. Annual Review of Environment and Resources 31: 93–122.
Sarkar, S., 2006. Ecological diversity and biodiversity as concepts for conservation planning: comments on Ricotta. Acta Biotheoretica 54: 133–140.
Schmitt, S., J. B. Weisz, N. Lindquist & U. Hentschel, 2007. Vertical transmission of phylogenetically complex microbial consortium in the vivipare sponge *Ircinia felix*. Applied and Environmental Microbiology 73: 2067–2078.
Schmitt, S., H. Angermeier, R. Schiller, N. Lindquist & U. Hentschel, 2008. Molecular microbial diversity survey of sponge reproductive stages and mechanistic insights into vertical transmission of microbial symbionts. Applied and Environmental Microbiology 74: 7694–7708.
Sharp, K. H., B. Eam, D. J. Faulkner & M. G. Haygood, 2007. Vertical transmission of diverse microbes in the tropical sponge Corticium sp. Applied and Environmental Microbiology 73: 622–629.
Siegl, A. & U. Hentschel, 2010. PKS and NRPS gene clusters from microbial symbiont cells of marine sponges by whole genome amplification. Environmental Microbiology Reports 2: 507–513.
Sipkema, D., M. C. R. Franssen, R. Osinga, J. Tramper & R. H. Wijffels, 2005. Marine sponges as pharmacy. Marine Biotechnology 7: 142–162.
Taylor, M. W., R. Radax, D. Steger & M. Wagner, 2007. Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. Microbiology and Molecular Biology reviews 71: 295–347.
Templado, J., M. Calvo, A. Garcia, A. A. Luque, M. Maldonado & L. Moro, 2004. Guía de Invertebrados y Peces Marinos protegidos por la legislación nacional e internacional. In N.y.P.N.s. (ed) Guía de Invertebrados y Peces Marinos protegidos por la legislación nacional e internacional. M.M.A. & C.S.I.C.: 214.
Unson, M. D. & D. J. Faulkner, 1993. Cyanobacterial symbiont biosynthesis of chlorinated metabolites from *Dysidea herbacea* (Porifera). Experientia 49: 349–353.
Unson, M. D., N. D. Holland & D. J. Faulkner, 1994. A brominated metabolite by the cyanobacterial symbiont of a marine sponge and accumulation of the crystalline metabolite in the sponge tissue. Marine Biology 119: 1–11.
Uriz, M. J., D. Rosell & M. Maldonado, 1992. Parasitism, commensalism or mutualism—the case of Scyphozoa (Coronatae) and Horny sponges. Marine Ecology-Progress Series 81: 247–255.
Usher, K. M., J. Kuo, J. Fromont & D. C. Sutton, 2001. Vertical transmission of cyanobacterial symbionts in the marine sponge Chondrilla australiensis (Demospongiae). Hydrobiologia 461: 15–23.
Vali, U., A. Einarsson, L. Waits & H. Ellegren, 2008. To what extent do microsatellite markers reflect genome-wide genetic diversity in natural populations? Molecular Ecology 17: 3808–3817.
Vellend, M. & M. A. Geber, 2005. Connections between species diversity and genetic diversity. Ecology Letters 8: 767–781.
Voultsiadou-Koukoura, H. E., A. Koukouras & A. Eleftheriou, 1987. Macrofauna associated with the sponge Verongia

aerophoba in the north Aegean Sea. Estuarine, Coastal and Shelf Science 24: 265–278.

Whittaker, R. H., 1972. Evolution and measurement of species diversity. Taxon 21: 231–251.

Yue, M., L. J. Zhang, F. X. Gu, X. L. Pan & G. F. Zhao, 2003. The relationships between plant community species diversity and soil factors. Ecosystems Dynamics, Ecosystem-Society Interactions, and Remote Sensing Applications for Semi-Arid and Arid Land, Pts 1 and 2 4890: 534–540.

Yue, T. X., et al., 2005. Considerable effects of diversity indices and spatial scales on conclusions relating to ecological diversity. Ecological Modelling 188: 418–431.

Zoetendal, E. G., A. D. L. Akkermans, W. M. Akkermnas-Van Vliet, J. A. G. M. De Visser & W. M. De Vos, 2001. The host genotype affects the bacterial community in the human gastrointestinal tract. Microbial Ecology in Health and Disease 13: 129–134.