

Past, Present and Future Connectivity of Mediterranean Cold-Water Corals: Patterns, Drivers and Fate in a Technically and Environmentally Changing World

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Abstract

Given the vastness of the oceans and the small size of larvae and other marine propagules, tracking marine organisms' dispersal particles is unrealistic. When propagules successfully recruit, a genetic profile can be tracked that integrates the movements of multiple generations. Molecular analyses thus provide an indirect means of estimating connectivity among subdivided populations, at any given spatial and/or temporal scale of interest. In general, it remains challenging to disentangle the relative influence of the various processes that cause the observed patterns of genetic differentiation among subpopulations, notably in far-off deep-sea environments. In the past decade only a handful of studies have reported on the genetic patterns in Mediterranean cold-water corals. This represents a substantial limitation for any researcher attempting to understand the dynamics of Mediterranean cold-water coral populations. It affects as well conservation decisions involving these vulnerable species and habitats. Until recently, hypotheses for gene flow of some deep-sea corals in the Atlantic have included moderate to high connectivity at broad and regional scales, through sporadic larval transport mediated by ocean currents, and simultaneous strong discontinuities between ocean basins

caused by vicariance or local adaptation. However, progress has been made that is allowing researchers to retrace past major modifications in the patterns of cold-water coral migrations at evolutionary time-scales, in and out of the Mediterranean Sea. Growing evidence of the influence of clonality and its effect on estimates of genetic diversity now stimulate researchers to engage in optimised sampling strategies. Improved estimates are possible to attain, provided a rigorous sampling strategy. Studies of the cosmopolitan corals *Lophelia pertusa* and *Madrepora oculata* have made evident that Atlantic populations are clearly differentiated from Mediterranean ones, suggesting that contemporary gene flow between the two genetic backgrounds is very limited, if at all present. Results support several non-mutually exclusive hypotheses: that subpopulations in the North East Atlantic were recolonised from Mediterranean refugia following the last glacial maximum; that Mediterranean *L. pertusa* reefs appear to have been relevant glacial refugia during the Pleistocene glaciations and the main source for North East Atlantic recolonisation; and that *M. oculata* in the NE Atlantic may have had multiple sources of post-Last Glacial Maximum colonisation, which remain elusive. The solitary *Desmophyllum dianthus* has been shown to share the same genetic identity between very distant populations (Mediterranean and NE Atlantic). Yet, it remains unclear if this is solely due to high contemporary gene flow or if the genetic resemblance is a remnant left by an Atlantic post-glacial recolonisation from a unique Mediterranean source with high population size. Future genome-wide studies with next generation sequencing techniques will undoubtedly help clarifying the interpretations of molecular data in terms of past and present cold-water corals migration pathways. This is strongly needed, as the development of effective conservation strategies relies on well-informed, knowledge-based, policies. These must include thorough species invento-

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ries, relatedness, connectivity metrics and clear identification of genetic units, all of which depending on the use of robust techniques. Indeed, information on the genetic connectivity of Mediterranean cold-water coral populations has proven to be key to the establishment of a protected site under the European Union's Natura 2000 Network of protected areas. We conclude with perspectives on how Next Generation Sequencing will strengthen inferences on connectivity of the majestic cold-water coral habitats in the coming years.

Keywords

Population genetics · Clonality · Connectivity · Next generation sequencing

31.1 Introduction

Connectivity designates many different aspects of movement in ecology. Landscape connectivity is defined as ‘the degree to which the landscape facilitates or impedes movement among resource patches’ (Taylor et al. 1993). More relevant to population genetics, connectivity is designated by Moilanen and Hanski (2001) as “typically related to the migration rate and gene flow among populations and colonization of empty habitat”. Before engaging into this synthesis it is essential to clarify that the choice of sampling, molecular and analytical methods impose important differences in the inferred level of population connectivity (past or present) and interdependence (demographic or evolutionary) revealed by molecular estimates (Waples and Gaggiotti 2006; Lowe and Allendorf 2010). In this chapter, the wording “past, present and future connectivity” embeds on one hand the evolutionary history, present day migration and future modification owing to environmental changes, and on the other the constant improvement of molecular tools used to infer those patterns, and the way this progress can modify our perception and inferences.

Numerous reports of fossils of several species of cold-water corals (CWCs) within and surrounding the Mediterranean Sea (Malinverno et al. 2010; Perrin and Bosellini 2012) have guided sampling for use as archives to retrace past climates. Yet, only scarce remotely operated vehicle (ROV) exploration has been performed thus far in canyons and seamounts (e.g. Danovaro et al. 2010; Fabri et al. 2017; Aymà et al., [this volume](#); Lastras et al., [this volume](#)), features susceptible of hosting living corals. Living-records, however, spread across the entire Mediterranean. These are dominated by *Madrepora oculata* (Orejas et al. 2009; Gori et al. 2013; Lastras et al. 2016; Fabri et al. 2017), which seems to better resist environmental changes (Wienberg et al. 2010), and *Desmophyllum dianthus*

(Freiwald et al. 2009; Orejas et al. 2009; Mytilineou et al. 2014). A diversity of species have been used to reconstruct the past history of the Mediterranean Sea (Montagna et al. 2006; Frank et al. 2011). Nevertheless, mainly three species (*Lophelia pertusa*, *M. oculata* and *D. dianthus*) could be collected with sufficient numbers of specimens to allow the indirect genetic reconstruction of past and present connectivity of Mediterranean CWCs and their present and past exchanges with Atlantic coral specimens.

One of the particularities of most corals is their ability to reproduce sexually and asexually. Partial asexuality (“clonality”) complicates both the dynamics and evolution of populations and the strategies needed to sample and to analyse molecular data to allow interpretations in a population genetics framework. It is thus important to explicitly take asexual reproduction into account in sampling designs, data analyses and interpretations (Halkett et al. 2005; Arnaud-Haond et al. 2007).

This chapter will first tackle clonality and its implication for adequate sampling, before engaging into a synthesis of previous work on the population genetics of CWCs (with a focus on scleractinians) in the Mediterranean Sea, and their connection to Atlantic populations. The scarce number of studies published thus far on Mediterranean CWCs and a number of important pitfalls associated with life history traits of corals led us to offer a synthesis of the limited available data. However, here we give a roadmap for future studies: We point the reader to optimised sampling schemes and identify new research avenues opened by the new generation of molecular data -offered by next generation sequencing (NGS)- and computational analyses.

31.2 Considerations for Sampling and Clonality

Partial asexuality (clonality) characterises most coral species: they can reproduce sexually through the production of gametes and recombination, or clonally through the production of colonies sharing the same genome (except for somatic mutations) by fragmentation or parthenogenesis. Clonality is an essential aspect to consider when engaging into a population genetics study on corals. It does not only affect the path for gene transmission, the dynamics of populations at different stages of colonisation or demographic equilibrium, but also their resistance and resilience, and their evolution. More pragmatically for the researcher, it also affects the way sampling should be planned and molecular data interpreted in a population genetics framework. Actually, the population genetics framework was designed and developed for pure sexual organisms and requires dealing with individual genotypes, thus recognising individuals at the demographic and genetic scale (reviewed in Arnaud-Haond et al. 2007).

31.2.1 Clonality and Sampling Strategy

In terms of sampling strategy, working on partially asexual organisms imposes strong constraints on the sampling design (Arnaud-Haond et al. 2007). When researchers are interested in appraising the imprint of clonality on the dynamics of populations, they should go for an explicit, standardised sampling scale (i.e. standardising the sampled area among reefs), and randomised spatial sampling at an accurate spatial scale, in order to be able to compare results obtained in different reefs or coral gardens. The sampling scale should be nested based on previous knowledge of the size of *genets* (i.e. the extent of a genetic individual issued from a single zygote that spreads spatially and temporally through clonality by producing modules, called *ramets*), or the dispersal of clonal propagules. When such knowledge is not available it is highly recommended to perform pilot studies with nested sampling scales in order to appraise the spatial extent of the influence of clonality for the species considered. When researchers are not necessarily interested in appraising the extent of clonality but are just focused on basic genetic analysis to estimate genetic diversity and structure, they may favor a coarse sampling of ramets with a haphazard strategy, and exclude replicates of the same genotypes (i.e. belonging to the same genet) if still occurring in the final dataset. Making the *a priori* choice of ignoring clonality can be imposed by technical limitations when sampling, which are particularly acute in deep-sea research. It is however not necessarily recommended, as clonality can have strong implications in the spatial distribution of polymorphism and in its temporal evolution. At least in such cases, the unique geographical coordinates of each colony should be kept in order to attempt assessing the scale of clonal spread/dispersal on the basis of the best possible sampling in hand. A notable practical example of this strategy was given for *Lophelia pertusa* reefs along the Bay of Biscay (Becheler et al. 2017). Despite a general low incidence of clonality, sampling colonies separated by at least 150 m showed identical genotypes suggesting a rather long dispersal distance.

The choice of sampling gear is also essential to allow isolating georeferenced colonies sampled on the sea floor, discriminating them on the deck and analysing them individually in the laboratory. Trawls, dredge or even ledges are thus to be avoided for various reasons. Leaving aside the interesting and fundamental debates on the ethic of trawling reefs for scientific purposes, fragments of colonies sampled through trawling or dredging cannot be easily teased apart on the deck, preventing the appraisal of clonality in the collected sample. In other words, sampling a coral reef or a coral garden with such gear can be compared to the sampling of tropical forests with a bulldozer, followed by the blind genotyping of broken branches.

Estimates of clonal versus sexual reproduction as well as genetic diversity would obviously suffer serious biases, with some of the individuals being overrepresented due to their large size or position in the middle of the gear trajectory. Submersible or ROV based sampling, with well annotated videos and/or organised sampling material (like drawers with individual and/or labelled cases; see Becheler et al. 2017; Orejas et al. 2017), allow a good traceability which is required. Additionally, accidental bycatch with longlines can provide interesting samples. These may be associated to approximate GPS coordinates and thus valuable, once the extent of clonality has been appraised during pilot or previous studies.

31.2.2 Clonality and Population Genetics: Beware of Bush Mosaicism and the Strategy with Genetic Replicates

A peculiarity of corals is their ability to form coral bushes (also termed “patches” and “thickets”). This capacity was confirmed for CWCs in the North Atlantic (Wilson 1979, Reed et al. 2007) and, more recently, through the description of mosaic colonies of *M. oculata* and *L. pertusa* growing on each other (Arnaud-Haond et al. 2017). In the same way, conspecific colonies of different colors have been observed on the field, for which mosaicism was confirmed by genotyping of *L. pertusa* (Hennige et al. 2014). The occurrence of such coral bushes implies that additional caution is necessary when sampling: to examine the shape of colonies and possible anastomosed branching is fundamental, in order to avoid mixing genets in the lab, which would mix genotypes, making it impossible to reconstruct individuals *a posteriori*.

Estimating gene flow requires the access to a random and representative sampling of the populations studied, with demographic units represented only once. Now, the delineation of what is a “demographic unit” depends on the path of sexual reproduction and on the independent or entangled fates of ramets. Once the colonies are discriminated and the level of clonality is assessed, one will engage in a population genetics analysis in a different way depending on the path of asexual reproduction. For most clonal plants including seagrasses, where modules –ramets- of the same genetic individual –genet- remain connected and potentially metabolically interdependent for some time (Salzman and Parker 1985; Alpert 1996; Arnaud-Haond et al. 2012), the choice of keeping or discarding replicates in the dataset before estimating population genetic structure can be debated. Each of these choices implies underlying assumptions: the lack of a relationship between the reproductive success and the size (in terms of number of ramets) of genets, none of which are usually tested for the species and populations at stake. For

269 corals, the question at hand is a little different: distinct colo- 318
 270 nies in the same area can be considered as independent meta- 319
 271 bolic and reproductive units, whether or not they share the 320
 272 same genotype, and thus be included for connectivity esti- 321
 273 mates. It is thus reasonable in most cases, provided sampled 322
 274 colonies were well discriminated in the field (see above), to 323
 275 engage into connectivity estimates based on the full dataset. 324

276 31.3 Case Studies 325

277 31.3.1 Context 326

278 Despite the fast technological progress that has allowed 327
 279 improved access to the deep-sea, these ecosystems remain 328
 280 hard to explore and information about connectivity is still 329
 281 scarce. For example, direct tracking of free living marine 330
 282 larvae is highly challenging in coastal species (but see 331
 283 Thorrold et al. 2007), and virtually impossible for species 332
 284 such as CWCs (but see recent progress in Orejas et al., [this 333](#)
 285 [volume](#)). The use of genetic markers is necessary to obtain 334
 286 an indirect mean of levels of connectivity. In this context, 335
 287 population genetics offers the theoretical framework to indi- 336
 288 rectly assess connectivity through the analysis of the distri- 337
 289 bution of genetic variation (“polymorphism”), in order to 338
 290 infer the degree of inter-dependence of discretely distrib- 339
 291 uted CWC reefs. 340

292 Contemporary genetic processes, such as migration and 341
 293 population size, contribute to explaining the present-day 342
 294 distribution of neutral genetic diversity (i.e. not subject to 343
 295 selection) within species ranges. Yet, past events, such as 344
 296 fluctuations of geographic ranges, demographic bottle- 345
 297 necks and/or expansions, can leave strong and persistent 346
 298 genetic signatures, still detectable today and thus imping- 347
 299 ing the present day study of the distribution of genetic 348
 300 polymorphism (Hewitt 2000, 2004). Understanding a pat- 349
 301 tern of genetic structure and appraising the present day 350
 302 level of genetic connectivity among populations, thus 351
 303 requires considering and discriminating both present and 352
 304 past events affecting the demography of species and the 353
 305 present day distribution of their genetic diversity as well as 354
 306 the evolutionary forces driving it (Bierne et al. 2011; 355
 307 Gagnaire et al. 2015). 356

308 Geological records and isotopic dating of sediment and 357
 309 coral remains have delivered most of the knowledge about 358
 310 the paleogeography of CWC. The Mediterranean Basin is an 359
 311 historical area for CWC presence (Freiwald et al. 2004; Di 360
 312 Geronimo et al. 2005; Taviani et al. 2005; Montagna and 361
 313 Taviani, [this volume](#); Rebesco and Taviani, [this volume](#); 362
 314 Taviani et al., [this volume](#); Vertino et al., [this volume](#)) where 363
 315 the occurrence of deep reefs has been recognised to be con- 364
 316 tinuous for over 480,000 years (McCulloch et al. 2010). The 365
 317 last 50,000 years may correspond to an active growth period 366

for reefs in the western basin of the Mediterranean Sea, as 318
 well as in the Gulf of Cádiz (GoC) and Moroccan margin 319
 (Schroder-Ritzrau et al. 2005), even if a relative decline is 320
 reported since the end of the Last Glacial Maximum (LGM) 321
 (Taviani et al. 2011), 10,000–12,000 years ago. An opposite 322
 situation was suspected in Northeast (NE) Atlantic, where 323
 climatic fluctuations were suggested to be the main driver of 324
 geographic distribution and development of reefs. In this 325
 region, Ice Ages hampered the growth of CWC reefs (Frank 326
 et al. 2011). 327

Studies of demography, population structure, and repro- 328
 duction of CWCs are rather scarce (but see Brooke and 329
 Young 2003; Waller 2005; Larsson et al. 2014; Pires et al. 330
 2014; Bramanti et al., [this volume](#); Lartaud et al., [this vol- 331](#)
 ume; Reynaud and Ferrier-Pagès, [this volume](#)), as generally 332
 applies with other deep-water organisms. However, it is well 333
 known that understanding the connectivity of marine popula- 334
 tions is vital for conservation and fisheries management, par- 335
 ticularly for the strategic design of marine reserves 336
 (Hedgecock et al. 2007) or for the sustainable use of marine 337
 resources (e.g. implementation of sea-based wind farms and 338
 their environmental impact on benthic communities, see for 339
 example the European project CoCoNET [http://www. 340](http://www.coconet-fp7.eu/)
[coconet-fp7.eu/](http://www.coconet-fp7.eu/)). 341

31.3.2 *Madrepora oculata* and *Lophelia* 342 *pertusa*: Out and 343 Into the Mediterranean 344

The two reef-building CWCs, *L. pertusa* and *M. oculata*, are 345
 suspected to have recolonised the NE-Atlantic from the 346
 Mediterranean at the end of the last glacial episode (De Mol 347
 et al. 2005; Henry et al. 2014), after a long period of scarcity 348
 or extirpation during the late Pleistocene (see Taviani et al., 349
[this volume](#); Vertino et al., [this volume](#); other chapters from 350
 the past section in this same volume). 351

In the work we summarise here, we aimed at addressing 352
 those hypotheses about past connectivity by unraveling the 353
 genetic differentiation, or the lack of, of CWC populations in 354
 the Mediterranean and in the Atlantic Ocean. 355

31.3.2.1 Sampling Locations and Strategy 356

As explained above, clonal organisms require an adequate 357
 sampling strategy, which is particularly hard to apply in the 358
 deep-sea. This is probably one of the main barriers to gather- 359
 ing robust information about the clonal architecture and pop- 360
 ulation genetics of CWC populations. In 2011, during the 361
 cruise BobEco (European Project CoralFish, Research Vessel 362
 RV *Pourquoi-Pas?*), the scientific team experimented a chal- 363
 lenging sampling strategy, allowing a standardised and ran- 364
 domised collection of 25–30 colonies per site of both *L.* 365
pertusa and *M. oculata* (Becheler et al. 2017). Five canyons 366

367 from the Bay of Biscay (European continental margin) and
 368 one location within the Logachev Mounds (Rockall bank)
 369 were sampled in this way. In 2012, the same strategy was
 370 used in two locations in South Iceland (European project
 371 Hermione, RV *Thalassa*). The sampling strategy consisted of
 372 a sampling quadrat of 200 * 100 m². Then, 30 X-Y coordi-
 373 nates were randomly generated within each quadrat (with
 374 0 < X < 200 m and 0 < Y < 100 m). They corresponded to the
 375 geographical coordinates of each sampling unit. Such stan-
 376 dard strategy, based on known and random spatial coordi-
 377 nates, allowed acquiring high-resolution quantitative
 378 information about the association among coral species, nota-
 379 bly its spatial extent (Arnaud-Haond et al. 2007), for three
 380 regions of the NE-Atlantic. All theoretical and practical
 381 details about this strategy can be found in Becheler et al.
 382 (2017).

383 Yet, such sampling is technically complex and time con-
 384 suming, requiring cruises partly devoted to genetic purposes.
 385 Most samples, notably those from the Mediterranean inte-
 386 grated in this study (Santa Maria di Leuca -Ionian Sea, and
 387 Canyon of Lacaze-Duthiers -Gulf of Lions, northwestern
 388 Mediterranean) were indeed collected in an “opportunistic”
 389 way, either during exploratory transects, or within sediment
 390 collected with cores or grabs. We could thus retrace popula-
 391 tion genetics and connectivity of Mediterranean and Atlantic
 392 reefs, but could not compare the clonal architecture in the
 393 Mediterranean and the Atlantic due to the limitation imposed
 394 by the opportunistic sampling strategy.

395 31.3.2.2 Present-Day Genetic Differentiation 396 Between Mediterranean and North-East 397 Atlantic

398 The genetic structure of both *L. pertusa* and *M. oculata* was
 399 assessed through the use of two types of nuclear markers, i.e.
 400 a set of respectively seven and six microsatellites, and inter-
 401 nal transcribed spacer (ITS) sequences (Becheler 2013). For
 402 both species, Atlantic populations appeared divergent from
 403 Mediterranean ones (Fig. 31.1). For example for *L. pertusa*
 404 the fixation index (F_{ST} , estimated with microsatellites)
 405 between a Mediterranean and an Atlantic population is
 406 around ten times higher than a F_{ST} between two Atlantic pop-
 407 ulations, and two to three times higher for *M. oculata*.

408 Yet, in the open NE-Atlantic, patterns of genetic structure
 409 are strikingly different between these two species. Atlantic
 410 populations of *L. pertusa* appeared as a large and single pan-
 411 mictic unit, i.e. one where all individuals are randomly mat-
 412 ing, with no clear genetic differences among reefs of the Bay
 413 of Biscay, Logachev Mounds and Iceland. In this context, it
 414 is worthy to mention that experiments in aquaria have
 415 recently reported that larval duration can reach 5 weeks and
 416 that larvae were active swimmers (Larsson et al. 2014).
 417 Results observed here support the hypothesis that high dis-
 418 persal potential is realised in the wild. Contrastingly, a

regional, pattern of genetic structure was observed among *M. oculata* reefs, with several differentiated units detected in the Atlantic.

The ability to disperse may be lower for *M. oculata* than for *L. pertusa*. In this case, the gene flow among regions would be too low to homogenise the genetically diverging units. Unfortunately, despite its apparently rather widespread distribution (Arnaud-Haond et al. 2017), *M. oculata* has thus far retained less attention than *L. pertusa* and knowledge is lacking about its larval biology. Another, non-mutually exclusive hypothesis can also be formulated: the influence of past events, such as the Last Glacial Maximum, which may have differently affected these two species, depending on their physiological tolerance (see Reynaud and Ferrier-Pagès, this volume) and consequent location of the refuges that would be at the origin of present day Atlantic populations.

501 31.3.2.3 Past Glacial Events and Their Influence 502 on Shared and Unique Polymorphism 503 Between the Mediterranean 504 and the Atlantic

505 The ITS sequences delivered complementary information to
 506 that obtained based on microsatellites. Their rate of evolu-
 507 tion being lower than for microsatellites (Hillis and Dixon
 508 1991; Balloux and Lugon-Moulin 2002), they may be infor-
 509 mative for larger temporal and spatial scales. For *L. pertusa*,
 510 the haplotype network (Fig. 31.1) revealed two main haplo-
 511 types, essentially found in the Mediterranean, Bay of Biscay
 512 and in Iceland. Closely related haplotypes separated by few
 513 mutations were found in low frequency around the main hap-
 514 lotypes and they originated from the Bay of Biscay, Ireland,
 515 and Iceland. This *L. pertusa* haplotype network is organised
 516 as a typical “star-like cluster” often recognised as a signature
 517 of ‘recent’ demographic expansion (Teixeira et al. 2011).
 518 Additionally, the main haplotypes are shared between the
 519 putative source(s) of recolonisation and recolonised areas.
 520 Satellite haplotypes are generated within populations having
 521 demographically expanded, which together with the signifi-
 522 cant results obtained using the Fu and Li tests (Fu and Li
 523 1993), support the hypothesis of a recent demographic
 524 expansion in NE Atlantic populations.

525 The network built with *M. oculata*’s ITS haplotypes
 526 shows a very different pattern, with a chaotic distribution of
 527 frequent and rare haplotypes, and private (endemic) haplo-
 528 types found in all regions. The more frequent haplotypes are
 529 numerous and can alternatively appear as shared between
 530 two, three, four or five regions. Notably, several Mediterranean
 531 haplotypes are shared by specimens collected in the Azores
 532 (central NE Atlantic), the Bay of Biscay, Ireland and in South
 533 Iceland, suggesting a possible common history and/or an
 534 ancient genetic connection, whereas they are not shared with
 535 the Mediterranean.

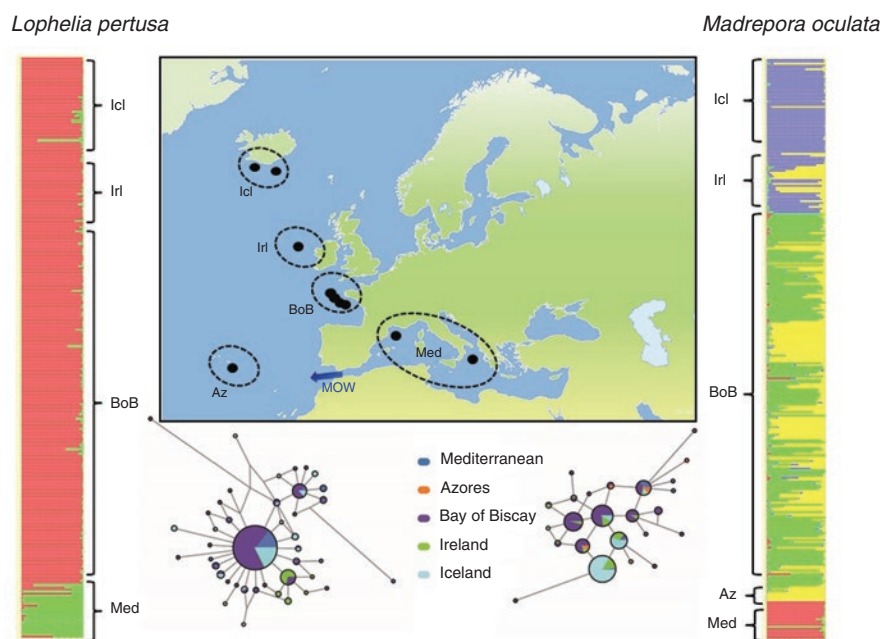


Fig. 31.1 Summarised insight of the known phylogeography of two relevant reef forming cold-water corals (CWCs), *Lophelia pertusa* (on the left) and *Madrepora oculata* (on the right). Black points are located on sampling sites included in this chapter. Bar plots on the left and the right correspond to the clustering analyses (performed with the free software TESS). Each multi-colored unitary bar corresponds to the probability that a genotype belonged to the corresponding cluster, fol-

lowing the idea that one color is for one given cluster. These analyses were based on multi-locus microsatellite genotypes. Below, the internal transcribed spacer (ITS) haplotype-networks are provided. Each circle corresponds to one haplotype, while its size is proportional to the frequency of this haplotype within the full dataset. These circles are divided in colored portions corresponding to the different biogeographic regions. *Icl* Iceland, *Irl* Ireland, *BoB* Bay of Biscay, *Az* Azores Islands, *Med* Mediterranean Sea

471 If signs of demographic expansions were also detected
 472 within *M. oculata*'s dataset, this would support a recent pop-
 473 ulation expansion/recolonisation in some reefs. The contrast-
 474 ing present-day genetic structure between *L. pertusa* and *M.*
 475 *oculata* and the polymorphism differentially shared among
 476 regions pleads for two different biogeographic scenarios: a
 477 clear support of a Mediterranean origin only for the first spe-
 478 cies, and a pattern calling for a more complex history for the
 479 other.

31.3.2.4 Biogeographic Scenarios: Two Reef-Building Species, Two Distinct Stories?

480 The genetic data obtained for *L. pertusa* thus supported the
 481 hypothesis of De Mol et al. (2005) of a post-glacial (i.e.
 482 <20,000 years ago) recolonisation of Atlantic reefs from a
 483 Mediterranean refuge. In line with the star-like network
 484 observed with ITS haplotypes (Fig. 31.1 bottom left), data
 485 suggests a (re) colonisation by the main Mediterranean haplo-
 486 type, followed by the accumulation of rare mutations during
 487 the post-glacial expansion of Atlantic reefs (the satellite haplo-
 488 types observed only in the Atlantic). The homogeneous genetic
 489 background found in the NE-Atlantic with microsatellites was
 490 also found, although in rare instances, in the Mediterranean
 491 (see bar plots on Fig. 31.1 left). This also suggests a
 492 Mediterranean source of recolonisation for the NE-Atlantic.
 493
 494

The possibly large dispersal ability of *L. pertusa* larvae may
 have maintained genetic homogeneity in the recolonised area
 of the NE-Atlantic reefs analysed in this study during the last
 millennia (Boavida et al., [under revision](#)).

Madrepora oculata clearly exhibits a more complex pat-
 tern likely resulting from marked different historical and
 present day connectivity. The decline of its populations dur-
 ing the LGM is very plausible. Nonetheless, the coexistence
 of several genetic backgrounds –and the high number of
 main haplotypes- suggests an alternative scenario of post-
 LGM recolonisation: despite the fact that the Mediterranean
 has likely been a glacial refuge for this species, additional
 sources of recolonisation in the Atlantic, which remain to be
 unraveled, may explain this pattern.

31.3.3 Large Scale Connectivity in *Desmophyllum dianthus*

Despite the Mediterranean-wide distribution of *D. dianthus*,
 from the Alborán Sea in the west to the Marmara Sea in the
 east, its low frequency of occurrence (but see recent data in
 Fourt et al., [this volume](#); Aymá et al., [this volume](#)) limits and
 challenges its study. Nevertheless, several studies have been
 conducted in biology, physiology and ecology revealing that

517 *D. dianthus* is an exceptional proxy for pH and temperature
518 of water masses in marine ecosystems; It has moderate
519 tolerance to elevated seawater temperature and to ocean
520 acidification, but there is a clear synergistic impact when
521 elevated temperature and pCO₂ are combined, resulting in
522 severe reduction of coral metabolism (e.g. Freiwald and
523 Roberts 2005 and references therein; Naumann et al. 2013;
524 Gori et al. 2016).

525 The presence of the solitary gregarious coral *D. dianthus*
526 (Vertino et al. 2014), or *D. cristagalli* (synonym, Milne
527 Edwards and Haime 1848), has been documented in the
528 Mediterranean since the Early- Middle Miocene. It dwells on
529 hard substrates occurring from the continental shelf to the
530 upper bathyal zone, commonly associated to scleractinian
531 reef framework-forming CWCs in what was described as a
532 “white coral” triad of *Lophelia-Madrepora-Desmophyllum*
533 (Pérès and Picard 1964; Zibrowius 1980; Roberts et al. 2009;
534 Chimienti et al., [this volume](#)). Macrohabitats of the coral
535 framework consist of continuous aggregations of branched
536 scleractinian coral colonies (primarily *M. oculata* and sec-
537 ondarily *L. pertusa*), whose skeletons are in mutual contact
538 with the exception of some small sediment pockets (Savini
539 et al. 2014). The solitary *D. dianthus* is thought to have been
540 influenced by the Mediterranean Sea’s geologic history in a
541 similar way as the reef-building corals mentioned here
542 above, and their co-occurrence is documented in several
543 places in the Mediterranean and the Atlantic. Hence, we also
544 addressed the hypotheses about past (and present) connectiv-
545 ity of *D. dianthus* populations in the Mediterranean and NE
546 Atlantic through the analysis of genetic polymorphism.

547 31.3.3.1 Sampling Locations and Strategy

548 Although solitary corals are not colony forming and do not
549 require a complex sampling strategy compared to their colo-
550 nial counterparts, particular attention is needed in case of
551 “aggregations” of individuals, where the presence of asexual
552 (clonal) individual is likely and may interfere with the inter-
553 pretation of molecular results. Since all the Mediterranean
554 samples of *D. dianthus* in this study were collected in an
555 “opportunistic” way, as explained above, it is essential to
556 apply adequate labels to each ‘ramet’. Samples have been
557 numbered consecutively, except for those forming an “aggre-
558 gation”, where the same number was applied, followed by a
559 letter further included in the label in order to identify poten-
560 tial clones during the genetic analyses.

561 31.3.3.2 Past and Present-Day Genetic 562 Polymorphism Between the 563 Mediterranean and Northeastern 564 Atlantic Ocean

565 Nuclear and mitochondrial genes have been used as molecu-
566 lar markers to determine genetic differentiation, phylogeog-
567 raphic patterns and gene flow within and among populations

of *D. dianthus* in the Mediterranean Sea and the northeastern
Atlantic Ocean. Nevertheless, the internal transcribed spacer
(ITS), the mitochondrial ribosomal small subunit (16S) and
the cytochrome c oxidase subunit I (COI) were not informa-
tive in terms of distinguishing between geographically and
bathymetrically isolated populations from global to regional
spatial scales (see Fig. 3 in Addamo et al. 2012; Fig. 31.2).
The occurrence of shared haplotypes between specimens
could indicate historical patterns of shared genetic diversity
(current or recent gene flow, incomplete lineage sorting or
retention of ancestral polymorphism), methodological bias
(using genes or regions with a substitution rate inadequate to
show divergence) or both (differences in the coalescence of
these genes combined with population’s divergence; Addamo
et al. 2012). To assess the population genetic structure and
the ability of this species to disperse into different regions
and habitats, contributing to the local gene pool (i.e. migra-
tion), nuclear microsatellite sequences have provided ade-
quate resolution. Markers with higher evolutionary rates –
such as microsatellites – have been developed for *D. dian-
thus* (Addamo et al. 2015). They allowed the assessment of
connectivity among populations dwelling at different depths
and to infer biological (e.g. reproduction strategy or larval
dispersal) and oceanographic (e.g. bathymetry or water cir-
culation) factors driving isolation. No statistically significant
levels of genetic differentiation have been found among geo-
graphic populations from the Mediterranean Sea and NE
Atlantic Ocean (Fig. 31.3), suggesting high connectivity
among deep-sea locations, with some exceptions for north-
ern individuals from the Cantabrian Sea and Ireland.

598 31.3.3.3 Biogeographic Scenarios for *D. dianthus* 599 Populations in the Mediterranean Sea 600 and the Atlantic Ocean

601 An interpretation of the shared genetic polymorphism
602 between these two marine regions is the hypothesis of a his-
603 torical recolonisation homogenising the gene pools in both
604 marine areas. During the Holocene, a colonisation could
605 have taken place from the Mediterranean Sea to the Atlantic
606 Ocean with a sufficiently large number of individuals not to
607 have resulted in a genetic differentiation through a founder
608 effect (i.e. the dramatic change –and loss- of genetic varia-
609 tion that occurs when a population is established in a new
610 area by a very small number of migrants not genetically rep-
611 resentative of the larger original population).

612 On the other hand, a physical connectivity along thou-
613 sands of miles between the Mediterranean and NE-Atlantic
614 Ocean sites may be attributed to the principal currents in
615 intermediate and deep waters (see Fig. 19.18 in Chimienti
616 et al., [this volume](#)). The intermediate and deep water currents
617 mainly characterising the Mediterranean Sea and the
618 NE-Atlantic Ocean are the Levantine Intermediate Water
619 (LIW), Adriatic Deep Water (AdDW), Aegean Deep Water

Fig. 31.2 (a) Study areas: Mediterranean Sea (orange=Adriatic Sea; red=Ionian Sea; yellow=strait of Sicily) and NE-Atlantic Ocean (light blue=Galicia, Cantabria and Ireland). Squares indicate the sampling locations. (b) Haplotype network of internal transcribed spacer (ITS) ribosomal DNA sequence haplotypes of *Desmophyllum dianthus* belonging to Mediterranean Sea populations (orange) and NE-Atlantic Ocean populations (light blue). Sizes of the circles are proportional to the number of samples presenting the same haplotype. (Adapted from Addamo et al. 2012)

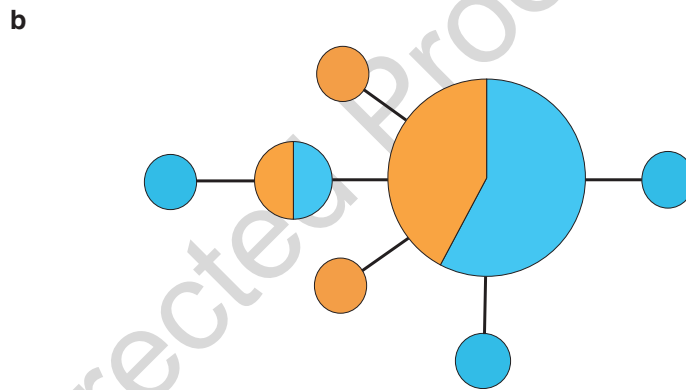
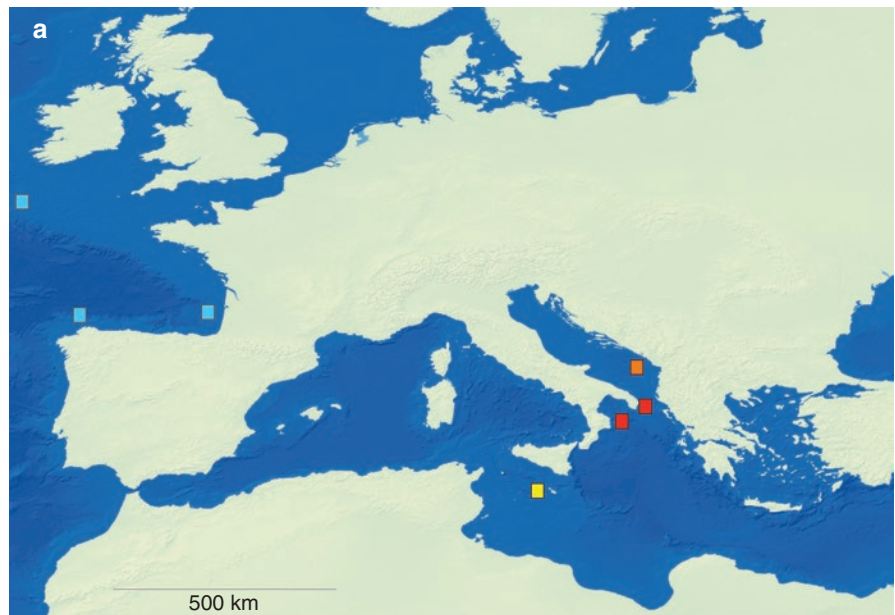
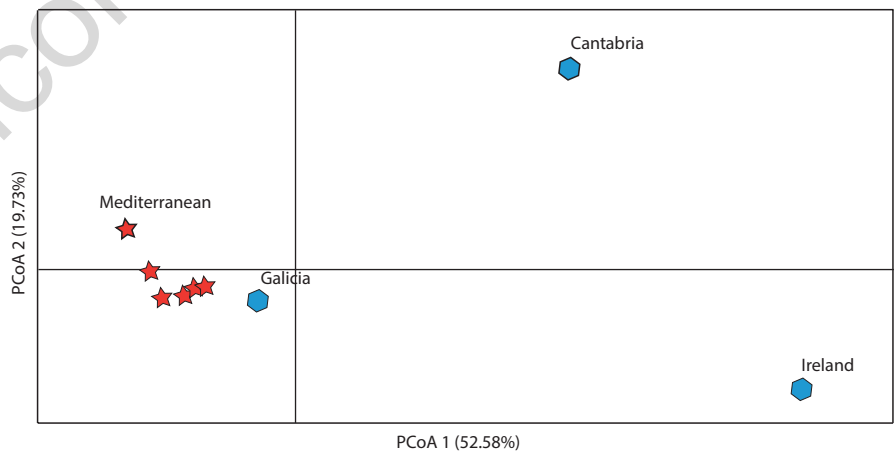


Fig. 31.3 Principal coordinate analysis (PcoA) projecting the relative genetic distances among *Desmophyllum dianthus* populations from the Mediterranean Sea (red stars) and the Atlantic Ocean (blue hexagons)



620 currents (AeDW), and Atlantic Water (AW) (Millot and
 621 Taupier-Letage 2005). The LIW circulates at approximately
 622 200–600 m along the northeastern slope of the Ionian Sea,
 623 penetrating into the southern Adriatic Sea, and then contin-
 624 ues along the slope to the Strait of Sicily, where most of it

outflows into the western Basin (at 400 m depth). The other
 625 two currents (AdDW, AeDW) first accumulate in the troughs
 626 (1000–1500 m) over which they are formed (in the southern
 627 Adriatic and southern Aegean Seas, respectively) before out-
 628 flowing into the western Basin through various openings. 629

630 These water masses are depth-stratified and may represent
 631 two distinct bathymetric levels that could create depth struc-
 632 turing in species diversity and community composition.
 633 Nevertheless, the intermediate and deep water masses circulate
 634 and can be identified at greater or lesser distances from
 635 their zone of origin. They continuously mix and, finally, out-
 636 flow at the Strait of Gibraltar as a rather homogeneous water
 637 (the so called Mediterranean Outflow Water, MOW), which
 638 is saltier and denser than Atlantic water (AW) in that location
 639 (Millot et al. 2006). Therefore, the Mediterranean Sea is a
 640 “machine” that transforms AW present at the surface just
 641 west of the Strait of Gibraltar into denser water that is recog-
 642 nised at 1000–1200 m in most of the northern Atlantic Ocean
 643 (Millot and Taupier-Letage 2005). The MOW is a saline
 644 water mass occupying the intermediate depths of the
 645 NE-Atlantic. It reaches a neutrally buoyant depth between
 646 800 and 1300 m (Price et al. 1993) and spreads into the North
 647 Atlantic: westward to the central Atlantic and northward fol-
 648 lowing the coasts of Portugal and Spain (Bozec et al. 2011;
 649 and references therein).

650 Deep-sea and offshore marine protected areas (MPAs)
 651 have been recently recognised by international and national
 652 organisations as efficient tools for protection of deep habitats
 653 (Cordes et al. 2016; Davies et al. 2017; OSPAR [https://www.
 654 ospar.org/news/ospar-ministers-protect-the-north-east-atlan-
 655 tic-deep-seas-treasures](https://www.ospar.org/news/ospar-ministers-protect-the-north-east-atlantic-deep-seas-treasures); Colombia Deep-water Coral
 656 National Natural Park designated in May 2013 by the
 657 Ministry of Environment [http://www.parquesnacionales.gov.
 658 co/portal/es/parques-nacionales/parque-nacional-natural-
 659 corales-de-profundidad](http://www.parquesnacionales.gov.co/portal/es/parques-nacionales/parque-nacional-natural-corales-de-profundidad)). Specifically, results from studies
 660 on genetic connectivity of *D. dianthus* populations in the
 661 Mediterranean Sea contributed to the classification of a
 662 Natura 2000 Site of Community Interest (SCI), the center-
 663 piece of the European Union’s conservation policy, in the
 664 southern Tyrrhenian Sea. This SCI will be used to character-
 665 ise and define the novel concept of cells of ecosystem func-
 666 tioning (Boero 2015) as conservation and management units,
 667 a key concept for the definition of networks of MPAs and for
 668 the identification of suitable areas for off-shore wind farms
 669 (Costantini et al. 2017; see also www.coconet-fp7.eu,
 670 CoCoNet EU project).

671 **31.4 A Complex Biogeographic History** 672 **Prevents Teasing Apart Remnants** 673 **of Past Connectivity from Present Day** 674 **Migration**

675 The reason for the results observed here (high homogeneity
 676 of *Desmophyllum dianthus* between the Atlantic and the
 677 Mediterranean compared to a strikingly strong differentia-
 678 tion for *Lophelia pertusa* and *Madrepora oculata*) remains
 679 to be elucidated. Subject to similar distributions and cur-

680 rents, these species may respond differently due to specific
 681 life history traits. Although largely unknown, these may
 682 include a different pelagic larval duration for *D. dianthus*
 683 and *M. oculata* relatively to *L. pertusa*, and/or the solitary
 684 versus reef forming nature of these three taxa. Yet, it is also
 685 likely that differences in their past biogeographical history
 686 have led to the present genetic structure patterns. In order to
 687 tease apart the evolutionary scenarios explaining individual
 688 species patterns, and the cause of their strikingly different
 689 levels of differentiation at the scale of European waters,
 690 molecular data are needed that would deliver a higher statis-
 691 tical power to feed data analyses and modelling methods.

692 CWCs in the Mediterranean Sea have a long-term history
 693 for which the origin is still mostly unknown (but see chapters
 694 of the Past section in this volume). Most Mediterranean
 695 deep-water fauna was likely extinct during the Miocene due
 696 to the Messinian Salinity Crisis (5.9 Myr ago, Garcia-
 697 Castellanos and Villasenor 2011; Freiwald, [this volume](#))
 698 after which the re-opening of the Gibraltar Strait is thought
 699 to have led to a recolonisation with Atlantic fauna (Bouchet
 700 and Taviani 1992; Hsü et al. 1977). The long-term persis-
 701 tence of some coral species in the Mediterranean Sea is how-
 702 ever indicated by fossil data (Fink et al. 2012, 2015; Perrin
 703 and Bosellini 2012). Most explorations of CWCs in the
 704 Mediterranean Sea have occurred during the past 15 years.
 705 The results from these studies reflect a dominance of fossil-
 706 derived data. Important events of colonisation are likely to
 707 have started in the late Pleistocene (Malinverno et al. 2010;
 708 Taviani et al., [this volume](#); Vertino et al., [this volume](#)). At the
 709 end of the Last Glacial Maximum (LGM) coral abundance
 710 appears to have declined in the Mediterranean Sea and adja-
 711 cent Gulf of Cadiz (Taviani et al. 2011), but episodes of local
 712 proliferation and contraction varied across basins and spe-
 713 cies (Fink et al. 2015). In the case of *M. oculata*, notable
 714 coral proliferation occurred in the Mediterranean Sea during
 715 a cold event after the onset of the deglaciation (a period
 716 known as the Younger Dryas 12.9–11.7 kyr ago; Fink et al.
 717 2015). This trend is opposite to the one reconstructed for *L.*
 718 *pertusa* in the North East Atlantic (Frank et al. 2011). There,
 719 corals disappeared from higher latitudes (approx. >50°N)
 720 during periods of major cooling (e.g. LGM), their presence
 721 being largely coincident with warmer conditions. The reap-
 722 pearance of *L. pertusa* in higher latitudes is suspected to
 723 have originated from a hypothesised refuge located in the
 724 Mediterranean (De Mol et al. 2005; Henry et al. 2014) and/
 725 or GoC, including the Moroccan margin (Wienberg et al.
 726 2010). Surprisingly, as summarised here above, results on *M.*
 727 *oculata* data point toward other putative refugia, such as
 728 mid-Atlantic seamounts, whereas *D. dianthus* only gives
 729 signs of present day or recent exchanges across major basins.
 730 The phylogenetic reconstruction of the long-term past his-
 731 tory of connectivity and recolonisation between the Atlantic
 732 and the Mediterranean is thus likely to be blurred by multiple

733 events with opposite direction. However, Bayesian recon- 773
 734 structions of high-density genome scans¹ using NGS may 774
 735 help reconstruct complex past scenarios. 775

736 Numerous cases of connectivity estimates have been 776
 737 blurred by errors in taxonomic identification or description, 777
 738 including artificial exclusion of species described as differ- 778
 739 ent on the basis of non-discriminant taxonomic characters, or 779
 740 in contrast, the inclusion of distinct taxa (Pante et al. 2015a; 780
 741 Altuna and Polisenio, [this volume](#)) or even by including semi 781
 742 species having developed a certain level of reproductive 782
 743 incompatibility (Bierne et al. 2011; Gagnaire et al. 2015). It 783
 744 is now clear that markers used in population genetic studies 784
 745 should allow ascertaining the taxonomic status of the taxa at 785
 746 stake. Teasing apart the signatures of past events such as the 786
 747 last glacial cycles from the ones of present day connectivity 787
 748 is not a trivial task, especially when relying on a handful of 788
 749 markers, as were the cases presented above. 789

750 31.5 Perspectives Arising From Access 773 751 to Genome Scan Data 774

752 Traditional phylogenetic and population genetic studies have 775
 753 relied on few molecular sequence markers. These include 776
 754 mitochondrial and nuclear genes, which can be amplified at 777
 755 low cost with nearly universal primers. However, they present 778
 756 some limitations, such as poor genome representation 779
 757 (Angeloni et al. 2012). The use of mitochondrial sequence 780
 758 markers is further limited in corals due to the remarkable low 781
 759 rate of mitochondrial genome evolution (Hellberg 2006; 782
 760 Huang et al. 2008; Shearer and Coffroth 2008). The intro- 783
 761 duction of microsatellites in population genetic studies 784
 762 promised great power to assess similarity between individu- 785
 763 als and closely related taxa in a cost-effective way, mainly 786
 764 due to their high polymorphism and high mutation rate 787
 765 (Hodel et al. 2016; Jarne and Lagoda 1996; Putman and 788
 766 Carbone 2014). Microsatellites have proven extremely use- 789
 767 ful when the questions at hand require a very large number of 790
 768 individuals to be analysed, but their power strongly depends 791
 769 on the strength of the genetic signal and of the density and 792
 770 resolution of the set of markers used (Selkoe and Toonen 793
 771 2006). They do not allow the same level of automatism, cross 794
 772 platform compatibility, and reliability as genome scans when 795

¹Genome scan is used here in its primary semantic meaning, thus fitting the definition given by Richard and Hawley (2010, "Human genome"; p 561): "A survey of genetic markers spread across the whole genome. A genome scan will usually involve hundreds of markers", rather than the one previously proposed in the Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine (2006), that narrows its scope to the screening for phenotype related markers ("Genome scan refers to a genetic research method in which the entire DNA of an organism is searched systematically for locations on the chromosomes that are inherited in the same pattern as a specific trait").

the hypothesis or scenario to be tested requires a high density 773
 of markers. The advent of NGS offers a novel opportunity to 774
 gather genome-scale sequence information, which may 775
 allow a more powerful estimate of genetic variation within 776
 and among populations and a much higher resolution in the 777
 reconstruction of past and present scenarios susceptible to 778
 explain the present day distribution of genetic polymor- 779
 phism. The NGS includes several techniques, most of which 780
 have in common the random sequencing of DNA, RNA or 781
 cDNA (Heather and Chain 2016). Hundreds to thousands of 782
 polymorphic genetic markers, behaving as independent loci, 783
 can be retrieved across the entire genome, in a relatively 784
 simple and affordable manner (price per sequenced Gigabase 785
 in Goodwin et al. 2016) even for species without a reference 786
 genome. Collectively, these techniques are called reduced- 787
 representation (RR) methods (e.g. Andrews et al. 2016) 788
 because they target a random subset of the genome (or tran- 789
 scriptome), rather than sequencing the entire genome (termed 790
 "whole-genome sequencing"), a more costly approach. Even 791
 with the typical small sample sizes as those often limiting 792
 deep-sea studies, by using thousands of independent markers 793
 across the genome, the recent (<10 years) application of 794
 NGS approaches to deep-water organisms has improved the 795
 power of estimates of demographic parameters in deep-sea 796
 octocorals and population histories (Everett et al. 2016). 797
 They also allowed detection of genomic areas that are under 798
 selection in deep-sea scallops (Van Wyngaarden et al. 2017), 799
 and have resolved deep-sea species radiations and phyloge- 800
 nies (Reitzel et al. 2013; Pante et al. 2015a; Herrera and 801
 Shank 2016). 802

803 One of the challenges of population genetic -and now 804
 805 genomic studies applied to the deep-sea remains the diffi- 806
 807 culty in obtaining a representative number of samples to 808
 809 make inferences about the evolutionary history of metapopu- 810
 811 lations. Whereas NGS may require fewer samples than clas- 812
 813 sical genetic analyses to obtain robust statistical inferences, 814
 815 a similar amount of sampling units and a rigorous sampling 816
 817 design will remain a requirement with NGS in order to deal 818
 819 with clonal diversity, and the consequent best sampling strat- 820
 821 egy to obtain a representative sample of a population. 822

823 Also, while RR techniques provide greater depth of cover- 824
 825 age (i.e. number of sequences or "reads"), when working on 825
 non-model organisms with small genomes (i.e. <0.5 Giga 826
 base pairs), such as those characterising many CWC species, 827
 assembling draft whole-genomes for reference may become 828
 another valuable approach. Low-coverage, cost-effective 829
 methods to produce draft *de novo* assemblies are already 830
 available (Malmstrom et al. 2017), opening a wider range of 831
 possible studies encompassing selection and adaptation 832
 (Ekblom and Wolf 2014), as well as the influence of second- 833
 ary contacts (Gagnaire et al. 2015). Finally, having a refer- 834
 ence genome in combination with a characterised 835
 transcriptome (RNA-Seq; Wang et al. 2009) will allow anno-

826 tating the genome for particular genes, permitting the distinc- 844
 827 tion between selected from neutral variation. Such an 845
 828 approach to pinpoint expressed genes may also assist the fine- 846
 829 tuned identification of appropriate conservation units. The 847
 830 combination of these novel techniques with statistical 848
 831 approaches that test environment-mediated selection and eco- 849
 832 logical niche modelling is likely to trigger a wave of seascape 850
 833 genomics studies (notably reviewed in Riginos and Liggins 851
 834 (2013); Riginos et al. (2016)), highly relevant for CWC con- 852
 835 servation and management. Regardless of the particular van- 853
 836 guard method chosen, for the days that lay ahead, NGS 854
 837 encompasses a range of enticing techniques that promise to 855
 838 set the scene for deciphering the elusive population contours 856
 839 of clonal lineages, CWC species and associated fauna.

840 The scenarios that could be put forward to explain the 857
 841 present-day geographic distribution of polymorphism among 858
 842 populations that we reviewed here for these three relevant 859
 843 species of CWC scleractinians, are still limited by the level 860
 861

of resolution of markers used attempting to indirectly recon- 844
 struct and rank complex biogeographical histories. A higher 845
 amount of information (i.e. loci) is necessary to increase the 846
 statistical power and unravel previously ignored patterns of 847
 moderate differentiation, if it exists. As summarised above, 848
 moderate levels of differentiation may exist for *Lophelia* 849
pertusa in the Atlantic or *Desmophyllum dianthus* across the 850
 studied range. Besides, denser sampling of the genome is 851
 necessary to reliably reconstruct and rank evolutionary sce- 852
 narios using up to date Bayesian methods, ideally with 853
 approximate dating. These methods may help to explain the 854
 present day patterns of differentiation and to improve under- 855
 standing regarding present day patterns of connectivity. For 856
 example, the recent work on the coral-associated polychaete 857
Eunice norvegica already shows the increased power gained 858
 through genome scans (Box 31.1). Similarly, ongoing 859
 genomic studies on some of the CWC flagship species *L.* 860
pertusa, *M. oculata* and *D. dianthus* promise to hasten our 861

Box 31.1: Taxonomic Uncertainties Reflect Past and Present Day Connectivity in the Polychaete *Eunice norvegica* Inhabiting Cold-Water Corals (CWC) Along European Margins

The recent (approx. 10 years) development of Next Generation Sequencing (NGS) methods now allows the use of an array of genetic markers to analyse the connectivity of populations. Analysis of nearly 4000 single nucleotide polymorphisms (SNP) on the CWC-associated polychaete *Eunice norvegica* (Fig. 31.B1) using Restriction Site Associated sequencing (RADseq, a kind of RR) has confirmed limited to non-existent gene flow between Atlantic and central Mediterranean samples (Fig. 31.B2). It completes a previous analysis based on maternally inherited

mitochondrial markers (Fig. 31.B3), thus excluding the possibility of male-exclusive migration. RADseq loci (sequenced sites along the genome) avoid issues of developing specific markers (such as microsatellites) not necessarily transferrable between two distant genetic backgrounds, and have increased resolution to reliably reflect the variability of patterns across the genome. The application of this sequencing technique also bolstered evidence of a common evolutionary history dating from before the Last Glacial Maximum (LGM) between individuals as distant as the high latitudes on the North East Atlantic and the central Mediterranean Sea. This new exciting data may represent a harbinger of distinct high latitudes and Mediterranean refugia for CWC habitats and associated species during past climatic fluctuations.



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Fig. 31.B1 Polychaete worms *Eunice norvegica*. (© Ifremer, Olivier Dugornay, Videocor cruise)

(continued)

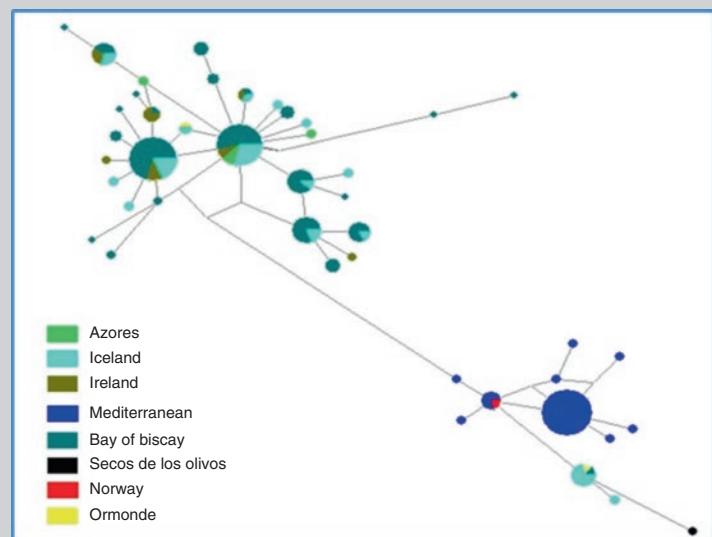
Box 31.1 (continued)

Fig. 31.B2 Result from admixture ancestry analysis considering two genetic groups



AUI

Fig. 31.B3 COI haplotype networks of 45 haplotypes. Each node corresponds to a haplotype, and is divided according to its count in each sampled population



862 understanding of highly contrasting pathways for CWC
 863 post-glacial colonisation at the European scale as reported
 864 here, to further disentangle existing putative cryptic CWC
 865 species diversity and to clarify species hypothesis (Addamo,
 866 [this volume](#)). Recent results indeed suggest that *L. pertusa*
 867 may be a junior synonym of the recently reassigned scientific
 868 name of *Desmophyllum pertusum* (Addamo et al. 2016).
 869 Here also, genome scans are expected to deliver more robust
 870 information to dig deeper into this intriguing similarity.

871 Discriminating the imprint of past *versus* present day
 872 mechanisms acting on the current distribution of genetic
 873 diversity is a prerequisite to understand present day drivers
 874 and patterns of connectivity across their geographic range.
 875 This information is central to development of informed
 876 conservation strategies. Teasing apart past and present drivers
 877 is needed to differentiate between the ones we can no
 878 longer act on from the ones that are relevant to take into
 879 account in the definition of sampling strategies, as well as

880 to “*promote evolutionary potential and evolutionary pro-*
 881 *cesses to better protect extant biodiversity and biodiversifi-*
 882 *cation*” (Olivieri et al. 2016). Despite the historical local
 883 and temporary extinctions in the Mediterranean and
 884 NE-Atlantic, linked to variations in productivity and oxy-
 885 gen levels (Fink et al. 2012, 2015), it is remarkable that
 886 CWC have so swiftly responded to drastic environmental
 887 changes (latitudinal shifts in the order of thousands of kilo-
 888 meters-wide for *L. pertusa* and *M. oculata*, as supported in
 889 the works cited and synthesised here). Coral reappearance
 890 has tracked these environmental fluctuations in just a few
 891 hundreds of years (Wienberg et al. 2010). Yet, with the
 892 cumulative and synergistic effects of environmental oscil-
 893 lations, habitat loss, overexploitation and pollution of the
 894 deep-sea, this ability to promptly track changes and re-
 895 establish/re-adjust their geographical and/or bathymetric
 896 distributions may be hampered, leading to an increased risk
 897 of extinctions (Harnik et al. 2012). Conservation of CWC

benefits from well-informed, knowledge-based decisions, for example when it comes to decide how to size and distribute MPAs. Knowledge must include thorough species inventories, which allow for an understanding of their relatedness (Addamo, [this volume](#); Altuna and Poliseno, [this volume](#)), their genetic variation and distribution, as well as pinpointing the genetic units and connectivity of CWC populations (Gagnaire et al. 2015; Lagabrielle et al. 2014; Pante et al. 2015b; Costantini et al. 2016).

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