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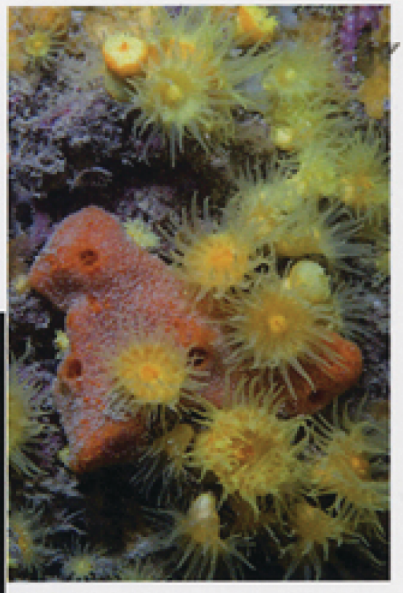

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# GENETIC DIFFERENTIATION OF THE TEMPERATE-SUBTROPICAL STONY CORAL *LEPTOPSAMMIA PRUVOTI* IN THE MEDITERRANEAN SEA

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## ABSTRACT

Data on genetic structure of corals living in the Mediterranean Sea are very scarce. *Leptopsammia pruvoti* Lacaze-Duthiers, 1897, is a gonochoric and brooding solitary coral that colonizes shaded rocky habitats. It is very abundant under overhangs, in caverns, and in small crevices, with typical densities of thousands of polyps per square meter. In the present study, a hierarchical survey of spatial models of genetic structure and potential gene flow was conducted within and among seven shallow water Mediterranean populations, which ranged from 2 to 872 km apart. Within each population, polyps were randomly collected from three patches 5 m apart. Seven allozyme loci indicated a genetic structure characterized by (1) a marked departure from Hardy–Weinberg equilibrium, with a considerable deficit of heterozygotes; (2) most genetic differentiation occurring between patches within populations ($F_{ST}$ = 0.118 to 0.206), rather than among distant populations ($F_{ST}$ = 0.052); and (3) no significant correlation between genetic differentiation and geographic distance. Localized recruitment with consequent biparental inbreeding or Wahlund effect, associated with a proportion of larvae that undergo long-distance dispersal, could explain the observed pattern of genetic structure.

*Keywords*: scleractinian corals, population genetic structure, inbreeding, Wahlund effect, larval dispersal, cup corals, Mediterranean Sea

## INTRODUCTION

Dispersal capability plays a fundamental role in determining metapopulation dynamics because it defines the extent of genetic neighborhoods as well as the level of gene flow between them. Genetic differences arise both within and among populations through the processes of genetic drift or natural selection. Broad dispersal of propagules with subsequent random mating will oppose and mitigate such local differentiation through the random mixing of alleles from differing local populations (Slatkin, 1993). In contrast, limited dispersal can give rise to small-scale genetic structure within populations as a result of isolation-by-distance. When gene flow is extremely limited, discrete breeding units, whose size is determined by the variance in gene flow, may develop and become

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differentiated from other such breeding units as a result of localized genetic drift or selection (Wright, 1978). Estimating the degree of ecological interconnectivity of spatially disjunct systems and the extent of self recruitment is important to the study of the evolutionary potential of a species (Taylor and Hellberg, 2003) and is a current focus of conservation science, as communities are under increasing adverse pressures from short- and long-term anthropogenic effects, ranging from over exploitation to global warming (Hoegh-Gulberg et al., 2007). Small and isolated populations are vulnerable to inbreeding depression, which might reduce their evolutionary potential and increase their risk of extinction (Saccheri et al., 1998). Studies using genetic markers have become vital due to the difficulties associated with directly observing the movement among populations in the natural environment (Hellberg et al., 2002). Although increasing numbers of genetic estimates of connectivity among populations are becoming available, generalizations about species that are likely to have genetically structured populations, and the process driving such differentiation, are still difficult to assess (Bohonak, 1999).

Many marine organisms are benthic as adults, dispersing primarily during the larval phase. The dispersal potential of these species may be related to traits such as egg type (e.g., pelagic vs. benthic: Shulman and Berminghan, 1995), larval development (direct vs. pelagic: Hellberg, 1996; Ayre and Hughes, 2000), length of the pelagic larval phase (Doherty et al., 1995; Riginos and Victor, 2001), and pelagic larval environment (inshore vs. offshore: Riginos and Victor, 2001). Comparative studies of marine gastropods (Janson, 1987), sea urchins (McMillan et al., 1992), sponge-dwelling shrimps (Duffy, 1993), sea stars (Hunt, 1993), solitary corals (Hellberg, 1996), and fishes (Riginos and Victor, 2001) have shown that as the length of the pelagic larval phase decreases, the degree of local genetic differentiation increases. However many exceptions, such as genetically differentiated populations of species with extensive larval durations, have also been documented (Taylor and Hellberg, 2003). Effective dispersal of larvae can be affected by physical barriers, lack of suitable habitats for settlement, larval viability, and the habitat-specific reproductive success. All these factors, either singly or in combination, appear crucial in determining the spatial scales of genetic structuring in marine invertebrates.

Corals are sessile invertebrates and show a variety of reproductive systems that may directly influence their potential for dispersal. They are either hermaphroditic or gonochoric, and brooders (corals that release sperm, but brood the eggs) or broadcasters (corals that release eggs and sperm in to the water; review in Carlon, 1999). While brooded planulae generally settle within a few hours of their release, planulae originating from spawning require several days after gamete release before they settle (Carlon, 1999). This suggests that the dispersal potential of brooded planulae is more restricted than that of planulae originating from spawning because of the shorter pre-competency period in brooders versus spawners. Nevertheless, a previous comprehensive study of the population genetics of scleractinian corals does not fully support this hypothesis (Ayre and Hughes, 2000). In addition to the pre-competency period, the competency period of planulae also affects the dispersal distance of coral planulae. Laboratory investigations of larval competency in corals show that the planulae of both spawners and brooders

may remain in the planktonic phase for weeks (Wilson and Harrison, 1998; Goffredo and Zaccanti, 2004, and references therein), and the duration of the planktonic phase differs among species, even among those with the same reproductive mode. This suggests that the dichotomy of spawners versus brooders does not always account for the differences in the competency periods of coral planulae. Asexual reproduction also exists in corals in a variety of forms, including fragmentation, budding, or polyp expulsion, and asexually produced planulae (Fautin, 2002).

*Leptopsammia pruvoti* Lacaze-Duthiers, 1897 is an ahermatypic, azooxanthellate, and solitary scleractinian coral, which is distributed in the Mediterranean basin and along the European Atlantic coast from Portugal to southern England. It is one of the most common organisms in semi-enclosed rocky habitats, under overhangs, in caverns, and small crevices at 0–70 m depth, with a mean population density of more than ten thousands individuals per square meter, i.e., more than two kilograms of $CaCO_3$ biomass $m^{-2}$ (Goffredo et al., 2007). Sea surface temperature and solar radiation do not significantly influence its biometry (skeletal density, corallite length, width, and height) and population density along a latitudinal gradient spanning 850 km on the western Italian coast (Goffredo et al., 2007). It is a gonochoric internal brooder (Goffredo et al., 2006). Fertilization takes place from January to April and planulation during May and June. When released, the planulae larvae have completed ontogenesis, have a length of 695–1,595 μm, and, in laboratory rearing conditions, swim by ciliary movement for several days (Goffredo et al., 2005). Evidence of asexual reproduction (either through polyp budding or fission) has not been observed (Goffredo et al., 2006, and references therein). The yellow color and high density of polyps make this species attractive for recreational divers, who represent an important income for coastal tourist resorts in the Mediterranean (Mundet and Ribera, 2001).

Here, the genetic structure of *L. pruvoti* was examined using allozyme electrophoresis. Allozyme electrophoresis is based on the fact that different alleles for enzymatic proteins may have different electrophoretic mobilities due to differences in protein net charge caused by amino acid differences. There are many potential differences between alleles that will not be detected by this approach (e.g., silent substitutions). Hence, the total number of alleles will be underestimated. An alternative useful approach for population genetic studies could be the use of microsatellite DNA markers. Despite their utility, microsatellites have been used in few studies in scleractinian corals (for example: Underwood et al., 2007, and references therein). Microsatellites may be rare in scleractinian corals and difficult to isolate (Ridgway, 2005; Shearer et al., 2005). Given the allozyme relative ease of use and the numerous comparable data sets from previously published studies, they still represent a powerful and relevant technique for understanding connectivity and population structure in many marine animal taxa (Ridgway, 2005). Here the genetic structure was compared across and within populations in the Western Mediterranean Sea, and dispersal patterns were inferred at two different spatial scales: large scale (i.e., among populations across a range greater than 800 km), and local scale (i.e., among patches within populations along a transect that was 10 m long). The hypothesized limited dispersal potential of azooxanthellate and brooded planulae (Har-

rison and Wallace, 1990; Carlon, 1999; Ayre and Hughes, 2000; Goffredo and Zaccanti, 2004), should lead to inbreeding, with departure from Hardy–Weinberg equilibrium due to heterozygote depression, along with low gene flow and genetic divergence (Wright, 1978; Carlon, 1999; Ayre and Hughes, 2000).

## MATERIALS AND METHODS

### *SAMPLING*

Samples of *L. pruvoti* were collected from seven sites in the Western Mediterranean basin (Fig. 1): three in the Ligurian Sea [one in the Gulf of Genoa (Punta della Targhetta, PTR), the other two south of Leghorn along the Tuscan coast (Calafuria, CLF, and Calignaia, CLG)]; three in the Southern Tyrrhenian Sea along the Calabrian coast (Bagnara Calabra, BGN; Scilla, SCL; and Lido il Gabbiano, LDG); and one in the Channel of Sicily, in Pantelleria Island (PNT). These seven localities ranged from 2 (CLF–CLG) to 872 (PTR–PNT) km apart. Specimens were collected from January 2006 to February 2007 by SCUBA diving, at a depth of 14–18 meters, where this species reaches high population density on the vault of crevices. Population density of *L. pruvoti* is homogeneous among sites along the Western Mediterranean (average population density of *L. pruvoti* at the depth of collection is: 10,155 individuals $m^{-2}$, SE = 1,317; 2,030 g $m^{-2}$, SE = 232; 15.4% cover, SE = 1.4; Goffredo et al., 2007). Given the estimated turnover time of *L. pruvoti* > 7 yr in populations of the Ligurian and Tyrrhenian seas, and in the Channel of Sicily (maximum life span > 30 yr; S. Goffredo, University of Bologna, unpubl. data), the range in the sampling dates was not considered to be significant with respect to differentiation within populations. At each site, polyps were randomly col-


Fig. 1. *Leptopsammia pruvoti*. Sampled populations (coordinates and abbreviations in decreasing order of latitude: PTR, Punta della Targhetta, 44º20′N, 9º9′E; CLF, Calafuria 43º29′N, 10º19′E; CLG, Calignaia 43º28′N, 10º21′E; BGN, Bagnara Calabra 38°16′N, 15º48′E; SCL, Scilla 38°14′N, 15º43′E; LDG, Lido il Gabbiano 38°14′N, 15º40′E; PNT, Pantelleria Island 36º46′N, 11°58′E).

lected from three quadrats (within a 43 $cm^2$ area cordoned off by a plastic frame) 5 m apart (we estimated the distance between patches within localities based on the number of body lengths between them, according to Hellberg, 1996). Benthic habitat was homogeneous between populations and patches; i.e., small crevices, rocky bottom, and coralligenous concretions (Ballesteros, 2006). All polyps collected were adult polyps, above the size at sexual maturity (3 mm polyp length; Goffredo et al., 2006; average length of collected polyps = 7.4 mm, SE = 0.2, total collected polyps = 308). Living polyps were transported in refrigerated units to the laboratories of the Department of Evolutionary and Experimental Biology of the University of Bologna. Specimens were dissected and examined under a binocular microscope. All oocytes and embryos were removed to avoid confounding the parental genotype with that of the progeny. Epibionts were scraped off the samples and each individual was put into a single tube devoid of seawater, which was stored at –80 ºC.

*LABORATORY ANALYSIS*

Tissue was taken from samples and then homogenized using an equal volume of 0.1 M Tris-HCl buffer pH 7.5 (containing 1 mM Na EDTA, 0.6 mM NAD, 0.5 mM NADP, 1 mM mercaptoethanol). The homogenates were centrifuged at 11,000*g* for 10 min at 4 ºC, to remove mucus and cellular debris. The supernatants were used for electrophoresis. Allozyme electrophoresis was carried out under different electrical conditions using cellulose acetate (Diploid dry) as substrate at 4 ºC. Staining was carried out in an oven at 37 ºC. Eighteen loci were initially tested using four different buffers: TEC, pH 7.5; Tris citrate, pH 7.2; and TEM, pH 7.4 and 7.8. The latter gave the best resolution. Pilot screening revealed seven polymorphic loci: malate enzyme (*Me-1* and *Me-2*, EC 1.1.1.40), hexokinase (*Hk-1* and *Hk-2*, EC 2.7.1.1), phosphoglucose isomerase (*Pgi*; EC 5.3.1.9), and malate dehydrogenase (*Mdh-1* and *Mdh-2*; EC 1.1.1.37). Alleles were numbered using the most common allele found in the CLF population as reference (allele 100). In order to avoid miscoring, all samples were run at least twice. Enzyme names and numbers followed the recommendations of the Commission on Biochemical Nomenclature (http://www.chem.qmul.ac.uk/iupac/jcbn/).

*STATISTICAL ANALYSIS*

Population genetic analyses were carried out using GENEPOP (version 3.3; Raymond and Rousset, 1995) and FSTAT software (version 2.9.3.2; Goudet, 2002). Measures used to assess genetic variation were the average number of alleles per locus, the percentage of polymorphic loci (95% criterion) and the comparison between observed ($H_o$) and expected ($H_e$) heterozygosity under Hardy–Weinberg equilibrium. Expected heterozygosity corresponds to Nei's (1973) gene diversity and is calculated using Levene's (1949) correction for small samples. The degree of genetic differentiation among populations was assessed by Wright's *F*-statistics (Wright, 1978) as calculated by Weir and Cockerham (1984). This statistic, using departures from expected levels of heterozygosity under complete panmixia, is based on three indexes: $F_{IS}$ (*f*), an estimate of the deficit of heterozygotes within populations (inbreeding coefficient); $F_{ST}$ ($\theta$), an estimate of the

deficit of heterozygotes among populations (indicating the genetic subdivision among populations); and $F_{IT}$, an estimate of the deficit of heterozygotes in the total population. Evidence for null alleles (i.e., consistent failure of activity at just one of the seven loci for some individuals) was not detected.

We used the approximation $N_em = [(1 / F_{ST})–1] / 4$ to calculate the effective number of migrants per generation, i.e., the gene flow ($N_em$) between pairs of populations (following, for example, Hellberg, 1996; Miller, 1997; Ayre and Hughes, 2000; Goffredo et al., 2004). This inference, based on Wright's "island model" (whose assumptions are that gene flow is bidirectional and at stable equilibrium, that the rate migration greatly exceeds that of mutation, and that the genetic markers employed are selectively neutral; Wright, 1978, and references therein), provides an unbiased estimator of gene flow that is relatively insensitive to moderate levels of selection (Slatkin and Barton, 1989; for a discussion on the caveats of using $F_{ST}$ to infer $N_em$, see Neigel, 2002, and references therein). Genetic differentiation between pairs of populations was expressed by Nei's measure ($D_{ST}'$) of unbiased genetic distances (Nei, 1986). In order to assess the influence of geographic distance on genetic separation in the populations, both the model of Slatkin (1993): $\log_{10}(M) = a + b \log_{10}$ (*geographic distance*), in which $M$ is equal to $N_em$ between pairs of populations, and the model of Rousset (1997) for two-dimensional habitats: $F_{ST} / (1 - F_{ST}) = a + b \ln$ (*geographic distance*) were used. A Mantel test (5,040 permutations) was used to assess the significance of any correlation between genetic distance and geographic distance, using the ZT software (Bonnet and Van de Peer, 2002). The geographic distance between pairs of populations was measured as the lowest nautical distance between localities measured on maps based on a scale from 1:24,000 to 1:1,000,000 (Hellberg, 1996).

Results of the hierarchical survey of spatial models of genetic structure were split between differentiation at large (2–872 km apart) and small (from 5–10 m apart) spatial scales as suggested by previous studies on corals, other invertebrates, and plants (for example: Rodriguez-Lanetty and Hoegh-Guldberg, 2002; Goffredo et al., 2004; Luna et al., 2005; Calderón et al., 2007).

## RESULTS

### *GENETIC DIFFERENTIATION AT LARGE SPATIAL SCALE*

The allelic frequencies at the seven polymorphic loci in seven Mediterranean populations of *L. pruvoti*, ranging from 2 to 872 km apart, showed that the total number of alleles per locus ranged from 15 (*Mdh-2* and *Hk-1*) to 22 (*Me-2*). The frequency of private alleles ranged from 12.5% in *Pgi* to 56.3% in *Me-1*.

The mean number of alleles per locus within each population ranged from 4.29 to 8.86. The percentage of polymorphic loci was 100% in all the populations. Observed heterozygosity was lower than expected heterozygosity under Hardy–Weinberg equilibrium in all populations, ranging from 3.2 times lower in the SCL sample ($H_o = 0.059$, $H_e = 0.187$) to 7.8 times lower at the LDG site ($H_o = 0.027$, $H_e = 0.212$; Table 1). Departure from Hardy–Weinberg equilibrium was enhanced by the genotypic frequencies at

each locus, showing general deficit of heterozygotes; the exceptions were the loci *Me-1* in the populations PTR and CLG, *Me-2* in the population PNT, *Hk-1* in the populations PTR, CLG, and BGN, and *Hk-2* in the populations CLG and PNT. The loci *Pgi*, *Mdh-1*,

Table 1

*Leptopsammia pruvoti*. Genetic variability in the seven Mediterranean populations (population abbreviations as in Fig. 1; Date, of sample collection; *N*, mean number of individuals examined per locus; *A*, mean number of alleles per locus; *P* percent of polymorphic loci in which the most common allelic frequency is <95%; $H_o$ and $H_e$, mean observed and expected heterozygosity, respectively; standard errors are in parentheses

| Population | Date | *N* | *A* | *P* | $H_o$ | $H_e$ |
|---|---|---|---|---|---|---|
| PTR | 17 Feb 2007 | 17.0 | 5.9 | 100 | 0.041 | 0.252 |
| | | (4.0) | (0.9) | | (0.010) | (0.022) |
| CLF | 6 Jan 2006 | 18.6 | 7.3 | 100 | 0.062 | 0.212 |
| | | (2.8) | (1.1) | | (0.009) | (0.018) |
| CLG | 6 Jan 2006 | 8.3 | 4.3 | 100 | 0.067 | 0.321 |
| | | (2.1) | (1.1) | | (0.018) | (0.028) |
| BGN | 23 Apr 2006 | 34.0 | 8.9 | 100 | 0.042 | 0.183 |
| | | (6.7) | (1.3) | | (0.007) | (0.014) |
| SCL | 22 Apr 2006 | 28.1 | 8.7 | 100 | 0.059 | 0.187 |
| | | (4.3) | (1.1) | | (0.008) | (0.017) |
| LDG | 24 Apr 2006 | 33.7 | 7.6 | 100 | 0.027 | 0.212 |
| | | (4.7) | (1.0) | | (0.005) | (0.019) |
| PNT | 2 Aug 2006 | 14.3 | 4.9 | 100 | 0.053 | 0.264 |
| | | (4.9) | (1.2) | | (0.019) | (0.023) |

Table 2

*Leptopsammia pruvoti*. Estimate of deviation from Hardy–Weinberg equilibrium ($F_{IS}$ estimates per population and locus), and *F*-statistics for the seven Mediterranean populations. Statistical significance was determined using Markov chain method (Guo and Thompson, 1992) and permutation tests followed by Bonferroni adjustments. *F*-statistics indicate deviation from Hardy–Weinberg equilibrium within the total population ($F_{IT}$), within populations ($F_{IS}$), and among populations ($F_{ST}$) Population abbreviations as in Fig. 1

| Population | *Me-1* | *Me-2* | *Hk-1* | *Hk-2* | *Pgi* | *Mdh-1* | *Mdh-2* |
|---|---|---|---|---|---|---|---|
| PTR | 0.500 | 0.810*** | 1.000 | 0.737*** | 0.776*** | 0.961*** | 1.000*** |
| CLF | 0.653*** | 0.611*** | 0.750*** | 0.631*** | 0.564*** | 1.000*** | 0.937*** |
| CLG | 1.000 | 0.792* | 1.000 | 0.464 | 0.587*** | 1.000*** | 1.000* |
| BGN | 0.809*** | 0.678*** | 0.706 | 0.676*** | 0.609*** | 0.940*** | 0.977*** |
| SCL | 0.889*** | 0.507*** | 0.426*** | 0.753*** | 0.442*** | 1.000*** | 0.862*** |
| LDG | 1.000*** | 0.654*** | 0.898*** | 0.807*** | 0.833*** | 1.000*** | 0.943*** |
| PNT | – | 0.200 | 1.000*** | 0.643 | 0.966*** | 0.955*** | 1.000*** |
| $F_{IT}(F)$ | 0.851*** | 0.643*** | 0.807*** | 0.723*** | 0.699*** | 0.978*** | 0.955*** |
| $F_{IS}(f)$ | 0.837*** | 0.635*** | 0.797*** | 0.714*** | 0.691*** | 0.976*** | 0.951*** |
| $F_{ST}(\theta)$ | 0.086** | 0.021 | 0.047 | 0.034* | 0.028** | 0.078*** | 0.071*** |

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 3

*Leptopsammia pruvoti*. $F_{ST}$ values (above the diagonal line), mean number of migrants per generation ($N_em$, in parentheses above the diagonal) and Nei's unbiased genetic distance ($D_{ST}'$, Nei, 1986; below the diagonal line) in the seven sampled Mediterranean populations. $F_{ST}$ statistical significance was determined using permutation tests followed by Bonferroni adjustments. Population abbreviations as in Fig. 1

| | PTR | CLF | CLG | BGN | SCL | LDG | PNT |
|---|---|---|---|---|---|---|---|
| PTR | | 0.023 | 0.028 | 0.053 | 0.030 | 0.068** | 0.038 |
| | | (10.595) | (8.706) | (4.466) | (8.160) | (3.439) | (6.403) |
| CLF | 0.021 | | 0.045 | 0.059** | 0.039* | 0.062** | 0.041 |
| | | | (5.275) | (3.956) | (6.177) | (3.775) | (5.815) |
| CLG | 0.023 | 0.039 | | 0.051 | 0.052* | 0.062* | 0.066 |
| | | | | (4.628) | (4.587) | (3.775) | (3.541) |
| BGN | 0.048 | 0.054 | 0.046 | | 0.050** | 0.063** | 0.045* |
| | | | | | (4.719) | (3.637) | (5.245) |
| SCL | 0.026 | 0.034 | 0.046 | 0.045 | | 0.058** | 0.057** |
| | | | | | | (4.055) | (4.160) |
| LDG | 0.061 | 0.055 | 0.057 | 0.056 | 0.051 | | 0.058** |
| | | | | | | | (4.057) |
| PNT | 0.034 | 0.038 | 0.059 | 0.045 | 0.054 | 0.054 | |

*$p < 0.05$, **$p < 0.01$.

and *Mdh-2* showed no exceptions, with significant lack of heterozygotes in all populations (Table 2). The high rate of inbreeding in the populations was shown by the mean value of $F_{IS}$, which was > 0 at all loci (these significant values ranged from 0.635 to 0.976, $p < 0.001$; Table 2). The $F_{IS}$ value over all loci, over all populations was 0.798 ($p < 0.001$).

$F_{ST}$ values departed significantly from zero in five of the seven loci; these ranged from 0.034 for the *Hk-2* locus to 0.086 for the *Me-1* one (Table 2). *Me-2* and *Hk-1* had $F_{ST}$ values not significantly different from zero. The mean $F_{ST}$ value calculated for all seven loci over all populations was 0.052 ($p < 0.001$), indicating genetic differentiation among populations. Analysis of the genetic subdivision between pairs of populations revealed some genetic fragmentation. The fragmentation varied from the case of the PTR population, which was separated only with LDG, to the case of LDG, which had significant $F_{ST}$ values with all populations. (Table 3). Estimated gene flow ($N_em$) was calculated using $F_{ST}$ values and indicated a mean value of 4.558 effective migrants per generation among the seven populations and a range of 3.439–10.595 effective migrants per generation for pairs of populations (Table 3).

Regression of $\log_{10}$ gene flow ($M$, migrants per generation) versus $\log_{10}$ geographic distance of separation (km; Slatkin model), and regression of $F_{ST}$ / (1–$F_{ST}$) against natural logarithm of geographic distances (Rousset's two-dimensional model) for all pairwise combinations of seven populations examined did not indicate a significant correlation between genetic isolation and geographic separation. Nei's unbiased genetic

Table 4

*Leptopsammia pruvoti*. F-statistics, gene flow, and Nei's unbiased genetic distance for patches in the seven Mediterranean populations (population abbreviations as in Fig. 1). *F*-statistics significance was determined using permutation tests followed by Bonferroni adjustments. *F*-statistics indicate deviation from Hardy–Weinberg equilibrium within the total population ($F_{IT}$), within the patches ($F_{IS}$) and among patches ($F_{ST}$). For each population 3 patches were analyzed, ranging from 5 to 10 m apart. $N_em$: average gene flow among patches (number of migrants per generation; in parentheses is the range for the single pairs of patches); $D_{ST}'$: average pairwise (minimum) genetic distance (Nei, 1986) among patches (in parentheses is the range for the single pairs of patches)

| Population | $F_{IT}(F)$ | $F_{IS}(f)$ | $F_{ST}(\theta)$ | $N_em$ | $D_{ST}'$ |
|---|---|---|---|---|---|
| PTR | 0.865** | 0.832** | 0.196** | 1.026 (0.758–5.279) | 0.172 (0.073–0.222) |
| CLF | 0.728** | 0.691** | 0.118** | 1.869 (1.102–4.086) | 0.108 (0.053–0.155) |
| CLG | 0.736** | 0.680** | 0.174 | 1.187 (0.489–1.789) | 0.153 (0.088–0.330) |
| BGN | 0.797** | 0.751** | 0.187** | 1.087 (0.888–1.234) | 0.177 (0.157–0.236) |
| SCL | 0.676** | 0.631** | 0.123** | 1.783 (1.084–3.961) | 0.100 (0.045–0.187) |
| LDG | 0.882** | 0.852** | 0.206** | 0.964 (0.708–1.074) | 0.172 (0.144–0.202) |
| PNT | 0.975** | 0.970** | 0.151** | 1.406 (1.117–1.941) | 0.137 (0.104–0.167) |

**$p < 0.01$.

distance ($D_{ST}'$) between populations pairs varied from 0.021 (the PTR–CLF pair) to 0.061 (the PTR–LDG pair).

*GENETIC DIFFERENTIATION AT SMALL SPATIAL SCALE*

The genetic structure was compared among different patches, ranging from 5 to 10 m apart, within each population (Table 4). Our analysis showed a deficit of heterozygotes at all loci within all patches in each population, with mean $F_{IS}$ values ranging from 0.631 for patches of SCL population to 0.970 for patches of PNT population ($p < 0.01$). For all loci, *F*-statistic analysis yielded $F_{ST}$ values that were significantly different from zero for patches within each population (significant $F_{ST}$ values ranged from 0.118 in CLF population to 0.206 in LDG population; $p < 0.01$), except for the patches of CLG population ($F_{ST} = 0.174$; $p > 0.05$). The $F_{ST}$ analysis indicates general genetic differentiation at small spatial scale. The estimate of gene flow ($N_em$) among patches in the seven populations, based on $F_{ST}$ values, showed average values ranging from 0.964 migrants per generation, among patches of LDG, to 1.869 migrants per generation, among patches of CLF. Within all populations, among pairs of patches, no significant correlation was found between gene flow and spatial distance of separation. Nei's unbiased average genetic distance ($D_{ST}'$) among patches varied from 0.100 in the SCL population to 0.177 in the BGN population (Table 4).

## DISCUSSION

Data on the genetic structure of Mediterranean scleractinians are almost absent in the literature. To the authors' knowledge, only one other study, involving another dendrophylliid scleractinian, the solitary coral *Balanophyllia europaea* (Risso, 1826) exists

(Goffredo et al., 2004). Although we used allozymes as genetic markers, the variability was high compared to other studies on scleractinian corals (see Sherman, 2008). Here, the solitary dendrophylliid coral *L. pruvoti* in the Mediterranean exhibited a complex pattern of genetic structure, both within single populations at a small spatial scale and across populations at a large spatial scale. Overall, significant deviation from Hardy–Weinberg equilibrium was found due to heterozygote deficiencies, and genetic differentiation was found among patches within a single population and across distant populations. These patterns can be a consequence of the reproductive traits of the species, hydrographic patterns, and temporal events.

*HARDY–WEINBERG EQUILIBRIUM*

High positive $F_{IS}$ values (and heterozygosities significantly lower than those predicted at Hardy–Weinberg equilibrium) are commonly found in marine organisms with sedentary adults and larval dispersal (Ayre et al., 1997). Heterozygotes deficits may be caused by unobserved null alleles, natural selection acting on the genetic markers, mating among relatives, or spatial or temporal structure within samples known as the Wahlund effect.

The genotypic frequencies of *L. pruvoti* revealed a marked departure from Hardy–Weinberg equilibrium, with a considerable homozygote excess. $F_{IS}$ was significantly positive, indicating a high rate of inbreeding (at large spatial scale: $F_{IS}$ value over all loci, over all populations = 0.798; at small spatial scale: $F_{IS}$ value over all loci, over all patches = 0.631–0.970 depending on single population). *B. europaea* has $F_{IS}$ values similar to *L. pruvoti* (at large spatial scale: $F_{IS}$ value over all loci, over all populations = 0.820; at small spatial scale: $F_{IS}$ value over all loci, over all patches = 0.808, measured in only one single population; Goffredo et al., 2004). *B. europaea* is a simultaneous hermaphrodite and it is hypothesized that self-fertilization characterizes its reproductive biology (Goffredo et al., 2002), causing the observed marked lack of heterozygotes (Goffredo et al., 2004). In the gonochoric *L. pruvoti*, biparental inbreeding may be a mechanism explaining the observed deficit of heterozygotes (Carlon, 1999). Larvae of this species are brooded, and when released have completed the ontogenesis (i.e., they have differentiated mouth and pharynx, the gastrovascular cavity is divided into compartments by mesenteric septa, and are azooxanthellate; Goffredo et al., 2006, and references therein). Planula brooding and the lack of algal symbionts are traits that may lead to a limited larval dispersal capacity (Harrison and Wallace, 1990; Goffredo and Zaccanti, 2004) and the retention of larvae inside the sea caves and crevices in the proximity of parental polyps increases the likelihood of matings between close relatives. The hypothesis that heterozygote deficiencies are a consequence of localized recruitment (via restricted dispersal of gametes or larvae) and consanguineous mating is formulated for other gonochoric brooding Anthozoa: both the Caribbean *Pseudopterogorgia elisabethae* Bayer, 1961, in open illuminated bottom habitat (Gutiérrez-Rodriguez and Lasker, 2004), and the Mediterranean red *Corallium rubrum* (Linnaeus, 1758), in semi-dark habitats, such as caves and smaller crevices (Costantini et al., 2007). Parent/offspring genetic comparisons are underway to obtain evidence of Mendelian inheritance and assessments of inbreeding levels, to better interpret the observed heterozygotes deficit.

The Wahlund effect may be another explanation for heterozygosity reduction in the populations. In fact, a general subpopulation structure has been detected in *L. pruvoti* at both large and small spatial scales, with $F_{ST}$ values significantly different from zero. Barriers to gene flow followed by genetic drift in the subpopulations, causing genetic subdivision, may derive from the possible limited dispersal capability of the brooded and azooxanthellate planulae larvae of *L. pruvoti*, as discussed above. The Wahlund effect may also result from the admixture of genetically distinct cohorts with different geographical/parental origins. The source population of settling larvae may vary from year to year, depending on oceanographic and environmental conditions. Evidence of genetic variation among settling larvae has been documented in marine invertebrates (Virgilio et al., 2006, and references therein). In those studies, extremely large variation in individual reproductive success resulted in only a small portion of the population contributing to the yearly recruitment. Moreover, spatiotemporal variation in oceanographic conditions may produce limited windows for larval survival, further amplifying the effect of variations in reproductive success. According to this hypothesis, natural populations may show detectable genetic heterogeneity among cohorts, because only a small proportion of the adults contribute to maintaining the population. The existence of inter-cohort genetic variation and its role in determining population genetic structure needs to be verified.

Another common cause of heterozygote deficit is failure (i.e., nonstaining) of certain alleles at a single locus ("null alleles"). As a result, some heterozygotes are genotyped as homozygotes and a few individuals may fail to stain either allele. In the case of null alleles, a heterozygote deficit might not be apparent across all populations because a mutation that causes null alleles generally will only occur in one or a few populations (Selkoe and Toonen, 2006). Even if few individuals at PTR and CLG sites appeared to be expressed at some locus (*Me-1* and *Hk-1*), the substantial and general homozygote excess detected for the analyzed loci across all population of *L. pruvoti* is not likely explained by the presence of null alleles, and also excludes that selection against heterozygotes is the cause of the high rate of inbreeding (Lewontin and Krakauer, 1973; Gutiérrez–Rodriguez and Lasker, 2004; José and Solferini, 2007).

*GENETIC DIFFERENTIATION*

There are now two major alternatives to $F_{ST}$ for the estimation of gene flow (Neigel, 2002). For questions about contemporary gene flow to particular sites, direct estimation based on mark-and-recapture or genetic identification of migrant sources is the preferred alternative. For questions about patterns of gene flow among multiple populations, usually addressed with $F_{ST}$, there is now an alternative approach based on likelihoods calculated by coalescent methods. The new likelihood methods are expected to be generally superior to those based on $F_{ST}$ and could soon replace $F_{ST}$ for estimation of gene flow (Beerli and Felsenstein, 2001). Even with the development of powerful alternatives, the use of $F_{ST}$ as a relative measure of population structure and for comparative estimates of gene flow is still practical. $F_{ST}$ has been the standard measure of population structure for several decades, and is often the only information we have about gene flow from past

studies. $F_{ST}$ remains a useful measure of the average effects of gene flow and continues to be important for comparative purposes (Neigel, 2002).

We found significant genetic differentiation among both the patches (small spatial scale) and populations (large spatial scale) examined. At small spatial scale (5–10 m apart) average gene flow among patches varied from 0.964 migrants per generation within the LDG population ($F_{ST} = 0.206$), to 1.869 migrants per generation within the CLF population ($F_{ST} = 0.118$); at large spatial scale (2–872 km apart) average gene flow among populations was 4.558 migrants per generation ($F_{ST} = 0.052$). These data indicate higher genetic differentiation at local scale compared to large spatial scale. A similar scenario is known for several cases: the black coral *Antipathes fiordensis* Grange, 1990, with negatively buoyant planulae in New Zealand's fiords (Miller, 1997), several species of brooding and spawning corals along the Great Barrier Reef (Ayre and Hughes, 2000), and the broadcast spawning soft coral *Sinularia flexibilis* (Quoy and Gaimard, 1833) (Bastidas et al., 2001). This pattern of genetic differentiation can be the consequence of larval properties and recruitment patterns. Planulae may often settle close to their parents (for example, see the hydrodynamic model of Black et al., 1991), but some proportion may remain competent and planktonic for long periods and may be widely dispersed (Ayre and Hughes, 2000). This kind of larval dispersal is known for the surface brooder gorgonian coral *P. elisabethae* in the Bahamas, in which 14% of the larvae settle in proximity to parental colonies contributing to local mating, and the rest of the larvae flow in the water column and are dispersed (Gutierrez-Rodriguez and Lasker, 2004). The high interconnection (genetic similarity) among distant shallow water populations of *L. pruvoti* may also be maintained by continual seeding or sporadic larval dispersal from deep populations during deep water renewal events (Miller, 1997). This theory assumes that deep water communities comprise a single, genetically uniform population. This seeding from deep water populations may promote panmixis, although analysis of samples from deep water will be necessary to confirm genetic relationships among these populations and their relationship in terms of gene flow with shallow water populations. Oceanographic processes or current patterns in the region may be influencing the genetic patterns observed. In particular the high mean number of migrants per generation detected between the PTR population and other three coastal populations along the west coast of Italy (CLF, CLG, and SCL) may be promoted by the circulation of water masses in the Western Mediterranean Sea. The flow of water from south to north along the western coast of Italy, and the so-called Liguro–Provenco–Catalan Current, which is the "Northern Current" of the Mediterranean basin along the southwestern European coasts, are the dominant circulation features in the region (Robinson et al., 2001).

In *L. pruvoti*, at both smaller (patches within a populations) and rangewide (distant populations) spatial scales, significant relationships between the degree of genetic differentiation among samples and their geographic distance were not found, but the coefficients of determination were much higher for the small (maximum $r^2 = 0.9708$) than for the large (maximum $r^2 = 0.0002$) spatial scale. This gives some evidence for a possible steppingstone gene flow model (Kimura and Weiss, 1964; Slatkin, 1993) at small spatial scale, even if only three patches were analyzed within each location. The absence of any

evidence for isolation-by-distance at large spatial scales may be due to insufficient time to reach equilibrium following historical changes in gene flow or colonization events (Slatkin, 1993). The equilibrium time for $F_{ST}$ (on which $M$, individuals per generation, is based) varies inversely with the migration rate among population, and under a stepping-stone model, migration decreases with distance (Crow and Aoki, 1984). For gene flow between localities, using an estimate of $N_e m = 4.558$ migrants per generation, $N_e$ = 129 individuals (based on a sampled locality area of 129 cm$^2$ and density of 1 individual cm$^{-2}$), a generation time of 7 yr, and Crow and Aoki's approximation [$t_{½} = \ln 2 / (2m + 1/N)$], it would take only 62 yr to go halfway to equilibrium following a change in the amount of gene flow between populations. This relatively rapid period (Hellberg, 1996) does not support the hypothesis of lack of equilibrium between genetic drift and migration at large spatial scale (Hellberg, 1996; Miller, 1997; Bastidas et al., 2001). Long-distance dispersal may be an alternative explanation for the absence of any patterns of isolation-by-distance at large spatial scale. Field experiments on the larval dispersion of *L. pruvoti* were not performed, but larvae released in laboratory rearing have been observed to swim by a ciliary movement for several days (Goffredo et al., 2006, and references therein). Further observations on larval behaviour and metamorphosis are necessary to quantify larval longevity and to infer the potential for long-distance dispersal in *L. pruvoti*. A related sympatric dendrophylliid species, *B. europaea*, has the capability to delay metamorphosis more than 50 d, which is an average delay of larval metamorphosis in scleractinian corals (Goffredo and Zaccanti, 2004; Hizi-Degany et al., 2007). For the dendrophylliid *Balanophyllia elegans* Verrill, 1864, a related species of the western coast of North America, it is suggested that groups of adult polyps attached to small boulders or the shells of abalone might be rafted for long distances (Hellberg, 1996). Rafting has important ecological, biogeographical, and evolutionary implication contributing to population connectivity. In some cases journeys may be very long, depending on currents and wind, contributing to population connectivity over a wide range of geographic scales (Thiel and Haye, 2006). Accumulation of rafts in convergence zones reduces the risk of founder population being too small. This becomes particularly important over long distances where singular founder events could provoke strong reduction of the genetic variability in the founded population. Intermittent rafting routes are found along temperate continental shores, where they are facilitated by giant kelps and seagrasses (Thiel and Haye, 2006).

According to Goffredo et al. (2007) the population density of *L. pruvoti* at 15–17 m depth tends to be homogeneous among sites in the Western Mediterranean Sea. From the data extrapolated from their study the average population density of sexual mature polyps (polyp with length >3 mm, sampled in this study) is 83.1 polyps (SE = 9.3) over 129 cm$^2$ surface area (which is the 43 cm$^2$ × 3 surface area sampled in this study at each site). From 8 to 34 polyps per site were genotyped, which corresponds to 10–41% of the polyps present on the sampled surface area. Differences in the number of assayed individuals may translate into differences regarding reliability of estimates heterozygosity. Even in the case of the CLG population, which is the population with less genotyped individuals, the results on the heterozygosity, genetic variability, and deviation from

Hardy–Weinberg equilibrium are comparable to the ones from the other populations. Preliminary data from microsatellite markers (30 samples per populations, 3 microsatellite loci; S. Goffredo, University of Bologna, unpubl. data) reflect the allozyme genetic patterns found in the other solitary dendrophylliid coral *B. europaea* (Goffredo et al., 2004;) and in *L. pruvoti* (this study), supporting that sample sizes and the allozyme markers used in these studies to describe population genetic structures revealed true genetic architecture rather than artifacts due to sampling or marker errors.

The genetic structure of *L. pruvoti* was characterized by: (1) marked departure from Hardy–Weinberg equilibrium, with a considerable deficit of heterozygotes and a high rate of inbreeding; (2) low levels of genetic differentiation and high levels of gene flow among widely separated populations, and relatively limited gene flow and high genetic fragmentations at smaller spatial scales; (3) no significant pattern of isolation by distance. This study represents a contribution to the field of marine connectivity, and provides information on patterns of genetic connectivity for an underrepresented geographic region.

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