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Scaling of processes shaping the clonal dynamics and genetic mosaic of seagrasses through temporal genetic monitoring

Becheler Ronan [1], Benkara Elsa [1], Moalic Yann [1], Hily Christian [2], Arnaud-Haond Sophie [1,*]

[1] IFREMER, DEEP, Ctr Brest, F-29280 Plouzane, France.
[2] IUEM, Lab Sci Environm Marin, Plouzane, France.

* Corresponding author Sophie Arnaud-Haond, email address : Sophie.Arnaud@ifremer.fr

Abstract :

Theoretically, the dynamics of clonal and genetic diversities of clonal plant populations are strongly influenced by the competition among clones and rate of seedling recruitment, but little empirical assessment has been made of such dynamics through temporal genetic surveys. We aimed to quantify 3 years of evolution in the clonal and genetic composition of Zostera marina meadows, comparing parameters describing clonal architecture and genetic diversity at nine microsatellite markers. Variations in clonal structure revealed a decrease in the evenness of ramet distribution among genets. This illustrates the increasing dominance of some clonal lineages (multilocus lineages, MLLs) in populations. Despite the persistence of these MLLs over time, genetic differentiation was much stronger in time than in space, at the local scale. Contrastingly with the short-term evolution of clonal architecture, the patterns of genetic structure and genetic diversity sensu stricto (that is, heterozygosity and allelic richness) were stable in time. These results suggest the coexistence of (i) a fine grained (at the scale of a 20 x 30 m quadrat) stable core of persistent genets originating from an initial seedling recruitment and developing spatial dominance through clonal elongation; and (ii) a local (at the scale of the meadow) pool of transient genets subjected to annual turnover. This simultaneous occurrence of initial and repeated recruitment strategies highlights the different spatial scales at which distinct evolutionary drivers and mating systems (clonal competition, clonal growth, propagule dispersal and so on) operate to shape the dynamics of populations and the evolution of polymorphism in space and time.

Keywords : clonality, seagrass, spatio-temporal genetic structure, Zostera marina

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Introduction

Clonality is a life history trait widely distributed among taxa and habitats, particularly in photosynthetic organisms. Partially clonal organisms are characterized by a mixed system allowing the combination of two reproductive strategies: the production of new genetically identical modules through vegetative growth or fragmentation, and the production of new genetic individuals through sexual recombination. As a consequence, their population dynamics and evolutionary trajectories are profoundly affected by their rate and mode of clonal reproduction. Populations of clonal plants are composed of genetic individuals, or genets occupying space and dispersing locally through the production of modular shoots, or ramets (Harper, 1977). As genets are able to persist through time and space, the composition and evolution of populations of clonal plants is largely affected by the level of intraspecific competition (Eriksson, 1989; Eriksson, 1993; Pan and Price, 2001; Travis and Hester, 2005).

Depending on the turnover of genets and intensity of inter-genet competition for space, two extreme recruitment strategies have been defined (Eriksson, 1993): (i) the “Initial Seedling Recruitment” (ISR) strategy, characterizing populations originating from a single event of colonization from one pool of seeds, followed by occupation of space, mostly through vegetative spread; and (ii) the “Repeated Seedling Recruitment” (RSR) strategy, describing the continuous input of new genets.

In stable environments, ISR is expected to be the predominant strategy. Density-regulated populations are shaped by increasing competition among genets through time, the more ecologically competent excluding the less fit (Eriksson, 1989; Eriksson, 1993). This competitive exclusion among genets may theoretically result in a decrease of clonal richness through time, and is considered to be a major driver of the temporal evolution of clonal diversity (Eriksson, 1993; Soane and Watkinson, 1979; Watkinson and Powell, 1993). Such a strategy is similar to the mechanisms suggested by species coexistence models (Huston, 1979), and was proposed to describe the dynamics of meadows of the seagrass *Posidonia oceanica* (Arnaud-Haond *et al*, 2010).

69 In contrast, a strategy closer to RSR is expected in areas which undergo frequent disturbances
70 (Eriksson, 1993), where ecological events locally remove individuals and free microsites for new
71 recruits. Populations of the seagrass *Cymodocea nodosa* have been proposed to persist due to an
72 RSR strategy (Ruggiero *et al*, 2005).

73 These strategies are two theoretical extremes, observed in nature in proportions depending mostly
74 on the species studied and on the rate and intensity of environmental perturbations and
75 demographic variations (Douhovnikoff *et al*, 2005; Pluess and Stocklin, 2004; Watkinson and Powell,
76 1993). Their relative importance has strong implications for the evolution of populations and species,
77 as spatio-temporal recruitment strategy affects both the preferential mechanism of space occupation
78 and migration (clonal spread *versus* fragment or seed dispersal), and the predominant entity that
79 persists will evolve under natural selection (the alleles or the clonal lineages, see Ayala, 1998). The
80 pattern of spatial distribution of genetic and clonal diversities and their respective evolution in time
81 will therefore be highly dependent on the balance between these two strategies. Numerous spatial
82 studies have been carried out for diverse species, reporting highly variable levels of clonal richness
83 that suggest a differential intensity between ISR and RSR strategies (Alberto *et al*, 2008; Coyer *et al*,
84 2004; Diaz-Almela *et al*, 2007; Escaravage *et al*, 1998; Olsen *et al*, 2004; Pluess and Stocklin, 2004).
85 The extreme and most demonstrative cases of ISR dominance are the monoclonal meadows of some
86 seagrass species including *Zostera marina* (Olsen *et al*, 2004; Reusch *et al*, 1999), *Cymodocea nodosa*
87 (Alberto *et al*, 2008), or *Posidonia oceanica* (Arnaud-Haond *et al*, 2012; Arnaud-Haond *et al*, 2007b).
88 Meadows of the seagrass *Z. marina* surveyed simultaneously for their demographic evolution and
89 genetic composition in Brittany were shown to be fluctuating mosaics of genetically-differentiated
90 patches with variable levels of genotypic and genetic diversity (Becheler *et al*, 2010). The clonal and
91 genetic composition of meadows suggested both ISR and RSR strategies may apply in variable
92 proportions as a function of the time elapsed since the last colonization and of the frequency and
93 extent of disturbances (Becheler *et al*, 2010). However, the thorough appraisal of the dynamics of
94 these genetic mosaics and of the recruitment strategies operating at different spatial and temporal

95 scales in natural meadows cannot be inferred from a single instantaneous snapshot. Such an
96 approach requires the assessment of the temporal evolution of clonal and genetic composition of
97 patches, an approach that has rarely been used on clonal organisms (but see Cronberg, 2002;
98 Hossaert-McKey *et al*, 1996; Travis and Hester, 2005).
99
100 Here, we aimed to provide an estimate of short-term temporal variation in clonal and genetic
101 composition at local and regional scales, in relation to the demographic changes recorded,
102 particularly the time elapsed since the last recorded recolonization or major disturbance event. The
103 seven meadows of *Zostera marina* studied in 2009 were sampled again in 2012, following the same
104 sampling strategy, and each ramet was genotyped using the same nine microsatellite markers. Our
105 objective was to compare the spatial and temporal scales of evolution in clonal diversity and spread
106 *versus* genetic diversity in order to infer the balance among recruitment strategies in natural
107 meadows.
108
109

110 **Material and methods**

111

112 *Sample collection and field observations*

113

114 Following the same sampling scheme and strategy as in Becheler *et al.* (2010), sampling units of
115 *Zostera marina* were collected in Spring 2009 and Spring 2012 from seven locations in Brittany,
116 France (Supplementary Figure S1) stretching from Saint-Malo to Arradon. Distances between
117 meadows ranged from 33 km (Molène–Roscanvel) to 442 km (Arradon–Saint-Malo). At each
118 location, two 20 * 30 m quadrats separated by several tens of meters were chosen, located in
119 continuous parts of the meadow being monitored by the REBENT survey (www.rebent.org), a
120 national network of survey for major coastal ecosystems. Sampling units, corresponding to one
121 ramet, were collected at thirty-five random coordinates within each quadrat, and their set of internal
122 leaves were stored in silica crystals until DNA extraction. In Arcouest and Molène, due to patchiness
123 of the meadow, the sampling area was not strictly 30*20 m^2 because the total area covered by the
124 patches had narrowed to less than this size. Deviation from random coordinate occurred when a gap
125 was found on the field at the pre-identified coordinate (therefore corrected to the one of the closest
126 existing ramet). The quadrat 1 of Arcouest was haphazardly sampled due to harsh meteorological
127 conditions. In general, as only approximate GPS positions were available together with visual cues,
128 the quadrats for 2012 were placed as close as possible to the quadrat positions in 2009 but might
129 have slightly shifted depending on the extent of the meadow, and the moving of sediments. The
130 estimated discrepancy between new and initial positions of quadrats was nevertheless estimated to
131 be relatively weak and unlikely to exceed 10 meters except possibly at the Arcouest site, where
132 coastal landscape had significantly changed.

133

134

135 *Microsatellite amplification and genotyping*

136

137 Total DNA was extracted using the classic CTAB method (Doyle and Doyle, 1988). Using identical
138 PCR conditions to Becheler *et al.* (2010), the nine microsatellite loci used in 2009 were amplified in
139 the present study. PCR-products were visualized using the same ABI-3100 FNVR automated
140 sequencer (Applied Biosystems) and scored using the microsatellite plugin of Geneious v5.6.4
141 (Biomatters®). Double blind reading was used to minimize the occurrence of scoring errors and verify
142 interpretation of the peaks.
143 To standardize the samples to the same number of sampling units (n = 30) before analyses,
144 incomplete genotypes were removed, and excess genotypes were randomly removed.

145

146 *Genetic and clonal data analysis*

147
148 The first step of the genetic analyses was the clonal discrimination, based on the probability that
149 identical multilocus genotype (MLG) arise from distinct events of sexual reproduction, as described in
150 (Arnaud-Haond *et al*, 2007a). When $P_{sex(FIS)}$ (estimated taking departure from Hardy-Weinberg into
151 account) falls below a threshold value fixed at 0.01, the two identical MLGs are considered as
152 belonging to the same clonal lineage. The dataset was also screened for the possible occurrence of
153 scoring errors and somatic mutations, in order to avoid overestimates of clonal diversity. Multilocus
154 lineages (MLL) were defined clustering slightly different MLG likely belonging to the same genets, as
155 detailed in Arnaud-Haond *et al.* (2007a) and in the Supplementary Information.

156 Clonal diversity was estimated with three parameters, previously used in (Becheler *et al*, 2010) and
157 recommended in (Arnaud-Haond *et al*, 2007a), as this set fully describes richness and diversity of the
158 clonal composition. R is the clonal richness, estimated as the ratio of the number of discriminated
159 genets within the sampling set with the number of sampling units (Dorken and Eckert, 2001). The
160 slope (β) of the Pareto distribution, describing the distribution of ramets within genets (see Arnaud-
161 Haond *et al*, 2007a for details), and sensitive to the presence or absence of dominant clonal lineages

162 is also provided, as well as the Simpson's Index (Pielou, 1969), an index of diversity sensitive to
163 dominant entities. Clonal discrimination and estimations of the parameters of clonal diversity and
164 structure were performed with Genclone 2.1 (Arnaud-Haond and Belkhir, 2007).

165 A single copy of each discriminated MLL was retained in the dataset used to assess genetic diversity
166 and structure.

167 Genetic diversity within quadrats was estimated as the mean number of alleles per locus (Â), with
168 observed (H_O) and unbiased (H_E) multilocus heterozygosity (Nei, 1978). A permutation procedure
169 (1000 permutations) was used to test whether a particular estimate of the overall inbreeding
170 coefficient (F_{IS}) was significantly different from 0 ($P < 0.01$). Genetic structure among samples was
171 estimated with θ (Weir and Cockerham, 1984) for 1000 permutations. This Fst-estimator was used to
172 assess the spatial genetic structure among locations and between quadrats from a single location. In
173 addition, a Mantel test including geographical distances among quadrats was carried out to test for
174 the 2D-Isolation-By-Distance model, crossing the logarithm of the geographical distance (in
175 kilometers) between quadrats and a derived index of genetic differentiation [$F_{st}/(1-F_{st})$], as
176 recommended by Rousset (1997). For each sampling quadrat, this same estimator of F_{st} was also
177 used on datasets gathered in 2009 (Becheler *et al*, 2010) and 2012 (the present study), to assess the
178 temporal genetic structure, between identical sampling quadrats.

179

180 Autocorrelation analyses were also performed to estimate the evolution of the pattern of clonal
181 extension and spatial genetic structure at the within-quadrat level. We used the kinship estimator
182 coefficient of Ritland (F_{ij}) as a genetic relatedness statistic (Ritland, 1996). We performed regression
183 analyses of mean F_{ij} against the Log_e of mean geographic distance, within each distance class. The
184 autocorrelation analyses were performed using F_{ij}: (i) first including all sampled ramets, and (ii) using
185 permutations (1000) in order to include only one ramet (and one of the possible corresponding
186 coordinates, randomly chosen for each permutation step) from each genet at each permutation, in
187 order to examine the dispersion through sexual propagules. The slopes of regressions (b) allowed us

188 to calculate the S_p-statistic (Vekemans and Hardy, 2004). Autocorrelation parameter estimations
189 were performed with GENCLONE 2.1 (Arnaud-Haond and Belkhir, 2007).
190 The clonal subrange CR was estimated, which corresponds to the maximum distance between two
191 identical MLGs belonging to the same clone, in meters, and is determined as the distance for which
192 the probability of clonal identity becomes null (Alberto *et al*, 2005; Harada *et al*, 1997). It therefore
193 provides an estimate of the minimal spatial extent of the largest observed clone in each quadrat.
194 The total number of genotypes occurring in the sampling quadrats in 2009 was approximated as
195 follows, with R the clonal richness (assessed from analyses) and N_{tot} the total numbers of ramets
196 within a quadrat (assessed using density counts in three subplots of 0.10 m^2 in the quadrat to
197 estimate overall density across the entire surface of 30 * 20 m quadrats, see Arnaud-Haond *et al.*
198 (2012) for a similar approach):
199 $G_{tot} = R * N_{tot}$
200 This allows an estimate of the expected percentage of each present MLG being sampled $\%G_{sampled}$,
201 corresponding to the sampling density at the genotypic level:
202 $\%G_{sampled} = (G / G_{tot}) * 100$
203 G being the number of MLGs detected in our sampling set of 30 ramets. This percentage estimate
204 was used to appraise the likelihood of sampling the same MLG twice (both in 2009 and 2012). If R
205 provides a reliable estimate of clonal richness, such percentage is extremely low, due to the very low
206 sampling density. Despite such low percentage, the repeated observation of persistent MLG sampled
207 both in 2009 and 2012, suggests an overestimation of clonal richness through R estimates. This
208 would imply the occurrence of a large dominance of space occupation by few clonal lineages, as
209 showed in both seagrass species *Posidonia oceanica* and *Cymodocea nodosa*, and confirm the
210 consistent overestimation of clonal richness estimates based on extremely low sampling densities as
211 usually performed in clonal plants (Arnaud-Haond *et al*, 2007a).
212
213 *Network analysis*

214

215 Network analysis was performed on the basis of the totality of genets in the dataset (n = 289) to
216 compare the shape and properties of the networks in 2009 (Becheler *et al*, 2010) and 2012 (the
217 present work), on both local and regional scales. Nodes in the network represent genets, while links
218 represent the genetic distance between two genets.
219 The genetic distance used is the "Shared Allele Distance" (Chakraborty and Jin, 1993), based on the
220 proportion of shared alleles between two individuals. It is estimated by:
221

222 $$P_{SA} = \frac{1}{2n_u} \sum_u S,$$

223

224 where the number of shared alleles S is summed over all loci u, and n_u is the number of loci.

225 Distance between individuals D_{SA}, ranges from 0 to 1:

$$D_{SA} = 1 - P_{SA}$$

226 A "fully connected" network was built, including all links among all genets, and then scanned to the
227 percolation threshold (Stauffer & Aharony 1994) as previously done for this type of data (Becheler *et*
228 *al*, 2010; Moalic *et al*, 2011; Rozenfeld *et al*, 2007). This method aims to analyze network topology at
229 the minimal genetic distance, allowing gene flow to spread throughout a giant network. Under the
230 effective percolation distance, a giant network collapses into smaller isolated clusters. Occasionally,
231 when the system is not fully hierarchically structured, several nodes will prematurely disconnect and
232 stand-alone outside of the giant cluster. This happens when genets are particularly genetically
233 distinct (and therefore distant) from all other genets of the system. Only when a secondary cluster
234 emerges, made of several nodes (genets), is the effective percolation threshold reached. The
235 percolation threshold reveals the first significant level of limitations to gene flow within the system.
236 Only links just above the effective percolation distance were used to analyze the topology and

237 features of the network. The clustering coefficient Ci of genet i is the ratio between the number of

238 existing links to the maximal number of potential links within the cluster. The clustering of genets

239 reveals the existence of substructures, grouping the closest genets. It is defined as:

$$C_i = \frac{E_i}{{E_i}^{(max)}} = \frac{2E_i}{k_i(k_i - 1)}$$

240 where E_i is the number of links existing among the neighbors of a given genet i, and the degree k_i of

241 genet i is the number of other genets linked to it. The clustering coefficient of the whole network

242 <CC> is defined as the average of all individual clustering coefficients in the system. Topologies and

243 features of the current network were also compared with the network obtained in 2009. In order to

244 visualize finer structures, the same methodology was performed to build a network for each location.

245 **Results**

246

247 *Short timescale variation of clonal diversity and architecture*

248

249 All replicates of identical MLG showed a significant p_{sex} ($p<0.01$), supporting the hypothesis they
250 belonged to the same genet and were therefore issued of a single event of sexual reproduction. In a
251 single instance two slightly different MLG were found to be likely derived from a single event of
252 reproduction and differed at only one allele as a product either of somatic or of *in vitro* mutation,
253 both MLG were merged into a single multilocus lineage (MLL; Arnaud-Haond *et al*, 2007a) and
254 considered as a single genet for further analysis.

255 Clonal diversity is variable in time, depending on quadrat more than site (Table 1; Figure 1). In
256 2009, the mean clonal richness R was 0.76 with a variance of 0.03, ranging from 0.48 to 1. In 2012,
257 the mean clonal richness had remained rather steady (0.71), with lower variance (0.01). The least and
258 most diverse quadrats remained the same, respectively Molène (R_{2009} = 0.48; R_{2012} = 0.51) and the
259 quadrat 1 of Roscanvel (R_{2009} = 1; R_{2012} = 0.89). On the overall dataset, the mean clonal richness of
260 2009 and 2012 are not significantly different (Wilcoxon test: W = 102; p = 0.38).

261 The Simpson Index values, D*, were very stable between 2009 and 2012 (Table 1), and no
262 significant evolution was observed between 2009 and 2012 (Wilcoxon test: W = 101.5; p = 0.39).
263 Contrastingly, the β-parameter of the Pareto distribution was the most variable of the descriptive
264 parameters estimated (Table 1; Figure 1), and its quasi unidirectional evolution showed a consistent
265 decrease of between 31% and 93% (except in Arcouest, quadrat 1, where there was an increase of
266 38%). The percentages of variation of β and the clonal subrange CR are highly correlated (R^2 = 0.77; p
267 < 0.01; negative slope), indicating that an increase of the size of genets (CR) is related to reduced
268 evenness in the distribution of ramets among genets (reflected by the β-parameter of Pareto). On the
269 overall dataset, the mean values of the β-parameter of 2009 and 2012 were significantly different
270 (Wilcoxon test: W = 139; p = 0.006), indicating a consistent increase in clonal dominance.

271 In five quadrats of the 13 sampled, despite the possible shift in coordinates between dates and
272 the low sampling densities, common MLGs were found in 2009 and 2012, with all P_{sex} values being
273 <0.001. One to six shared clonal lineages were found in five quadrats (Table 1). Assuming R provided
274 a reliable estimate of genotypic richness (but see Arnaud-Haond *et al*, 2007a), the total number of
275 genotypes expected to occur in each quadrat was estimated to be between 45900 in Molène and
276 344172 in the quadrat 1 of Saint-Malo, resulting in a sampling coverage of 0.01% to 0.04% genotypes
277 (Supplementary Table S1).
278 For most of the locations, the shoot density was found to be relatively stable between 2009 and
279 2012 (Supplementary Table S2). It should be noted that a full colonization event had happened
280 between 2008 and 2009 in quadrat 2 of Sainte-Marguerite, offering the opportunity to follow the first
281 year of clonal structure evolution, which could have affected the fluctuation in density.
282
283
284 *Temporal variation of genetic composition of quadrats*
285
286 Genetic diversity descriptors showed more stable patterns through time than clonal diversity
287 ones (Table 1; Figure 1). The mean heterozygosity was steady, with 0.46 in 2009 to 0.45 in 2012,
288 associated with a constant variance (0.004 in 2009 and 2012). No significant differences in
289 heterozygosity were found between 2009 and 2012 (Wilcoxon test: W = 98; p = 0.50). Similarly,
290 values of allelic richness of 2012 are not significantly different from values of 2009 (Wilcoxon test: W=
291 104.5; p = 0.32).
292
293 *Spatio-temporal genetic structure and differentiation, among and within populations*
294
295 Results in 2012 were similar to those observed in 2009 at local and regional scales. Spatial genetic
296 structure was observed at all scales (Supplementary Table S3), all F_{st}-values being significantly

297 different from 0 -except between the two sampling quadrats of Arradon. This reveals a strong genetic
298 differentiation on different spatial scales: from the regional scale, where F_{st}-values vary between 0.01
299 and 0.35, to the local scale, where the differentiation between the two quadrats produces F_{st}-values
300 varying from 0 to 0.05. Temporal F_{st}-values were all significant and were systematically higher than
301 inter-quadrat F_{st}-values (Table 2), ranging from 0.04 to 0.18.
302 A Mantel-test carried out among all pairs of quadrats was significant ($r^2 = 0.06$; $p < 0.05$), presenting a
303 pattern very similar to the one observed in 2009. Yet, as explained in Becheler *et al.* (2010), this
304 cannot indicate a strict IBD pattern, since the hierarchical sampling, resulting in two clouds of dots,
305 drives this apparent tendency (Supplementary Figure S2). No such correlation was observed within
306 each of the clouds corresponding to the pairs of quadrats from the same location ($r^2 = 0.03$; $p = 0.73$)
307 or to pairs of quadrats among locations ($r^2 = 0.001$; $p = 0.71$).
308
309
310 *Network analysis*
311
312 The global network topology built on the base of genets in 2012 (Figure 2) was highly similar to the
313 one obtained in 2009, with identical percolation threshold (Dpe = 0.45). We observe the same
314 organization with three central locations (Callot, Arcouest and Saint-Malo) and four peripheral ones
315 (Arradon, Roscanvel, Molène and Sainte-Marguerite). The clustering coefficient <CC> was slightly
316 higher than that recorded in 2009 (0.37 *vs*. 0.30), suggesting an increase of the hierarchical
317 differentiation among clusters (mainly corresponding to sets of samples within each location).
318 In order to see whether this result was due to an increase in differentiation at the regional or local
319 scale, networks were also constructed at the levels of localities. This analysis also revealed an
320 increase of clustering within every locality except for Saint-Malo (0.43 in 2012 *vs.* 0.52 in 2009). Slight
321 increases of a few percent were observed in Arradon and Roscanvel (+1% and +2%, respectively) and
322 strong increases were observed in Molène and Callot (+34% for both), Sainte-Marguerite (+53%), and

323 Arcouest (+47%). All values of <CC> are given in the Supplementary Table S4. Additionally, no clusters
324 of MLG corresponding to quadrats delineation are observed in 2012, in contrast to what was
325 observed for most of the locations in 2009.
326
327
328

329 **Discussion**

330 The snapshot of spatial genetic structure previously recorded at different scales suggested the
331 existence of temporally fluctuating mosaics of genets shaping patches within meadows (Becheler *et*
332 *al*, 2010). This temporal survey aims to confirm this hypothesis. The comparison of clonal
333 architecture and genetic composition between 2009 and 2012 provides insight into the possible dual
334 dynamics of the meadows. First, an increasing dominance of large genets was observed, indicating
335 the existence of a core of stable genets. This contrasts with the strong temporal genetic structure
336 between the 3 years of monitoring, suggesting the concomitant occurrence of a turnover of genets.
337 In the light of the recent demographic history of the quadrats, we propose a scenario explaining the
338 evolution observed.
339
340
341 1. *Prevalence of the Initial Seedling Recruitment strategy: toward the emergence of large clones*
342
343 Evenness of clonal distribution decreased in the large majority of quadrats (Table 1), while the clonal
344 subrange showed an overall increase. These points highlight an increased dominance of large MLLs.
345 The large MLLs varied in size, reaching at least 19 meters in two quadrats (Table 1), and revealed the
346 persistence of a stable core within the seagrass meadows, implanted for several years or decades, as
347 horizontal rhizome elongation rate for *Zostera marina* is estimated between 22 and 31 $cm.year^{-1}$
348 (Marba and Duarte, 1998). In addition, genets that persisted in time were revealed by the resampling
349 of identical genets across a three year period. Considering the low density of sampling and the low
350 percentage of genotypes covered by our analysis, repeated sampling of some genets in time implies
351 a rather strong pattern of clonal dominance. This dynamic of persistence and increased dominance of
352 clones competing for space is exemplified by the analysis of quadrats showing stable density after a
353 recent colonization, for example in Sainte-Marguerite (Q2) where a decrease in clonal richness and
354 evenness of respectively 15% and 49%, was accompanied by an almost doubled clonal subrange. A

355 very similar scenario occurred in the 2 quadrats of Callot, where a large increase of density occurred
356 in 2008 (Supplementary Table S2), constituting a potential high input of new genets and leading to an
357 increase of clonal richness for 2009. These elements highlight the prevalence of an initial seedling
358 recruitment strategy (Eriksson, 1993).

359

360 A similar study was performed on a meadow of the terrestrial clonal plant *Lathyrus sylvestris*,
361 studied in two successive years (Hossaert-McKey *et al*, 1996), where a similar pattern of clonal
362 dominance fitted with a predominantly ISR strategy (Figure 3a). In contrast to the present work, a
363 large spatial but low temporal genetic structure was observed and interpreted as the result of
364 successive colonizations at the edge of the population having formed mosaics of genets (i) colonizing
365 space through vegetative elongation and (ii) producing seedling through reproduction among flowers
366 of the same genet or its relatives, recruiting closed to the maternal plants (Hossaert-McKey, 1988;
367 Hossaert-McKey and Jarry, 1992; Hossaert-McKey *et al*, 1996).
368 This pattern bears some similarity to the evolution through time of stable cores of large genets
369 reported here, revealing a strategy of ISR and trimming of genets through the differential capacity of
370 spatial spread and possible intraspecific competition in *Z. marina* meadows. Under relatively stable
371 demographic conditions, differential clonal spread would therefore play a major role in shaping the
372 clonal composition and differentiation of quadrats separated by only several tens of meters, as
373 previously suggested by Becheler *et al.* (2010). The temporal component is however strikingly
374 different in these two studies: whereas in case of *Lathyrus sylvestris*, the temporal differentiation
375 was less marked than the spatial one, results show the opposite in *Z. marina* meadows. Such a
376 contrasted pattern may be attributed to the contrasted mechanisms and dynamics of dispersal in the
377 sea compared with on land. This leads us to propose a slightly different scenario (Figure 3b) to
378 explain our findings, taking into account the large dispersal potential of seagrasses through drifting
379 shoots or seeds in the marine environment (Harwell and Orth, 2002; Kendrick *et al*, 2012).

380

381

382 *2. A cloud of transient genets revealed by the temporal aspect of genetic patchiness*

383

384 Reported in various marine taxa (Arnaud-Haond *et al*, 2008; Hedgecock and Pudovkin, 2011; Johnson
385 and Black, 1982; Jones *et al*, 1999; Selkoe *et al*, 2010), genetic patchiness is a paradoxical
386 combination of high dispersal potential and strong genetic structure at local scales, characterized by
387 three main features : (i) a fine-grained genetic structure comparable to or apparently exceeding that
388 observed at a large scale; (ii) the fuzziness of population contours; and (iii) rapid temporal variations.
389 The two first criteria were already met in for *Zostera marina* meadows in Brittany (Becheler *et al*,
390 2010), as well as in San Francisco Bay (Ort *et al*, 2012), underlining the importance of clonality in
391 favoring fine-grained genetic structure and spatial patchiness in organisms with mixed mating
392 systems. Here, the temporal genetic structure exceeding the spatial one (Table 2) underlines a rather
393 fast modification of genetic composition of patches, in line with the third characteristic describing
394 genetic patchiness . The strong temporal F_{st} observed in this study emphasize the equal importance
395 of dispersal and settlement of 'non-dominant clones' and lead us to hypothesize that a second
396 compartment of genets could exist, subject to turnover within short time periods and generating
397 significant variation in the genetic composition of quadrats through time (temporal F_{st}, table 2).
398 Independent of longer-term meadow stability, the dynamics of *Z. marina* meadows are
399 punctuated by annual variations in density, with a rather synchronized peak of recruitment of new
400 ramets in early spring, filling gaps left by an annual winter drop in density (Marba *et al*, 1996). Spring
401 increase in density can be caused by the local clonal spread of genets that have persisted through
402 winter, or new waves of recruits through ramet and seedling dispersal/settlement over broader
403 spatial scales. While the existence of persistent and widely-spread genets reported here supports the
404 first of these possible explanations, the temporal pattern of differentiation also suggests the co-
405 occurrence of the latter. An annual cloud of recruits may disperse in synchronized waves settling
406 over spatial scales encompassing both quadrats, tending to bring quadrats genetically closer. This

407 likely annual turnover of transients genets illustrates the occurrence of a parallel repeated seedling
408 recruitment strategy (Eriksson, 1993) in *Z. marina* meadows. Following a typical pattern of genetic
409 patchiness, the clouds of recruits may originate from a limited pool of plants or patches in the
410 meadow and differ among years, therefore explaining the large temporal differentiation observed
411 among quadrats. A recent work (Broquet *et al*, 2012) analytically demonstrates that "chaotic
412 patchiness can be produced by neutral demographic processes alone", including collective dispersal
413 (Selkoe *et al*, 2006) and genetic drift. This is well illustrated by the successive recruitment events
414 proposed to explain the empirical results reported here.
415
416 *Conclusion*
417
418 This study provides the first short-term temporal assessment of the dynamics of clonal and genetic
419 composition of seagrass meadows at fine-grained, local and regional scales. Results revealed a typical
420 pattern of genetic patchiness, with small scale genetic structure despite large-scale dispersal
421 potential, and high temporal differentiation. Some mechanisms underlying this observation appear
422 slightly different from those of other marine species, however, as clonal growth and differential
423 fitness of genets appear, as for terrestrial clonal plants, to be the root of the fine-grained
424 differentiation observed between quadrats within meadows. At fine grained scale, results support an
425 Initial Seedling Recruitment Strategy and the temporal persistence of part of the genetic pool. We
426 therefore propose a scenario involving two compartments of genets to explain the results reported
427 here (Figure 3b). The first compartment is a core of persistent and growing genets (ISR) contributing
428 to the spatial differentiation within meadows, which may either result from a different time or source
429 of colonization or from genetic divergence through differential exclusion of genets in the distinct
430 quadrats. The second compartment is an annual cloud of transient genets, mostly alternating on an
431 annual basis (RSR), although data reported here does not exclude the possibility that some of those
432 genets would occasionally succeed on the longer term and finally contribute to the persistent core.

433 These scenarios imply a central role of the frequency of perturbations and clonal life history traits, in
434 particular spatial spread and selection acting on clonal lineages, in the evolution of the clonal and
435 genetic composition of meadows. These observations confirm the ecological concept of population
436 based on distribution continuity as best adapted to clonal plants (Becheler et al., 2010), but suggest
437 the possible existence of a spatial scale larger than sampling sites and within which allelic frequencies
438 may remain stable in time. These results underline the importance of addressing distinct interlocked
439 spatial and temporal scales simultaneously in order to gain understanding of the dynamics of a
440 population system as a whole. This is particularly important in those seagrass meadows apparently
441 following permanent non-equilibrium dynamics at a local scale, possibly combined with long-term
442 stability at a global scale.
443

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451

452 Supplementary information is available at the Heredity website

453

454 Data archiving

455 Genotype data have been submitted to Dryad: doi to be added on acceptance

456

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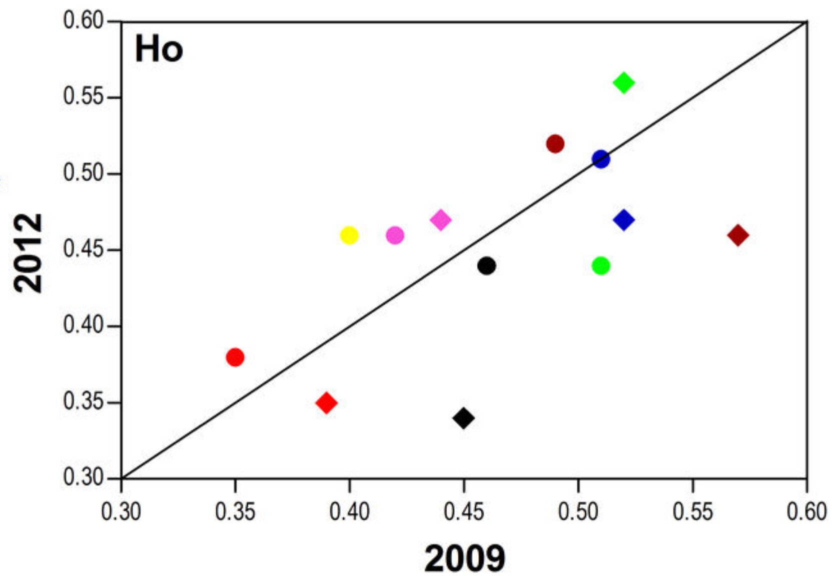
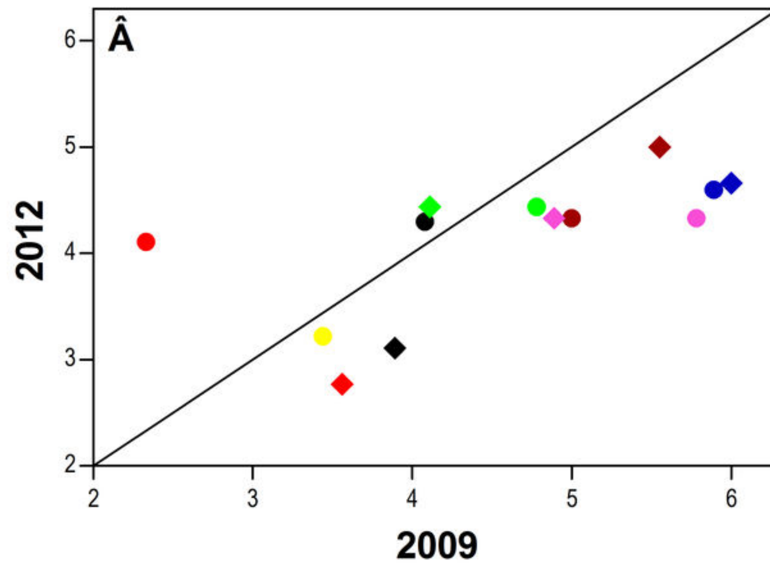
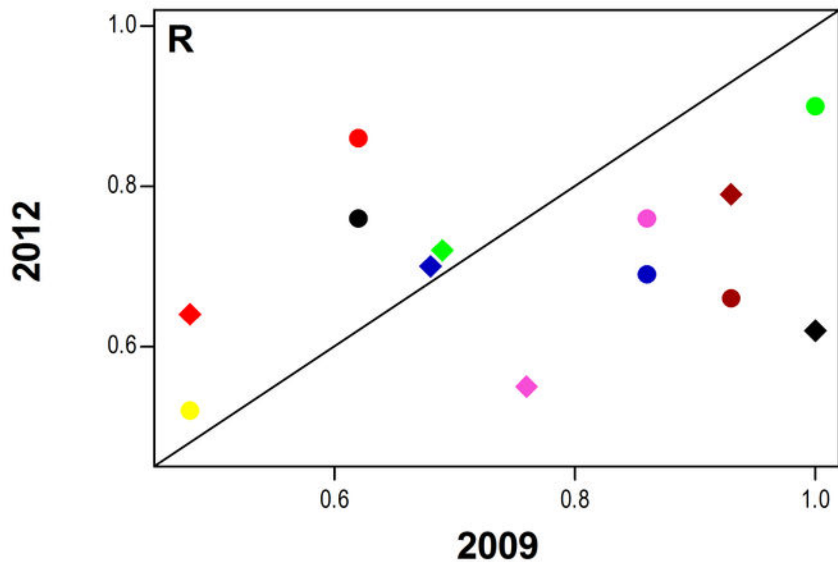
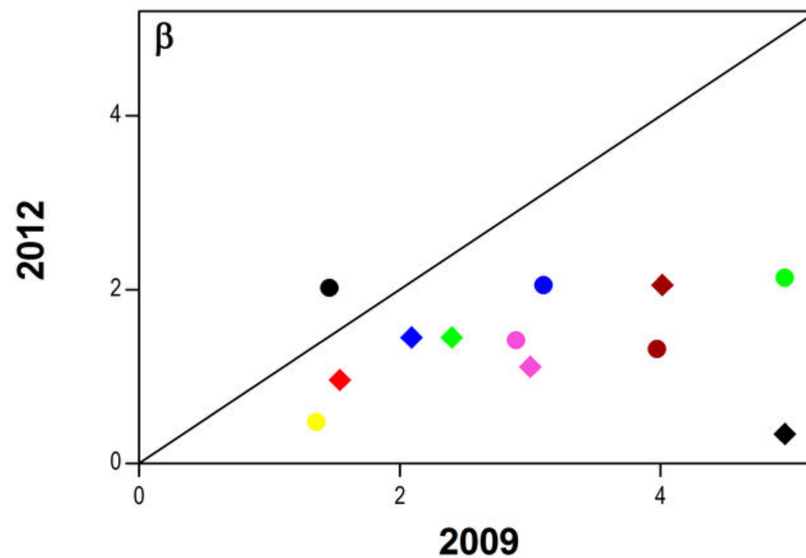
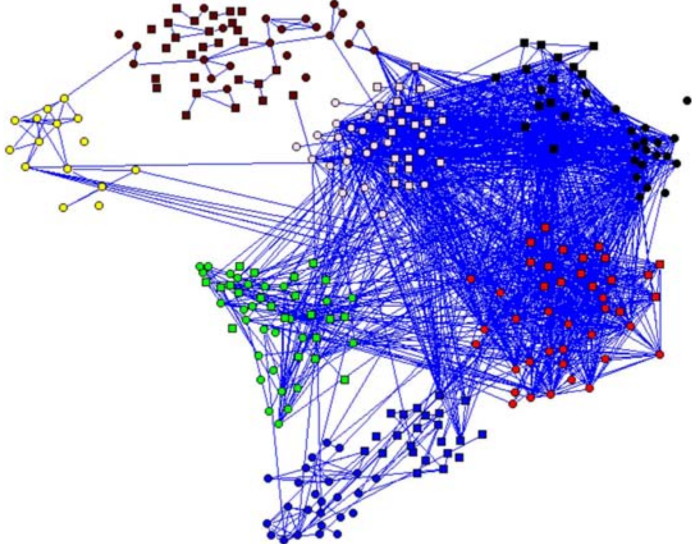
- Arradon
- Roscanvel
- Molène
- Sainte-Marguerite
- Callot
- Arcouest
- Saint-Malo











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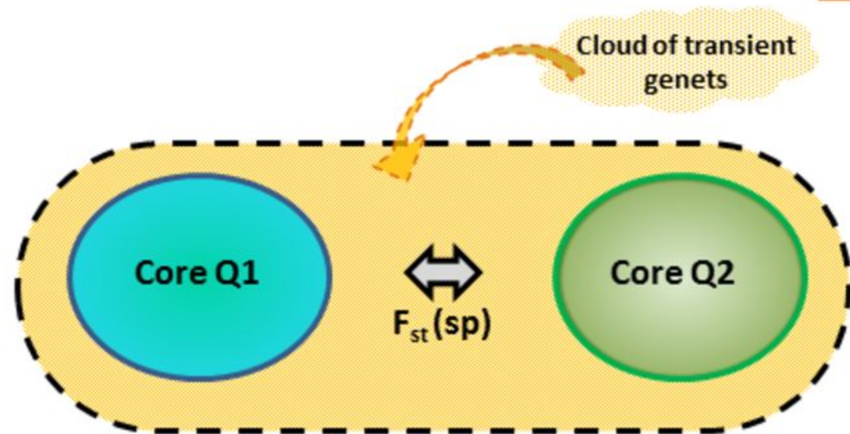

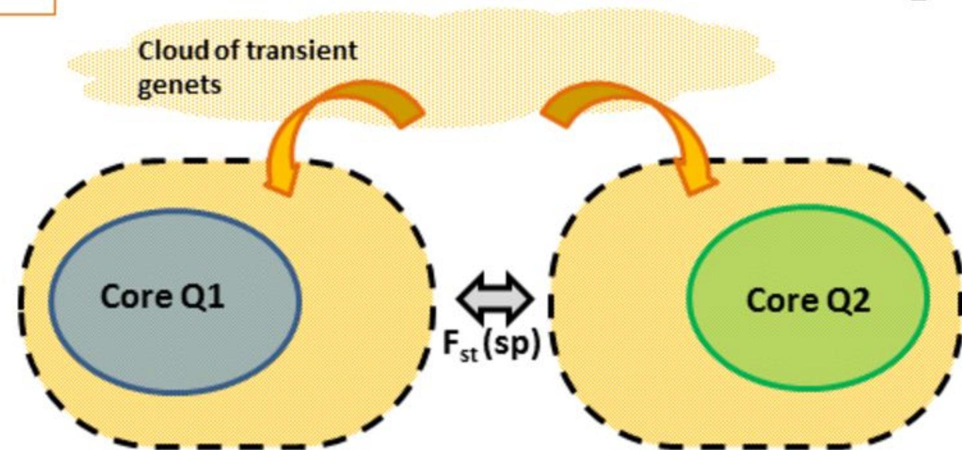

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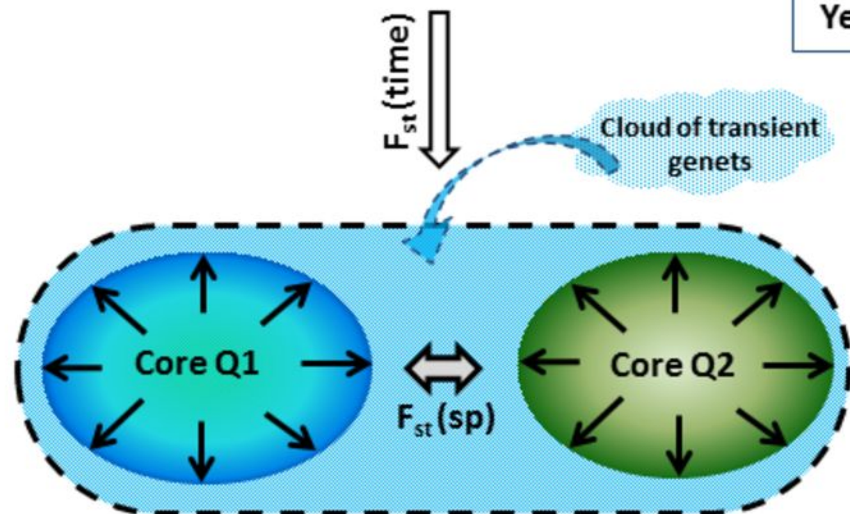

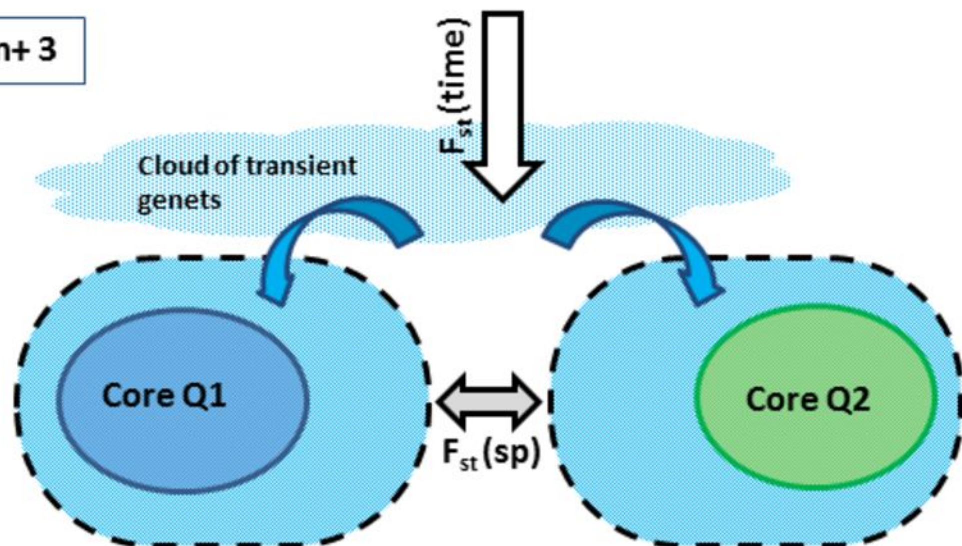

Table 1 Parameters describing clonal structure and genetic diversity in 2009 and 2012. Samples were standardized at 30 ramets. G: number of identified MLLs. R: clonal richness. D* and ED*: Simpson index and its equitability index. β: slope of Pareto distribution. Sp: statistic of spatial autocorrelation. CR: clonal subrange in meters. Gsh: number of clones shared between 2009 and 2012. Parameters of genetic composition: the two parameters we assessed were heterozygosity and allelic richness. He: expected heterozygosity without bias (Nei 1978). Ho: observed heterozygosity. FIS -values were estimated after 1000 permutations of alleles within the quadrat. The mean number of alleles per locus Â was also estimated. Bold values are significant ($p < 0.05$).

location	quadrat	year	clonal structure								genetic composition			
			N	G	R	D*	β	Sp	CR	Gsh	He	Ho	Fis	Â
Arradon	1	2012	30	21	0.68	0.98	2.05	**0.02**	9.7	0	0.5	0.51	-0.02	4.6
		2009	30	26	0.86	0.99	3.10	**0.04**	5.6		0.52	0.51	0.03	5.89
	2	2012	30	22	0.72	0.98	1.45	0.00	13.0	2	0.5	0.47	0.05	4.66
		2009	30	21	0.69	0.97	2.09	0,00	14.6		0.54	0.52	0.03	6.00
Roscanvel	1	2012	30	27	0.89	0.99	2.14	**0.01**	6.4	0	0.47	0.44	0.06	4.44
		2009	30	30	1.00	1.00	4.95	0,00	0.0		0.52	0.51	0.01	4.78
	2	2012	30	22	0.72	0.97	1.45	0.003	5.0	1	0.52	0.56	-0.07	4.44
		2009	30	21	0.69	0.97	2.40	**0.02**	12.0		0.5	0.52	-0.05	4.11
Molène	1	2012	30	16	0.51	0.86	0.48	0.02	11.9	6	0.4	0.46	**-0.14**	3.22
		2009	30	15	0.48	0.85	1.36	-	-		0.4	0.40	0.01	3.44
Sainte-Marguerite	1	2012	30	20	0.65	0.97	1.32	0.02	11.2	0	0.48	0.52	-0.08	4.33
		2009	30	28	0.93	0.99	3.97	0,00	2.5		0.47	0.49	-0.04	5,00
	2	2012	30	24	0.79	0.99	2.05	0.01	6.0	0	0.5	0.46	**0.09**	5,00
		2009	30	28	0.93	0.99	4.01	-0.01	3.0		0.55	0.57	-0.04	5.55
Callot	1	2012	30	23	0.75	0.97	1.42	**0.04**	14.0	0	0.43	0.46	-0.06	4.33
		2009	30	26	0.86	0.99	2.89	0,00	5.3		0.46	0.42	**0.08**	5.78
	2	2012	30	17	0.55	0.96	1.11	**0.02**	18.9	0	0.44	0.47	-0.06	4.33
		2009	30	23	0.76	0.98	3.00	0.01	7.8		0.45	0.44	0.03	4.89
l'Arcouest	1	2012	30	23	0.75	0.98	2.02	-	-	0	0.41	0.44	**-0.09**	4.44
		2009	30	19	0.62	0.91	1.46	-	17.0		0.41	0.46	**-0.13**	4.11
	2	2012	30	19	0.62	0.84	0.34	0.01	19.1	0	0.33	0.34	-0.04	3.11
		2009	30	30	1.00	1.00	4.95	**0.03**	0.0		0.4	0.45	**-0.12**	3.89
Saint-Malo	1	2012	30	26	0.86	0.98	-	0.01	2.7	1	0.36	0.38	-0.07	4.11
		2009	30	19	0.62	0.95	2.05	0.02	18.6		0.29	0.35	**-0.21**	2.33
	2	2012	29	19	0.64	0.95	0.96	**0.05**	10.3	5	0.31	0.35	**-0.13**	2.77
		2009	30	15	0.48	0.89	1.54	0.01	10.2		0.4	0.39	0.04	3.56

Table 2 Genetic differentiation in space (between quadrats from the same location, inter-quadrat Fst) and in time (temporal Fst). Fst-values were calculated after 1000 permutations (Weir & Cockerham, 1984). *P < 0.05; **P < 0.01; ***P < 0.001

Location	Temporal Fst (2009 VS 2012)	Inter-quadrat Fst (2009)	Inter-quadrat Fst (2012)
Arradon Q1	0.15***	0.08***	0.00
Arradon Q2	0.04***		
Roscanvel Q1	0.13***	0.02*	0.01*
Roscanvel Q2	0.10***		
Molène	0.11***	-	-
Sainte-Marguerite Q1	0.09***	0.01	0.04***
Sainte-Marguerite Q2	0.01*		
Callot Q1	0.18***	0.12***	0.01
Callot Q2	0.08***		
Arcouest Q1	0.10***	0.06**	0.02*
Arcouest Q2	0.12***		
Saint-Malo Q1	0.17***	0.07**	0.05***
Saint-Malo Q2	0.14***		