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The concept of population in clonal organisms: mosaics of temporally colonized patches are forming highly diverse meadows of *Zostera marina* in Brittany

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Abstract:

Seagrasses structure some of the world's key coastal ecosystems presently in decline due to human activities and global change. The ability to cope with environmental changes and the possibilities for shifts in distribution range depend largely on their evolvability and dispersal potential. As large-scale data usually show strong genetic structure for seagrasses, finer-grained work is needed to understand the local processes of dispersal, recruitment and colonization that could explain the apparent lack of exchange across large distances. We aimed to assess the fine-grained genetic structure of one of the most important and widely distributed seagrasses, *Zostera marina*, from seven meadows in Brittany, France. Both classic population genetics and network analysis confirmed a pattern of spatial segregation of polymorphism at both regional and local scales. One location exhibiting exclusively the variety 'angustifolia' did not appear more differentiated than the others, but instead showed a central position in the network analysis, confirming the status of this variety as an ecotype. This phenotypic diversity and the high allelic richness at nine microsatellites (2.33–9.67 alleles/locus) compared to levels previously reported across the distribution range, points to Brittany as a centre of diversity for Z. marina at both genetic and phenotypic levels. Despite dispersal potential of several 100 m, a significant pattern of genetic differentiation, even at fine-grained scale, revealed 'genetic patchiness'. Meadows seem to be composed of a mosaic of clones with distinct origins in space and time, a result that calls into question the accuracy of the concept of populations for such partially clonal species.

Keywords: clonality • dispersal • ecotype • network analysis • population • *Zostera marina*

45 **INTRODUCTION**

46

47 Seagrasses are the structural basis of key coastal marine ecosystems (Orth *et al.* 2006)
48 supporting high biodiversity and biomass (Orth *et al.* 1984). These ecosystems provide a great
49 number of goods and services, including primary production, the supply of food for
50 megaherbivores, habitats for resident faunas and stabilization of sediments (Hemminga &
51 Duarte 2000). Many of these are experiencing a decline on a worldwide scale, probably due to
52 anthropogenic disturbances and climate change (Waycott *et al.* 2009). The ability of these
53 species, and of the ecosystems they supply, to survive future environmental changes may
54 largely depend on their genetic adaptability (Booy *et al.* 2000; Frankham 2005). The attempt
55 to predict, and possibly prevent, changes in the geographical pattern of persistence, local
56 extinction or range shifts should be based on a good understanding of the implications of
57 genetic diversity for the resistance and resilience of local populations, as well as on reliable
58 estimates of dispersal among geographic areas. Dispersal is likely to determine the balance
59 between migration-drift and local adaptation, and in turn the likelihood of meadows to survive
60 or to be naturally recolonized if locally extinct.

61 In temperate latitudes, meadows are generally monospecific, and the dominant family is
62 Zosteraceae, which contains five monophyletic species, including the eelgrass *Zostera marina.*
63 This species is widely distributed in the northern hemisphere, and present in the Pacific and
64 Atlantic Oceans, as well as in the Mediterranean and Black Seas. It is the dominant seagrass
65 on the coasts of Brittany (France) where it shares (Den Hartog & Hily 1997) a dominant role
66 with algae in structuring one of the most important coastal ecosystems and providing habitat
67 for a large number of species (Hily & Bouteille 1999).

68 Eelgrass reproduces sexually through the production of propagules, and clonally through

69 the vegetative production of ramets *via* rhizome elongation. This partial clonality has multiple
70 biological and methodological implications. In particular, estimations of diversity in these
71 populations require distinction between genet (genetic individuals, where all tissue has
72 originated from one zygote; Eriksson, 1993) and ramet (a potentially independent part of the
73 genet that often represents the sampling unit; Eriksson 1993; Arnaud-Haond *et al.* 2007a).
74 Clonal diversity has been shown experimentally to enhance resistance and resilience of
75 experimental quadrats of *Z. marina* to perturbations (Ehlers *et al.* 2008; Hughes & Stachowicz
76 2004; Reusch *et al.* 2005). If these experimental results are extrapolated to natural populations
77 (Arnaud-Haond *et al.* 2010; Hughes & Stachowicz 2009), the level of clonal diversity is found
78 to be associated with an enhanced resistance to environmental perturbations. A large
79 biogeographic survey (Olsen *et al.* 2004) reported moderate to high values of clonal richness
80 for two locations in Brittany, (Carantec: 0.54; Morgat: 0.90).
81 Genetic diversity, i.e. the allelic richness and/or heterozygosity observed in meadows, is
82 influenced by numerous factors, such as effective population size, spatial pattern of dispersal
83 and recruitment success of immigrant propagules (dependent on competition and local
84 adaptation) and the biogeographical history of populations (Olsen *et al.* 2004). At the scale of
85 its entire distribution, these authors reported an allelic diversity hotspot for *Z. marina* in the
86 North Sea-Wadden Sea region, where populations are characterized by high allelic richness.
87 This region is also a diversity hotspot for *Z. noltii* (Coyer *et al.* 2004). In contrast, East
88 Atlantic meadows of *Z. marina,* including the two locations in Brittany studied here, exhibited
89 much lower levels of allelic richness, suggesting a narrower adaptive potential. Yet those two
90 particular samples from Brittany showed distinct levels of clonal and allelic richness, calling
91 for further analysis of the spatial variability and fine-grained pattern of clonal and genetic
92 composition that had thus far only been performed on samples from the Baltic Sea.

93 Moreover, the possibility of heterogeneity in intra-meadow clonal and genetic
94 composition had never been explored, to the best of our knowledge, in such phylogeographic
95 analyses on seagrasses (Alberto *et al.* 2008; Arnaud-Haond *et al.* 2007b; Coyer *et al.* 2004;
96 Olsen *et al.* 2004). Some benthic marine invertebrates show contrasted patterns of genetic
97 diversity and structure at a very fine-grained scale (Arnaud-Haond *et al.* 2008; Johnson &
98 Black 1984), likely due to the chaotic nature of dispersal in the marine environment
99 (Roughgarden *et al.* 1988). Given the large-scale dispersal potential of most marine
100 angiosperms through seed or shoot dispersal, combined with their benthic nature and
101 extensive clonal propagation once settled, a similar phenomenon might occur in seagrass
102 meadows.
103 Finally, the occurrence of the variety '*Z. angustifolia*', already described in the UK, has
104 also been seen in Brittany, particularly in the Morbihan Gulf (France, REBENT). Whether this
105 particular morph corresponds to a distinct species, as suggested by some authors (Percival *et*
106 *al.* 1996; Provan *et al.* 2008), or to an ecotype (De Heij & Nienhuis 1992; Den Hartog 1970),
107 is still unknown as no genetic studies have been reported so far that address this issue. This
108 topic is of central importance for understanding gene flow and local adaptation processes in *Z.*
109 *marina* meadows across its distribution range.
110
111 In the present work, we used nine microsatellites to (i) identify the particular morph "*Z.*
112 *marina v. angustifolia"*, in order to test its status as a genetically distinct taxon or ecotype; (ii)
113 investigate the genetic diversity and genotypic structure along the coasts of Brittany; (iii) test
114 for the importance of fine-grained variation (intra meadow versus regional scale) of these
115 characteristics; and (iv) assess the dispersal potential at local (i.e. fine-grained) and regional
116 scales.

117 **MATERIAL AND METHODS**

118

119 *Sample collection*

120

121 Eelgrass samples were collected in February to April 2009 from 7 intertidal meadows
122 along the coast of Brittany (Fig. 1), stretching from Saint-Malo to Arradon. Distances between
123 meadows ranged from 33 km (Molène - Roscanvel) to 442 km (Arradon - Saint-Malo). For
124 each location, two 20 * 30m quadrats separated by several tens of meters were chosen, located
125 in continuous parts of the meadow monitored as part of the REBENT survey (REseau
126 BENThique, a French network specialized in the survey of major coastal ecosystems including
127 eelgrass meadows; www.rebent.org). Approximately 35 sampling units (SU) were collected
128 according to randomly drawn coordinates (Table 1). In Molène, due to high patchiness of the
129 meadow, only one quadrat was sampled, with 20 SU according to random coordinates and the
130 12 more collected at haphazardly in the patchy end of the meadow. Annual observations from
131 REBENT indicated Saint-Malo as one of the sites where the variety "*angustifolia*" is observed
132 across years. The field observations indeed showed the typical *Z. marina v. angustifolia*
133 variety in both quadrats of this meadow, with dwarf shoots exhibiting narrow leafs almost
134 comparable in size and shape to the dwarf *Z. noltii*.
135 The base of each leaf bundle, including the shoot apical meristem, was preserved in silica
136 crystals until DNA extraction.

137

138 *DNA extraction, isolation, microsatellite and ITS amplification and loci scoring*

139

140 The Fast DNA®SPIN kit for soil was used for DNA extraction according to the protocol

141 provided by the manufacturer (MP Biomedicals, France). Nine microsatellite loci (Genbank
142 accession numbers: AJ009899, AJ009901, AJ009902, AJ009905 and AJ249303 to AJ249307;
143 Reusch *et al.* (1999) Reusch (2000)) were PCR-amplified using fluorescently labeled primers
144 (GA12, GA19, GA20, GA17D, GA16, GA2, GA23, GA35 and GA17H). PCR products were
145 visualized using an ABI-3100 FNVR automated sequencer (Applied Biosystems) and scored
146 using STRand software (http://www.vgl.ucdavis.edu/informatics/strand.php). A double blind
147 reading was made by two different users and gels were re-scored when discrepancies were
148 recorded.

149 To standardize the samples at 30 individuals before estimations of clonal and genetic
150 composition, excess individuals were removed at random.

151 To test whether the variety "*angustifolia*" corresponded to a species or to an ecotype, we
152 also compared sequences of ITS markers (1100 bp) of two samples exhibiting the typical
153 morphology of the variety "*angustifolia*" (Saint-Malo) with two samples from Arradon and
154 from Arcouest locations exhibiting the typical morphotype of *Z. marina*. ITS-PCRs were
155 performed using the universal primers Jo6 and TW5 (White et al., 1990 in Diekmann *et al.*
156 2001).

157

158 *Genetic and clonal data analysis*

159

160 In order to identify genetic individuals (i.e. to discriminate genets from ramets on the
161 basis of their Multi Locus Genotype: MLG), we used a "barcoding" type approach based on 9
162 microsatellite loci.

163 For clonal organisms, two questions must be answered: (i) do all the replicates of the
164 same MLG really belong to the same genet (i.e. are they all issued from the same sexual
165 reproduction event)? and (ii) does each distinct MLG really belong to a distinct genet?

166 To answer the first question, when the same MLG is encountered n times in a sample of N
167 sampling units, the probability that the identical MLGS originate from different sexual
168 reproductive events (p_{sex}) should be assessed (Arnaud-Haond *et al.* 2007a). Below a threshold
169 value fixed at 0.01, identical MLGs may be considered as belonging to the same genet.
170 Estimates of p_{sex} are derived on the basis of allelic frequencies estimated using the round robin
171 method (Parks & Werth 1993), with a sub-sampling approach to limit the overestimation of
172 the rare alleles. Allelic frequencies for each locus are estimated on the basis of a sample pool
173 composed of all the MLGs discriminated, while excluding the loci for which allelic frequency
174 is estimated. This procedure is repeated for each locus, taking into account Wright's
175 inbreeding coefficient estimated after the exclusion of identical MLG (Young *et al.* 2002).

176 Once the clonal membership of identical MLG is ascertained using $p_{sex,}$ slightly distinct
177 MLGs belonging to the same genet may, nevertheless, still occur in the dataset, either due to
178 the existence of somatic mutation or scoring errors (Arnaud-Haond *et al.* 2007a;
179 Douhovnikoff & Dodd 2003). If ignored, this would lead to an overestimation of the number
180 of clones in the sample analyzed. The two-step approach proposed by Arnaud-Haond *et al.*
181 (2007a) was applied to test whether these slightly distinct (at one or two loci) MLGs belong to
182 the same genet by: (i) screening each MLG pair presenting an extremely low distance; (ii)
183 using p_{sex} on the set of identical loci in order to estimate the probability that the slightly
184 distinct MLG could actually be derived from distinct reproductive events. When p_{sex} is lower
185 than 0.01, the two identical MLG can be considered to belong to the same genet or Multi Locus
186 Lineage (MLL; Arnaud-Haond *et al.* 2007a).

187 Estimates were calculated using the software GENCLONE 2.1 (Arnaud-Haond & Belkhir
188 2007).

189 For each quadrat, clonal diversity was estimated by:

190 $$R=\frac{G-1}{N-1}$$

191 where G is the number of MLLs in the sample and N is the number of SUs analyzed, as
192 recommended by Dorken & Eckert (2001) and Arnaud-Haond *et al.* (2005). The minimum
193 value for clonal diversity in a monoclonal stand is always 0, independent of sample size, and
194 the maximum value is still 1 when each analyzed sample corresponds to a distinct MLL. The
195 complement of the Simpson index (Pielou 1969) for genotypic diversity in each quadrat,
196 representing the probability of encountering distinct MLLs when randomly taking two
197 sampling units, was estimated as:

$$D=1-\sum_{i=1}^{G} p_i^2$$

200 where p_i^2 is the frequency of the ith MLL (its estimation is given by: $p_i^2 = [n_i(n_i-1)] / [N(N-1)]$
201 where N is the number of ramets sampled and n_i is the number of sample units sharing the
202 i^{th} MLL). Moreover, we estimated the Simpson's evenness index to describe clonal equitability:

203 $$ED^* = \frac{(D-D_{min})}{(D_{max}-D_{min})}$$

204 with D_{min} and D_{max} being the approximate minimum and maximum values of Simpson's
205 complement index given the sample size N and the sample clonal richness G, estimated as:

206 $$D_{min} = [\frac{(2N-G)\times(G-1)}{N^2}]\times\frac{N}{(N-1)} \text{ and } D_{max} = \frac{(G-1)}{G}\times\frac{N}{(N-1)}$$

207 The β of the Pareto distribution, representing the negative slope of the power law usually
208 describing the distribution of ramets into groups of clonal size (Arnaud-Haond *et al.* 2007a),
209 was also estimated as this metric seems less sensitive than other estimators to the relative
210 density of sampling units *versus* shoots in the sampled meadow. All clonal diversity and
211 structure parameters were calculated with GENCLONE 2.1 (Arnaud-Haond & Belkhir 2007).

212

213 A single copy of each discriminated MLL was retained in the dataset used to assess genetic
214 diversity and structure. Genetic diversity within quadrats was estimated as the mean number
215 of alleles per locus (Â), with observed (H_O) and unbiased (H_E) multilocus heterozygosity (Nei
216 1978). Linkage disequilibrium was tested according to Black & Krafsur (1985). A
217 permutation procedure (1000 permutations) was used to test whether a particular estimate of
218 the overall inbreeding coefficient (F_{IS}) or linkage disequilibrium was significantly different
219 from 0 ($p < 0.01$).

220 Genetic structure among populations was estimated with θ (Weir & Cockerham 1984). A
221 Mantel test including geographical distances among populations was carried out to test for the
222 two dimensional "Isolation By Distance" (IBD) model (Rousset 1997). These parameters were
223 estimated using GENETIX (Belkhir *et al.* 2004).

224

225 At the within-quadrat level, autocorrelation analyses were performed to test for the
226 existence of restriction to dispersion at the intra-meadow scale, and to estimate the extent of
227 clonality. We used the kinship estimator coefficient of Ritland (F_{ij}) as a genetic relatedness
228 statistic (Ritland 1996). We performed regression analyses of mean F_{ij} against the Log_e of
229 mean geographic distance, within each distance class. This allowed us to test the adequacy of
230 IBD models in each quadrat. The autocorrelation analyses were performed using Ritland's
231 coefficient of kinship: (i) first including all SUs, where it is mostly influenced by the spatial
232 extent of clones/clonal lineages (i.e. the genetic neighborhood of SUs belonging to the same
233 MLL) and (ii) using permutations (1000) in order to include only one ramet (and one of the
234 possible corresponding coordinates, randomly chosen for each permutation step) from each
235 genet at each permutation, in order to examine the dispersion through sexual propagules. The
236 slopes of regressions (*b*) allowed us to calculate the *Sp* statistic (Vekemans & Hardy 2004).

237 This statistic corresponds to the spatial autocorrelation profile, varying from 0 (no limitation
238 to gene dispersal at the scale of the sampling) to +∞ (theoretical case, where the structure is
239 maximal). Its equation is the following:

240
$$Sp = \frac{-b}{1 - \hat{F}_{(1)}}$$

241 with $\hat{F}_{(1)}$ the kinship value for the first distance class. Autocorrelation parameter
242 estimations were performed with GENCLONE 2.1 (Arnaud-Haond & Belkhir 2007).
243 The clonal subrange CR was estimated for each quadrat to describe the spatial
244 components of the clonal population. It corresponds to the minimal estimates of the maximum
245 distance between two identical genotypes belonging to the same clone, in meters, and is
246 determined as the distance for which the probability of clonal identity becomes null (Alberto
247 *et al.* 2005; Harada *et al.* 1997).
248

249 *Network analysis*
250 Network analysis is a graphic, holistic and non-parametric method that has proven useful
251 in the illustration and analysis of population structure (Fortuna *et al.* 2009; Rozenfeld *et al.*
252 2007; Rozenfeld *et al.* 2008). In this study, networks based on genetic distances were used (i)
253 to ascertain the relative position of the variety "*angustifolia*" (sampled in Saint-Malo) against
254 other sampling locations, in a global network including all genets from all locations, and (ii) to
255 illustrate the distribution of genetic distances at a finer scale (i.e. among genets from distinct
256 quadrats within sampling locations). Individual-based networks of genetic distances were built
257 at the global scale (all quadrats) and at local scales (for each sampling locality), illustrating the
258 connection of some genets (agents) depending on their genetic distance (link). The distances
259 used have proven successful in assigning unknown individuals to their correct subpopulations

260 (Estoup *et al.* 1995) and is classically known as the "Shared Allele Distance", (Chakraborty &

261 Jin 1993), although it actually reflects the proportion of non shared alleles:

262 $$D_{SA_I} = 1 - \frac{\sum uS}{2\mathrm{u}}$$

263 with S the number of shared alleles and u the number of loci. D_{SA_I} spans from 0 to 1.

264 Networks are built including links for all distances, which are subsequently removed in

265 decreasing order, until reaching the effective percolation point, Dpe (Rozenfeld *et al.* 2007;

266 Stauffer & Aharony 1994), below which the network fragments into small clusters. This

267 phenomenon can be interpreted as the first level of limitation to gene flow. The precise

268 calculation of the Dpe is made with the standard methodology for a finite system, proposed by

269 Stauffer & Aharony (1994) and consisting of calculating the average cluster size excluding the

270 largest one:

271 $$\langle S \rangle = \frac{1}{N} \sum_{s<Smax} s^2 n_s$$

272 as a function of the last distance value removed. N is the total number of nodes not

273 included in the largest cluster and n_s is the number of clusters containing s nodes. Once this

274 effective percolation threshold is reached, we analyzed the network topology and its

275 characteristics (Table 4; Fig. 4).

276

277 *Global and local property estimates of the network*

278 The clustering coefficient C_i of the node i is the ratio between the number of existing links

279 with the maximal number of potential links within the cluster. It is defined as:

280 $$Ci = \frac{Ei}{Ei^{(\max)}} = \frac{2Ei}{ki(ki-1)}$$

281 with Ei the number of links existing among the neighbors of the node i, and the degree ki
282 of a given node i the number of other nodes linked to it. The clustering coefficient of the
283 whole network $<CC>$ is defined as the average of all individual clustering coefficients in the
284 system. The clustering of nodes is interpreted as the existence of hierarchical substructure,
285 with clusters of genets within which members are more closely related to one another than
286 they are to other genets outside the particular cluster.

287 The *betweenness centrality* (Freeman 1977) of node i, $bc(i)$, is the fraction of shortest
288 paths between pairs of nodes that pass through node i. Let σ_{st} denote the number of shortest
289 paths connecting nodes s and t and $\sigma_{st}(i)$ denote the number of those passing through the node
290 i; then:

291 $$bc(i) = \sum_{s \neq t \neq i} \frac{\sigma st(i)}{\sigma st}.$$

292 Higher values of *betweenness centrality* in genetic networks have been interpreted as the
293 importance of a given agent (a population or cluster of individuals) in relaying gene flow
294 across the system, i.e. to and from other agents (Rozenfeld *et al.* 2008).

295 **RESULTS**

296

297 *ITS polymorphism in Z. marina and Z. marina v. angustifolia*

298

299 Of six genets from the first quadrats in Saint-Malo, Arradon and Arcouest, only two
300 haplotypes were observed, and these differed at only one nucleotide substitution out of 1100
301 base pairs of ITS 1 & 2. This difference distinguished one genet from Arradon from the five
302 others, which shared the most common haplotype.

303

304 *Clonal structure and diversity*

305

306 The clonal diversity of the 13 quadrats showed high variability both within and among
307 locations (Table 2). The minimum values were observed in the Molène meadow and quadrat 2
308 at Saint-Malo (R = 0.48; β = 1.36 and R = 0.48; β = 1.54, respectively), whereas quadrat 1 at
309 Roscanvel and quadrat 2 at Arcouest showed highest clonal richness (R = 1.00; $\beta \geq 4.95$ for
310 both). Mean clonal richness was 0.72. The largest discrepancies within location were observed
311 in Arcouest (R=0.62 to 1), Roscanvel (R=0.69 to 1) and Saint-Malo (R = 0.48 to 0.62).
312 The clonal subrange was also highly variable (largest clones in quadrat 1 of the Saint-
313 Malo meadow: CR = 18.61m; shortest clones in quadrats 1 of Roscanvel and 2 of Arcouest:
314 CR = 0.00m). A high within-location variability was observed here also: those quadrats with
315 the highest clonal diversity, and therefore a minimum clonal subrange, were found sharing a
316 site with a second quadrat ranking among the highest in terms of clonal subrange (CR =12.04
317 in quadrat 2 at Roscanvel and CR = 17.01 in quadrat 1 at Arcouest).

318

319

320 *Genetic diversity and Hardy-Weinberg equilibrium*

321

322 Genetic composition analyzed with a single representative of each MLL was highly
323 variable among locations (Table 2), mostly due to the extreme composition of Saint-Malo
324 (heterozygosity of 0.35 and allelic richness of 2.33 in quadrat 1) and Sainte-Marguerite
325 (heterozygosity of 0.6 and allelic richness of 9.67 in quadrat 2). The estimates appeared more
326 stable among quadrats within these locations, as well as among the other locations, despite
327 high variance in clonal diversity estimates, which tends to support the idea of sexual and
328 panmictic entities in the remaining quadrats, once replicates are removed.

329 Similarly, departures from HWE and the occurrence of linkage disequilibrium (LD) were
330 generally weak once replicates were removed, except for quadrat 2 in Sainte-Marguerite,
331 showing heterozygote deficiency (F_{IS} = 0.14, $p < 0.001$) and quadrats 2 of Arcouest and 1 of
332 Saint-Malo, with an excess of heterozygosity (respectively F_{IS} = -0.12; $p < 0.01$ and F_{IS} = -
333 0.21; $p < 0.01$). Here most values were similar among quadrats within location except for
334 Saint-Malo with the important heterozygote excess mentioned here in one quadrat and no
335 significant departure observed in the other.

336 Slightly significant preferential matching between alleles was detected for the majority of
337 quadrats, with 6.7 to 19 % of LD values significantly different from 0. Here again, quadrat 1
338 of Saint-Malo and quadrat 2 of Sainte-Marguerite departed from the average with 50 and 58 %
339 of significant values, respectively. To assess whether significant values resulted from a
340 "locus" or "population effect", we therefore removed these two quadrats. For each pair of loci
341 (total of 36 pairs), no significant LD value was found for 11 pairs, 1 significant value was
342 found for 15 pairs, 2 for 5 pairs, 3 for 4 pairs, and only the pair GA2 / GA17H showed 6
343 significant LD values, indicating a significant and possibly physical linkage disequilibrium
344 between these two loci. Finally, a positive and significant relation between F_{IS} and LD values

345 was found ($R^2 = 0.31$; slope = 0.25; $p = 0.049$) indicating that most LD values may be
346 statistical and due to departure from random mating rather than to physical proximity of loci,
347 in agreement with the lack of significant results observed in a previous study with large-scale
348 sampling across the distribution range of the species (Olsen *et al.* 2004).
349
350 *Genetic structure among locations & differentiation*
351
352 When the dataset was analyzed at the genet level (i.e. including only one copy of each
353 recognized MLL), this revealed wide genetic differentiation among the 13 locations. All F_{ST}
354 values per pair of sampling quadrats were significantly different from 0 ($p < 0.05$; Table 3).
355 The minimum value was observed between quadrats Callot 1 and Saint-Malo 2 ($F_{ST} = 0.039$; p
356 < 0.05) and F_{ST} reached 0.33 ($p < 0.01$) between Molène and quadrat 2 at Arcouest. Within
357 locations, all quadrat pairs were also significantly different and sometimes exceeded some of
358 the inter-location estimates. The minimum was observed among the quadrats at Roscanvel
359 ($F_{ST} = 0.019$; $p < 0.05$) and the maximum among the quadrats at Callot ($F_{ST} = 0.12$; $p < 0.01$).
360 A Mantel test carried out among all pairs of populations was significant (slope : 0.04; R^2 =
361 0.11; $p < 0.01$; Fig. 2), but such significance can not be interpreted as an indication of a strict
362 IBD pattern, as it is mostly driven by the sampling scheme, which results in two clouds of dots
363 representing the intra *versus* inter location distances among quadrats. No relation was
364 observed at either of these two scales (among pairs within location: slope = -0.19, $R^2 = 0.63$,
365 n.s.; pairs among locations: slope = -0.002, $R^2 = 0.004$, n.s.), suggesting that distance may not
366 be the predominant factor acting at the regional spatial scale.
367 The limitation to dispersal, as estimated through autocorrelation analysis, was indeed
368 highly variable among meadows (for an example see Fig. 3). The value of *Sp* was significantly
369 different from 0 for four out of 13 quadrats (quadrat 1 at Arradon, $Sp = 0.04$, $p < 0.001$;

Arcouest , *Sp* = 0.03, p < 0.001; and Saint-Malo, *Sp* = 0.02, p < 0.05; and quadrat 1 at Roscanvel, *Sp* = 0.02, p < 0.05).

Network topology of Z. marina individuals

The effective percolation threshold (Dpe) was seen to be about 0.45 (data not shown), below which the network lost its integrity and the clusters broke down into 2 distinct clusters. On the global network just above this percolation threshold (Fig. 4) there is a first cluster composed of genets sampled in Molène and Sainte-Marguerite (on the top left part) and a second cluster of genets sampled the other localities. Within the giant cluster above the percolation point, as well as inside this secondary cluster emerging below it, the genets from Saint-Malo have a central position, along with the highest average value of *betweenness centrality* (<BC>=0.0099) (global average value of BC is 0.0048, see table 4 for network characteristics).

Network topologies at the fine-grained spatial scale (Fig. S1) revealed two clusters for each location, related to the sampling quadrats, except for Roscanvel and Sainte-Marguerite, which showed clustering values that were half those of the four other locations (0.17 and 0.16 respectively *versus* an average value of 0.32). It should be noted that no conclusion can be drawn about Molène as there was only one quadrat.

389 **DISCUSSION**

390

391 *Z. marina v. angustifolia: an ecotype rather than a protospecies?*

392

393 Considered alternatively as an ecotype or as a distinct species, *Z. angustifolia* was first
394 reported in the UK, the Morbihan Gulf and Arcachon Bay (France). The annual observations
395 by the REBENT network also detected this morph at Saint-Malo over a number of years. Its
396 predominant occurrence in spring led us to consider it as an annual variety of *Z. marina* (Hily,
397 *personnal observation*). No differences in ITS-sequence were observed between the variety
398 "*angustifolia*" (sampling units from Saint-Malo) and the typical variety of *Z. marina* (SUs
399 from other locations). The occurrence of private alleles was no higher in Saint-Malo than in
400 the other locations (only two private alleles in the first quadrat). Also, pairwise F_{ST} values
401 involving quadrats from Saint-Malo ranked among the average pairwise comparisons.
402 Network analysis agreed with this result, showing a complete mixture of Saint-Malo MLLs
403 with all other MLLs, and even highlighting a central position of genets from Saint-Malo (Table
404 4 and Fig. 5). The values of *betweenness centrality* also suggest a higher genetic relatedness of
405 genets from the meadow of Saint-Malo to those of most other locations. Our data therefore
406 support *Z. marina v. angustifolia* in Saint-Malo as an ecotype, rather than a distinct species.

407 It was not clear at this point in the analysis whether this morph with a cyclical "bloom"
408 arose due to the annual growing of shoots from persistent rhizomes or to annual episodes of
409 germination of dormant seeds. The former seems to be a more likely explanation, considering
410 the limited clonal diversity, high clonal subrange and heterozygote excess observed in Saint-
411 Malo. These indices indeed tend to support the occurrence of large and persistent clones. In
412 the neighborhood near the city (several tens of meters from the walls of Saint-Malo), the

413 extreme exposure, together with a high level of local clam digging during equinox tides,
414 suggests an increased anthropogenic influence on this meadow. The level of disturbance may
415 therefore be high, allowing only the recruitment and persistence of specific genotypes able to
416 cope with extreme conditions. Similarly, Diaz-Almela *et al.* (2007) highlighted an increase of
417 CR in impacted populations of *Posidonia oceanica,* compared with reference non-impacted
418 populations, and a higher resistance to disturbances resulting from fish-farming in meadows
419 showing a high CR value. This suggests that large clones have a higher fitness, potentially
420 conferring a competitive advantage for spatial colonization, and enhanced phenotypic
421 plasticity (Diaz-Almela *et al.* 2007). This hypothesis is consistent with the excess of
422 heterozygosity (in a scenario of heterosis), as well as with low clonal and allelic richness.
423 Interestingly, *Z. marina v. angustifolia* is also generally observed in harsh conditions in the
424 residual ponds within meadows of *Z. noltii* in Arcachon bay, France (Auby, *personnal*
425 *communication),* where it is subjected to strong variations in temperature, salinity, pH and
426 oxygen concentration during low tide. All these elements suggest that *Z. marina v.*
427 *angustifolia* is an ecotype that is revealed above a perturbation threshold, leading to an
428 extreme in the expression of the phenotypic plasticity of *Z. marina* that allows survival in such
429 stressful and fluctuating environmental conditions. A further point of inquiry would be to
430 compare these two types in terms of resistance to "wasting disease", a development of the
431 slimemold-like protist *Labyrinthula macrocystis* in *Zostera* leaves at various sites (Hily *et al.*
432 2002).
433
434 *Clonal architecture and genetic variability.*
435
436 The clonal richness observed in Brittany is quite variable among sites but remains high (R

437 ranges from 0.48 to 1) in comparison to previous studies (Olsen *et al.* 2004; Reusch *et al.*
438 1999). These values are comparable to those observed in the populations representing Brittany
439 in the large biogeographic survey performed by Olsen *et al.* (2004) where the neighboring
440 populations of Carantec and Morgat had R values of 0.54 and 0.90, respectively. The
441 heterogeneity of clonal richness values for locations in Brittany indicates a notable variation in
442 the pattern of investment in sexual *versus* clonal reproduction at the regional scale.

443 Contrastingly, estimates of allelic diversity in the present study are strikingly different
444 from those found by Olsen *et al.* (2004). A much higher allelic richness was consistently
445 observed, ranging from 2.33 (quadrat 1 in Saint-Malo) to 9.67 (quadrat 2 in Sainte-
446 Marguerite) alleles/locus (Table 2). Allelic richness evidenced here is comparable to, or even
447 double, the highest values reported in the Wadden Sea (4.10 alleles per locus) that led these
448 previous authors (Olsen *et al.* 2004) to consider this region as a hotspot of diversity for the
449 species. The values are also at least comparable to, and sometimes strikingly higher than,
450 those observed in the supposed center of origin located in the Northern Pacific (the mean of Â
451 for this region reaches 5.89; from Olsen *et al.* 2004). The Wadden Sea-North Sea region
452 exhibits a linear coastal distance equivalent to Brittany. Such a difference could be due to
453 sampling strategy and scale, as the authors Olsen *et al*. (2004) took samples according to
454 linear transects, a strategy that has been shown to be more prone to overestimate than
455 underestimate diversity (Arnaud-Haond *et al.* 2007a). The higher values, which were observed
456 consistently here, may therefore be attributable to the larger number of sampling locations
457 analyzed. The sampling effort made by Olsen *et al.* (2004) was indeed greater in the region of
458 the Wadden Sea (nine sampling areas) than in Brittany (two sampling areas), for which only
459 one meadow was studied for allelic richness, potentially meaning that the sample in this
460 previous study was not representative of the meadows at the scale of the Brittany coasts.

461 These new results reveal a hotspot of *Z. marina* genetic diversity in Brittany compared
462 with other populations over the distribution range as a whole. Moreover, the discrepancy with
463 the first estimates obtained from the neighboring location of Morgat points toward a possibly
464 significantly heterogeneous distribution of genetic polymorphism at the regional scale. This
465 was further confirmed by analyzing the genetic structure at both regional and fine-grained
466 spatial scales.
467
468 *Mosaic pattern of genetic differentiation at regional and local scales*
469
470 A rather highly structured pattern was revealed at both regional and fine-grained spatial
471 scales. This significant and generalized differentiation was consistent even at the fine-grained
472 spatial scale, among quadrats separated by less than 100 m, although these values tended to be
473 smaller than those observed among locations (Table 3; Fig. 2). This was confirmed by
474 network analysis, which showed the occurrence of two clusters in each location,
475 corresponding to the two quadrats, except in Roscanvel and Sainte-Marguerite. F_{ST} values
476 among quadrats agreed with this finding as they were also the smallest in these two locations.
477 These results suggest a strong limitation to dispersal without a real pattern of gradual IBD, as
478 shown by the lack of significance of the Mantel test at the regional scale.
479 Spatial autocorrelation analysis at the local scale allows a quantitative estimation of the
480 spatial scale over which clonality affects the SGS (spatial genetic structure), as
481 autocorrelograms performed at the ramet-level and at the MLL-level merge at the distance
482 corresponding to the clonal subrange (Alberto *et al.* 2005). In agreement with the patterns of
483 high SGS obtained when including all sampling units, the clonal subranges observed in Saint-
484 Malo, Arradon, Roscanvel and Arcouest (Table 2) provide a minimal estimate of ten or

485 possibly several tens of meters, as these estimates are confined to our sampling areas. This
486 suggests that clonal propagation *via* rhizomatic elongation accounts for dispersal at the scale
487 of up to several tens of meters, as previously reported for *Cymodocea nodosa* (Alberto *et al.*
488 2005) *Z. noltii* (Ruggiero *et al.* 2005) and *Posidonia oceanica* (Arnaud-Haond *et al.* 2007b).

489 In the case of seagrasses, two modes of dispersal exist besides strict clonal elongation: (i)
490 long distance dispersal *via* unrooted shoots, in species such as eelgrass that have easily
491 breaking rhizomes (Hall *et al.* 2006; Harwell & Orth 2002; Orth *et al.* 2006) when exceptional
492 climatic events such hurricanes possibly favor long distance dispersal (Kendall *et al.* 2004);
493 and (ii) medium distance dispersal via seeds (Orth *et al.* 2006), with the formation of gas
494 bubbles that adhere to the seed coat of *Zostera sp*, giving buoyancy (Churchill *et al.* 1985).
495 These authors followed drifting seeds and reported a dispersal that may exceed 200m, large
496 enough to encompass distances similar to those among neighboring quadrats.

497 Considering these rather large estimates of dispersal potential and the lack of limitation to
498 gene flow evidenced in 9 quadrats, a relative genetic homogeneity may be expected at the
499 local scale. Yet, SGS is detected in 4 quadrats and the genetic differentiation among quadrats
500 of the same location is significant (Table 3) and appears clearly in network analysis (Fig. S1).
501 Such a combination of relatively high dispersal potential and stronger or similar genetic
502 differentiation at the very fine spatial scale compared to the regional one was previously
503 described as the paradox of the chaotic genetic patchiness, and the pattern has been
504 extensively reported for marine benthic invertebrates (Arnaud-Haond *et al.* 2008; Edmands *et*
505 *al.* 1996; Johannesson *et al.* 1995; Johnson & Black 1982; Johnson *et al.* 1993; Watts *et al.*
506 1990), and fishes (Doherty *et al.* 1995; Hedgecock *et al.* 1994; Lacson & Morizot 1991). This
507 pattern of genetic mosaic at both temporal and spatial scales may be explained by several
508 hypotheses. Distinct origin or differential survivorship of recruits, as well as the "sweepstake"

509 hypothesis based on a differential reproductive success leading to instantaneous genetic drift
510 (Hedgecock 1994), have been proposed. In the case of *Z. marina,* which is also a partially
511 clonal species, other factors linked to the specific pattern of temporal recruitment and clonal
512 growth are likely to be involved.
513
514 *Recruitment dynamics and the concept of population*
515
516 According to our results, dispersal does not balance the effect of genetic drift in eelgrass
517 meadows. Three explanations can be advanced for this, the first being that (i) the hypotheses
518 on which the estimates of autocorrelation patterns are based are not met, potentially leading to
519 an overestimation of the dispersal potential. For example, the dispersion is assumed to be
520 isotropic (i.e. equivalent in all directions of 2D space); this is a large assumption, particularly
521 in coastal environments where current regimes are highly complex (Siegel *et al.* 2003). In
522 cases where the conditions required to interpret spatial autocorrelation are met, this apparent
523 discrepancy between expected and realized dispersal may be explained by (ii) low propagule
524 production. This is not in agreement with the extreme clonal richness observed, which reveals
525 an important implication of sexual reproduction in the quadrats of Roscanvel, Arcouest and
526 Sainte-Marguerite. The third hypothesis (iii) is that of low recruitment success of dispersed
527 propagules, possibly due to spatial competition exerted by already-established clones against
528 drifting immigrant propagules. This hypothesis is consistent with previous studies, showing
529 that recruitment in the sea may follow a chaotic distribution (Roughgarden *et al.* 1988) and
530 that the more impacted areas in seagrass meadows (i.e. a lower density of shoots) exhibit
531 greater recruitment success, probably due to a decrease in intraspecific competition (Reusch
532 2006). The influence of the outcompetition of migrants by some fitter clones is also supported

533 by the observation of the highest level of clonal richness in the very recently (re)colonized
534 meadow of Sainte-Marguerite.

535 Two dynamic strategies have been proposed for the settlement and growth of clonal plant
536 meadows (Eriksson 1993): Initial Seedling Recruitment (ISR) and Repeated Seedling
537 Recruitment (RSR). The colonization of an area results either mostly from the recruitment of an
538 initial cohort occupying space through clonal growth (ISR) or from continuous colonization of
539 patches (RSR). For a low level of environmental and demographic fluctuations, the
540 predominant strategy may be ISR, due to the advantage for a seedling to be the first arrived and
541 to acquire "strength in number"" by growing ramets to colonize space through clonal growth
542 before another new recruit arrives. In this case of stable environmental conditions, and thereby
543 demographic conditions, relatively low clonal diversity may also result from competitive
544 exclusion of initially-settled clones, as suggested for species coexistence models (Huston
545 1979) and in the case of the dynamics of *Posidonia oceanica* meadows (Arnaud-Haond *et al.*
546 2010). For an intermediate level of environmental and demographic fluctuations, which
547 reduces the intensity of competitive exclusion, the number of free microsites favors the
548 settlement of new recruits, thereby allowing the turn-over of patches and enhancing genotypic
549 diversity, as described in the experimental approach by Reusch *et al.* (2006). In such cases, the
550 pattern of highest clonal diversity, and probably lowest genetic structure, would reveal the
551 tuning of dynamic strategy toward RSR.

552 As for *Z. marina*, the range of clonal and genetic diversities at the regional scale therefore
553 suggests that both strategies may apply in variable proportions depending on both the time
554 elapsed since the last colonization and the levels of periodic disturbance in the meadows
555 studied. As suggested for *Posidonia oceanica* (Arnaud-Haond *et al.* 2007b), the heterogeneity
556 of spatial and temporal patterns demonstrated here highlight a potentially serious limitation of

557 the use of genetic differentiation as a tool to predict recolonization potential. Such results
558 mean we should be cautious about drawing conclusions from genetic data alone in the absence
559 of further ecological information about local adaptation and/or intra specific competition for
560 space for example.

561 Finally, according to the genetic definition, the genetically differentiated quadrats of *Z.*
562 *marina* would not be considered as belonging to the same population. Yet the pattern reported
563 here leads us to question whether two quadrats belonging to the same continuous meadow at a
564 distance of a few meters should be considered as belonging to distinct populations. This
565 population genetic concept was initially developed for species with exclusively sexual
566 reproduction and may not be relevant for clonal organisms, as suggested by Bahri *et al.*
567 (2009). The ecological population as defined by Camus & Lima (2002) ("*a group of*
568 *individuals of the same species that live together in a area of sufficient size to permit normal*
569 *dispersal and/or migration behaviour and in which numerical changes are largely determined*
570 *by birth and death processes"*), based on their discrete distribution, may be a more objective
571 concept for application to clonal organisms. It should however be noted that, in order to be
572 meaningful in an evolutionary sense, such a concept would rely on the assumption that
573 distance and fragmentation are the main *proxies* for assessing the efficiency of gene flow.

574

575 *Conclusion*

576 This is, to our knowledge, the first time that the detailed screening of within-meadow
577 variance in clonal and genetic composition and differentiation has been performed. This work
578 reveals:

579 - High heterogeneity of clonal and genetic diversities at the regional scale, and the
580 possibility that Brittany (France) could be considered as a hotspot for the genetic diversity of
581 *Z. marina* at the scale of the entire species distribution range;

582 - Strong genetic structure at regional scales revealing dispersal limitations that
583 could potentially influence the future of *Z. marina* populations;

584 - Mosaic structure (genetic patchiness) at the local scale, supporting a Repeated
585 Seedling Recruitment strategy that is likely driven by perturbations opening windows for
586 recruitment;

587 - Large phenotypic plasticity, allowing *Zostera* development in a wide range of
588 environmental conditions. As our results confirm the hypothesis that *Z. marina v. angustifolia*
589 is an ecotype, this phenotypic plasticity is probably characteristic of highly stressful
590 environments.

591

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600

601

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790 **Figure legends**

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792 **Figure 1** Cartography of the 7 intertidal meadows of *Z. marina,* studied here. For each
793 location, two quadrats were determined for sample collection (approximately 35 sampling
794 units were taken from each quadrat).

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797 **Figure 2** Isolation-by-distance for *Z. marina*. The dashed line corresponds to the
798 significant regression combining the two distance scales, indicating that pairs within a location
799 are less distinct than pairs among locations. The left-hand full line corresponds to the
800 regression with pairs within a location (local scale), and the right-hand full line to the
801 regression with pairs among locations (regional scale).

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803 **Figure 3** Spatial autocorrelation analysis of *Z. marina* in quadrat 1 of Arradon. (a) clonal
804 structure and subrange. Kinship estimates from all ramet pairs or only for pairs between
805 ramets showing a different multilocus genotype, and probability of clonal identity (proportion
806 of pairs between ramets with identical MLGs), with confidence limits (for P = 0.975 and P =
807 0.025) based on 1000 permutations of spatial coordinates. (b) A single ramet per multiramet
808 genet was randomly selected to create a 100-genet data file to generate the confidence limits
809 for the correlogram.

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815 **Figure 4** Network topology of the 7 meadows of *Z. marina* studied, based on the Shared
816 Alleles Distance between genets. Only links with distances smaller than or equal to the
817 percolation distance (Dpe = 0.45) are presented. For greater readability, nodes representing
818 genets are not arranged according to their geographic coordinates. For each location, genets of
819 quadrat 1 are represented by ellipses and genets of quadrat 2 by boxes. Colors correspond to
820 sampling locations.
821
822 **Figure S1** Network topologies of *Z. marina* genets at the local scale, based on the Shared
823 Alleles Distance between genets. Only links with values smaller than or equal to the effective
824 percolation distance (Dpe) are presented. Nodes representing genets of quadrat 1 are
825 represented by ellipses and genets of quadrat 2 by boxes. The color legend is the same as that
826 used in Figure 4. A = Arradon, B = Roscanvel, C = Molène, D = Sainte-Marguerite, E =
827 Callot, F = Arcouest, G = Saint-Malo.

828 **Tables**

829 **Table 1** Locations, correspondence with the points surveyed by REBENT network and
830 number of sampling units (SU). For Molène, we also give the number of haphazardly-sampled
831 SU. The inter-quadrat distances were calculated with GPS coordinates.

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Site	Quadrate	Number of SU	Latitude	Longitude	Distance (meters)
Saint-Malo	Q1	35	48°38’923 N	02°01’992 W	85
	Q2	35	48°38’958 N	02°02’038 W	
L’Arcouest	Q1	34	48°49'428 N	03°01'162 W	70
	Q2	34	48°49'425 N	03°01'218 W	
Callot	Q1	35	48°41’064 N	03°54’968 W	30
	Q2	35	48°41’052 N	03°54’982 W	
Sainte-Marguerite	Q1	35	48°35’811 N	04°37’389 W	75
	Q2	35	48°35’830 N	04°37’443 W	
Molène	Q1	32 (12)	48°23’760 N	04°56’934 W	-
Roscanvel	Q1	35	48°19’934 N	04°32’209 W	100
	Q2	35	48°19’984 N	04°32’182 W	
Arradon	Q1	34	47°36’911 N	02°49’636 W	80
	Q2	35	47°36’914 N	02°49’574 W	

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834 **Table 2** Parameters of clonal structure: for each quadrat; samples were standardized with
835 30 ramets. G: number of identified MLLs. R: clonal richness. D* and ED*: Simpson index and
836 its equitability index. β: slope of Pareto distribution. Grey cells indicate values calculated
837 following a procedure of minimal estimation. CR: clonal subrange. Parameters of genetic
838 composition: the two parameters we assessed were heterozygosity and allelic richness. H_e:
839 expected heterozygosity without bias (Nei, 1978); H_o: observed heterozygosity. F_{IS} and LD
840 values were estimated after 1000 permutations of alleles within the quadrat. The mean number
841 of alleles per locus Â was also estimated. Grey values: ns; * : p < 0.05; ** : p < 0.01; *** : p <
842 0.001.

Meadow	quadrate	clonal structure							genetic composition				
		N	G	R	D*	ED*	β	CR	He	Ho	Fis	Â	LD
Arradon	**Q1**	30	26	0.86	0.99	0.65	3.10	5.59	0.52	0.51	0.03	5.89	0.06
	Q2	30	21	0.69	0.97	0.86	2.09	14.56	0.54	0.52	0.03	6.00	0.00
Roscanvel	**Q1**	30	30	1.00	1.00	-	4.95	0.00	0.52	0.51	0.01	4.78	0.06
	Q2	30	21	0.69	0.97	0.88	2.40	12.04	0.50	0.52	-0.05	4.11	0.03
Molène	**Q1**	30	15	0.48	0.85	0.51	1.36	-	0.40	0.40	0.01	3.44	0.05
Sainte-Marguerite	**Q1**	30	28	0.93	1.00	0.52	3.97	2.50	0.54	0.50	0.08*	6.67	**0.50**
	Q2	30	28	0.93	0.99	0.00	4.01	3.04	0.69	0.60	0.14***	9.67	**0.58**
Callot	**Q1**	30	26	0.86	0.99	0.65	2.89	5.32	0.46	0.42	0.08*	5.78	0.06
	Q2	30	23	0.76	0.98	0.85	3.00	7.76	0.45	0.44	0.03	4.89	0.08
l'Arcouest	**Q1**	30	19	0.62	0.91	0.45	1.46	17.01	0.41	0.46	-0.13*	4.11	0.04
	Q2	30	30	1.00	1.00	-	4.95	0.00	0.40	0.45	-0.12**	3.89	0.07
Saint-Malo	**Q1**	30	19	0.62	0.95	0.80	2.05	18.61	0.29	0.35	-0.21**	2.33	0.07
	Q2	30	15	0.48	0.89	0.70	1.54	10.20	0.40	0.39	0.04	3.56	0.00

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846 **Table 3** Matrix of genetic distance (F_{ST}) and geographic distance (kilometers). The
847 geographic distance is expressed in kilometers and ranged from 0.03km for the two quadrats
848 at Callot to 442km between Saint-Malo and Arradon. Fst values are calculated following
849 Weir&Cockerham (1984), for each pair of samples. Grey values: $p < 0.05$. All other values are
850 significant with a probability $p < 0.01$.

		Arr 1	Arr 2	Ros 1	Ros 2	Mol	SMar 1	SMar 2	Cal 1	Cal 2	Arc 1	Arc 2	SMal 1	SMal 2
Arradon	**Q1**	-	0.08	0.15	0.13	0.22	0.30	0.24	0.08	0.12	0.17	0.19	0.19	0.14
	Q2	0.08	-	0.14	0.13	0.25	0.28	0.21	0.12	0.06	0.13	0.13	0.21	0.14
Roscanvel	**Q1**	195	195	-	0.02	0.23	0.21	0.17	0.10	0.15	0.12	0.13	0.19	0.09
	Q2	195	195	0.1	-	0.24	0.23	0.20	0.09	0.14	0.13	0.14	0.23	0.11
Molène	**Q1**	198	198	33	33	-	0.14	0.14	0.14	0.28	0.28	0.33	0.28	0.22
Sainte-Marguerite	**Q1**	239	239	35	35	41	-	0.02	0.22	0.29	0.25	0.28	0.32	0.22
	Q2	239	239	35	35	41	0.08	-	0.20	0.24	0.20	0.23	0.25	0.17
Callot	**Q1**	299	299	134	134	101	60	60	-	0.12	0.10	0.16	0.10	0.04
	Q2	299	299	134	134	101	60	60	0.03	-	0.11	0.07	0.19	0.12
l'Arcouest	**Q1**	367	367	202	202	169	128	128	68	68	-	0.06	0.14	0.06
	Q2	367	367	202	202	169	128	128	68	68	0.07	-	0.17	0.10
Saint-Malo	**Q1**	442	442	277	277	244	203	203	143	143	75	75	-	0.07
	Q2	442	442	277	277	244	203	203	143	143	75	75	0.085	-

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854 **Table 4** Network values of Betweenness Centrality (*BC*) and Clustering coefficient (*CC*).
855 Each value corresponds to one pair of quadrats from the same location. The *BC* column
856 corresponds to the average value of location inside the global network; while the *CC* column
857 corresponds to the average value of *CC* inside local networks.

	BC (*1000)	CC
Arradon	0.73	0.42
Roscanvel	5.93	0.17
Molène	5.03	0.33
Sainte-Marguerite	6.02	0.16
Callot	2.68	0.33
Arcouest	5.15	0.31
Saint-Malo	9.95	0.52
Average	4.9	0.32

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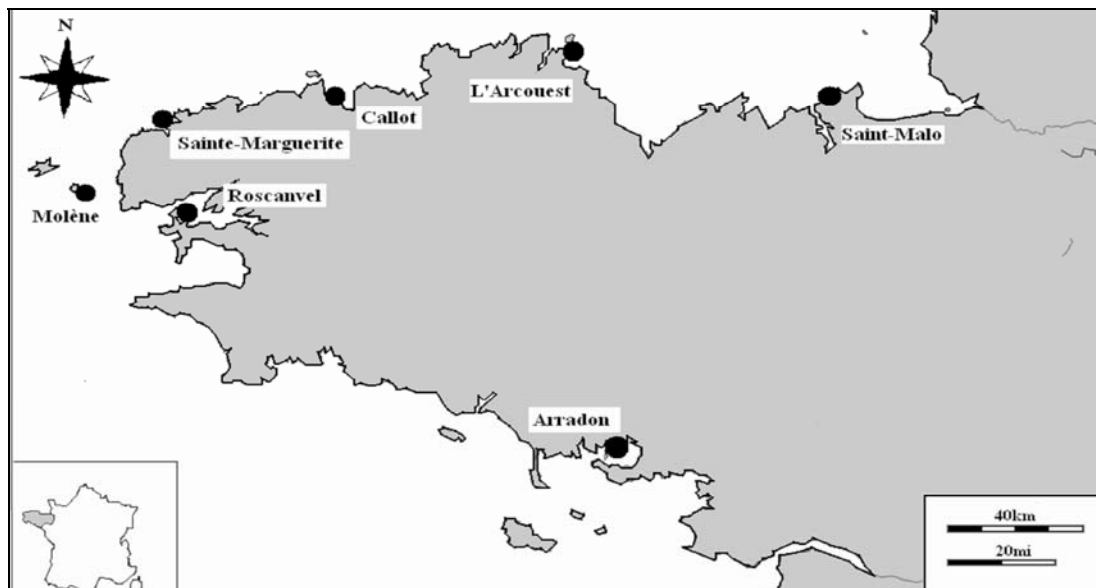
860 Fig.1



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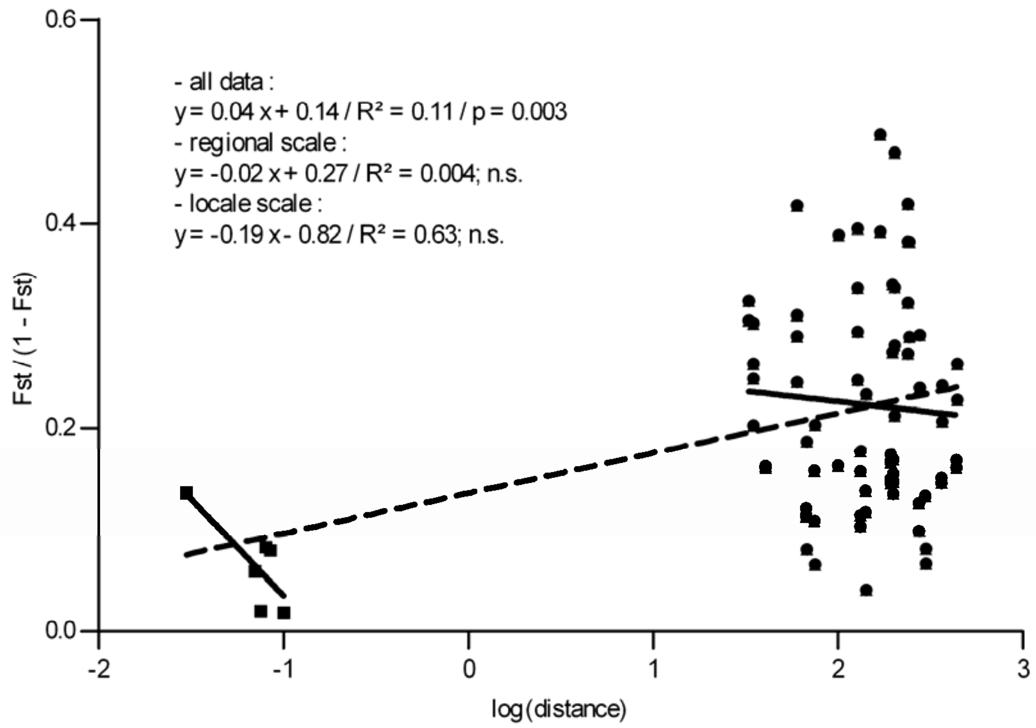
863 Fig. 2



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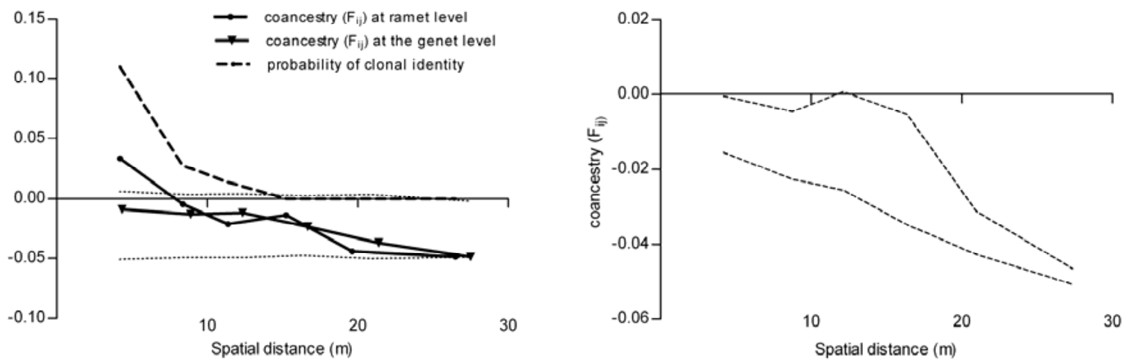
866 Fig. 3



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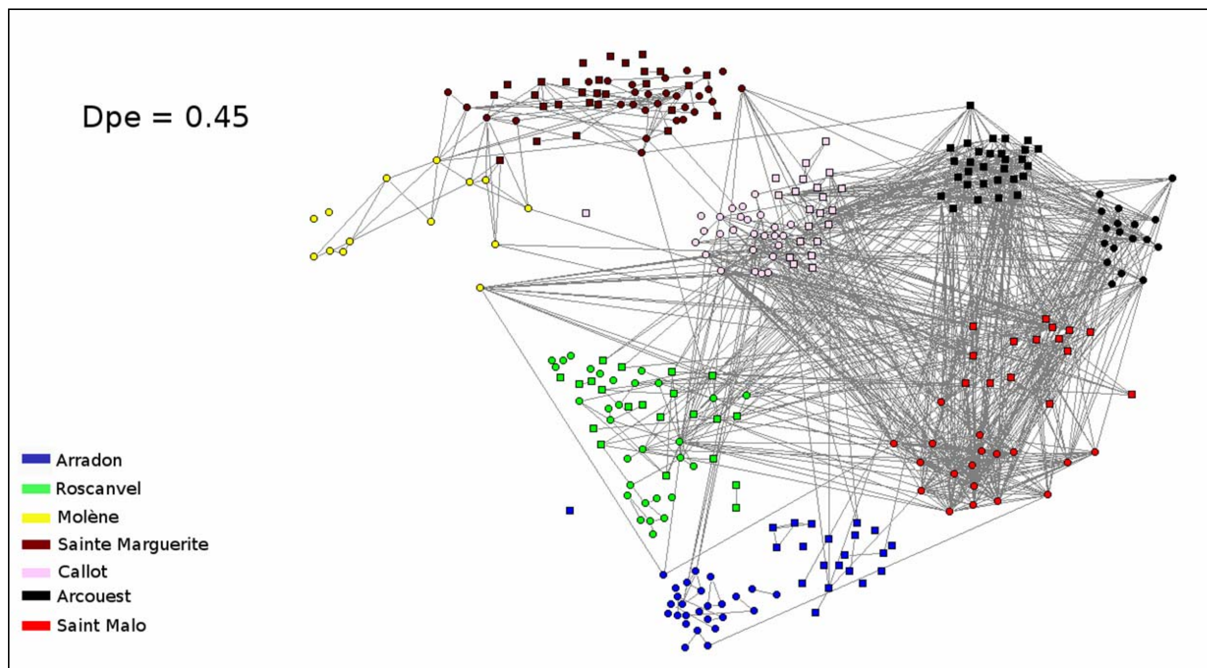
872 Fig. 4



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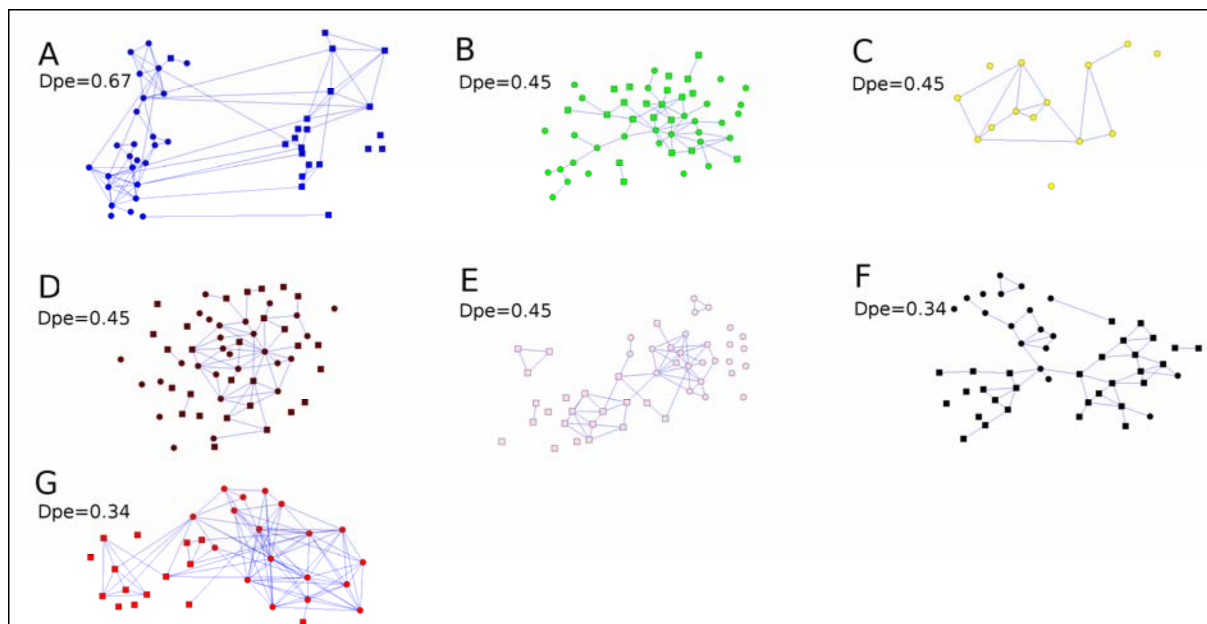
875 Fig. S1



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