

1 **Time and space: genetic structure of the cohorts of the common sea urchin**  
2 ***Paracentrotus lividus* in Western Mediterranean**

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## 20 **Summary**

21 Spatio-temporal variability in settlement and recruitment, high mortality during the first  
22 life-history stages, and selection may determine the genetic structure of cohorts of long-  
23 lived marine invertebrates at small scales. We conducted a spatial and temporal analysis  
24 of the common Mediterranean sea urchin *Paracentrotus lividus* to determine the genetic  
25 structure of cohorts at different scales. In Tossa de Mar (NW Mediterranean),  
26 recruitment was followed over 5 consecutive springs (2006-2010). In spring 2008,  
27 recruits and two-year old individuals were collected at 6 locations along East and South  
28 Iberian coasts separated from 200 to over 1100 km. All cohorts presented a high genetic  
29 diversity based on a fragment of mtCOI. Our results showed a marked genetic  
30 homogeneity in the temporal monitoring, and a low degree of spatial structure in 2006.  
31 In 2008, coupled with an abnormality in the usual circulation patterns in the area, the  
32 genetic structure of the southern populations studied changed markedly, with arrival of  
33 many private haplotypes. This fact highlights the importance of point events in  
34 renewing the genetic make-up of populations, which can only be detected through  
35 analysis of the cohort structure coupling temporal and spatial perspectives.

36

## 37 **INTRODUCTION**

38 The last few years have seen an increase in the number of studies aimed at discerning  
39 temporal genetic structure in marine invertebrates with large dispersal abilities. Long-  
40 lived larval phases theoretically ensure connectivity over large distances in species with  
41 benthic adult phases, determining the genetic composition of populations (Hedgecock  
42 1986; Watts et al. 1990; Sponaugle et al. 2002; Cowen et al. 2006). However, the  
43 composition and survival of these dispersing stages depend on many factors, such as  
44 reproductive success of adult populations, sperm densities, settlement and recruitment  
45 variability, food availability, environmental conditions, currents, etc. These factors may  
46 change between reproductive events, considerably altering the genetic compositions of  
47 cohorts within a site (Planes and Lenfant 2002; Hogan et al. 2010). As a consequence,  
48 genetic exchange between populations may be variable over small temporal and spatial  
49 scales, varying from one site and from one generation to the next (Grosberg and Levitan  
50 1992; Cowen et al. 2000; Hellberg et al. 2002; Hogan et al. 2010). A comprehensive  
51 study of dispersal patterns thus requires the analysis of multiple cohorts in time and in  
52 space (Selkoe et al. 2006), a crucial information that is overlooked when populations are  
53 sampled and analyzed without considering the underlying cohort structure.

54

55 Marine broadcast spawners with external fertilization have potentially a high fecundity,  
56 which can be offset by large variance in reproductive success (Cushing 1990; Levitan  
57 2005). This random variance may affect the effective population size ( $N_e$ ), leading to a  
58 reduction in genetic diversity of cohorts of recruits relative to adult populations  
59 (Hedgecock 1994). Several factors can determine this variance between reproductive  
60 events. Selection at pre-zygotic stages can play a crucial role in fertilization success  
61 (Metz and Palumbi 1996; Palumbi 1999; Zigler et al. 2005). The effect of selection may  
62 vary from one generation to the next depending on factors such as sperm density (i.e.,  
63 Levitan 2002, 2004). During the dispersal phase, larvae may gather in non-homogenous  
64 pools resulting in spatial genetic patchiness (Johnson et al. 1993; Li and Hedgecock  
65 1998). In addition, the crucial settlement and recruitment steps depend not only on  
66 predictable factors, such as substrate type, but also on other variable aspects such as  
67 adult density or food availability (Harrold et al. 1991; Tomas et al. 2004). Besides,  
68 many marine invertebrates present high mortalities over the first years due, among  
69 others, to physical and biological disturbances, predation, competition or selective  
70 pressures (i.e., Gosselin and Qian 1996; Hunt and Scheibling 1997). If some or all of  
71 these processes vary in space and time, fine-scale patchy genetic structure can build up  
72 even in the absence of restricted dispersal (Waples 1998; Banks et al. 2007; Hedgecock  
73 et al. 2007; Botsford et al. 2009). In the long term this genetic signal will be blurred,  
74 since adult populations usually comprise a mixture of several larval pools issued from  
75 different reproductive and dispersal events.

76

77 Sea urchins present a benthic adult phase while dispersal is generally achieved through  
78 planktonic larvae that can remain in the water column from several days to months,  
79 potentially ensuring gene flow between distant populations. Large variations in  
80 settlement and recruitment in sea urchins have been observed from one year to the next  
81 for reasons not fully understood (Ebert 1983; Hereu et al. 2004). In effect, many recent  
82 studies have shown a marked short-scale temporal genetic structure in some species of  
83 sea urchins (Edmands et al. 1996; Moberg and Burton 2000), but not in others (Flowers  
84 et al. 2002). The present paper addresses the study of cohort genetic structure, in space  
85 and time, in the common or purple Mediterranean sea urchin *Paracentrotus lividus*  
86 (Lamarck). This species presents an Atlanto-Mediterranean distribution, including the  
87 islands of Macaronesia (Boudouresque and Verlaque 2001). The common sea urchin is

88 the most abundant echinoid in the shallow Mediterranean sublittoral, and it is relevant  
89 both ecologically, as it is a keystone herbivorous (Boudouresque and Verlaque 2001)  
90 and commercially, as it is harvested for human consumption (Guidetti et al. 2004).

91

92 *Paracentrotus lividus* is an external fertilizer that produces planktotrophic larvae with a  
93 life span of 20-40 days (Pedrotti 1993) that ensures dispersal over thousands of  
94 kilometers. However, changes in gene frequencies have been detected associated to a  
95 hydrological front close to the Gibraltar boundary (Duran et al. 2004; Calderón et al.  
96 2008). Besides, recent studies suggest also a certain degree of genetic structure within  
97 the Mediterranean basin (Maltagliati et al. 2010). The long dispersing stage should  
98 theoretically enable the larval pool to homogenize during its pre-competent phase.  
99 Nevertheless, multiple factors affect the spatial, bathymetric and temporal structure of  
100 the larval pool, resulting in variable settlement and recruitment at small scales (Hereu et  
101 al. 2004; Tomas et al. 2004). In previous studies, neutral (microsatellite) markers have  
102 revealed a shallow differentiation between cohorts in *P. lividus* at a single locality,  
103 Tossa de Mar, in North-Western Mediterranean (Calderón et al. 2009). In another study  
104 at the same locality with a different temporal scale, no population structure was revealed  
105 through the analysis of a fragment of mtCOI of cohorts of different ages, including  
106 recruits (Calderón and Turon 2010). However, selection acting upon the gamete  
107 recognition protein binding may have an important effect upon pre-zygotic stages,  
108 potentially determining a small-scale temporal structure of cohorts (Calderón and Turon  
109 2010). These previous results suggest that there is a large effective number of  
110 progenitors (and thus a poor influence of sweepstake events) although pre-zygotic  
111 events may be important (gamete recognition mechanisms).

112

113 Temporal genetic processes can be examined by sequential sampling through time or by  
114 evaluating genetic data with respect to the age structure of the population sampled at a  
115 single point in time. Calderón and Turon (2010) took the latter approach to investigate  
116 the genetic variation of *Paracentrotus lividus* present in Tossa de Mar. In this approach,  
117 cohorts of different ages were analyzed, including recruits of the year (in 2006 and  
118 2007), as well as 7 cohorts of different ages forming the current adult population (i.e., as  
119 identified by the band pattern, see Calderón and Turon 2010 for details). Previous data,  
120 therefore, had the caveats of having been obtained from a single locality and using  
121 mostly cohorts sampled when they were already several years old, missing all processes

122 that occurred before the year of sampling. In the present work we wanted to use the  
123 alternative, sequential approach to assess cohort variability in this species by sampling  
124 the recruits arrived over 5 consecutive years (2006-2010) at this same locality. At the  
125 same time, and in order to include a spatial perspective, we conducted a spatial survey  
126 at 6 different locations along South and East Iberian coast, sampling individuals  
127 recruited in spring 2006 and in spring 2008.

128

## 129 **MATERIAL AND METHODS**

### 130 **Sampling design**

131 Sampling was designed to capture the structure of cohorts at different temporal and  
132 spatial scales. *Paracentrotus lividus* presents two recruitment episodes in North-  
133 Western Mediterranean, a major event during spring and a minor event during the  
134 autumn (Lozano et al. 1995; Tomas et al. 2004; Ourens et al. 2011). Sampling was  
135 therefore conducted in June, in order to collect the newly arrived recruits of the main  
136 annual cohort. Samples were obtained by scraping off algae from 20 x 20 cm squares (at  
137 least 5 scrapings per sampling). In all localities samples were obtained in shallow (3-6  
138 m deep) sublittoral communities on rocky shores, with well-developed algal  
139 assemblages. They were then preserved in 96% ethanol at -20°C before being carefully  
140 sorted under the stereomicroscope to look for recruits (ca. 1 mm in diameter).

141

142 First, in order to assess the temporal genetic variability of recruitment in Tossa de Mar  
143 (41°43.1'N, 2°56.2'E; Fig. 1), where previous studies on this species were undertaken,  
144 recruits were sampled at exactly the same rocky wall over 5 consecutive springs [2006-  
145 2010; data from 2006 and 2007 were obtained from Calderón and Turon (2010)].  
146 Second, in June 2008 sampling was also conducted at 5 additional locations along South  
147 and East Iberian coasts (separated between 200 and >1100 km; Fig. 1), from similar  
148 habitats to those sampled in Tossa de Mar. In this year, and in order to couple a spatial  
149 and a temporal survey, we collected both recruits, following the methodology described  
150 above, and two-year old individuals (recruited in spring 2006 and collected in June  
151 2008) from the underside of boulders. Available data suggest that individuals between  
152 20 and 30 mm in diameter most likely belong to this age category (Calderón and Turon  
153 2010). We therefore targeted sea urchins within this size range, and their age was  
154 subsequently confirmed by analyzing the band pattern in interambulacral plates revealed  
155 after immersion in xylene under the stereomicroscope (following the method detailed in

156 Calderón and Turon 2010). Sea urchins belonging to the cohort recruited in spring 2006  
157 were retained for genetic analyses. Previous studies have evidenced that the analysis of  
158 band patterns observed in the inside of tests, corresponding to dense deposits of CaCO<sub>3</sub>  
159 incorporated during growing periods, is an accurate method to estimate age in this  
160 species (Turon et al. 1995; Calderón et al. 2009; Calderón and Turon 2010).

161

162 Genomic DNA was directly extracted from whole recruits using DNeasy Tissue Kit  
163 (Qiagen, Valencia, CA) for samples collected between 2006 and 2008 and REExtract-  
164 N-Amp<sup>TM</sup> Tissue PCR (Sigma-Aldrich, Madrid, Spain) for samples collected in 2009  
165 and 2010. For two-year old individuals, gonads (or Aristote's lantern when gonads were  
166 absent) were obtained and used for genomic DNA extraction with REALPURE  
167 extraction kit (Durviz) following manufacturer's instructions. A fragment of the  
168 mitochondrial Cytochrome oxidase I (hereafter COI) was amplified with a specific  
169 primer *P. liv*-F (5'-TCC CAC TAA TGA TTG GAG CA-3'), designed with the  
170 program Primer3 v. 0.4.0 (Rozen and Skaletsky 2000), and the universal primer eCOI-R  
171 (Arndt et al. 1996) using conditions adapted from Duran et al. (2004). PCR products  
172 were vacuum-cleaned (Millipore) and sequenced using ABI PRISM Big Dye v. 3.1  
173 (Applied Biosystems). All sequences were edited and aligned using BioEdit v. 7.0.5.3  
174 (Hall 1999) and deposited in GenBank (accession n. XXXX-XXXX, submitted)

## 175 **Sequence analyses**

176 Analyses involved temporal comparisons of the cohorts recruited along 5 consecutive  
177 springs (2006-2010) in Tossa de Mar as well as spatial comparisons of the 2006 cohort  
178 (collected in 2008) and the 2008 cohort between localities. Furthermore, the cohorts  
179 from 2006 and from 2008 were compared within locations.

180 Haplotype and nucleotide diversities were calculated with DnaSP v.5.10.00 (Librado  
181 and Rozas 2009). The significance of the differences observed in haplotype diversities  
182 between cohorts was determined using an *ad hoc* randomization routine written in  
183 Turbo Pascal. Given the large variation in sample size (from 11 to 29 individuals, see  
184 Table 1), size-corrected haplotypic richness was calculated using the program Contrib  
185 1.02 (Petit et al. 1998). Arlequin v.3.11 (Excoffier et al. 2005) was used to calculate  
186 genetic divergence ( $F_{ST}$ ) between cohorts based on haplotype frequencies. The  
187 significance of comparisons was assessed by performing 10 000 permutations, and *P*-

188 values were corrected for multiple comparisons based on the false discovery rate (FDR)  
189 control following Benjamini and Yekutieli's (2001) method (B-Y), according to which  
190 the critical value is determined by:

$$\alpha / \sum_{i=1}^k (1/i)$$

191  
192 where  $k$  is the number of hypothesis tests performed and  $\alpha$  is the experiment-wise error  
193 rate sought (Narum 2006) which we set at 0.05.

194  
195 Recent studies have questioned the suitability of the commonly used estimator  $F_{ST}$  to  
196 assess population differentiation, as it is highly dependent on the variability of the  
197 marker used (Hedrick 2005; Jost 2008). Therefore, we also computed the new index  
198  $D_{est\_Chao}$  (Chao et al. 2008; Jost 2008), hereafter  $D$ , using the program SPADE (Chao et  
199 al. 2008; Chao and Shen 2010). This program further calculated a confidence interval  
200 around the obtained value by 1 000 bootstrap replicates. We set this confidence interval,  
201 using the normal approximation, at the appropriate  $P$ -value following the FDR  
202 correction as explained above. If 0 is included within this confidence interval, no  
203 evidence for significant differentiation is demonstrated. Further, a MDS analysis was  
204 used to graphically represent the differentiation among cohorts based on  $D$  estimates.

205 Finally, a Mantel test was performed to determine whether a correlation exists between  
206 genetic differentiation of a single cohort at different locations (using both  $F_{ST}$  and  $D$ )  
207 and geographic distances between locations. Distances between sampling sites were  
208 calculated using Google Earth (<http://earth.google.com/>) by measuring the shortest path  
209 by sea between localities.

## 210 **RESULTS**

211 A total of 290 individuals (including 199 recruits and 91 two-year old individuals) were  
212 analyzed (Table 1). A fragment of COI 541-bp long was sequenced, showing 79  
213 variable positions of which 64 corresponded to changes in third positions. Most  
214 sequence changes were neutral, with only 11 amino acid changes determined by all the  
215 substitutions found. Thirty-nine of the variable positions presented substitutions only  
216 once in our data set. Global haplotype diversity was high ( $H=0.894\pm 0.016$ ) and  
217 nucleotide diversity was low ( $\pi=0.00638\pm 0.00029$ ). We identified a total of 108

218 haplotypes of which 77 (71%) were singletons. One single haplotype was present in 89  
219 individuals (ca. 31%) individuals.

220

221 We analyzed a total of 117 recruits arrived at Tossa de Mar over 5 consecutive springs  
222 (2006, 2007, 2008, 2009 and 2010). The fragment of COI sequenced corresponded to 38  
223 haplotypes, globally revealing high haplotype diversity ( $H=0.821\pm 0.033$ ) and low  
224 nucleotide diversity ( $\pi=0.0049\pm 0.00043$ ), as was also observed for each cohort  
225 independently (Fig. 2). No significant differentiation was observed in haplotype  
226 diversity between cohorts (randomization test, all pairwise comparisons  $P>0.125$ ).  
227 Haplotypic richness did not show large variations between cohorts of recruits arrived at  
228 Tossa de Mar (Table 1). Except for Calafat, all populations presented higher haplotypic  
229 richness in the cohorts recruited in 2008 than in two-year old cohorts (recruited in 2006;  
230 Table 1).

231

232 Concerning the spatial study along the Iberian coast, from 38 to 58% of the sea urchins  
233 collected in 2008 actually corresponded to two-year old individuals recruited in spring  
234 2006 according to the band pattern observed in the inside of the tests. Two-year old  
235 individuals were not found in Tarifa. A significant change in haplotype diversity  
236 between cohorts was observed only in one of the 5 localities where both cohorts were  
237 available (randomization test, Jávea,  $P=0.038$ ), where the two-year old individuals had a  
238 significantly higher diversity than the recruits of 2006 (Fig. 3), as reflected also by a  
239 higher haplotypic richness of the former in spite of a lower sample size (Table 1).

240

241 Temporal cohort differentiation was studied at Tossa de Mar. No significant  
242 differentiation in haplotype frequencies was observed in any pairwise comparison of  
243 cohorts recruited between 2006 and 2010 based on  $F_{ST}$  (mean value=-0.0119) and the  
244 same results were obtained when differentiation was computed based on Jost's  $D$  (mean  
245 value=0.0124; Table 2).

246

247 We also computed genetic differentiation between cohorts coupling the temporal and  
248 spatial perspective. The comparison of haplotype frequencies between the two cohorts  
249 (2006 sampled in 2008 and 2008, Table 3) recruited at the same location in the 5  
250 localities for which we have data of both years yielded significantly different results  
251 based on  $F_{ST}$  in Jávea and Carboneras, with Cádiz being marginally significant

252 ( $P=0.051$ , Table 3), or in Jávea, Carboneras and Cádiz according to  $D$ . It is noteworthy  
253 that no haplotype was shared between the Carboneras samples of 2006 and 2008 (thus  
254  $D=1$ ). No significant differentiation was observed in Tossa de Mar or Calafat based on  
255 either estimator (Table 3).

256

257 Concerning spatial differentiation, no significant differentiation was observed in  
258 comparisons between two-year old individuals recruited in 2006 from populations  
259 separated up to 1100 km (Table 4), and results were coherent for both  $F_{ST}$  and  $D$   
260 estimators. Interestingly, however, a large number of significant differentiations were  
261 observed when recruits arrived and collected in 2008 at the same geographic locations  
262 were compared to each other (Table 5). Average differentiation between two-year old  
263 cohorts ( $F_{ST}=0.0136$ ;  $D=0.111$ ) was markedly smaller than that between cohorts of  
264 recruits ( $F_{ST}=0.0467$ ;  $D=0.801$ ). Finally, a significant differentiation based on haplotype  
265 frequencies [ $F_{ST}=0.0123$ ,  $P<0.01$ ;  $D=0.270$  (0.067, 0.506)] was observed when  
266 comparing all recruits arrived in 2008 as a single cohort with all two-year old  
267 populations pooled together.

268

269 A graphical representation of the genetic structure can be obtained by combining the  $D$   
270 values estimated between all cohorts and localities in a MDS plot (Fig. 4). This figure  
271 further evidences the higher differentiation found in the recruits of 2008. Samples of  
272 2008 from Jávea and Carboneras (on the first axis) and from Tenerife and Cádiz (on the  
273 second axis) are set apart from a tight group containing all samples from Tossa de Mar  
274 and all samples from the two-year old individuals (plus the 2008 recruits from Calafat).

275

276 Finally, a Mantel test did not reveal a significant correlation between genetic  
277 differentiation and geographic distance between cohorts for the global dataset ( $F_{ST}$ :  
278  $r=0.177$ ,  $P=0.092$ ;  $D$ : 0.221,  $P=0.064$ ). Nonetheless, when cohorts were analyzed  
279 separately, this same result was obtained for the cohort recruited in 2006 ( $F_{ST}$ :  $r=-0.349$ ,  
280  $P=0.886$ ;  $D$ :  $r=-0.406$ ,  $P=0.880$ ), but a significant correlation was observed for the  
281 cohort recruited in 2008 ( $F_{ST}$ :  $r=0.602$ ,  $P=0.034$ ;  $D$ :  $r=0.488$ ,  $P=0.043$ ).

282

283 **DISCUSSION**

284

285 The data presented in this study constitute an approach to the spatial and temporal  
286 genetic structure of cohorts of the common Mediterranean sea urchin *Paracentrotus*  
287 *lividus*. Our results showed that the partition of genetic variability between temporal and  
288 spatial components is a dynamic feature, and that conclusions extracted either from a  
289 single point in time (e.g., a cohort monitored at different localities) or a single point of  
290 space (e.g., different cohorts sampled at a given locality) may be misleading. Although  
291 our work is based on a single marker (mtDNA sequence data) and more variable genetic  
292 markers (e.g., microsatellites) could reveal more subtle genetic patterns, it is clear that  
293 important structuring in the genetic composition of the recruiting cohorts can be missed  
294 unless sampling in space and time is made at scales wide enough to encompass  
295 potentially infrequent episodes.

296

297 The use of  $F_{ST}$ -related statistics as a measure of population differentiation has been  
298 recently challenged (Hedrick 2005; Jost 2008) because of its dependency on within-  
299 population diversity, leading to counter-intuitive results when markers are highly  
300 variable. Alternatives to these statistics have been proposed and discussed (Hedrick  
301 2005; Jost 2008, 2009; Ryman and Leimar 2009). In the present study, we follow the  
302 criterion in Meirmans and Hedrick (2011), who advocate a combined use of  $F_{ST}$  and  
303 other estimators such as  $D$  to obtain the maximum possible information based on the  
304 properties of the different statistics.  $F_{ST}$  and related measures may be more suited to  
305 analysis of demographic parameters, such as migration rate, while  $D$  has the right  
306 properties to measure differentiation based on allele frequencies (Jost 2009). In  
307 addition,  $F_{ST}$  measures have been used for decades and reporting them is useful for  
308 comparative purposes (Meirmans and Hedrick 2011). In the present study, both  
309 statistics provided similar results for the 2006 cohort although, globally,  $D$  estimator  
310 yielded more significant outcomes, especially for the 2008 cohort, and more accurately  
311 detected differentiation between cohorts (i.e., two cohorts with no shared allele have a  $D$   
312 value of 1), which was the primary interest of this paper.

313

314 A summary of our results based on the  $D$  estimator of genetic differentiation is  
315 presented in Fig. 5. An additional measure of the differentiation among adult  
316 populations was obtained by reanalyzing data to obtain  $D$  values from Duran et al.  
317 (2004) from 6 so-called adult populations within the same spatial area (localities 1, 2, 4,  
318 5, 6, and 7 in Duran et al. 2004) using the same marker. Those populations were

319 sampled without regard to their age structure and thus represent a mixture of cohorts  
320 potentially averaging out yearly differences. Variability between those adult populations  
321 is ca. 20 times higher than that found between cohorts from Tossa de Mar (Fig. 5).  
322 Likewise, spatial differentiation within a single recruitment event (spring 2006 or spring  
323 2008) was higher than values of inter-cohort comparisons in Tossa de Mar (Fig. 5).  
324 Taken together, these results would lead to the conclusion that the spatial component  
325 plays a stronger role than the temporal component in differentiation between cohorts  
326 within this species. However, the situation was drastically different between 2006 and  
327 2008. *D* values between cohorts arrived at different localities during the recruitment  
328 event in 2008 were 8 times higher than in 2006, and differentiation in temporal  
329 comparisons between the two cohorts at a given locality was 40 times higher than the  
330 inter-cohort comparisons in Tossa de Mar (Fig. 5).

331  
332 Had we only undertaken the temporal study in Tossa de Mar and the spatial analysis of  
333 the cohort recruited in 2006, we would have concluded that temporal variation within  
334 localities was negligible and that differentiation among localities for a single cohort,  
335 although higher, was not significant at this spatial scale. However, the analysis of the  
336 2008 cohort clearly showed that marked spatial and temporal cohort differentiation can  
337 occur at some years at least. Our results were largely dependent on patterns occurred in  
338 the southern localities studied (Cádiz, Tarifa, Carboneras and, to a lesser extent, Jávea),  
339 while genetic composition of recruits of northern localities (Calafat and, in particular,  
340 Tossa de Mar), remained constant within the time-frame studied (see Fig. 3). A  
341 thorough analysis of the composition of the 2008 cohort evidenced the arrival of a large  
342 number of private haplotypes, mainly in Carboneras (12 out of 12 haplotypes) and  
343 Cádiz (9 out of 15). This suggests that individuals recruited in 2008 at these localities  
344 most likely belonged to different genetic pools, apparently with a marked spatial  
345 heterogeneity. This heterogeneity is probably behind the significant relationship  
346 between genetic differentiation and geographic distance (Mantel test) detected in 2008,  
347 but not in the cohort recruited in 2006, which was much more homogeneous.

348  
349 The contrasting patterns of genetic structure in 2006 and 2008 raise questions about the  
350 reasons behind such variation and which the “anomalous” year is (if any can be  
351 described as such). To advance possible answers to these questions, we sought for  
352 hydrological features in the zone studied. The sampled shores are located in the Balearic

353 Sea and the Alborán Sea, two sub-basins of the Western Mediterranean whose  
354 circulation patterns are well known (López-Jurado et al. 1996; Pinot et al. 1996;  
355 Bouffard et al. 2010). In short, in the Iberian coast, a predominantly southward current  
356 flows from the Gulf of Lions to the Alborán Sea, keeping Atlantic waters restricted to  
357 the southern part of the basin. However, alterations of this pattern have been described.  
358 For instance, an intense anticyclonic eddy was established in 1998 in the Balearic Sea,  
359 which reversed the usual pattern of southward circulation along the littoral (Pascual et  
360 al. 2002). This abnormality had important effects on larval distribution of several  
361 species of fish (Olivar et al. 2003). Interestingly, in spring 2008, another powerful  
362 anticyclonic gyre became established in the Balearic Sea (Bouffard et al. 2010),  
363 allowing Atlantic waters to reach further North than usual along the Iberian  
364 Mediterranean shores (Tintoré, pers. comm.). This abnormality in 2008 nicely fits our  
365 results, as the genetic patterns in the southern populations were changed (particularly in  
366 Carboneras), while no changes were observed in the north (Calafat and Tossa).  
367 Although evidence is purely correlational, the fact that 2008 was an abnormal year as  
368 regards patterns of circulation in the study area strongly points towards hydrological  
369 features as the cause of the genetic structures found. These results highlight the potential  
370 role of punctual, infrequent episodes of altered circulation in providing a genetic  
371 renewal to established populations.

372

373 Of course, other factors can also determine changes in the genetic composition of  
374 cohorts recruiting in a given area between consecutive reproductive events. Some  
375 populations may experience systematically a higher degree of self-recruitment than  
376 others due to micro or mesoscale topographic patterns. Reproductive periods in  
377 *Paracentrotus lividus* may be slightly variable from year to year (e.g., Lozano et al.  
378 1995) and from one area to another (Ourens et al. 2011). As a result, larval batches can  
379 be subject to various prevailing oceanographic conditions at different points of space or  
380 time. This may explain the marked variability in the strength of the yearly cohorts  
381 which has been repeatedly noted in this species (López et al. 1998; Tomas et al. 2004).  
382 Differences also exist between different habitats (Tomas et al. 2004) but were  
383 minimized here by sampling the same type of habitat in all localities. In addition,  
384 selection can be acting at different pre-zygotic (e.g., Calderón and Turon 2010) or post-  
385 zygotic stages, and selection pressures themselves may vary spatially and temporally  
386 (e.g., years of rich or poor planktonic food sources for the larvae). Finally, it must be

387 noted that we have analyzed only the main recruitment event occurring in June of every  
388 year, but *P. lividus* has a second, smaller, recruitment in autumn in the study area  
389 (López et al. 1998; Tomas et al. 2004). It would have been instructive to assess whether  
390 this second recruitment event showed patterns similar to the June episode. Overall,  
391 longer time series coupled with circulation data and models are necessary to reliably  
392 establish a causal link between the different potential factors and the realized  
393 recruitment of species with long-lived larvae in the South-East Iberian shores.

394

395 In Tossa de Mar, combining the present work with data from Calderón and Turon  
396 (2010) we could monitor the cohort of 2006 at the year of recruitment, and after one  
397 (2007) and two (2008) years. In this cohort, no significant change occurred in genetic  
398 diversity over time (mean±SD: 0.847±0.047, 0.819±0.082, 0.825±0.059, in 2006, 2007  
399 and 2008, respectively, all randomization tests  $P>0.5$ ). Likewise, no differences in allele  
400 frequencies were found (overall  $F_{ST}=0.028$ , overall  $D=0.117$ , not different from 0). This  
401 result suggests that genetic composition of cohorts does not experience important  
402 changes as the cohort grows older. Large mortalities occur during the first stages of  
403 benthic life in most marine invertebrates in general (Gosselin and Qian 1996; Hunt and  
404 Scheibling 1997), and *Paracentrotus lividus* in particular (Turon et al. 1995; López et  
405 al. 1998). A reduction of genetic diversity over these crucial first stages of benthic  
406 existence would therefore be expectable (e.g., Pini et al. 2011). However, our results do  
407 not support this idea, possibly due to the huge numbers of recruits that can settle in a  
408 given locality (López et al. 1998).

409

410 Previous studies have shown contradictory results about the temporal structure in sea  
411 urchins (Palumbi and Wilson 1990; Edmands et al. 1996; Moberg and Burton 2000; but  
412 see Flowers et al. 2002). Sweepstake events, whereby only a random subset of adult  
413 individuals contributes to each reproductive event (Hedgecock 1994), can explain  
414 stochastic changes in recruits' composition. In our case, however, sweepstake effects  
415 were not detected for *Paracentrotus lividus* using nuclear and mitochondrial markers  
416 (Calderón et al. 2009; Calderón and Turon 2010). It might be noted, however, that those  
417 studies were performed in Tossa de Mar, where a marked constancy in the genetic  
418 make-up of the cohorts in the 5 years monitored has been found in the present study.  
419 This pattern may be far from general, as shown in other localities of the present study.  
420 Thus, a stochastic component of variability related to arrival of different pools of larvae

421 may be determinant to obtain genetically diverse populations, at least in some areas and  
422 over long temporal frames. Care must be taken when interpreting results of connectivity  
423 derived from single time-point genetic analyses between populations of this  
424 commercially interesting species for which massive collection is leading to population  
425 depletion in many areas (Guidetti et al. 2004).

426

427 In conclusion, our study showed a hidden source of genetic variability that could only  
428 be unveiled by tracing the genetic structure of cohorts over a spatial and temporal  
429 scenario. This is likely applicable to other marine species. The widely used approach of  
430 studying populations by combining different cohorts neglects this important source of  
431 variability. Relatively high genetic homogeneity among cohorts recruiting over the  
432 years at a given locality may be common. However, episodes of genetic “renewal” do  
433 occur, which contribute to the diversity and variability of the populations by seeding  
434 them with larvae coming from different sources. This temporal variability may be  
435 linked to changes in current patterns, to differences in reproduction timing and success,  
436 or to a combination of these and other factors. Analyses focusing on cohorts of settling  
437 recruits can yield important spatial and mechanistic insights into patterns of larval  
438 dispersal (Selkoe et al. 2006). Further research should try to couple genetic variability  
439 of cohorts at larger spatio-temporal scales with biological and oceanographic features.  
440 *Paracentrotus lividus* is an ideal case study for the analysis of the implications of  
441 dispersal and genetic diversity of cohorts, for its long-lived larval strategy, for its  
442 ecological importance as a keystone herbivore and because it is an economic resource  
443 fished in much of its distribution range.

444

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452

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598

599 **Figure legends**

600

601 **Fig. 1** Sampling locations for *Paracentrotus lividus* along East and South Iberian coast. Tossa de Mar was  
602 sampled over 5 consecutive springs (June 2006-2010). The 5 additional locations were only sampled in  
603 June 2008, both for recruits and two-year old individuals

604

605 **Fig. 2** Haplotype diversity of 5 cohorts of recruits arrived at Tossa de Mar in 5 consecutive years (spring  
606 2006-spring 2010). Vertical bars represent standard deviations

607

608 **Fig. 3** Values of haplotype diversity between two different cohorts (2006, two-years old, in black) and  
609 2008 (recruits of the year, in grey) at the 6 localities studied. Bars represent standard deviations. Asterisk  
610 mark significant differences in haplotype diversity assessed by a randomization test

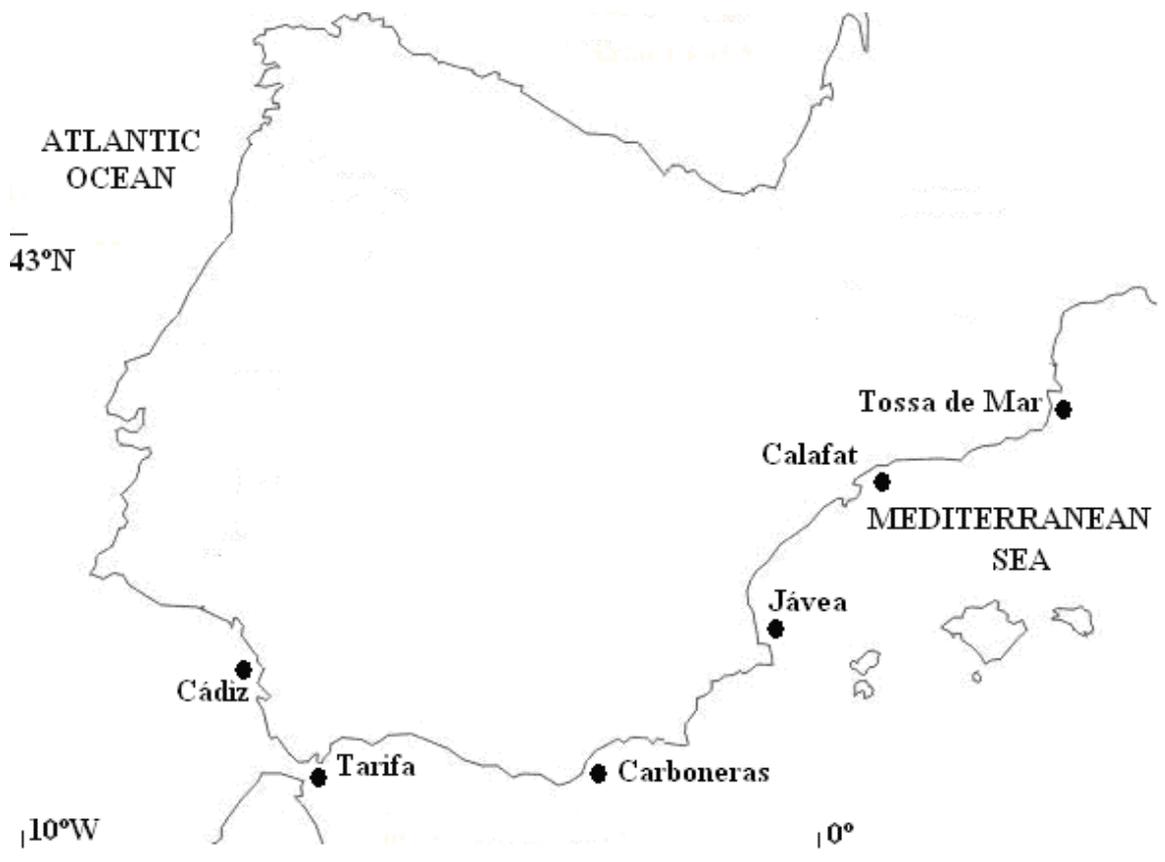
611

612 **Fig. 4** MDS plot obtained from the dissimilarity matrix of  $D$  values among all cohorts and localities. The  
613 inset shows an enlarged view of the cluster of points indicated. The stress of the final configuration was  
614 0.038

615

616 **Fig. 5** Representation of  $D$  values of dissimilarity obtained among adult populations (data from Duran et  
617 al. 2004) and from the different sets of cohort comparisons performed in the present study. Bars represent  
618 standard errors

Fig. 1



**Fig. 2**

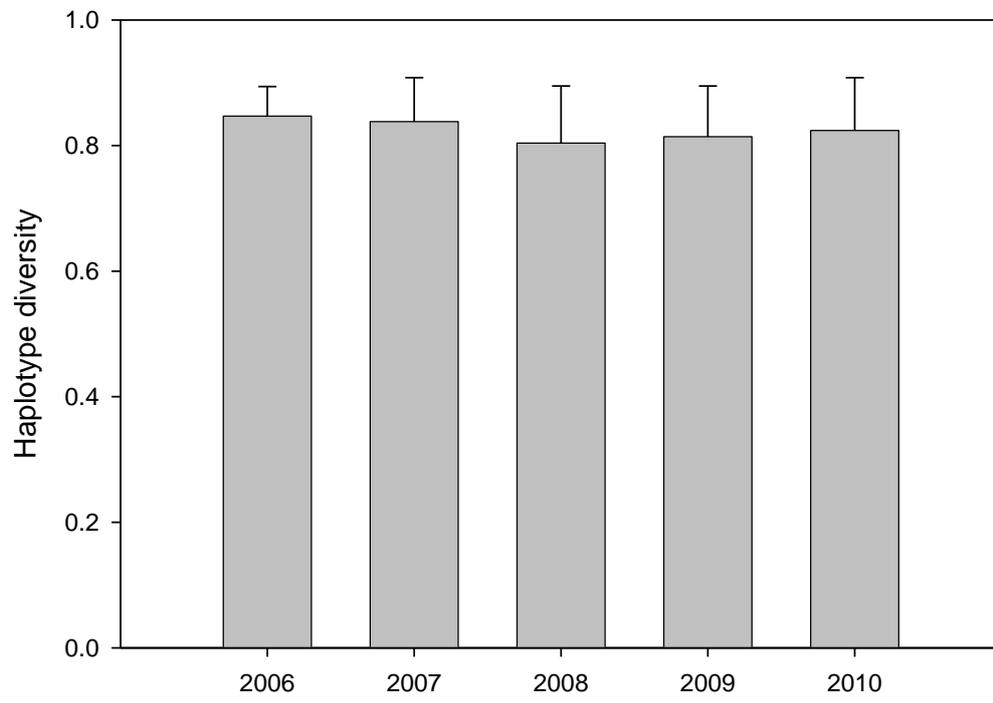


Fig. 3

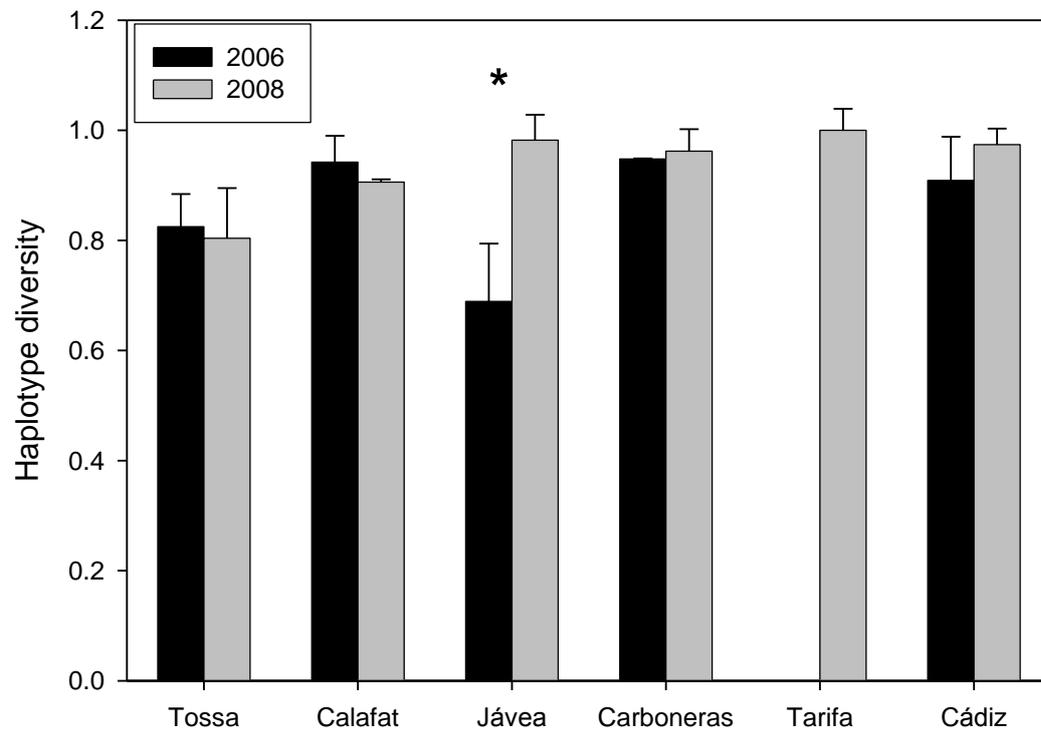


Fig. 4

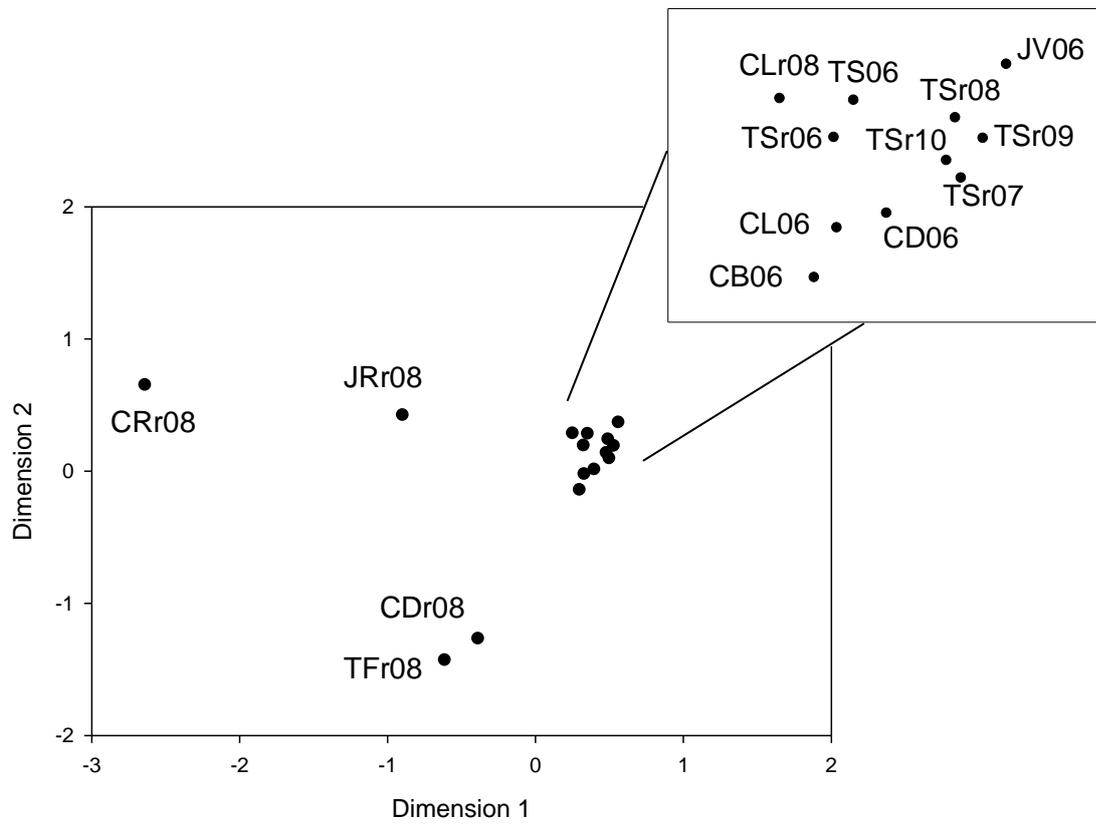
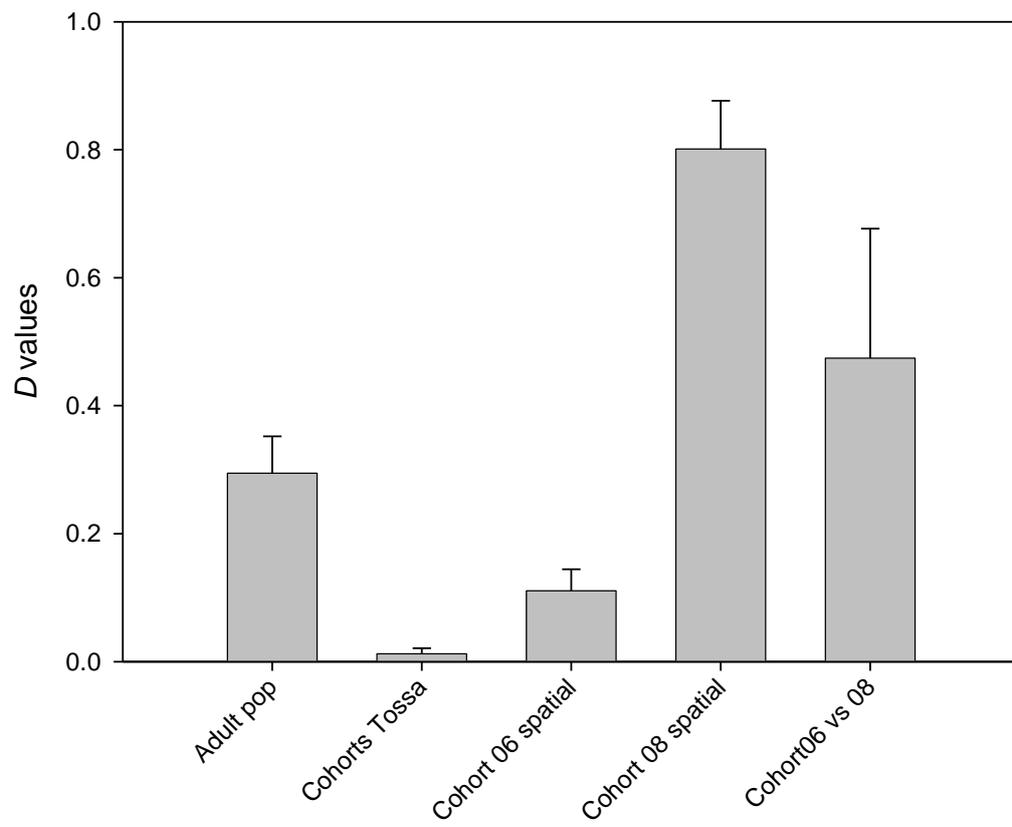


Fig. 5



Location	Year of recruitment	Year of collection	Code	Sample size	Number of haplotypes (private haplotypes)	Haplotypic richness
Tossa de Mar	2006	2006	TSr06	28	11 (4)	5.238
Tossa de Mar	2007	2007	TSr07	27	15 (5)	6.205
Tossa de Mar	2006	2008	TS06	29	13 (7)	5.376
	2008	2008	TSr08	18	9 (6)	5.392
Tossa de Mar	2009	2009	TSr09	23	12 (5)	5.783
Tossa de Mar	2010	2010	TSr10	21	12 (3)	6.024
Calafat	2006	2008	CL06	16	12 (4)	7.789
	2008	2008	CLr08	19	12 (7)	6.767
Jávea	2006	2008	JV06	20	7 (3)	3.937
	2008	2008	JVr08	11	10 (5)	9.000
Carboneras	2006	2008	CB06	22	16 (8)	8.006
	2008	2008	CBr08	15	12 (12)	8.267
Cádiz	2006	2008	CD06	12	9 (3)	7.333
	2008	2008	CDr08	18	15 (9)	8.764
Tarifa	2008	2008	TFr08	11	11 (5)	10.000
<b>Total number of recruits</b>				191	82	
<b>Total number of two-year old adults</b>				99	41	
<b>Total number of individuals</b>				290	108	

Table 1. Locations of collection, year of recruitment, collection and sample sizes for the cohorts analyzed. Code lists short names given to each sample: two upper case letters designate locality, two numbers refer to year of recruitment, and lower case “r” means that the sample in question consisted of recruits of the year. Note that the 2006 cohort at Tossa de Mar was collected in 2006 (TSr06) and two years later (TS06). Number of haplotypes (haplotypes exclusive to one population in brackets) and haplotypic richness (corrected for sample size) are also presented.

<b>Tossa de Mar</b>	<b>2006</b>	<b>2007</b>	<b>2008</b>	<b>2009</b>	<b>2010</b>
<b>2006</b>	0	0.077 (0, 0.395)	0 (0, 0.310)	0.047 (0, 0.356)	0 (0, 0.299)
<b>2007</b>	0.0115 (0.1942)	0	0 (0, 0.292)	0 (0, 0.181)	0 (0, 0.171)
<b>2008</b>	-0.0099 (0.5604)	-0.0128 (0.7496)	0	0 (0, 0.245)	0 (0, 0.225)
<b>2009</b>	0.0058 (0.2871)	-0.0117 (0.7545)	-0.0213 (0.8749)	0	0 (0, 0.152)
<b>2010</b>	-0.0086 (0.5738)	-0.0196 (0.9453)	-0.0256 (0.9529)	-0.0275 (0.9999)	0

Table 2. Cohort differentiation between temporal samples from Tossa de Mar. Only recruits collected at the same year of arrival (spring 2006 to spring 2010) are analyzed. Lower diagonal are  $F_{ST}$  estimates ( $P$ -values in brackets). Upper diagonals represent  $D$  values (confidence intervals bounded between 0 and 1 in brackets). Following a FDR correction,  $P$ -values for significance (and confidence interval limits) were set at 0.017.

Locality	$F_{ST}$ ( <i>P</i> -value)	$D$ (IC <sub>95%</sub> )
Tossa	-0.01258 (0.6498)	0 (0, 0.256)
Calafat	-0.0067 (0.5102)	0 (0, 0.419)
Jávea	0.1323 ( <b>0.0063</b> )	0.713 ( <b>0.233, 0.930</b> )
Carboneras	0.04523( <b>0.0039</b> )	1 ( <b>1,1</b> )
Cádiz	0.03505 (0.0512)	0.659 ( <b>0.241, 0.854</b> )

Table 3.  $F_{ST}$  and  $D$  estimates of genetic differentiation between the cohorts of 2006 (sampled in 2008) and 2008 at 5 localities;  $P$ -values for  $F_{ST}$  and confidence interval for  $D$  are presented in brackets. Significant values at  $P=0.05$  are presented in bold.

	<b>TS06</b>	<b>CL06</b>	<b>JV06</b>	<b>CB06</b>	<b>CD06</b>
<b>TS06</b>	0	0.095 (0, 0.516)	0.097 (0, 0.367)	0.220 (0, 0.585)	0.079 (0, 0.526)
<b>CL06</b>	0.0096 (0.2499)	0	0.229 (0, 0.648)	0 (0, 0.351)	0 (0, 0.345)
<b>JV06</b>	0.0243 (0.1197)	0.0466 (0.0613)	0	0.302 (0, 0.656)	0.085 (0, 0.557)
<b>CB06</b>	0.0257 (0.0653)	-0.0109 (0.7795)	0.0583 (0.0233)	0	0 (0, 0.303)
<b>CD06</b>	0.0066 (0.2888)	-0.0214 (0.9192)	0.0164 (0.2289)	-0.0221 (0.9227)	

Table 4. Differentiation between samples from different Iberian localities for the cohort of 2006 sampled in 2008. Codes as in Table 1. Lower diagonal shows  $F_{ST}$  estimates ( $P$ -values in brackets). Upper diagonal represents  $D$  values (confidence intervals bounded between 0 and 1 in brackets). Following a FDR correction,  $P$ -values for significance (and confidence interval limits) were set at 0.017.

	<b>TSr08</b>	<b>CLr08</b>	<b>JVr08</b>	<b>CBr08</b>	<b>TFr08</b>	<b>CDr08</b>
<b>TSr08</b>	0	0.028 (0, 0.427)	<b>0.657</b> <b>(0.162, 0.884)</b>	<b>1 (1, 1)</b>	<b>1 (1, 1)</b>	<b>0.789</b> <b>(0.446, 0.948)</b>
<b>CLr08</b>	-0.0018 (0.4057)	0	<b>0.566</b> <b>(0.057, 0.796)</b>	<b>1 (1, 1)</b>	<b>1 (1, 1)</b>	<b>0.776</b> <b>(0.463, 0.908)</b>
<b>JVr08</b>	0.0745 <b>(0.0146)</b>	0.0298 (0.1123)	0	<b>1 (1, 1)</b>	<b>1 (1, 1)</b>	<b>0.845</b> <b>(0.586, 0.901)</b>
<b>CBr08</b>	0.1188 <b>(0.0006)</b>	0.06644 <b>(0.0008)</b>	0.0284 (0.0572)	0	<b>1 (1, 1)</b>	<b>1 (1, 1)</b>
<b>TFr08</b>	0.1039 <b>(0.0038)</b>	0.0492 (0.0278)	0.0091 (0.4741)	0.0196 (0.1465)	0	0.357 (0, 0.537)
<b>CDr08</b>	0.0886 <b>(0.0009)</b>	0.0461 (0.0111)	0.0174 (0.1316)	0.0320 <b>(0.0072)</b>	-0.0016 (0.5355)	0

Table 5. Differentiation between samples from different Iberian localities for the cohort of 2008 sampled the same year. Codes as in Table 1. Lower diagonal represents  $F_{ST}$  estimates ( $P$ -values in brackets). Upper diagonal represents  $D$  values (confidence intervals bounded between 0 and 1 in brackets). Values in bold indicate significant comparisons. Following a FDR correction,  $P$ -values for significance (and confidence interval limits) were set at 0.015.