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**SNP-based parentage analyses over two successive generations demonstrates the feasibility of efficient production of inbred lines in the Pacific oyster (*Crassostrea gigas*) by self-fertilization of simultaneous hermaphrodites despite severe inbreeding depression**

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1 **SNP-based parentage analyses over two successive generations demonstrates the**  
2 **feasibility of efficient production of inbred lines in the Pacific oyster (*Crassostrea gigas*)**  
3 **by self-fertilization of simultaneous hermaphrodites despite severe inbreeding**  
4 **depression.**

5  
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15  
16 **Running title: Self-fertilization in *Crassostrea gigas***

17

18 **Abstract**

19

20 The Pacific oyster, *Crassostrea gigas*, is a species in which true male and true female well as  
21 hermaphrodite individuals have been reported. Among the latter, most are sequential, with  
22 one or more sex changes throughout their live, while a few (<2%) are simultaneous  
23 hermaphrodites (SH) which may self-fertilize. Sex determinism and its functional bases  
24 remain unclear in this species. In this study, one SH oyster was found among a mature  
25 broodstock and used to produce a progeny by self-fertilization. Several thousand offspring  
26 were obtained for this family (SF1) but only 73 SF1 oysters survived following an episode of  
27 mass mortality at the spat stage. Six of them were conditioned for reproduction and three  
28 were found to be SH, and one produced a limited but viable progeny by self-fertilization  
29 (SF2). Oysters were sampled and genotyped with 226 SNP markers, confirming that the SF1  
30 and the SF2 oysters were produced by self-fertilization over two successive generations. To  
31 our knowledge, it is the second study reporting the feasibility of production on an inbred line  
32 of Pacific oysters by self-fertilization using a simultaneous hermaphrodite as primary genitor,  
33 and the first study to generate two successive generations of selfing. Observed mean  
34 inbreeding coefficient of SF2 oysters produced by selfing for two generations was 0.61,  
35 ranging from 0.46 to 0.80. This coefficient was lower than the expected theoretical value  
36 (0.8125), suggesting that the most homozygous offspring died at early stages due to  
37 inbreeding depression. Our study demonstrates that inbred lines of Pacific oysters can be  
38 produced by self-fertilization but may exhibit higher heterozygosity than theoretically  
39 expected due to better survival of the most heterozygous offspring.

40

41 *Keywords:* self-fertilization; inbreeding; Pacific oysters; hermaphroditism; *Crassostrea gigas*

## 42 1. Introduction

43

44 Crossing between related individuals, including selfing, gynogenesis and androgenesis, is  
45 used in numerous plant and animal species to produce isogenic or highly inbred lines. Such  
46 lines are key resources that are widely used to study the effect of the environment on  
47 phenotypes, mapping quantitative trait loci or for genetic improvement through heterosis.  
48 Gynogenesis and androgenesis can successfully produce isogenic lines in only one or two  
49 generations (e.g. in the rainbow trout: (Quillet et al., 2007)), but those approaches remain  
50 unsuccessful in many species. For oyster species, gynogenesis was successful in *Crassostrea*  
51 *gigas* (Guo and Gaffney, 1993; Li and Kijima, 2006), while Li et al. (2004) managed to  
52 induce haploid androgenesis embryos that died before reaching the D-shaped stage.  
53 Alternatively, self-fertilization of hermaphroditic species results to faster inbreeding than  
54 crossing between siblings (Falconer and Mackay, 1996). Self-fertilization has been reported  
55 in mollusk species especially in functional simultaneous hermaphrodites, such as giant clams  
56 *Tridacna derasa*, *T. squamosa*, *T. crocea* (Zhang et al., 2020), scallops *Argopecten irradians*  
57 (Zheng et al., 2012), *Argopecten purpuratus* (Martinez et al., 2007), *Argopecten circularis*  
58 (Ibarra et al., 1995), *Aequipecten tehuelchus* (Narvarte and Pascual, 2003) or *Pecten maximus*  
59 (Beaumont, 1986; Beaumont and Budd, 1983). For successive hermaphrodite species, such as  
60 cupped oysters, several articles also reported attempts to produce inbred progenies using  
61 cryopreserved sperm to fertilize sex-reversed females. Lannan (1971) first reported  
62 successful fertilization of ova in *C. gigas* using this approach, but no information was given  
63 about the faith of the obtained embryos. A similar approach was also successful in *C.*  
64 *virginica* and self-fertilization was confirmed using microsatellite genotyping of the resulting  
65 eyed-larvae, but none managed to survive beyond the larval stage (Yang et al.,  
66 2015). Survival beyond settlement and metamorphosis of larvae resulting from selfing using

67 cryopreserved sperm remained to be demonstrated due to high mortality of inbred progenies.  
68 This likely to be related to the strong inbreeding depression observed at early life stages  
69 resulting from the high genetic load that has been well demonstrated in *C. gigas* through the  
70 study of segregation distortion (Launey and Hedgecock, 2001). Meanwhile, inbred lines have  
71 been successfully developed by brother-sister mating for genetic improvement through  
72 crossbreeding (Hedgecock and Davis, 2007; Yin and Hedgecock, 2019).

73 World oyster production jumped from 3.6 million tons in 2000 to 5.7 million tons 2017  
74 (FAO, 2019), and the Pacific oyster is the one of the main oyster species cultivated  
75 worldwide. Its sex determinism and associated molecular mechanisms have been investigated  
76 but remains unclear (Dheilly et al., 2012; Han et al., 2021; Yue et al., 2018; Zhang et al.,  
77 2014), notably because this species shows dioecy, sequential/successive hermaphrodites as  
78 well as a low frequency (<2%) of simultaneous hermaphrodites (Broquard et al., 2020; Guo  
79 et al., 1998). To our knowledge, only one previous study reported the production of oysters  
80 by using a simultaneous hermaphrodite. Thus, Hedgecock et al. (1995) used Pacific oysters  
81 produced by selfing, confirmed using allozyme markers, to generate inbred lines indicating  
82 that selfing oysters can survived beyond the larval stage, and that they are capable to produce  
83 a new generation by crossing males and females . In our study, we report the production of  
84 viable adult Pacific oysters over two successive generations of selfing of simultaneous  
85 hermaphrodites. The main objective was to demonstrate the feasibility to produce inbred  
86 oysters by self-fertilization over successive generations, confirm parentage using molecular  
87 DNA markers and estimate the level of inbreeding of the resulting progeny.

88

## 89 **2. Materials and methods**

90 Unless specified, all crosses and rearing of the biological material was conducted at Ifremer's  
91 shellfish hatchery in La Tremblade, Charente Maritime, France.

## 92 2.1. Broodstock

93 The pedigree of the oysters is given in supplementary Table 1 and illustrated in Fig.1. Bi-parental  
94 crosses were performed in March 2013 by crossing wild oysters samples in the Marennes-Oléron  
95 Bay in 2012 as described by Azéma et al. (2017). Among the resulting full-sib families, some  
96 of them (including family "G0-H17") proved to be highly resistant to OsHV-1 and were  
97 therefore selected for the next generation. Among those, G0-H17 oysters were conditioned in  
98 a 240 L tank in January 2015: seawater temperature was gradually increased to 21°C over  
99 one week. Seawater flow was 400 L/h, and a cultured phytoplankton diet (*Isochrysis galbana*,  
100 *Tetraselmis suecica*, and *Skeletonema costatum*) was provided *ad libitum* (i.e. 50,000  
101 cells/mL). Mass spawning was obtained following a thermal shock on March 2015 using 14  
102 full-sib G0-H17 oysters, producing the G1-H17 progeny (Fig.1). Individual sex of each G0-  
103 H17 oyster used in this cross was not recorded but we assumed that each offspring resulted  
104 from two parents, i.e., one male and one female as reported by the pedigree provided in  
105 supplementary table 1.

106

## 107 2.2. First selfing-cross using a simultaneous hermaphrodite to produce family SF1

108 In June 2016, oysters of the G1-H17 progeny were ready to reproduce (i.e. presenting mature  
109 gonads), and a new generation of the H17 progeny was planned to be produced by stripping  
110 the gonad of the progenitors. This step required the sex determination with a microscope. One  
111 G1-H17 oyster was found to be a simultaneous hermaphrodite (SH1), i.e. presenting both  
112 male and female gametes with a higher proportion of sperm than ova. Gametes were  
113 collected by stripping the gonad, and then diluted in seawater in a 1 L beaker for 5 min to

114 allow self-fertilization (Fig.1). The ratio eggs/sperm was much higher (rough estimation of  
115 1/10,000) than the one basically used in hatchery practices (1/100). Then, gametes and early  
116 embryos were sieved on 100- $\mu$ m and then 20- $\mu$ m mesh screens to separate large and small  
117 tissue debris, respectively, including the sperm, from eggs. Unfertilized and fertilized eggs,  
118 retained on the 20- $\mu$ m mesh screen, were transferred in a 30 L tank filled with 25°C using  
119 UV-treated (40 mj/cm<sup>2</sup>) and filtered (5  $\mu$ m) seawater. Gill tissue of the SH1 was sampled and  
120 stored in ethanol 100% at ambient temperature for further DNA analyses. Following larval  
121 development, eyed-larvae were settled on cultch from day 15 to day 18 post-fertilization.  
122 Around 5000 larvae successfully settled but a mortality outbreak due to *Vibrio europaeus*  
123 (formerly described as *V. tubiashii* for mortality reported in France (Dégremont et al., 2021;  
124 Travers et al., 2014)) led to high mortality at 1 mm size. In December 2016, 73 surviving SF1  
125 oysters were counted.

126

127 *2.3. Second selfing-cross using a simultaneous hermaphrodite from family SF1 to*  
128 *produce family SF2*

129 In January 2018, as described for the production of the G1-H17, six SF1 oysters were  
130 conditioned in a 240 L tank so that ripe oysters were obtained in March 2018. All were  
131 opened, sampled for DNA analyses and their sex was determined using a microscope. Three  
132 SF1 oysters (SH2, SH3, and SH4) were found to be simultaneous hermaphrodites. For each  
133 one, self-fertilization was done as described for the production of SF1. Two crosses failed to  
134 generate larvae, whereas a few D-larvae (<2000) were obtained from SH2, producing family  
135 SF2 (Fig. 1). Around 150 eyed pediveliger larvae were observed and settled from day 16 to  
136 day 23 post-fertilization. Seed (n~70) were transferred at Ifremer's nursery in Bouin (Vendée,  
137 France) in May 2018. Fifty individuals (weighing 15g) were sacrificed in June 2019. For each  
138 individual, a piece of gills was sampled and stored in ethanol 100% for further DNA analysis.

139

140       2.4. *Genotyping*

141   A SNP-genotyping array (Illumina Infinium), comprising 226 markers and developed by  
142   Lapègue et al. (2014) was used to genotype oysters in order to perform parentage assignment  
143   and to estimate inbreeding coefficient of SF2 oysters. The list of those markers is provided in  
144   supplementary Table 2. DNA extraction and genotyping were performed by Labogena (Jouy-  
145   en-Josas, France). For the parent of the SF1 family, and the parent of the SF2 family, DNA  
146   extraction and SNP-genotyping were done in duplicates. In total, SNP-genotyping was done  
147   for 50 SF2, six SF1 and one G1-H17 oysters (supplementary Table 1).

148   Parentage assignment and genetic parameters for each parent (SH1 and SH2) and their  
149   progenies (i.e. number of alleles per locus, observed and expected heterozygosity,  
150   Polymorphic Information Content...) were determined using Cervus 3.0.7 (Kalinowski et al.,  
151   2007). The theoretical inbreeding coefficient  $F$  at each generation  $t$  was calculated to be 0.25  
152   for SH1 (assuming a first generation of crossing between full brother and sister), and 0.625  
153   and 0.8125 for SF1 and SF2 oysters from Falconer and Mackay (1996):

154   
$$F_t = \Delta F (1 - F_{t-1}) + F_{t-1}$$

155   Where  $\Delta F$  is the rate of inbreeding, expected to be 0.5 for self-fertilization.

156    $F_t$  was also estimated from the observed genotypes for each SF1 and SF2 oysters, as  $\Delta F$   
157   measures the fraction of heterozygosity that disappears each generation, and so, could be  
158   higher or lower than 0.5.

159   The consensus sequences of each 226 SNP from the SNP-genotyping arrays were compared  
160   against the National Center for Biotechnology Information (NCBI) by searching for  
161   similarities at the nucleotide level using BLASTN. The E-value cutoff was  $1e-10$ . Only



162 markers without doubt on their position were kept (206 SNPs), and then, we get the physical  
163 position and the name of the linkage group on which each marker is located.

164 Finally, PLINK v1.9 software (Purcell et al., 2007) was used to compute observed and  
165 expected homozygous genotype counts for each individual of SF1 and SF2 families, as well  
166 as SH1 oyster of the G1-H17 family, and reports method-of-moments F coefficient estimates  
167 using the 206 mapped SNPs.

168

### 169 **3. Results and discussion**

170 In our study, the four simultaneous hermaphrodite oysters were identified using microscope  
171 by observing a few eggs bathing in sperm involving a low fecundity and resulting into limited  
172 number of offspring (Table 1). However, it might be possible to observe simultaneous  
173 hermaphrodites with many eggs and a few sperm, which will still be enough to fertilize eggs.  
174 These oysters could show a higher fecundity, and a higher likelihood to obtain larger  
175 progenies by selfing, but such oysters would need to observe large numbers of mature oysters  
176 and it may therefore be practically difficult to identify by microscopic observation of gonad  
177 samples. An alternative approach would be to maintain the gametes in seawater for one or  
178 several hours, and check the presence of swimming sperm or/and the polar body extrusions  
179 and the first cleavages of fertilized eggs.

180 Out of four SH oysters observed in our study, two successfully produced viable D larvae  
181 (approximately 20,000 and 2000 larvae for the SF1 and SF2 families, respectively), and  
182 subsequently spat (Table 1). The duration of their larval development was similar to those  
183 observed for bi-parental crosses or mass spawns that were raised at the same time of the SF1  
184 and SF2 families (data no shown). Unfortunately, abnormal mortality caused by pathogenic  
185 bacteria of *C. gigas* was observed for the SF1 oysters at 1 mm size as well as for other oyster

186 families produced by mating at least two parents, reducing drastically the number of SF1  
187 oysters at the spat stage from 5000 to less than 100 (Table 1). For the SF2 oysters, although  
188 the number of spat was low, they show relatively good survival from the spat stage  
189 suggesting that oysters produced by self-fertilization may not exhibit higher mortality than  
190 outbred oysters in our controlled facilities from the spat stage. This is in agreement with  
191 results reported by Plough (2018), who observed no appreciable genetic mortality of inbred  
192 spat 40 days after settlement.

193 The genotypes from the SNP array are given in supplementary Table 3. All SF1 and SF2  
194 oysters were unambiguously assigned to their respective presumed parents, confirming that  
195 viable inbred adult oysters can be obtained by selfing and for the first time, over two  
196 successive generations. To date, only Hedgecock et al. (1995) used oysters generated by self-  
197 fertilization of simultaneous hermaphrodites collected in the wild, to produce a new  
198 generation of bi-parental inbred families. Their study has confirmed the pedigree using 24  
199 allozyme markers. One of the two studied lines presented a mean fixation index significantly  
200 greater than expected. Otherwise, previous studies focusing on oysters were limited to larval  
201 stage and based on cryopreservation of male gametes to fertilize reverted females (Lannan,  
202 1971; Yang et al., 2015).

203 Summary genetic statistics obtained by CERVUS for the SH1 and SH2 oysters and their  
204 progenies are provided for each locus in supplementary Table 4. The SH1 oyster, assuming  
205 that it was produced from a cross using full brother and sister, showed 53 and 161  
206 heterozygous and homozygous SNPs, respectively (Table 2). For the two next generations  
207 produced by selfing, the mean numbers of heterozygous SNPs decreased from 53 to 41 for  
208 the SF1 oysters and 28 for the SF2 oysters (Table 2) while it should theoretically decrease by  
209 half at each generation with selfing. Thus, mean observed inbreeding coefficient reached 0.42  
210 and 0.61 for the SF1 and SF2 oysters, respectively, when using the fraction of heterozygosity

211 that disappears at each generation, and 0.62 and 0.74 when calculated from PLINK (Table 2)  
212 (Fig.2). The higher value of the latter may be explained by the low number of SNP used.  
213 While the expected theoretical inbreeding coefficient would have been 0.8125 for SF2  
214 oysters, the lower observed inbreeding coefficient may result from lower survival of  
215 individuals showing high homozygosity due to inbreeding depression resulting from the high  
216 genetic load in *C. gigas* as demonstrated by Launey and Hedgecock (2001). Indeed, without  
217 considering the SF1 oysters which had only 6 oysters genotyped, segregation distortion were  
218 observed for almost all heterozygous SNPs in SF2 oysters (Supplementary Table 4-sheet  
219 SF2) suggesting recessive lethal or nearly lethal alleles closely linked to the SNP (Launey  
220 and Hedgecock, 2001). For example, seven SNPs (AM858323\_344, AM859785\_261,  
221 CU684109\_266, CU997918\_398, EW777868\_135, EW779181\_638 and EW779181\_749),  
222 shown in red bold in Supplementary Figure 1 and mapped on LG1 and LG3, had observed  
223 heterozygosity >0.90 (Supplementary Table 4-sheet SF2) whereas the frequency in progenies  
224 should be closed to 0.5 for each polymorphic SNP. Beside the abnormal mortality caused by  
225 pathogenic bacteria of *C. gigas* observed for the SF1 oysters, most of the mortality for the  
226 SF1 and SF2 families occurred before and during metamorphosis which is first reported by  
227 Launey and Hedgecock (2001) showing that most deleterious genes mostly affect the larval  
228 stage or around the time of metamorphosis. However, our study suggests that selfing may be  
229 an efficient and rapid method of purging in *C. gigas* (Abu Awad and Roze, 2020). Proceeding  
230 successive self-fertilization should purge the lethal alleles, and when a highly inbred line or  
231 nearly isogenic line is obtained, although inbreeding depression reduce their fitness, cross  
232 between brothers and sisters would increase hatching and survival at the larval stage in  
233 subsequent generations as observed in *Argopecten irradians* (Zheng et al., 2012). Similarly,  
234 Brouwer and Osborn (1997) compared the expected and observed heterozygosity over four  
235 generations of selfing in diploid alfalfa (*Medicago sativa*) using 40 RFLP loci. Authors

236 concluded that “inbreeding (...) proceeds more slowly than expected (...) and that inbreeding  
237 depression may limit the level of homozygosity obtainable” and that “tests of genotype and  
238 allele ratios indicated that the deviations may be due to selection favoring heterozygotes”, as  
239 theoretically described by Hayman and Mather (1953) and observed when inbreeding  
240 numerous species (e.g. *Drosophila melanogaster*: Rumball et al. (1994); *Gallus gallus*  
241 *domesticus*: Mina et al. (1991)).

242 Interestingly, three of six SF1 oysters, produced by selfing, were also simultaneous  
243 hermaphrodites suggesting inheritance for this reproductive characteristic. Simultaneous  
244 hermaphrodites are scarcely observed in diploid *C. gigas* (Broquard et al., 2020; Guo et al.,  
245 1998) but are more frequent in triploid progenies (Normand et al., 2009). Synchronous mass  
246 spawning behaviour, in particular for females (Antonio and Camacho, 2019; Enriquez-Diaz  
247 et al., 2009), and strong inbreeding depression due to high genetic load (Launey and  
248 Hedgecock, 2001; Plough et al., 2016) are likely to explain why simultaneous hermaphrodites  
249 remain rare in *C. gigas* even if this reproductive characteristic might be heritable. If the  
250 occurrence of simultaneous hermaphrodites increased for the progenies of one parent  
251 showing this characteristic, this should ease the obtention of highly inbred oysters which  
252 takes, in theory, 7 generations of successive selfing to reach an inbreeding coefficient of 0.99  
253 against more than 20 generations of crosses between full-sibs (Falconer and Mackay, 1996).  
254 Further research is needed on this topic to confirm the inheritance of simultaneous  
255 hermaphroditism in *C. gigas*.

256

257 Our study demonstrates that the development of highly inbred lines of *C. gigas* is possible  
258 by using self-fertilization of simultaneous hermaphrodites using successive generations of  
259 selfing. This method is faster and technically easier to implement than using cryopreserved

260 sperm from a successive hermaphrodite that change sex to females. The development of  
261 highly inbred lines in *C. gigas* will depend on the number of generations needed to purge the  
262 genetic load that favour survival of the most heterozygous individuals. Such lines would be  
263 of great academic and applied interest to study genetics and epigenetics of traits in *C. gigas*.  
264 In any case, parentage assignment using molecular markers will be needed to avoid confirm  
265 pedigrees and to ensure absence of uncontrolled fertilization or mixing of progeny during the  
266 rearing of inbred lines.

267

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275

276 **References**

- 277 Abu Awad, D., Roze, D., 2020. Epistasis, inbreeding depression, and the evolution of self-fertilization.  
278 Evolution 74, 1301-1320.
- 279 Antonio, Í.G., Camacho, A.P., 2019. Reproduction of the introduced oyster *Crassostrea gigas*  
280 (Bivalvia: Ostreidae) cultured on rafts in Spain. Acta Zoologica 100, 257-267.
- 281 Azéma, P., Lamy, J.-B., Boudry, P., Renault, T., Travers, M.-A., Dégregmont, L., 2017. Genetic  
282 parameters of resistance to *Vibrio aestuarianus*, and OsHV-1 infections in the Pacific oyster,  
283 *Crassostrea gigas*, at three different life stages. Genetics Selection Evolution 49, 23.
- 284 Beaumont, A.R., 1986. Genetic aspects of hatchery rearing of the scallop, *Pecten maximus* (L.).  
285 Aquaculture 57, 99-110.
- 286 Beaumont, A.R., Budd, M.D., 1983. Effects of self-fertilisation and other factors on the early  
287 development of the scallop *Pecten maximus*. Marine Biology 76, 285-289.
- 288 Broquard, C., Martinez, A.-S., Maurouard, E., Lamy, J.-B., Dégregmont, L., 2020. Sex determination in  
289 the oyster *Crassostrea gigas* - A large longitudinal study of population sex ratios and  
290 individual sex changes. Aquaculture 515, 734555.
- 291 Brouwer, D.J., Osborn, T.C., 1997. Molecular marker analysis of the approach to homozygosity by  
292 selfing in diploid alfalfa. Crop Science 37, crops1997.0011183X003700040049x.
- 293 Dégregmont, L., Morga, B., Maurouard, E., Travers, M.-A., 2021. Susceptibility variation to the main  
294 pathogens of *Crassostrea gigas* at the larval, spat and juvenile stages using unselected and  
295 selected oysters to OsHV-1 and/or *V. aestuarianus*. Journal of Invertebrate Pathology 183,  
296 107601.
- 297 Dheilly, N.M., Lelong, C., Huvet, A., Kellner, K., Dubos, M.P., Riviere, G., Boudry, P., Favrel, P., 2012.  
298 Gametogenesis in the Pacific oyster *Crassostrea gigas*: a microarrays-based analysis  
299 identifies sex and stage specific genes. Plos One 7.
- 300 Enriquez-Diaz, M., Pouvreau, S., Chavez-Villalba, J., Le Pennec, M., 2009. Gametogenesis,  
301 reproductive investment, and spawning behavior of the Pacific giant oyster *Crassostrea*  
302 *gigas*: evidence of an environment-dependent strategy. Aquaculture International 17, 491-  
303 506.
- 304 Falconer, D.S., Mackay, T.F.C., 1996. Introduction to quantitative genetics, Fourth edition ed.  
305 Addison Wesley Longman, Essex, UK.
- 306 FAO, 2019. Fisheries and aquaculture software. FishStatJ - software for fishery statistical time series.  
307 FAO Fisheries and Aquaculture Department, Rome  
308 <http://www.fao.org/fishery/statistics/software/fishstatj/en>.
- 309 Guo, X., Gaffney, P.M., 1993. Artificial gynogenesis in the Pacific oyster, *Crassostrea gigas*: II.  
310 Allozyme inheritance and early growth. Journal of Heredity 84, 311-315.
- 311 Guo, X.M., Hedgecock, D., Hershberger, W.K., Cooper, K., Allen, S.K., 1998. Genetic determinants of  
312 protandric sex in the Pacific oyster, *Crassostrea gigas* Thunberg. Evolution 52, 394-402.
- 313 Han, Z., Li, Q., Xu, C., Liu, S., Yu, H., Kong, L., 2021. QTL mapping for orange shell color and sex in the  
314 Pacific oyster (*Crassostrea gigas*). Aquaculture 530, 735781.
- 315 Hayman, B.I., Mather, K., 1953. The progress of inbreeding when homozygotes are at a  
316 disadvantage. Heredity 7, 165-183.
- 317 Hedgecock, D., Davis, J.P., 2007. Heterosis for yield and crossbreeding of the Pacific oyster  
318 *Crassostrea gigas*. Aquaculture 272, S17-S29.
- 319 Hedgecock, D., McGoldrick, D.J., Bayne, B.L., 1995. Hybrid vigor in Pacific oysters: an experimental  
320 approach using crosses among inbred lines. Aquaculture 137, 285-298.
- 321 Ibarra, A.M., Cruz, P., Romero, B.A., 1995. Effects of inbreeding on growth and survival of self-  
322 fertilized catarina scallop larvae, *Argopecten circularis*. Aquaculture 134, 37-47.
- 323 Kalinowski, S.T., Taper, M.L., Marshall, T.C., 2007. Revising how the computer program cervus  
324 accommodates genotyping error increases success in paternity assignment. Molecular  
325 Ecology 16, 1099-1106.

326 Lannan, J.E., 1971. Experimental self-fertilization of the Pacific oyster, *Crassostrea gigas*, utilizing  
327 cryopreserved sperm. *Genetics* 68, 599-601.

328 Lapègue, S., Harrang, E., Heurtebise, S., Flahauw, E., Donnadiou, C., Gayral, P., Ballenghien, M.,  
329 Genestout, L., Barbotte, L., Mahla, R., Haffray, P., Klopp, C., 2014. Development of SNP  
330 genotyping arrays in two shellfish species. *Molecular Ecology Resources* 14, 820-830.

331 Launey, S., Hedgecock, D., 2001. High genetic load in the Pacific oyster *Crassostrea gigas*. *Genetics*  
332 159, 255-265.

333 Li, Q., Kijima, A., 2006. Microsatellite analysis of gynogenetic families in the Pacific oyster,  
334 *Crassostrea gigas*. *Journal of Experimental Marine Biology and Ecology* 331, 1-8.

335 Li, Q., Hisatsune, T., Kijima, A., 2004. Induction of haploid androgenesis in Pacific oyster by UV  
336 irradiation. *Marine Biotechnology* 6, 291-297.

337 Martinez, G., Mettifogo, L., Perez, M.A., Callejas, C., 2007. A method to eliminate self-fertilization in  
338 a simultaneous hermaphrodite scallop. 1. Effects on growth and survival of larvae and  
339 juveniles. *Aquaculture* 273, 459-469.

340 Mina, N.S., Sheldon, B.L., Yoo, B.H., Frankham, R., 1991. Heterozygosity at protein loci in inbred and  
341 outbred lines of chickens. *Poultry Science* 70, 1864-1872.

342 Narvarte, M.A., Pascual, M.S., 2003. Fertilization, larval rearing and post-larval growth of the  
343 Tehuelche scallop *Aequipecten tehuelchus* D'Orb., 1846. *Aquaculture* 217, 259-274.

344 Normand, J., Ernande, B., Haure, J., McCombie, H., Boudry, P., 2009. Reproductive effort and growth  
345 in *Crassostrea gigas*: comparison of young diploid and triploid oysters issued from natural  
346 crosses or chemical induction. *Aquatic Biology* 7, 229-241.

347 Plough, L.V., 2018. Fine-scale temporal analysis of genotype-dependent mortality at settlement in  
348 the Pacific oyster *Crassostrea gigas*. *Journal of Experimental Marine Biology and Ecology*  
349 501, 90-98.

350 Plough, L.V., Shin, G., Hedgecock, D., 2016. Genetic inviability is a major driver of type III survivorship  
351 in experimental families of a highly fecund marine bivalve. *Molecular Ecology* 25, 895-910.

352 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de  
353 Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association  
354 and population-based linkage analyses. *The American Journal of Human Genetics* 81, 559-  
355 575.

356 Quillet, E., Dorson, M., Le Guillou, S., Benmansour, A., Boudinot, P., 2007. Wide range of  
357 susceptibility to rhabdoviruses in homozygous clones of rainbow trout. *Fish & Shellfish*  
358 *Immunology* 22, 510-519.

359 Rumball, W., Franklin, I.R., Frankham, R., Sheldon, B.L., 1994. Decline in heterozygosity under full-sib  
360 and double first-cousin inbreeding in *Drosophila melanogaster*. *Genetics* 136, 1039-1049.

361 Travers, M.-A., Mersni Achour, R., Haffner, P., Tourbiez, D., Cassone, A.-L., Morga, B., Doghri, I.,  
362 Garcia, C., Renault, T., Fruitier-Arnaudin, I., Saulnier, D., 2014. First description of French *V.*  
363 *tubiashii* strains pathogenic to mollusk: I. Characterization of isolates and detection during  
364 mortality events. *Journal of Invertebrate Pathology* 123, 38-48.

365 Yang, H., Wang, Y., Guo, X., Tiersch, T.R., 2015. Production of inbred larvae through self-fertilization  
366 using oocytes and cryopreserved sperm from the same individuals after sex reversal in  
367 eastern oyster *Crassostrea virginica*. *Aquaculture Research* 46, 2153-2165.

368 Yin, X., Hedgecock, D., 2019. Bayesian hierarchical modeling of yield in incomplete diallel crosses of  
369 the Pacific oyster *Crassostrea gigas*. *Aquaculture* 510, 43-50.

370 Yue, C., Li, Q., Yu, H., 2018. Gonad transcriptome analysis of the Pacific oyster *Crassostrea gigas*  
371 identifies potential genes regulating the sex determination and differentiation process.  
372 *Marine Biotechnology* 20, 206-219.

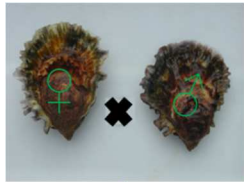
373 Zhang, N., Xu, F., Guo, X., 2014. Genomic analysis of the Pacific oyster (*Crassostrea gigas*) reveals  
374 possible conservation of vertebrate sex determination in a mollusc. *G3: Genes|Genomes|Genetics* 4, 2207-2217.

375

376 Zhang, Y., Ma, H., Li, X., Zhou, Z., Li, J., Wei, J., Zhou, Y., Lin, Y., Noor, Z., Qin, Y., Yu, Z., 2020. Analysis  
377 of inbreeding depression on performance traits of three giant clams (*Tridacna derasa*, *T.*  
378 *squamosa*, and *T. crocea*) in the South China Sea. *Aquaculture* 521, 735023.  
379 Zheng, H., Li, L., Zhang, G., 2012. Inbreeding depression for fitness-related traits and purging the  
380 genetic load in the hermaphroditic bay scallop *Argopecten irradians irradians* (Mollusca:  
381 Bivalvia). *Aquaculture* 366-367, 27-33.

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March 2013  
Stripping : one female x one male producing the G0-H17 family



March 2015  
Mass spawning of 14 full-sibs\* producing the G1-H17 family



June 2016  
Self fertilization by stripping one simultaneous hermaphrodite of the G1-H17 family, producing the SF1 family



March 2018  
Self fertilization by stripping one simultaneous hermaphrodite of the SF1 family producing the SF2 family

Figure 1 Pedigree of the SF-1 and SF-2 oyster families produced by self-fertilization in June 2016 and March 2018, respectively (\* no data on sex ratio recorded)

Table 1 Summary of the four simultaneous hermaphrodite oysters (SH) used for self-fertilization

Oysters	Gametes*	Self-Fertilization	Family name	D larvae number	Larval survival	Days post fertilization for settlement	Spat number
SH1	♀ / ♂ ♂ ♂ ♂	success	SF1	20,000	moderate	D16-D18	5000
SH2	♀ / ♂ ♂ ♂ ♂	success	SF2	2,000	moderate	D16-D23	70
SH3	♀ / ♂ ♂ ♂ ♂	fail					
SH3	♀ / ♂ ♂ ♂ ♂	fail					

\*The proportion given are highly approximative, but the number of sperms was much higher than the number of eggs, resulting a « low » fecundity for the four SH.

Table 2 Number of heterozygote SNPs (min, max and mean) and inbreeding coefficients for the two families of *C. gigas* produced by self-fertilization (SF1 and SF2), and their parents

Family	id	Number of SNP		Inbreeding coefficient Ft			
		Heterozygote	Homozygote	expected	From $\Delta F^*$	Plink	
G1-H17	SH1 (parent of SF1)		53	161	0.25		0.51
SF1	SH3	min	36	181	0.63	0.49	0.67
		max	48	169	0.63	0.32	0.54
		mean	41	176	0.63	0.42	0.62
SF1	SH2 (parent of SF2)		39	178	0.63	0.45	0.64
SF2	OVA19_68	min	14	203	0.81	0.80	0.87
		max	38	179	0.81	0.46	0.65
		SH2 mean	mean	28	189	0.81	0.61

\* The inbreeding coefficient Ft was calculated from the SNP array with  $\Delta F$  obtained from the fraction of heterozygosity that disappears at each generation