

ORIGINAL ARTICLE

O father where art thou? Paternity analyses in a natural population of the haploid–diploid seaweed *Chondrus crispus*

SA Krueger-Hadfield^{1,2,4}, D Roze¹, JA Correa³, C Destombe¹ and M Valero¹

The link between life history traits and mating systems in diploid organisms has been extensively addressed in the literature, whereas the degree of selfing and/or inbreeding in natural populations of haploid–diploid organisms, in which haploid gametophytes alternate with diploid sporophytes, has been rarely measured. Dioecy has often been used as a proxy for the mating system in these organisms. Yet, dioecy does not prevent the fusion of gametes from male and female gametophytes originating from the same sporophyte. This is likely a common occurrence when spores from the same parent are dispersed in clumps and recruit together. This pattern of clumped spore dispersal has been hypothesized to explain significant heterozygote deficiency in the dioecious haploid–diploid seaweed *Chondrus crispus*. Fronds and cystocarps (structures in which zygotes are mitotically amplified) were sampled in two 25 m² plots located within a high and a low intertidal zone and genotyped at 5 polymorphic microsatellite loci in order to explore the mating system directly using paternity analyses. Multiple males sired cystocarps on each female, but only one of the 423 paternal genotypes corresponded to a field-sampled gametophyte. Nevertheless, larger kinship coefficients were detected between males siring cystocarps on the same female in comparison with males in the entire population, confirming restricted spermatial and clumped spore dispersal. Such dispersal mechanisms may be a mode of reproductive assurance due to nonmotile gametes associated with putatively reduced effects of inbreeding depression because of the free-living haploid stage in *C. crispus*.

Heredity (2015) **114**, 185–194; doi:10.1038/hdy.2014.82; published online 17 September 2014

INTRODUCTION

The variation in reproductive systems found in both plant and animal taxa has profound impacts on mating system evolution and population genetic structure. Mating systems can exert a strong influence on the evolutionary ecology of a species and, as such, have been extensively reviewed in the literature (for plants, see Karron *et al.*, 2012; for animals, see Shuster, 2009). Outcrossing species generally exhibit larger, more genetically diverse populations, whereas species undergoing inbreeding tend to exhibit smaller effective population sizes with lower genetic diversity and reduced effective recombination. In seed plants, an understanding of the suite of life history changes accompanying the transition from outcrossing to selfing has recently been advanced by comparative analyses within lineages with both selfing and outcrossing species and by investigating the effects of key floral traits on variation in selfing rates (reviewed in Karron *et al.*, 2012). However, the initial transition to selfing will result in high levels of inbreeding depression. Inbreeding depression is thought to play a key role in opposing the evolution of self-fertilization (Sletvold *et al.*, 2013 and references therein). Once the deleterious mutations have been purged from the population by selection, there will no longer be a cost to selfing and it can become adaptive. For example, if a species is

limited in their opportunities to breed by seasonality, pollen limitation, low densities or short lifespans, selfing can be a form of reproductive assurance (Hardy *et al.*, 2004; Kalisz *et al.*, 2004).

In diploid-dominant angiosperms, gametophytes may be retained, but are few-celled and always unisexual. In contrast, ferns, mosses, fungi and many algae alternate between diploid and haploid stages, in which the haploid stage is prolonged and multicellular. Different mating systems may be distinguished in haploid–diploid species (reviewed in Soltis and Soltis, 1992): (1) intragametophytic selfing, in which the fusion of gametes produced by the same gametophyte results in homozygosity at all loci (Klekowski, 1973), (2) intergametophytic selfing, in which cross-fertilization occurs between two gametophytes produced by spores from the same sporophyte (analogous to selfing in seed plants and animals; Klekowski, 1969) and (3) intergametophytic crossing, in which cross-fertilization occurs between two gametophytes produced by spores from two different sporophytes (analogous to outcrossing in plants and animals). Intergametophytic selfing results in reduced heterozygosity and potentially inbreeding depression, just as selfing does in diploid organisms. Moreover, a prolonged haploid phase may favor purging of deleterious mutations as selection can act directly on the genotypes

¹CNRS, UPMC University Paris 06, Sorbonne Universités, UMI 3614, Evolutionary Biology and Ecology of Algae, Station Biologique de Roscoff, Roscoff, France; ²Departamento de Ecología, Center for Advanced Studies in Ecology and Biodiversity, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile and ³Departamento de Ecología, Center of Applied Ecology and Sustainability, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

⁴Current address: College of Charleston, Grice Marine Laboratory, Charleston, SC 29412, USA.

Correspondence: Dr SA Krueger-Hadfield, College of Charleston, Grice Marine Laboratory, Charleston, SC 29412, USA.

E-mail: kruegersa@cofc.edu

or Professor M Valero, CNRS, UPMC University Paris 06, Sorbonne Universités, UMI 3614 Evolutionary Biology and Ecology of Algae, Station Biologique de Roscoff, CS 90074, 29688 Roscoff, France.

E-mail: valero@sb-roscoff.fr

Received 29 November 2013; revised 11 August 2014; accepted 11 August 2014; published online 17 September 2014

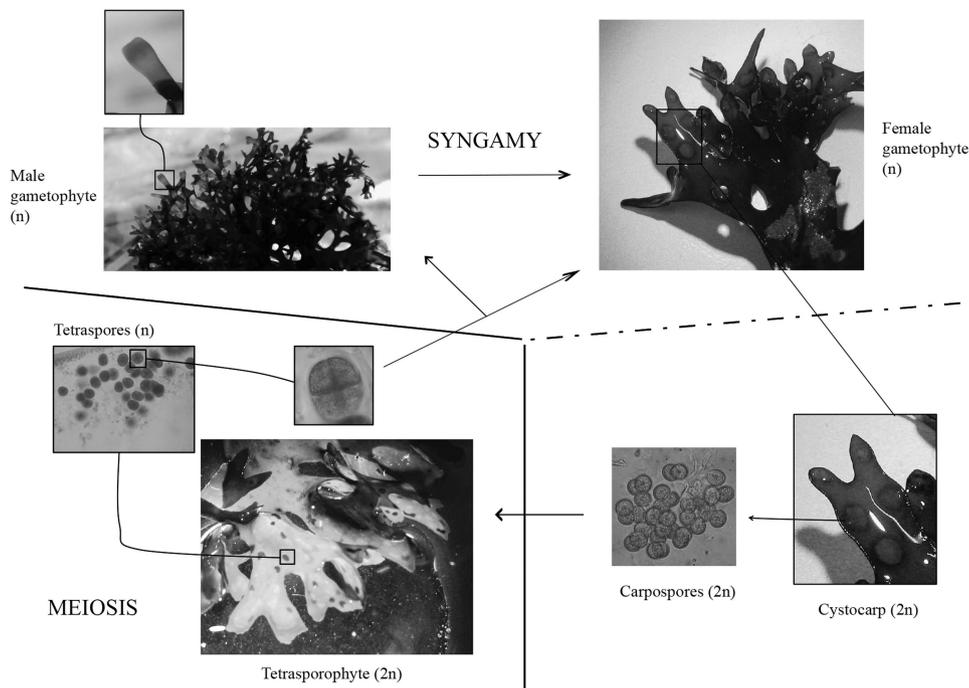


Figure 1 The life cycle of *Chondrus crispus* is typical of the Florideophyceae. Nonmotile spermata fertilize the female gamete (carpogonium) that is retained on the haploid female gametophyte (*syngamy*). A female may have many cystocarps of varying size and age depending on when carpogonia became mature on a particular part of the thallus and when carpogonia were fertilized. Within the cystocarp (the structure that develops on the female thallus following fertilization), the zygote is mitotically amplified producing thousands of diploid carpospores. The diploid carpospores produce the diploid free-living tetrasporophyte. *Meiosis* occurs in the tetrasporophyte-releasing haploid tetraspores that form the free-living haploid female and male gametophytes (photos: SA Krueger-Hadfield).

of haploid gametophytes eliminating deleterious alleles (Otto and Goldstein, 1992), potentially reducing inbreeding depression.

Selfing has been explored in ferns (see, for example, de Groot *et al.*, 2012) and mosses (for example, inbreeding depression in the sporophyte stage, Taylor *et al.*, 2007), but surprisingly few studies have addressed selfing rates in fungi (reviewed in Billiard *et al.*, 2012) or algae. Moreover, few studies have searched for correlates of sexual reproduction and life history traits in haploid–diploid organisms (but see review of mosses in Crawford *et al.*, 2009). Dioecy, or separate sexes, is often used as a proxy for the mating system in haploid–diploid organisms as the evolution of different sexes is likely driven by selection for outcrossing (Bell, 1997). Yet, dioecious haploid gametophytes can still undergo intergametophytic selfing, especially in populations with strong spatial structure.

Red seaweeds (Florideophyceae) are particularly interesting models to address questions related to evolutionary ecology of haploid–diploid organisms. Haploid gametophytes alternate with diploid tetrasporophytes; however, the female gamete is retained on the female thallus (Figure 1). Within the cystocarp, the zygote is mitotically amplified hundreds to thousands of times resulting in genetically identical diploid spores, called carpospores. This polyembryonic process is directly analogous to cloning in diploid organisms. Most of the literature available on red seaweeds suggest male gamete dispersal, and therefore fertilization is extremely limited in both space and time (Searles, 1980; Santelices, 1990). First, red algal sperm (spermata) are non-flagellated and, therefore, lack the ability to swim the final, crucial distance to the female. Second, spermata are viable for a matter of hours upon release (for example, <6 h in *Gracilaria gracilis*, Destombe *et al.*, 1990). Thus, in red florideophycean seaweeds, the three free-living stages, non-motile dispersal propagules (that is, spermata,

tetraspores and carpospores) and zygotic amplification following fertilization can have dramatic impacts on mating system evolution.

Paternity analyses (or analyses of progeny arrays) have been a very efficient method with which to study mating systems (see, for example, Zipperle *et al.*, 2011). Moreover, paternity analyses enable the detection of apomixis or intragametophytic selfing that would otherwise be difficult to detect by analyzing population structure alone. Surprisingly, only Engel *et al.* (1999) utilized paternity analyses to characterize the mating system of the red alga *G. gracilis*. Contrary to the expected association of inbreeding with the haploid–diploid life cycle (Otto and Goldstein, 1992), *G. gracilis* gamete encounters were clearly allogamous (Engel and Destombe, 2002; Engel *et al.*, 2004). Despite the paradigm of low red algal fertilization success, many different males, even males from different tide pools, were found to have fertilized the females studied (Engel *et al.*, 1999).

The aim of this study was to investigate the patterns of gamete encounters in a natural population of the red seaweed *Chondrus crispus* Stackhouse, a species characterized by high levels of intergametophytic selfing (Krueger-Hadfield *et al.*, 2011, 2013a). In this study, we addressed the following questions using cystocarps as records of fertilization events: (1) do tetraspores (leading to gametophytes) disperse, recruit and germinate into clumps of sibling haploid gametophytes; (2) is the mating system mostly inbred and (3) are males as extremely rare as previously thought in *C. crispus* (but see Tveter-Gallgher *et al.*, 1980)? We examined the levels of relatedness among life history stages in order to determine whether clumped spore dispersal, as hypothesized by Krueger-Hadfield *et al.* (2013a), led to significant heterozygote deficiency. In addition, we discuss the roles and evolutionary implications of the intertidal shorescape,

Table 1 The females and sampled cystocarps from (a) the high 5 m grid and (b) the low 5 m grid with (1) the code for each female, (2) the total number of cystocarps sampled per female, (3) the total number of loci used to genotype the cystocarps, (4) the number of different sires, (5) the number of sires who fertilized a female more than once, with the number of cystocarps sired by each different male given in parentheses, (6) the number of cystocarps identical to the maternal genotype and (7) the number of cystocarps that did not amplify at one or more loci

Female	No. of cystocarps	No. of successfully genotyped cystocarps	No. of loci	No. of different sires	No. of sires with > 1 cystocarp	No. of cystocarps with maternal genotype
<i>(a)</i>						
B-9-81	26	26	4	23	2 (3,2)	0
C-1-114*	24	21	5	18	3 (2,2,2)	0
C-9-123*	36	30	5	20	6 (4,2,2,3,3,2)	0
D-9-165*	22	22	5	21	1 (2)	0
F-9-249	16	11	4	11	0	0
G-5-283	18	17	4	14	1 (4)	0
G-6-285*	35	35	5	34	1 (2)	0
G-5-304*	20	16	5	12	3 (2,2,3)	0
G-6-306*	26	19	5	14	2 (5,2)	0
Total	223	197	42	167	19	0
<i>(b)</i>						
A-4-8*	13	13	5	12	1 (2)	0
A-8-16*	27	20	5	20	0	0
A-9-18	20	20	4	20	0	0
A-9-39*	11	7	5	6	1 (2)	0
A-1-43*	21	19	5	18	1 (2)	0
B-5-94*	25	18	5	14	0	4
D-7-161*	10	8	5	8	0	0
D-1-171*	15	15	5	14	0	1
E-4-198*	11	11	5	11	0	0
E-8-205*	16	15	4	13	0	2
E-4-219*	20	18	5	17	0	1
E-6-222*	23	14	5	13	0	1
E-7-225	13	9	4	9	0	0
F-4-239*	14	10	5	10	0	0
G-1-275*	17	13	5	13	0	0
G-6-285*	12	8	5	8	0	0
H-5-325*	22	17	5	16	1 (2)	0
H-6-327*	14	13	5	12	0	1
H-3-343	25	12	5	12	0	0
J-6-412	15	12	4	10	2 (2,2)	0
Total	344	272	96	256	6	10

The symbol ** denotes a female used in the calculation of kinship coefficients. For seven females, only four loci were used to genotype as one locus failed to amplify in a majority of the cystocarps.

intergametophytic selfing and polyandry in red florideophycean seaweeds.

MATERIALS AND METHODS

Sampling design and cystocarp excision

Following the sampling methodology applied in our previous study (Krueger-Hadfield *et al.*, 2013a), two 5 × 5 m grids were sampled within a high shore (3.6 m above mean low water) and a low shore (2 m above mean low water) intertidal stand of *C. crispus* at the Port de Blosson (48° 73' N, 3° 97' W) in Roscoff, France, in February 2010. A frond was sampled, if present, every 25 cm, whereby a maximum of 441 fronds could be sampled within each grid at each shore height.

Haploid fertilized female gametophytes, haploid male gametophytes and diploid tetrasporophytes were identified by the presence of reproductive organs, if present. During field sampling, 13 males in the high grid and 29 males in the low grid were morphologically identified by the presence of distinct pinkish-white bands below the apices (Tveter-Gallagher *et al.*, 1980). However, if

vegetative, ploidy was determined using the acetal–resorcinol reaction test in which reproductive female gametophytes and tetrasporophytes were used as ploidy controls (Krueger-Hadfield *et al.*, 2013a).

The cystocarp is unique to Florideophycean red algae and is composed of the carposporophyte (that is, the structure that generates carpospores following fertilization) and enveloping haploid, gametophytic tissue (that is, the pericarp). The cystocarp becomes visible several weeks after fertilization and a female may bear cystocarps of varying age resulting from different fertilization events. Preliminary tests of cystocarps ranging in age from young (that is, recently fertilized) to old (that is, partially opened structure in which most of the carpospores had already been liberated) demonstrated dark red cystocarps produced reliable amplification of both maternal and paternal alleles at each locus because of sufficient paternal DNA present as the carpospores were mature but not yet released (SA Krueger-Hadfield, personal observation). For each fertilized female gametophyte, these fully developed cystocarps were preferentially selected and excised from the female thallus under a dissecting microscope (×10–20) using a microscalpel.

Surrounding maternal, haploid tissue was removed as much as possible, though the pericarp could not be entirely removed. Cystocarps were stored at -20°C .

Nine females from the high 5 m grid and 20 females from the low 5 m grid were chosen as they had >10 cystocarps, enabling the calculation of kinship coefficients and other metrics where maximizing the number of mothers increases accuracy more than increasing the number of offspring per mother (Table 1 and Supplementary Figure 1). In total, 223 cystocarps were analyzed from the high grid and 344 from the low grid (Table 1).

DNA extraction and genotyping

Total genomic DNA was extracted using the Nucleospin 96 plant kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions except for the cell lysis buffer in which samples were left at room temperature for at least 2 h rather than heating to 65°C for 30 min. Silica gel-dried thallus tissue was ground to a powder using a mixer mill (Retsch GmbH, Haan, Germany), whereas cystocarps were manually broken open using a pointed probe. Each sample was eluted in 100 μl elution buffer.

Krueger-Hadfield *et al.* (2013a) genotyped and analyzed the individual gametophytic and tetrasporophytic fronds collected in the 5×5 m grids. In order to genotype the cystocarps, the five most polymorphic microsatellite loci in both the 5×5 m grids were selected: Chc_04, Chc_23, Chc_24, Chc_31 and Chc_40 (Supplementary Figure 2; Krueger-Hadfield *et al.*, 2013a).

PCRs were performed for each locus separately following the protocols described in Krueger-Hadfield *et al.* (2013a). However, as opposed to 2 μl of frond DNA template, 5 μl of cystocarpic DNA template was used per reaction. Of each PCR product, 2 μl diluted to 1:10 for individual fronds and neat for the cystocarps was added to 10 μl of loading buffer containing 0.3 μl of size standard (GeneScan 600 Liz, Applied Biosystems, Foster City, CA, USA) plus 9.7 μl of Hi-Di formamide (Applied Biosystems). Samples were electrophoresed on an ABI 3130 xl capillary sequencer (Applied Biosystems). Genotypes were scored manually using GENEMAPPER ver. 4 (Applied Biosystems). For a discussion of allele binning and locus error rates, see Krueger-Hadfield *et al.* (2013a).

Paternity analyses

The haploid genotype of each gametophyte can be readily identified with hypervariable microsatellite loci. The maternal multilocus genotype (*m*MLG) was determined by genotyping vegetative tissue from the haploid female thallus. Then, the paternal gametophytic multilocus genotypes (*p*MLGs) were unambiguously inferred by subtracting the *m*MLG from the cystocarpic genotypes across all loci (Figure 2). Unlike diploid-dominant seed plants and animals, the true father of each cystocarp can be identified exactly, without the need for maximum likelihood estimators to determine diploid sires that contributed one, but not both alleles at a locus to the progeny.

We performed paternity analyses by directly comparing the haploid *p*MLGs to (1) the *m*MLGs in order to determine the frequency of homozygous cystocarps, (2) each *p*MLG that fertilized cystocarps on the same female in order to determine the number of males who may have fertilized a female more than once, (3) all the *p*MLGs in order to determine the number of males that fertilized more than one female and (4) all gametophytic MLGs, reproductive and vegetative, scored at the Port de Blosson (Krueger-Hadfield *et al.*, 2013a) in order to locate putative sires using the Multilocus Matches option in GenAEx ver. 6.41 (Peakall and Smouse, 2006, 2012). This method does not, however, account for scoring errors; however, see a discussion of scoring errors in Krueger-Hadfield *et al.* (2013a).

The mean proportion of cystocarpic sires to cystocarps was computed in each shore level separately using maximum likelihood estimators, assuming a binomial distribution. However, the assumption of one binomial distribution that can describe the pattern of paternity across all 29 females used in this study may not be valid (Young-Xu and Chan, 2008). Thus, a test for overdispersion was computed following Roumagnac *et al.* (2004) and Sparks *et al.* (2008) and then the fit of both the binomial and β -binomial models were compared using a likelihood ratio test (see Supplementary Figure 2 and Supplementary Note 1). Using the model with the best fit, the two shore levels were then compared using the same likelihood approach as implemented to compare the two distribution models (see Supplementary Note 1).

Testing for genotyping errors

Although a direct comparison approach, such as the one employed above, does not permit the calculation of genotyping errors, which might lead to a mismatch between a candidate parent and its offspring, incorporating ploidy and known levels of inbreeding are not possible with existing software (see Supplementary Note 2). We verified that the *m*MLG was present in every one of the cystocarps genotyped from each female. This is a very robust test for genotyping errors that could be calculated by the number of cystocarps in which 'nonsense' alleles amplified, but did not correspond to the known *m*MLG.

A second type of genotyping error may arise in which a mismatch occurs between a putative sire and field-sampled gametophyte at any given locus. If genotypes are determined with 100% accuracy, then a mismatch between a *p*MLG and a field-sampled gametophyte would imply that the field-sampled gametophyte is not the sire. If genotypes contain errors, a mismatch might be due to a typing error, mutation or null alleles in the offspring or the putative sires. Thus, each putative mismatch at one or two loci as identified by the Multilocus Matches option in GenAEx was checked for each *p*MLG and field-sampled gametophyte pair to verify allele calls (see Supplementary Note 2).

Finally, a third type of genotyping error could arise because of the difficulty in amplifying paternal DNA from the cystocarp. It is very difficult to remove all maternal tissue (that is, the pericarp surrounding the spores). Nonamplification of a paternal allele may be because of technical errors and lead to overestimation of homozygosity (that is, higher value of F_{IS}). However, there was no evidence of short allele dominance at any of the microsatellites used in this study (Krueger-Hadfield, 2011). Alternatively, high levels of inbreeding could lead to inbreeding depression in which spores with very high levels of homozygosity do not often germinate and are, therefore, not found in the tetrasporophytic subpopulation. In order to determine whether levels of homozygosity were similar between the field-sampled tetrasporophytes and cystocarps (which produce the spores that lead to tetrasporophytes), bootstrap estimates of average homozygosity were determined for the tetrasporophytes and cystocarps from each shore level and *t*-tests were conducted between comparisons using bootstrap estimates of standard error (1000 bootstrap replicates across loci) and Bonferroni correction (Sokal and Rohlf, 1995) using R, ver. 3.03. (R Core Team, 2014).

Parental tetrasporophytes

In order to determine the parentage of the sampled gametophytes and to describe tetraspore dispersal distances, a C++ program (available on request) was used to determine whether the sampled tetrasporophytes could have produced the female and male gametophytes sampled in the field and reconstructed during paternity analyses by comparing the alleles present in the gametophytes with those present in the tetrasporophytes.

Kinship coefficients

In order to determine whether related males and females were exchanging gametes, kinship coefficients were calculated (Loiselle *et al.*, 1995; Ritland, 1996). The kinship coefficient (*r*) between two individuals estimates the probability that two alleles at a locus, sampled at random from each individual, are identical by descent (Malécot, 1948). In a haploid-diploid species undergoing random mating, the kinship coefficient between haploid full-sibs is 0.5, whereas it is also 0.5 between diploid full-sibs (sharing the same haploid parents) and 0.25 between half-sibs. It is important to note that inbreeding would increase these values. The kinship coefficient between a diploid parent and its offspring is 0.5, as diploids produce haploids via meiosis. In contrast, each haploid parent contributes its entire genome to each diploid offspring because haploid gametes are produced via mitosis (Figure 1).

The estimators of Ritland (1996) and Loiselle *et al.* (1995) were used to calculate kinship coefficients between different pairs of female and male gametophytes in the high and low shore stands of *C. crispus*. The main difference between both estimators is that the Ritland estimator gives relatively more weight to rare alleles in the population. The exact numerical values obtained using both estimators should be taken with caution, as their reliability is highly dependent on the choice of population allele frequencies (p_i). For this reason, and in order to estimate this potential bias, the estimators were

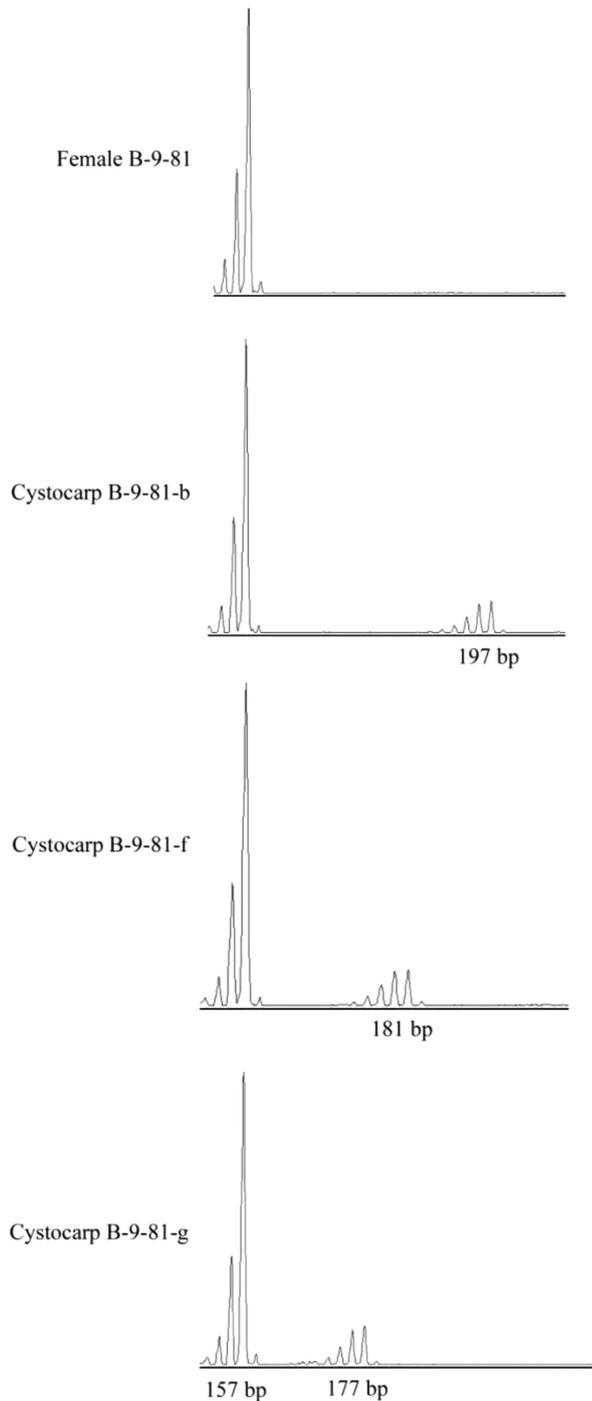


Figure 2 An example of the allelic peaks obtained from the female B-9-81 from the high 5 m grid and three cystocarps from locus *Chc_40*. The allele size of the female was 157 base pairs (bp). Each cystocarp shown here has a different allele from the maternal gametophyte from which the paternal genotype was reconstructed by subtracting the maternal 157 allele.

computed using two different *reference allele frequencies*: (1) allele frequencies from both sampled grids (high and low) and (2) allele frequencies from the high (respectively low) grid for individuals sampled from the high (respectively low) grid. Thus, there was an estimate for the entire shore and an estimate within shore height. In general, estimates of kinship coefficients may be biased upward because pairs of individuals may come from other parts of the population where allele frequencies may be different. Nevertheless, our results

allowed us to contrast patterns of kinship between different types of pairs of individuals (see below).

Cystocarps with missing information at one or more loci were removed, along with their corresponding female, before kinship calculations. A total of six high grid and 16 low grid females (females denoted by an * in Table 1) and associated progeny were retained to calculate average kinship coefficients for three different types of kinship pairs, using a *MATHEMATICA* notebook (Wolfram Research, Champaign, IL, USA), available upon request. The first type was of female–male mating pairs ($\text{♀}_a - \text{♂}_{ga}$). The kinship coefficient was calculated for each female–male pair and then averaged over all males that fertilized the same female to obtain a value for each female. The second type of pair consisted of two males that fertilized carpoegonia (eggs) on the same female ($\text{♂}_{ga1} - \text{♂}_{ga2}$). Again, the values obtained were averaged over all pairs of males to obtain a single value for each female. Finally, the third type of pair consisted of two males that fertilized different females from the same grid ($\text{♂}_{ga} - \text{♂}_{ga\text{allpop}}$). The average of these values was obtained for each female's fertilizing males.

Four different types of comparison were analyzed using permutation tests conducted by randomly resampling kinship coefficients between each group using R. An empirical type I error rate was calculated based on 10 000 permutations. Multiple comparisons were performed after Bonferroni correction (Sokal and Rohlf, 1995; see Supplementary Note 3 for detailed explanation of each comparison). The first comparison was made in order to investigate whether the reference allele frequency used led to different kinship coefficients among the three kinship pairs. Second, the Ritland and Loiselle estimators were compared for each kinship pair in order to evaluate the differences, if any, between both estimators. Third, comparisons were made between different kinship pairs within each shore level stand in order to investigate levels of relatedness between kinship pairs. Finally, fourth, the levels of relatedness between each type of kinship pair were contrasted between shore heights.

RESULTS

Genotyping errors

There were 26 cystocarps from the high shore stand and 72 from the low shore stand that did not amplify at one or more loci after at least two PCR attempts (Table 1). These cystocarps were removed from the data set before subsequent analyses. None of the cystocarps exhibited nonsense alleles in which other allele(s) amplified, but not the maternal allele. In other words, all amplifications resulted in either no amplification or the amplification of one allele matching the maternal allele. However, only 10 cystocarps exhibited complete homozygosity (that is, genetically identical to the *mMLG*) and were found exclusively in the low shore (Table 1). In addition, putative sires differing at one or at two loci exhibited alleles that were different from one another by more than a few base pairs, indicating different genotypes rather than genetic mismatches.

Although no difference was detected between low shore cystocarps and tetrasporophytes ($t = 1.70$, $P > 0.08$, $\alpha = 0.008$), high shore tetrasporophytes were more homozygous than the high shore cystocarps ($t = -3.02$, $P < 0.002$, $\alpha = 0.008$; Table 2). Because of fewer tetrasporophytic *MLGs*, the difference between high shore cystocarps and tetrasporophytes might be because of a sampling effect (that is, only 57 tetrasporophytes versus 197 cystocarps genotyped from the high shore). There were no differences between the cystocarpic or the tetrasporophytic homozygosities between tidal heights (cystocarps: $t = -2.06$, $P > 0.04$, $\alpha = 0.008$; tetrasporophytes: $t = 2.56$, $P > 0.01$, $\alpha = 0.008$). Nevertheless, the cystocarpic genotyping technique was verified, as there was not an overestimate of homozygosity because of nonamplification of paternal alleles within the cystocarpic DNA (Table 2).

Table 2 The frequency of homozygotes at each locus in the tetrasporophytes and the cystocarps in (a) the high and (b) the low 5 m grids

Locus	Tetrasporophytes	Cystocarps
<i>(a)</i>		
Chc_04	0.561	0.270
Chc_23	0.492	0.340
Chc_24	0.472	0.270
Chc_35	0.439	0.298
Chc_40	0.333	0.121
Average \pm s.e.	0.461 \pm 0.033	0.261 \pm 0.034
<i>(b)</i>		
Chc_04	0.352	0.449
Chc_23	0.394	0.495
Chc_24	0.183	0.318
Chc_35	0.268	0.453
Chc_40	0.169	0.294
Average \pm s.e.	0.275 \pm 0.042	0.404 \pm 0.038

Paternity analysis

The 5 loci chosen for cystocarpic analyses were polymorphic, with 41 alleles at *Chc_04*, 88 at *Chc_23*, 61 at *Chc_24*, 31 at *Chc_35* and 52 at *Chc_40* ranging from 156 to 470 base pairs.

In total, 167 and 256 sires were distinguished based on their distinct genotypes in the high and low stands, respectively ($n_{\text{total}}=423$, Table 1). Added to the 42 phenotypically male gametophytic fronds sampled in Krueger-Hadfield *et al.* (2013a), there were a total of 465 males found at the Port de Blosson. However, *none* of the sampled 42 phenotypically male fronds in the 5 m grids fertilized the cystocarps analyzed. Moreover, only one paternal genotype could be unequivocally assigned to a sampled gametophyte that was vegetative at the time of collection (C-8-122). This putative male was compatible with four cystocarps found on female C-9-123, located 25 cm away (Supplementary Figure 1). Thus, 422 of the reconstructed *p*MLGs were not sampled in the 5 m grids, thereby rendering it impossible to calculate intermate distances and directly explore dispersal distances.

With the exception of female F-9-249, most females in the high grid had more than one cystocarp sired by the same male, whereas few males sired more than one cystocarp low on the shore (Table 1), confirming indirect estimates of gene flow in Krueger-Hadfield *et al.* (2013a). On a female frond, different cystocarps sired by the same male were not always located next to one another or on the same dichotomy. Finally, no males fertilized carpogonia on more than one sampled female, regardless of shore height.

Although there was unexplained variation when fitting the binomial model, the β -binomial model was of no better fit ($P>0.12$ at both shore heights, see Supplementary Note 1). Standard errors were, therefore, computed assuming a binomial distribution. Nevertheless, the analysis of paternal genotypes revealed that multiple paternity was common among the progeny array. On average, the ratio of males to cystocarps was 0.85 (s.e. = 2.79×10^{-4} , $n=9$) in the high shore stand and 0.94 (s.e. = 4.98×10^{-5} , $n=20$) in the low shore stand. In other words, in the high shore, a female with 10 cystocarps had at least 8 different sires, whereas in the low shore, there were at least 9 different sires. This difference between shore heights was significant in that the low shore had significantly more cystocarpic sires than the high shore ($\chi^2=366.84$, d.f. = 1, $P<0.001$).

Parental tetrasporophytes

None of the sampled tetrasporophytes could have produced the male or female gametophytes analyzed (that is, the combination of alleles in each gametophyte analyzed was not present in any of the genotyped tetrasporophytes). Therefore, it was not possible to calculate tetraspore dispersal distance or describe dispersal patterns (that is, clumping) directly.

Kinship coefficients

The whole shore and 5 m grid *reference allele frequencies* led to little difference when the Loiselle and the Ritland estimators were used for the three kinship pair types (Figure 3, Table 3 and Supplementary Note 3). Similarly, there were no differences, after Bonferroni correction, between the two estimators for each kinship pair in the high and low shores (Figure 3, Table 3 and Supplementary Note 2). As there were no differences between reference allele frequencies and the cystocarps that were sampled within the 5 m grids, the 5 m reference allele frequencies were used for subsequent comparisons.

The kinship coefficients were similar between female–male ($\sigma_{\text{fa}} - \sigma_{\text{fa}}$) and male–male ($\sigma_{\text{ma1}} - \sigma_{\text{ma2}}$) kinship pairs using both estimators (all P -values nonsignificant after Bonferroni correction; Figure 3, Table 3 and Supplementary Note 3). In contrast, both female–male and male–male kinship pairs were more closely related to each other than to all the males in the population ($P<0.001$ with an $\alpha=0.003$; Figure 3, Table 3 and Supplementary Note 3). However, there were no differences between shore levels for each kinship pair (all P -values nonsignificant after Bonferroni correction; Supplementary Note 3). In both grids, even though the average values demonstrated the occurrence of inbreeding, the kinship coefficients were highly variable between females (using the female–male kinship coefficients; Figure 3 and Table 3) varying from 0.1 (less than half-sibs) to 0.5 (full sibs and, thus, selfing).

DISCUSSION

Paternity analyses enable the direct study of a mating system by providing information on dispersal, the parentage of the progeny and the relatedness between the parents. In *C. crispus*, Krueger-Hadfield *et al.* (2013a) hypothesized that significant heterozygote deficiencies were driven by the mating system and not by spatial substructuring or null alleles. The technique of using cystocarps as the result of fertilization events to reconstruct haploid *p*MLGs corroborated indirect methods (for example, F -statistics) by revealing high levels of inbreeding as well as documenting multiple paternity. The consequences of multiple paternity and inbreeding in a dioecious alga will be discussed with broader reference to other haploid–diploid organisms and the intertidal shorescape.

Reproductive modes in *C. crispus*

This is the first study to verify cross-fertilization in *C. crispus* and one of the few such studies in benthic macroalgae. In total, 469 cystocarps (81% of all genotyped cystocarps) were found to have one allele matching the maternal genotype (that is, dam) and the other allele corresponding to a male gametophyte (that is, sire). The cystocarps that did not amplify at one or more loci ($n_{\text{high+low}}=98$) were most likely the result of poor DNA quality and not null alleles as Krueger-Hadfield *et al.* (2013a) demonstrated low null allele frequency at the loci used in this study and Krueger-Hadfield (2011) did not find any evidence of short allele dominance.

Ten cystocarps exhibited *p*MLGs that fully matched the *m*MLG. Because of the very low occurrence (<3% of all genotyped

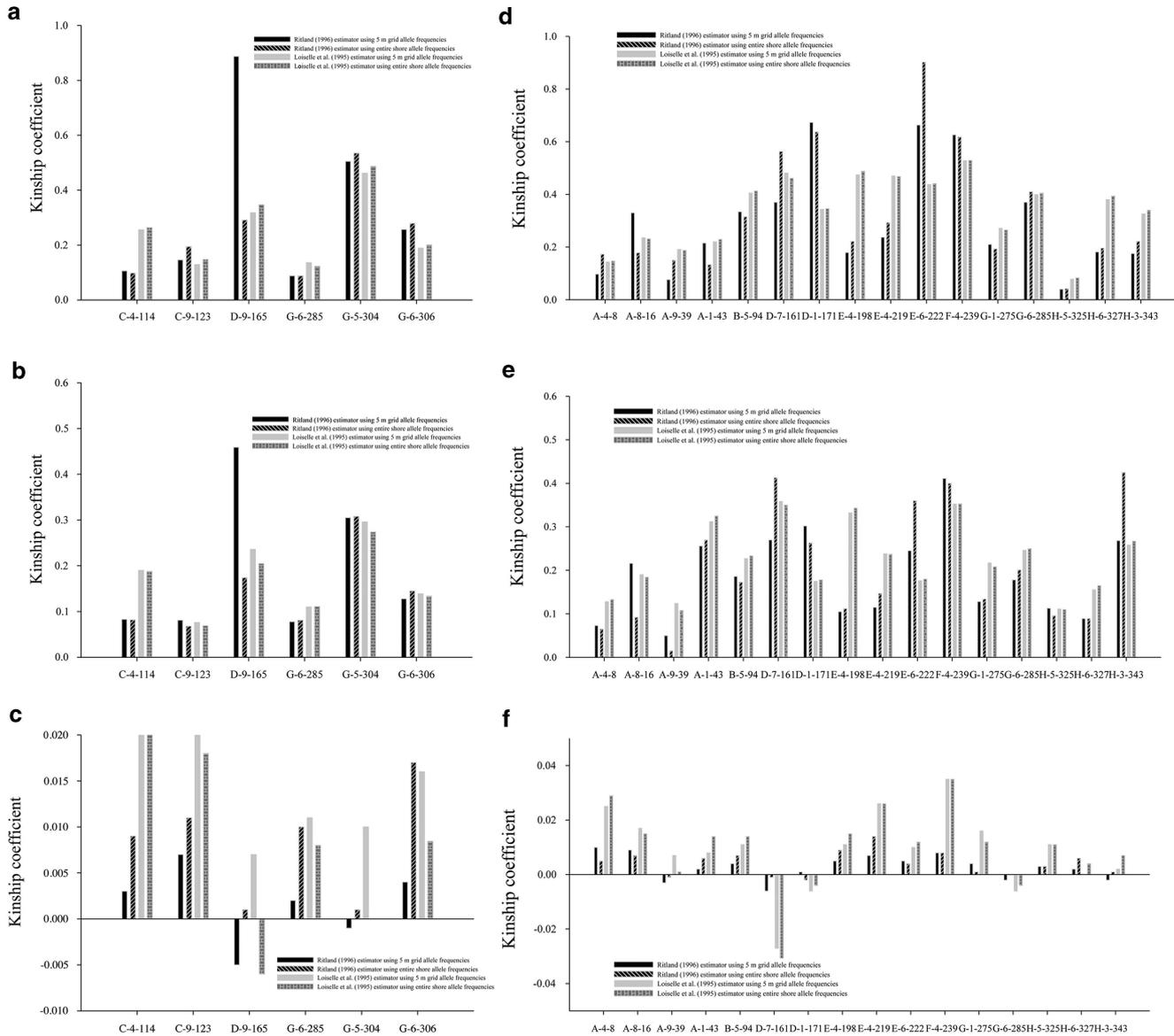


Figure 3 The kinship coefficients calculated using the estimators of Ritland (1996) and Loiselle *et al.* (1995) using the allele frequencies from each 5 m grid and the entire shore (both high and low 5 m grids) for (a) the high female–male pairings ($\varphi_a - \delta_{\varphi a}$), (b) the high male–male pairings ($\delta_{\varphi a1} - \delta_{\varphi a2}$), (c) the high male–male_{allpop} pairings ($\delta_{\varphi a} - \delta_{\varphi a\text{allpop}}$), (d) the low female–male pairings ($\varphi_a - \delta_{\varphi a}$), (e) the low male–male pairings ($\delta_{\varphi a1} - \delta_{\varphi a2}$) and (f) the low male–male_{allpop} pairings ($\delta_{\varphi a} - \delta_{\varphi a\text{allpop}}$). In haploid–diploid species, the kinship coefficients between haploid or diploid full-sibs is 0.5, between half-sibs is 0.25 and between parental genet and offspring is 0.5. Note that kinship coefficient axes vary for each pairing.

cystocarps), this might simply be due to genotyping errors where the paternal genotype did not amplify. However, as high levels of inbreeding were evident in populations of *C. crispus*, the probability that a zygote produced by intergametophytic selfing is homozygous at all five loci is not negligible. Accordingly, 9 of the 213 field sampled tetrasporophytes were found to be fixed homozygotes at the 8 microsatellite loci used to genotype populations at the Port de Bloscon (Krueger-Hadfield *et al.*, 2013a). Seven of the fixed homozygous tetrasporophytes were sampled high on the shore and the probability that the two alleles at the eight loci used were identical by descent was ~ 0.16 (Krueger-Hadfield, 2011). On the other hand, these 10 cystocarps might be evidence of parthenogenesis or self-fertilization (that is, intragametophytic selfing as documented in Klekowski, 1973) occurring at low frequency in natural populations, although this is speculative. Such a low frequency would not have been possible to

detect by Krueger-Hadfield *et al.* (2013a) using population genetic analyses. Parthenogenesis is known to occur in other red seaweeds, such as *Mastocarpus papillatus* (Krueger-Hadfield *et al.*, 2013b), but is often obligate (Zupan and Wes, 1988). In *C. crispus*, these 10 cystocarps occurred on females whose gametes had also been fertilized by other males. Fredericq *et al.* (1992) described the occurrence of a bisexual gametophytic *C. crispus* frond. A bisexual frond might undergo a combination of intragametophytic (that is, fertilization by its own spermatium) and intergametophytic selfing (that is, fertilization by a sibling male gametophyte) and/or outcrossing (that is, fertilization by an unrelated male gametophyte). Detailed field sampling and controlled crossing experiments in *C. crispus* are necessary to determine whether monoecious gametophytes exist and whether they are capable of self-fertilizing while also accepting conspecific spermatia.

Table 3 The average kinship coefficients calculated using the estimators of Ritland (1996) and Loiselle *et al.* (1995) using the allele frequencies from the 5 m grids and the entire shore (both high and low 5 m grids) for (1) the high female–male pairings ($\sigma_{\varphi_a} - \sigma_{\varphi_a}$), (2) the high male–male pairings ($\sigma_{\varphi_{a1}} - \sigma_{\varphi_{a2}}$), (3) the high male–male_{allpop} pairings ($\sigma_{\varphi_a} - \sigma_{\varphi_{allpop}}$), (4) the low female–male pairings ($\sigma_{\varphi_a} - \sigma_{\varphi_a}$), (5) the low male–male pairings ($\sigma_{\varphi_{a1}} - \sigma_{\varphi_{a2}}$) and (6) the low male–male_{allpop} pairings ($\sigma_{\varphi_a} - \sigma_{\varphi_{allpop}}$)

Shore location	Pairing	Ritland, 1996		Loiselle <i>et al.</i> , 1995	
		5 × 5 m	Shore	5 × 5 m	Shore
High	Female–male ($\sigma_{\varphi_a} - \sigma_{\varphi_a}$)	0.332	0.248	0.248	0.262
	Male–male ($\sigma_{\varphi_{a1}} - \sigma_{\varphi_{a2}}$)	0.189	0.143	0.175	0.164
	Male–male _{allpop} ($\sigma_{\varphi_a} - \sigma_{\varphi_{allpop}}$)	0.002	0.008	0.015	0.008
Low	Female–male ($\sigma_{\varphi_a} - \sigma_{\varphi_a}$)	0.299	0.328	0.336	0.339
	Male–male ($\sigma_{\varphi_{a1}} - \sigma_{\varphi_{a2}}$)	0.188	0.203	0.225	0.226
	Male–male _{allpop} ($\sigma_{\varphi_a} - \sigma_{\varphi_{allpop}}$)	0.003	0.004	0.009	0.010

Multiple paternity and relatedness

Multiple paternity has been a common feature observed across taxonomic groups. Even in dioecious haploid–diploid organisms such as mosses, multiple paternity can occur in which more than half of the sporophytes developed on a female plant have different paternal genotypes (for example, *Sphagnum lescurii*, Szövényi *et al.*, 2009). At least 6, and as many as 34, different males fertilized each female gametophyte in *C. crispus*. Multiple mating may be unavoidable, but quantitative studies of the variation in multiple paternity in natural populations of benthic marine organisms are rare (in addition to Engel *et al.*, 1999, see Johnson and Yund, 2007 in the colonial ascidian *Botryllus schlosseri*).

Distance from the female had a significant effect on male gametophyte reproductive success in the red seaweed *G. gracilis* (Engel *et al.*, 1999) and the moss *Polytrichum formosum* (Van der Velde *et al.*, 2001). As the parental tetrasporophytes and the paternal gametophytes were not exhaustively sampled within the 5 m grids, it was not possible to directly quantify dispersal. However, high levels of inbreeding (mean F_{is} : high: 0.398, low: 0.207, Krueger-Hadfield *et al.*, 2013a) and relatedness among females and males support dense recruitment of related individuals in *C. crispus*. This alga occupies a dense, nearly monospecific band in the mid-intertidal. This high population density with relatively low sampling intensity likely accounted for the field sampling of only one of the 423 different cystocarpic sires. However, if fertilization regularly occurs at scales of <25 cm, our sampling strategy would have missed many males as we sampled a frond, if present, every 25 cm. Nevertheless, Krueger-Hadfield *et al.* (2013a) observed a generalized pattern in which kinship coefficients decreased with increasing distance until individuals were less genetically similar than at random after performing spatial analyses in the same high and low shore stands at the Port de Blocon. In the high shore stand, in particular, significant negative slopes over distances classes <1 m suggested related individuals group together. Incorporating these results with the levels of relatedness between sires and dams demonstrates evidence of restricted spermatial dispersal at fine scales of <25 cm.

Restricted spermatial dispersal likely results in correlated paternity, in which progeny may share the same sire and/or derive from related

sires. In red seaweeds, correlated paternity originating from the codispersion of spermatia would likely result in a small subset of males siring the majority of cystocarps on the same female. However, in *C. crispus*, many different males of varying levels of relatedness to the female gametophyte sired cystocarps, suggesting spermatia did not arrive in a single event from a single source. Hardy *et al.* (2004) demonstrated that correlated paternity in the model plant *Centaurea corymbosa* was because of limited mate availability driven by limited pollen dispersal distances, heterogeneity in pollen production and phenology and a self-incompatibility system. Unlike *C. corymbosa*, mate availability was not as limited in *C. crispus*. Rather, males are plentiful, but limited dispersal coupled with heterogeneity in spermatial production and phenology likely resulted in the high ratios of sires to cystocarps. Unsurprisingly, based on previous spatial analyses, there was no decrease in among-sibship correlated paternity with distance, suggesting that spermatial pool spatial genetic structure may have been weak or, alternatively, our sampling density was not sufficient (Supplementary Note 2).

We had hypothesized an increase in relatedness with increasing shore height, as the high shore can be characterized as more isolated because of cyclical emersion and immersion during tidal cycles. *C. crispus* populations located high on the shore were less genetically diverse, exhibited increased levels of genetic structure and were characterized by higher levels of inbreeding in comparison with the low shore populations (Krueger-Hadfield *et al.*, 2011, 2013a). Indeed, many male gametophytes sired more than one cystocarp on the same female, although the overall proportion of sires to cystocarps was significantly lower in the high shore stand as compared with the low shore stand, suggesting less gene flow and gamete retention at this shore height. The high shore end of an intertidal species' distribution can be characterized as more isolated because of cyclical emersion and immersion during tidal cycles and lower density. Populations located high on the shore were less genetically diverse, exhibited increased levels of genetic structure and were characterized by higher levels of inbreeding in comparison with the low shore populations (Krueger-Hadfield *et al.*, 2011, 2013a). In other words, high shore populations exhibit similarities with the latitudinal margins of *C. crispus*, whereas low shore populations exhibit similarities with the center of the latitudinal range.

However, there was no difference detected between the high and low shore female–male and male–male kinship pairs. Because of lower densities and less genetic variability in the high shore stand, fewer female fronds were sampled and there was less power than in the low shore stand. Alternatively, in the high shore, the female gametes may be fertilized by the closest males (that is, from within the same population) and thus not available for gametes brought up the shore with the rising tide. In order to explore the discrepancy between our previous and current studies, all fronds located within very restricted spatial scales (that is, 0.25 m²) need to be sampled. This will enable a more detailed characterization of dispersal events to explore the impacts of clumped spore dispersal on population dynamics as well as understanding the observed stochasticity among females.

Clumped dispersal in the intertidal shorescape

Unlike bryophytes (see, for example, Szövényi *et al.*, 2009) and *G. gracilis* (Engel *et al.*, 1999), multiple paternity in *C. crispus* was not associated with selection (that is, female choice) for less inbred sporophytic progeny and, thus, may be completely passive. Clumped spore dispersal likely promoted intergametophytic selfing. In natural *C. crispus* populations as well as laboratory culture, individual gametophytic holdfasts have been found to be composed of multiple

genotypes supporting this hypothesis (SA Krueger-Hadfield *et al.*, unpublished data), although no chimeric fronds were detected. Merging of related individuals may promote survival in *C. crispus* sporelings (Tveter and Mathieson, 1976). Similar patterns are evident in other organisms, such as the ascidian *Botryllus schlosseri* (Grosberg and Quinn, 1986, merged clusters of highly related genets in the monoecious beech *Nothofagus pumilio* (Till-Bottraud *et al.*, 2012) and the kelp *Lessonia berteroana* (Segovia *et al.*, 2014) and genetically chimeric blades arising from meiosis after conchospore (spores produced by the sporophyte) germination in the red alga *Pyropia* (*Porphyra*, Bangiales, Zhang *et al.*, 2013). Clumped propagule dispersal may also be a mechanism by which reproductive assurance is achieved when dispersal is limited or populations are isolated (for example, fertilization in kelp species in which pheromones are detectable over millimeter scales, Muller *et al.*, 1985). However, clumping arises, these patterns may lead to reduced gene flow and populations may become subject to negative consequences, such as inbreeding.

Unlike diploid-dominant organisms, haploid–diploid seaweeds may exhibit reduced effects of inbreeding depression following mating among related individuals because of efficient purging of deleterious recessive alleles in the haploid stage (Otto and Goldstein, 1992). The isomorphic life cycles of red seaweeds in the genera *Gracilaria* and *Chondrus* may result in a lack of inbreeding depression as all genes could putatively be expressed in both ploidy levels. However, differences in growth rates and survivability have been demonstrated between haploids and diploids (for example, *G. chilensis*, Guillemain *et al.*, 2008, 2013). Richerd *et al.* (1993) and Engel and Destombe (2002) did not find evidence of inbreeding depression in *G. gracilis* and demonstrated the possibility of a self-incompatibility complex. The occurrence of inbreeding depression warrants empirical investigation in *C. crispus* in which high levels of intergametophytic selfing have been demonstrated (Krueger-Hadfield *et al.*, 2011, 2013a, and this study) and viable spores were produced following full-sib crosses (SA Krueger-Hadfield *et al.*, unpublished data).

CONCLUSIONS

Polyandry was a common phenomenon in a natural population of the red seaweed *C. crispus*. Unlike some bryophytes and *G. gracilis*, the only other red seaweed for which detailed information on the mating system is available, polyandry in *C. crispus* may not have evolved to enable post-fertilization selection for less inbred progeny, but rather as a consequence of clumped tetraspore dispersal coupled with limited spermatial dispersal. The very stark difference in the tidal distribution of *G. gracilis* (discrete genets in tide pools) versus *C. crispus* (genets composed of multiple genotypes forming a dense monospecific band) may be an important factor in the two very different mating systems observed. Outcrossing may be the result of extensive gene flow in *G. gracilis*, whereas very high densities in *C. crispus* lead to high levels of inbreeding. Thus, the ecology of these two species likely has influenced mating system evolution and warrants comparative investigations in other intertidal, haploid–diploid species with similar distributions. Empirical tests of inbreeding depression as well as a more detailed study of microgeographic kin structure must be undertaken in order to understand the mating system evolution as well as the intertidal shorescape on the evolutionary ecology of *C. crispus*. Finally, because of the high levels of inbreeding occurring in these populations of a dioecious, haploid–diploid seaweed, dioecy may not be a good proxy for the mating system in these organisms in which forms of inbreeding and/or selfing can occur.

DATA ARCHIVING

Tetrasporophyte and gametophyte genotypes were published in Krueger-Hadfield *et al.* (2013a): DRYAD entry doi: 10.5061/dryad.751p3. Microsatellite primer sequencers were deposited in GenBank and accession numbers published in Krueger-Hadfield *et al.* (2011, 2013a). Cystocarp genotypes for the present article available from the Dryad Digital Repository: doi:10.5061/dryad.07950. Data archival location: microsatellite primer sequences were deposited in GENBANK (KC188839–KC188841, KC188843).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank everyone from the Station Biologique de Roscoff who participated in the 5 m grid sampling, especially P Potin and J Coll n; S Mauger and M Callouet for aid in developing the protocol for cystocarpic DNA extraction; M Perennou and G Tanguy of the sequencing platform at the Station Biologique de Roscoff; and J Brodie, C Engel and G Pearson for insightful discussions; and Olivier Hardy and three anonymous reviewers for improving the manuscript. This project was supported by a PhD fellowship funded by CNRS and R gion Bretagne (ARED 211-B2-9: PLOKA) and a Grant-In-Aid-of-Research from the Phycological Society of America (2009) and GIS-Europ le Mer (2010) to SA Krueger-Hadfield and ASSEMBLE EU FP7 research infrastructure initiative. This study constitutes a contribution from the Associated International Laboratory between France and Chile: ‘Dispersal and Adaptation of Marine Species’ (LIA DIAMS).

- Bell G (1997). The evolution of the life cycle in brown seaweeds. *Biol J Linn Soc* **60**: 21–38.
- Billiard S, L pez-Villavicencio M, Hood ME, Giraud T (2012). Sex, outcrossing and mating types: unsolved questions in fungi and beyond. *J Evol Biol* **25**: 1020–1038.
- Crawford M, Jesson LK, Garnock-Jones PJ (2009). Correlated evolution of sexual system and life-history traits in mosses. *Evolution* **63**: 1129–1142.
- de Groot GA, Verduyn B, Wubs EJ, Erkens R, During H (2012). Inter- and intraspecific variation in fern mating systems after long-distance colonization: the importance of selfing. *BMC Plant Biol* **12**: 3.
- Destombe C, Godin J, Remy JM (1990). Viability and dissemination of spermatia of *Gracilaria verrucosa* (Gracilariales, Rhodophyta). *Hydrobiologia* **204/205**: 219–223.
- Engel CR, Wattier R, Destombe C, Valero M (1999). Performance of non-motile male gametes in the sea: analysis of paternity and fertilization success in a natural population of a red seaweed, *Gracilaria gracilis*. *Proc R Soc London B Biol* **266**: 1879–1886.
- Engel CR, Destombe C (2002). Reproductive ecology of an intertidal red seaweed, *Gracilaria gracilis*: influence of high and low tides on fertilization success. *J Mar Biol Assoc UK* **37**: 189–192.
- Engel CR, Destombe C, Valero M (2004). Mating system and gene flow in the red seaweed *Gracilaria gracilis*: effect of haploid–diploid life history and intertidal rocky shore landscape on fine-scale genetic structure. *Heredity* **92**: 289–298.
- Fredericq S, Brodie J, Hommersand MH (1992). Developmental morphology of *Chondrus crispus* (Gigartinales, Rhodophyta). *Phycologia* **31**: 542–563.
- Grosberg RK, Quinn JF (1986). The genetic control and consequences of kin recognition by the larvae of a colonial marine invertebrate. *Nature* **322**: 456–459.
- Guillemain M-L, Faugeron S, Destombe C, Viard F, Correa JA, Valero M (2008). Genetic variation in wild and cultivated populations of the haploid-diploid red alga *Gracilaria chilensis*: how farming practices favor asexual reproduction and heterozygosity. *Evolution* **62**: 1500–1519.
- Guillemain M-L, Sepulveda RD, Correa JA, Destombe C (2013). Differential ecological responses to environmental stress in the life history phases of the isomorphic red alga *Gracilaria chilensis* (Rhodophyta). *J Appl Phycol* **25**: 215–224.
- Hardy OJ, Gonz lez-Mart nez SC, Colas B, Fr ville H, Mignot A, Olivieri I (2004). Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae). II. Correlated paternity within and among sibships. *Genetics* **168**: 1601–1614.
- Johnson SL, Yund PO (2007). Variation in multiple paternity in natural populations of a free-spawning marine invertebrate. *Mol Ecol* **16**: 3253–3262.
- Kalish S, Vogler DW, Hanley KM (2004). Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature* **430**: 884–887.
- Karron JD, Ivey CT, Mitchell RJ, Whitehead MR, Peakall R, Case AL (2012). New perspectives on the evolution of plant mating systems. *Ann Bot* **109**: 493–503.
- Klekowski EJ (1969). Reproductive biology of the Pteridophyta. II. Theoretical considerations. *Bot J Linn Soc* **62**: 347–359.
- Klekowski EJ (1973). Genetic load in *Osmunda regalis* populations. *Am J Bot* **60**: 146–154.

- Krueger-Hadfield SA, Collén J, Daguin C, Valero M (2011). Distinguishing among gents and genetic population structure in the haploid-diploid seaweed *Chondrus crispus* (Rhodophyta). *J Phycol* **47**: 440–450.
- Krueger-Hadfield SA (2011) Structure des populations chez l'algue rouge haploïde-diploïde *Chondrus crispus*: système de reproduction, différenciation génétique et épidémiologie. PhD thesis, UPMC-Sorbonne Universités and la Pontificia Universidad Católica de Chile.
- Krueger-Hadfield SA, Roze D, Mauger S, Valero M (2013a). Intergametophytic selfing and microgeographic genetic structure shape populations of the intertidal red seaweed *Chondrus crispus* (Rhodophyta). *Mol Ecol* **22**: 3242–3260.
- Krueger-Hadfield SA, Kübler JE, Dudgeon SR (2013b). Reproductive effort in the red alga, *Mastocarpus papillatus*. *J Phycol* **49**: 271–281.
- Loiselle BA, Sork VL, Nason J, Graham C (1995). Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *Am J Bot* **82**: 1420–1425.
- Malécot G (1948). *Les mathématiques de l'hérédité*. Masson and Cie: Paris.
- Muller DG, Maier I, Glassmann G (1985). Survey on sexual pheromone specificity in Laminariales (Phaeophyceae). *Phycologia* **24**: 475–477.
- Otto SP, Goldstein DB (1992). Recombination and the evolution of diploidy. *Genetics* **131**: 745–751.
- Peakall R, Smouse PE (2006). GENALEX 6.2: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* **6**: 288–295.
- Peakall R, Smouse PE (2012). GenALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* **28**: 2537–2539.
- R Core Team (2014). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing: Vienna, Austria. URL: <http://www.R-project.org/>.
- Richard S, Destombe C, Cuguen J, Valero M (1993). Variation in reproductive success in a haplo-diploid red alga, *Gracilaria verrucosa*: effects of parental identities and crossing distance. *Am J Bot* **80**: 1379–1391.
- Ritland K (1996). Estimators for pairwise relatedness and individual inbreeding coefficients. *Genet Res* **67**: 175–185.
- Roumagnac P, Pruvost O, Chiroleu F, Hughes G (2004). Spatial and temporal analyses of bacterial blight of onion caused by *Xanthomonas axonopodis* pv. *allii*. *Phytopathology* **94**: 138–146.
- Santelices B (1990). Patterns of reproduction, dispersal and recruitment in seaweeds. *Oceanogr Mar Biol* **28**: 177–276.
- Searles RB (1980). The strategy of the red algal life history. *Am Nat* **115**: 113–120.
- Segovia NI, Vásquez JA, Faugeron S, Haye PA (2014). On the advantage of sharing a holdfast: density dependent effects on the occurrence of fusion of individuals in the kelp *Lessonia nigrescens*. *Mar Ecol* (in press) doi:10.1111/maec.12206.
- Shuster SM (2009). Sexual selection and mating systems. *Proc Natl Acad Sci USA* **106**: 10009–10016.
- Sletvold N, Mousset M, Hagenblad J, Hansson B, Ågren J (2013). Strong inbreeding depression in two Scandinavian populations of the self-incompatible perennial herb *Aradidopsis lyrata*. *Evolution* **67**: 2876–2888.
- Sokal RR, Rohlf FJ (1995). *Biometry: The Principles and Practice of Statistics in Biological Research* 3rd edn. W. H. Freeman and Co.: New York.
- Soltis DE, Soltis PS (1992). The distribution of selfing rates in homosporous ferns. *Am J Bot* **79**: 97–100.
- Sparks AH, Esker PD, Antony G, Campbell L, Frank EE, Huebel L *et al.* (2008) Ecology and Epidemiology in R: Spatial Analysis. The Plant Health Instructor. doi: 10.1094/PHI-A-2008-0129-03. <http://www.apsnet.org/edcenter/advanced/topics/EcologyAndEpidemiologyInR/SpatialAnalysis/Pages/default.aspx>.
- Szövényi P, Ricca M, Shaw AJ (2009). Multiple paternity and sporophytic inbreeding depression in a dioicous moss species. *Heredity* **103**: 394–403.
- Taylor PJ, Eppley SM, Jesson LK (2007). Sporophytic inbreeding depression in mosses occurs in a species with separate sexes but not in a species with combined sexes. *Am J Bot* **94**: 1853–1859.
- Till-Bottraud I, Fajardo A, Rioux D (2012). Multi-stemmed trees of *Nothofagus pumilio* second-growth forest in Patagonia are formed by highly related individuals. *Ann Bot* **110**: 905–913.
- Tveter E, Mathieson AC (1976). Sporeling coalescence in *Chondrus crispus* (Rhodophyceae). *J Phycol* **12**: 110–118.
- Tveter-Gallagher E, Mathieson AC, Cheney DP (1980). Ecology and developmental morphology of male plants of *Chondrus crispus* (Gigartinales, Rhodophyta). *J Phycol* **16**: 257–264.
- Van der Velde M, During HJ, Van de Zande L, Bijlsma R (2001). The reproductive biology of *Polytrichum formosum*: clonal structure and paternity revealed by microsatellites. *Mol Ecol* **10**: 2423–2434.
- Young-Xu Y, Chan KA (2008). Pooling overdispersed binomial data to estimate event rate. *BMC Med Res Methodol* **8**: 58.
- Zhang Y, Yan X-h, Aruga Y (2013). The sex and sex determination in *Pyropia haitanensis* (Bangiales, Rhodophyta). *PLoS One* **8**: e73414.
- Zipperle AM, Coyer JA, Reise K, Stam WT, Olsen JL (2011). An evaluation of small-scale genetic diversity and the mating system in *Zostera noltii* on an intertidal sandflat in the Wadden Sea. *Ann Bot* **107**: 127–133.
- Zupan JR, West JA (1988). Geographic variation in the life history of *Mastocarpus papillatus* (Rhodophyta). *J Phycol* **24**: 223–229.

Supplementary Information accompanies this paper on Heredity website (<http://www.nature.com/hdy>)