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Analysis of sexual phenotype and prezygotic fertility in natural populations of *Fucus spiralis, F. vesiculosus* (Fucaceae, Phaeophyceae) and their putative hybrids

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In the genus *Fucus*, the character dioecy/hermaphroditism has undergone multiple state changes and hybridization is possible between taxa with contrasting mating systems, e.g. between the dioecious *Fucus vesiculosus* and the hermaphrodite *F. spiralis*. In the context of mating system evolution, we evaluated the potential consequences of hybridization by studying the variation in sexual phenotype and prezygotic fertility. Firstly, as a result of hybridization between the two sexual systems, gender variation may arise depending on the relative importance of genes with large *versus* small phenotypic effects. We thus qualitatively examined the extent of gender variation within and among individual hybrids in comparison with both parental species. Secondly, if hybridization breaks up co-adapted gene complexes, hybrid fertility may be reduced in comparison with both parental species. Therefore, we also quantified male and female prezygotic fertility in parental species and their hybrids in order to test for reduction in hybrid fitness. A total of 89 sexually mature individuals (20 *F. spiralis*, 40 *F. vesiculosus*, 10 hermaphrodite hybrids and 19 dioecious hybrids) were sampled in two geographically distant regions (France and Portugal) and six conceptacles per individual were observed. Within-individual variation was very restricted qualitatively – only one hybrid carried a conceptacle with a different sexual phenotype from the five others – as well as quantitatively. This suggests a simple genetic system for sex determination involving a few genes with major effects. In addition, analyses showed no significant decrease in hybrid fertility compared with parental species. Moreover, hybrids exhibited all sexual phenotypes, suggesting several generations of hybridization and backcrossing and, therefore, that hybrids are reproductively successful. Finally, the occurrence of sterile paraphyses in female and hermaphrodite individuals was interpreted as a relic of male function and suggests that, as in higher plants, evolution from hermaphroditism to dioecy may be the most parsimonious pathway.

Key words: Dioecy, fucoid seaweed, hermaphroditism, hybridization, male and female function, mating system evolution, sexual allocation, sperm/egg ratio

Introduction

Analysing variation in reproductive strategies in hybridizing taxa provides an excellent opportunity for directly studying the factors that influence the evolutionary pathway between different reproductive systems (Barrett, 1998; Charlesworth, 1999; Hewitt, 2001; Dorken *et al.*, 2002; Dorken & Barrett, 2003). While changes in the mating system can influence the degree of reproductive isolation of the parental species, reproductive isolation may also evolve due to divergent selective pressures in different habitats (e.g. Sakai & Weller, 1999;

Barrett *et al.*, 2001; Dorken & Barrett, 2003). In stable, highly stressful habitats, selfing may present a real advantage compared to outcrossing (for review, see Takebayashi & Morrell, 2001), by (i) increasing reproductive assurance and (ii) maintaining local adaptation (e.g. via co-adapted gene complexes). On the other hand, in habitats where competition is strong, outcrossing may limit inbreeding depression. Differences in mating system may sometimes operate as a genetic barrier maintaining the two species in the two different environments.

Fucus, a genus of ecologically successful brown seaweeds, is one of the rare groups where hybridization in natural populations has been

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reported between taxa possessing contrasting mating systems. Hybridization between cosexual hermaphrodite and unisexual dioecious species has been suggested by the observation of intermediate thallus morphologies in the field between cosexual *F. spiralis* and unisexual *F. ceranoides* (Gard, 1910), as well as field observations and/or laboratory crosses between *F. spiralis* and unisexual *F. vesiculosus* (Sauvageau, 1909; Kniep, 1925; Burrows & Lodge, 1951; Scott & Hardy, 1994) and between cosexual *F. evanescens* and unisexual *F. serratus* (Lein, 1984; Rice & Chapman, 1985; Coyer *et al.*, 2002), and has been recently confirmed in natural populations using molecular markers for the latter two species pairs (Coyer *et al.*, 2002; Wallace *et al.*, 2004, Engel *et al.*, 2005).

Vernet & Harper (1980) advanced the hypothesis of contrasting selective pressures – abiotic on the upper shore and biotic on the lower shore – driving divergence in mating systems in *F. spiralis* and *F. vesiculosus*, which co-occur throughout most of their distributional range. Typically, the vertical distribution of the species is discontinuous (parapatric situation) where hermaphrodite *F. spiralis* is present in the high intertidal zone and dioecious *F. vesiculosus* in the mid-intertidal zone. However, individuals of the two species can be found in contiguous stands (sympatric situation) in the transition zone and on shores with complex topology. On average, due to their overlapping but distinct vertical distributions on the shore, *F. spiralis* individuals are emersed at low tide for longer than *F. vesiculosus* individuals, and are therefore subjected to different selective pressures in response to abiotic stress. Although laboratory studies of emersion stress reveal little difference in tolerance between *F. spiralis* and *F. vesiculosus* (e.g. Dring & Brown, 1982), field studies suggest that (sublethal) emersion stress mediates competitive interactions (perhaps between early post-settlement or juvenile stages). For example, it has been shown that *F. vesiculosus* can extend its vertical range upshore when *F. spiralis* is removed (Hawkins & Hartnoll, 1985; Chapman & Johnson, 1990); however, *F. spiralis* is competitively excluded by *F. vesiculosus* in the midshore region (Chapman, 1990; see reviews by Chapman, 1995; Davison & Pearson, 1996).

In *Fucus*, the character dioecy/hermaphroditism has undergone multiple state changes (Serrão *et al.*, 1999). Although the sister taxa to *Fucus* are hermaphrodite, the scattered phylogenetic positions of hermaphrodite and dioecious species suggests that either mating system changed independently several times, possibly by relatively simple mechanisms (Serrão *et al.*, 1999) as in the flowering plant genus *Silene* (Desfeux *et al.*, 1996),

and in angiosperms generally (for review see Charlesworth, 2002). In land plants, based on modelling, empirical and phylogenetic studies, dioecy generally appears to be the derived state arising from cosexual ancestors (Charlesworth, 1999). In many dioecious species for which hermaphrodite relatives are known, evidence for gender instability (i.e. sexual variation among flowers within the same individual) and, in particular, rudiments of structures of the opposite sex in flower morphology, were first observed by Darwin (1877) and provide further support for this evolutionary pathway (Webb, 1999; Charlesworth, 2002).

In *Fucus*, reproductive organs are grouped within conceptacles borne on fertile structures (receptacles); by analogy with flowering plants, conceptacles are cosexual in hermaphrodite species but unisexual (male or female) in dioecious species. Male gametes are enclosed in antheridia borne by branched filaments with chromatophores, called paraphyses, whereas female gametes are enclosed in oogonia (reviewed by Fritsch, 1945, p. 368 and illustrations). Fritsch also observed that paraphyses were present in female conceptacles although less ramified than the paraphyses in male conceptacles. Moreover, in this group, the sexual phenotype has been described as one of the most stable characters of a genus that otherwise shows a high degree of vegetative morphological plasticity (Burrows & Lodge, 1951; Pérez-Ruzafa *et al.*, 1993; Pérez-Ruzafa, 2001). The only reported gender instability in this genus concerns subdioecious individuals (with both cosexual and unisexual conceptacles) that were observed in typically unisexual *F. ceranoides* (see references cited in Pérez-Ruzafa, 2001), although this has also been cited as evidence of hybridization with *F. spiralis* (Gard, 1910; Lein, 1984). However, the extent of gender instability within individuals has never been quantified at the population level. Moreover, as a result of hybridization between the two sexual systems, theoretical studies by Charlesworth & Charlesworth (1978a, 1978b) show that gender variation among conceptacles may be discrete or continuous depending on whether a few genes with large, or many genes with small, phenotypic effects control sexual phenotype. Hybrids may thus give rise to qualitatively and/or quantitatively intermediate sexual phenotypes. In addition, as hybridization may break up co-adapted gene complexes, hybrid fertility may be reduced in comparison with either parental species.

In this paper, we first examined the extent of gender variation within and among individual hybrids in comparison with both *F. vesiculosus* and *F. spiralis* species. Secondly, we quantified

male and female prezygotic fertility in parental species and their hybrids in order to test for reduction in hybrid fitness.

Materials and methods

Sampling

To compare hybrids with their parental species, we used individuals previously characterized by Engel *et al.* (2005) for their sexual phenotype (hermaphrodite *v.* dioecious) and genetic status (hybrid *v.* parental species). In the aforementioned study, the sexual phenotype of each individual was established by qualitative observation of a single receptacle per individual. In parallel, individuals were genotyped for five microsatellite loci developed by Engel *et al.* (2003) and the occurrence of genetically intermediate individuals was determined on the basis of genotypic assignment tests, using the software package STRUCTURE (Pritchard *et al.*, 2000) where the proportion of each species' genome was estimated for each individual. Individuals for which the proportion of one of the genomes was between 0.1 and 0.9 were considered as putative hybrids (Engel *et al.*, 2005, fig. 3).

In the present study, a total of 89 sexually mature individuals were selected (20 hermaphrodite *F. spiralis*, 10 hermaphrodite individuals classified as hybrids, 40 dioecious *F. vesiculosus* – 20 males and 20 females – and 19 dioecious hybrids – 10 males and 9 females) for a quantitative estimation of their fertility. Individuals were sampled from two types of situations: one called 'parapatric', where the habitats of the two species were separated on the shore; and one called 'sympatric', where both taxa were found in contiguous stands. The same sampling was repeated in two geographically distant regions, at Cape Gris-Nez in the North of France and at Viana do Castelo in the North of Portugal. The details of the sample size for each taxon and sexual phenotype within each region are shown in Table 1.

Variation in sexual phenotype and male and female fertility within individuals

For each parental taxon and their hybrids, sexual phenotype was characterized for six conceptacles (three in each of two receptacles) per individual. To search for sexual inconstancy (i.e. variation in gender within individuals), we investigated the extent of variation in male and female fertility within individuals. For each of the 89 selected individuals,

Table 1. Means and standard errors (SE) for individual male and female fertilities for *Fucus* spp. calculated over the different taxa and population types

a. Male fertility[a]

Taxon	Mean ± SE	Region	Situation	*n*	Mean ± SE
Male *F. vesiculosus*	1677 ± 42	Gris Nez	Parapatric	5	1548 ± 63
		Viana	Parapatric	5	1727 ± 81
		Gris Nez	Sympatric	5	1831 ± 116
		Viana	Sympatric	5	1601 ± 51
Hermaphrodite *F. spiralis*	162 ± 13	Gris Nez	Parapatric	5	130 ± 14
		Viana	Parapatric	5	216 ± 40
		Gris Nez	Sympatric	5	127 ± 13
		Viana	Sympatric	5	175 ± 21
Male hybrids	1899 ± 99	Gris-Nez		5	1409 ± 110
		Viana		5	2388 ± 108
Hermaphrodite hybrids	119 ± 13	Gris-Nez		5	86 ± 10
		Viana		5	151 ± 22

b. Female fertility[b]

Taxon	Mean ± SE	Region	Situation	*n*	Mean ± SE
Female *F. vesiculosus*	47.32 ± 1.73	Gris Nez	Parapatric	5	36.54 ± 2.95
		Viana	Parapatric	5	57.85 ± 3.47
		Gris Nez	Sympatric	5	47.40 ± 3.15
		Viana	Sympatric	5	47.48 ± 3.24
Hermaphrodite *F. spiralis*	36.41 ± 2.16	Gris Nez	Parapatric	5	31.02 ± 2.39
		Viana	Parapatric	5	23.35 ± 1.33
		Gris Nez	Sympatric	5	48.27 ± 6.75
		Viana	Sympatric	5	42.99 ± 3.22
Female hybrids	35.01 ± 1.95	Gris-Nez		4	33.79 ± 3.12
		Viana		5	35.99 ± 2.52
Hermaphrodite hybrids	30.73 ± 1.97	Gris-Nez		5	22.06 ± 1.81
		Viana		5	39.41 ± 2.71

n: number of sampled individuals.
[a] Density of antheridia per conceptacle (number of antheridia mm^{-2} conceptacle section).
[b] Density of oogonia per conceptacle (number of oogonia mm^{-2} conceptacle section).

two freeze-dried receptacles were re-hydrated in sea-water for 10 min, and 60-μm frozen sections were cut with a cryo-microtome in the mid-basal part of the receptacle to standardize measurements between conceptacles. Three conceptacles per receptacle were observed under a microscope with Spot Basic Software (Diagnostic Instruments Inc., 1996–2003). Female fertility was estimated as the density of oogonia per section of conceptacle (number of oogonia mm^{-2} conceptacle section) and male fertility as the density of antheridia per section of conceptacle (number of antheridia mm^{-2} conceptacle section). In contrast to hermaphrodite individuals, it was not feasible to count all antheridia in sections of unisexual male conceptacles; consequently, we used an estimate of this number (mean density of antheridia over three different unitary areas). The intra- and inter-individual components of fertility variation were quantified for both parental taxa and their hybrids. Since sympatric situations tended to experience more genetic admixture (Engel *et al.*, 2005), the analyses were carried out separately for parapatric and sympatric situations. Within these different situations, the proportional contribution of intra-individual and inter-individual variance to the overall variation in male and female fertilities was estimated using a nested model ANOVA:

$$Y_{ijn} = \mu + \alpha_i + \beta_{ij} + e_{ijn}$$

where Y_{ijn} is the fertility of the nth conceptacle of receptacle j nested in the ith individual, μ is the individual mean, α_i is the random contribution of the ith individual, β_{ij} is the random contribution of the jth receptacle of the ith individual and e_{ijn} is the error term. In order to obtain a rough estimate of the relative contribution of *within* individual variation to the overall variance, we added receptacle and conceptacle (i.e. error term) variances.

Variation for male and female fertilities between both parental taxa

Prior to testing for fertility reduction in hybrids, the pattern of variation in male and female fertilities was evaluated within and between the two parental species in order to investigate the effect of environmental variation (i.e. difference between regions) on sexual allocation to male and female functions. Moreover, species differences may be reinforced in sympatric compared to parapatric situations (Servedio & Noor, 2003). Male and female individual fertilities (i.e. fertilities averaged over individuals) were compared between species, distributional situations (parapatric v. sympatric) and regions (Gris Nez v. Viana) using the following mixed-model ANOVA:

$$Y_{ijkn} = \mu + A_i + B_j + \delta_k + (AB)_{ij} + (A\delta)_{ik} + (B\delta)_{jk} + (AB\delta)_{ijk} + e_{ijkn}$$

where Y_{ijkn} is the fertility of the nth individual of the ith species belonging to the jth population type and sampled in the kth region, μ is the species mean, A_i is the fixed effect of the ith species, B_j is the fixed effect of the jth population type, and δ_k is the random effect of the kth region. $(AB)_{ij}$, $(A\delta)_{ik}$ and $(B\delta)_{jk}$ are, respectively, the interaction effect of 'species × population type', 'species × region' and 'population type × region'. $(AB\delta)_{ijk}$ is the 'species × population type × region' interaction effect and e_{ijkn} is the error term.

Comparison between hybrids and parental species

The putative reduction of male or female individual fertility in hybrids compared to parental species was tested using the following mixed-model ANOVA:

$$Y_{ijn} = \mu + A_i + \beta_j + (A\beta)_{ij} + e_{ijn}$$

where Y_{ijn} is the fertility of the nth individual of the ith taxon (parental v. hybrid), μ is the taxon mean, A_i is the fixed effect of the ith taxon, β_j is the random effect of region, $(A\beta)_{ij}$ is the interaction effect between taxon and region and e_{ijn} is the error term.

Finally, to summarise the information for both male and female fitness components, the pattern of sex allocation in male and female functions estimated as Sperm/Egg ratio (S/E) was compared between hermaphrodites and dioecious taxa. For hermaphrodites, this ratio was obtained for each individual by multiplying the number of antheridia in the section by 64 (number of sperm per antheridium) and the number of oogonia in the section by 8 (number of eggs (ovule) per oogonium). For the dioecious taxa, an approximation was calculated using the total number of sperm and the total number of ovules produced in each situation in each region, following the suggestion of Vernet & Harper (1980). In addition, for the 10 hermaphrodite hybrid individuals, the evolution of S/E ratio was reported according to the proportion of *F. vesiculosus* genome estimated from the genotype data of Engel *et al.* (2005). The effect of the dioecious species genotypes on the pattern of sex allocation was tested using Spearman rank correlation.

All ANOVAs were conducted using the general linear model procedure of MINITAB (version 13.2 MiniTab Inc. 1994, State College USA). Male fertility (density of antheridia) and female fertility (density of oogonia) were log-transformed in order to meet the normality and homoscedasticity requirement of ANOVA. Multiple comparisons of means were performed using the GT2 method recommended for unequal sample sizes (Sokal & Rohlf, 1995, p. 244).

Results

While male conceptacles were easily recognizable, it was more difficult to distinguish between female and hermaphrodite conceptacles because of the occurrence of numerous sterile paraphyses in many cross-sections. Since sterile paraphyses appear to represent aborted male structures, their occurrence was recorded for each conceptacle.
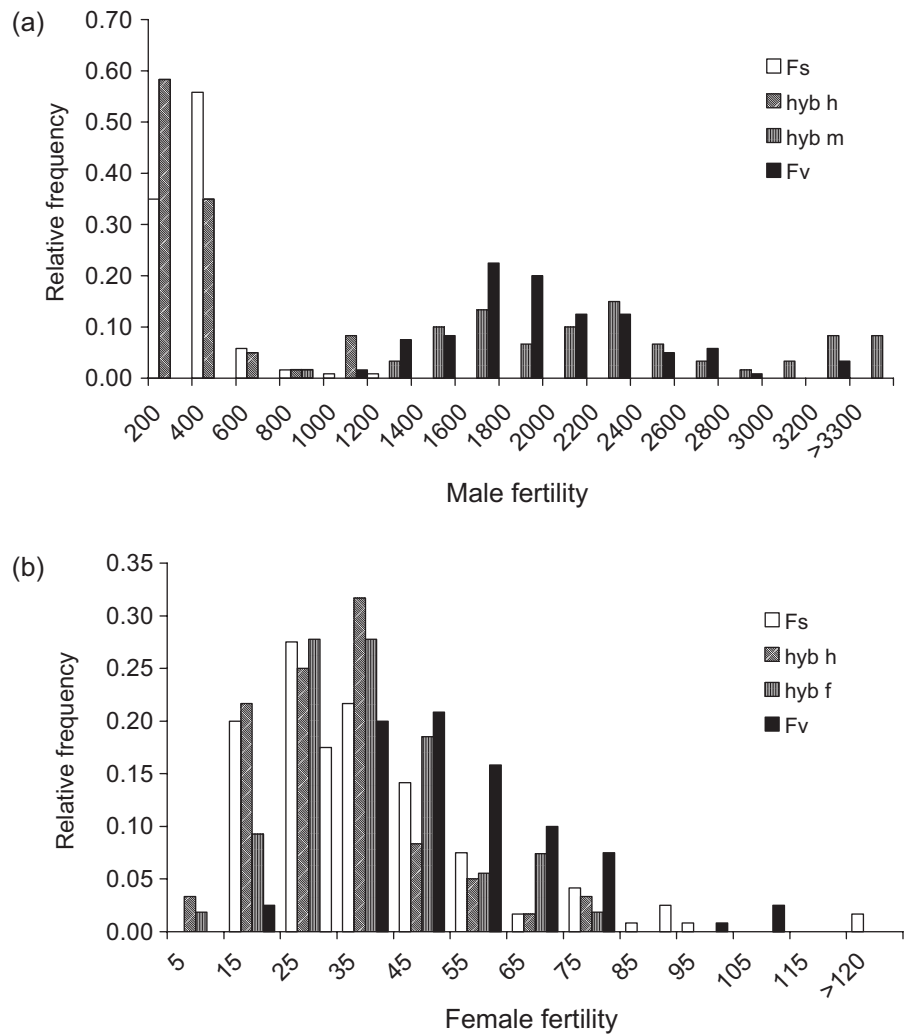
Fig 1. Frequency distribution of (a) male fertility and (b) female fertility estimated from 534 conceptacles from hermaphrodite individuals (Fs: *Fucus spiralis* and hyb h: hermaphrodite hybrids) and dioecious individuals (Fv: *F. vesiculosus*, hyb m: male hybrids and hyb f: female hybrids). See Table 1 for the definition of male and female fertility.

Sterile filaments were never observed in male individuals, but they were present in at least one out of the six sampled conceptacles per individual in females and hermaphrodites.

The frequency distribution of male and female fertilities estimated from the 534 observed conceptacles is given in Fig. 1. The distribution of male fertility was clearly multimodal and discriminated cosexual from unisexual individuals (Fig. 1a). The density of antheridia varied from 0 to 973 antheridia per mm^2 conceptacle section area for *F. spiralis* and hermaphrodite hybrids, and from 691 to 3920 in *F. vesiculosus* and unisexual hybrids. Consequently, the mean male fertility of both hermaphrodite taxa was ten times smaller than in dioecious taxa (Table 1a). This difference in fertility between unisexual and cosexual phenotypes was not found for the female function. The distribution of female fertility appeared unimodal and overlapped between the four taxa (Fig. 1b). The density of oogonia varied greatly among conceptacles (from 4 to 168 in hermaphrodites and from 5 to 103 in dioecious taxa). Inspection of mean values

suggested that female fertility tended to be lower in hermaphrodite taxa (Table 1b), although the effect seems to depend on the region and distributional situation (see below).

Variation of sexual phenotype within individuals

Among the 89 individuals studied, variation in sexual phenotype among conceptacles within an individual was observed only in a single hermaphrodite hybrid individual that exhibited one male-sterile (i.e. female) conceptacle while the other five conceptacles were clearly hermaphrodite. The only other qualitative patterns of sex inconstancy revealed concerned the presence/absence of sterile paraphyses in hermaphrodite and female individuals. This inconstancy was observed in both parental species and hybrids.

Variation in male and female fertility within individuals

Quantitative variation in sexual phenotype (i.e. variation in male or female fertility) was generally not significant among receptacles, whereas it was

Table 2. Result of nested ANOVA on the relative contribution (%) of between *versus* within-individual effects on the variance of male and female fertility, analyzed separately for parapatric and sympatric situations in each of the two parental species (*Fucus vesiculosus*, Fv; *F. spiralis*, Fs) and in unisexual and hermaphrodite hybrids.

a. Male fertility[a]

Group	Between-individual effect		Within-individual (receptacle) effect		SE
	%	F	%	F	%
Male *Fv* Parapatric	6.81	1.48	0[b]	0.91	93.19
Male *Fv* Sympatric	48.46	4.41*	16.86	2.46*	34.68
Hermaphrodite *Fs* Parapatric	28.97	4.27*	0[b]	0.75	71.03
Hermaphrodite *Fs* Sympatric	39.95	3.85*	12.04	1.75	48.01
Male hybrids	71.26	11.23***	6.54	1.88	22.20
Hermaphrodite hybrids	30.85	3.56*	0.84	1.04	68.31

b. Female fertility[a]

Group	Between-individual effect		Within–individual (receptacle) effect		SE
	%	F	%	F	%
Female *Fv* Parapatric	52.51	8.58**	0[b]	0.87	47.49
Female *Fv* Sympatric	44.74	4.16*	14.83	2.10*	40.43
Hermaphrodite *Fs* Parapatric	36.52	3.59*	10.63	1.60	52.85
Hermaphrodite *Fs* Sympatric	36.94	2.79	30.36	3.78**	32.70
Female hybrids	10.50	0.51	16.63	1.68	72.86
Hermaphrodite hybrids	58.26	6.91**	8.72	1.79	33.02

Significant *F*-values: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.
[a] See Table 1 for the definition of these variables. [b] Negative values close to zero due to larger within than among group variance.

significant among individuals (see nested ANOVA, Table 2), indicating that within-individual variation was less important than variation among-individuals. Over the 12 ANOVAs, variation among receptacles was significant in only three analyses, whereas variation between individuals was significant in all but three analyses (Table 2). However, even when non-significant values are removed, the proportion of total variation explained by inter-individual effects varied greatly among taxa (from 29% to more than 70%, Table 2) revealing some weak but interesting patterns, summarized below.

Within-individual variance of male fertility tended to be larger in sympatric than in parapatric situations in the two parental species (Table 2a). However, no increase in within-individual variation was detected in hybrids in comparison with either parental species. Indeed, the lowest contribution of within-individual variance to the overall (within plus between) variance of male fertility was observed in male hybrids. Finally, significant within-individual variation was observed only in dioecious species (significant contribution of receptacles in sympatric situations, but not in parapatric situations, Table 2a).

In contrast to male fertility, within-individual variance of female fertility tended to be larger in the hermaphrodite species. Indeed, in sympatric situations, the contribution of the within-individual term obscured the contribution of the between-individual term (Table 2b). In hybrids, contrasting results were obtained depending on the sexual phenotype. In female hybrids, no significant variation among individuals was observed while inter-individual variation was highly significant in hermaphrodite hybrids, explaining more than 50% of the overall variance in female fertility (Table 2b).

Variation in male and female fertility between parental taxa

The most obvious difference between parental species concerned the pattern of sex allocation between male and female functions. The density of antheridia of *F. vesiculosus* was much higher than in *F. spiralis* (Table 1a and Table 3a). Although there was a trend for *F. vesiculosus* to have greater densities of oogonia than *F. spiralis*, it was not consistently significant across all situations and regions (Table 3b, see also Fig. 1b and Table 1b). The much larger difference in male than in female fertility meant that the two species were easily distinguishable by their mean S/E ratio values (385.4 ± 35.8 for *F. vesiculosus*, and 44.3 ± 6.1 for *F. spiralis*).

Comparison between hybrids and parental species

When comparing hybrids with *F. vesiculosus* or *F. spiralis* for male (Table 4a) and female (Table 4b) functions, there was no significant taxon effect.

Table 3. Results of mixed model ANOVAs on effects of species, distributional situation (parapatric/sympatric) and region (Gris-Nez/Viana), on male (a) and female fertility (b)

a. Male fertility[a]

Source	df	MS	F	p
Species	1	12.3639	273.30	0.038
Distributional situation	1	0.0001	0.01	0.943
Region	1	0.0476	0.84	0.484
Species × Distributional situation	1	0.0043	8.63	0.209
Species × Region	1	0.0452	91.74	0.066
Distributional situation × Region	1	0.0117	23.67	0.129
Species × Distributional situation × Region	1	0.0005	0.02	0.894
Error	32	0.0274		
Total	39			

b. Female fertility[a]

Source	df	MS	F	p
Species	1	0.1945	2.86	0.340
Distributional situation	1	0.1140	17.58	0.149
Region	1	0.0085	0.84	0.968
Species × Distributional situation	1	0.0800	1.24	0.466
Species × Region	1	0.0680	1.06	0.491
Distributional situation × Region	1	0.0065	0.10	0.804
Species × Distributional situation × Region	1	0.0645	3.43	0.073
Error	32	0.0188		
Total	39			

[a] See Table 1 for the definition of these variables.

Table 4. Results of mixed model ANOVAs comparing male and female fertility between (a) *Fucus vesiculosus* and unisexual hybrids and (b) *F. spiralis* and hermaphrodite hybrids

Male fertility[a]	(a) *F. vesiculosus v.* unisexual hybrids				(b) *F. spiralis v.* hermaphrodite hybrids			
Source	df	MS	F	P	df	MS	F	p
Taxon	1	0.0072	0.07	0.834	1	0.1014	42.97	0.096
Region	1	0.1040	0.03	0.496	1	0.1603	67.94	0.077
Taxon*Region	1	0.1011	12.49	0.002	1	0.0024	0.06	0.814
Error	26	0.0081			26	0.0420		
Total	29				29			

Female fertility[a]								
Source	df	MS	F	P	df	MS	F	p
Taxon	1	0.1193	11.51	0.182	1	0.0317	0.17	0.748
Region	1	0.0306	2.96	0.335	1	0.0830	0.46	0.621
Taxon Region	1	0.0104	0.64	0.430	1	0.1813	6.42	0.018
Error	25	0.0161			26	0.0282		
Total	28				29			

[a] See Table 1 for the definition of these variables.

However, in two of the four cases, the divergence between parental taxa and hybrids varied depending on the region (*cf.* significant Taxon × Region interaction, Table 4). Hybrid fertility was lower than parental fertility in Gris-Nez but higher in Viana. Hybrid male fertility was higher than *F. vesiculosus* male fertility in Viana (GT2 multiple comparison of means tests, $p < 0.05$). The same trend was observed in Viana with female fertility

higher in hermaphrodite hybrids than in *F. spiralis*, however multiple comparisons were inconclusive. Therefore, there was no overall trend for hybrid superiority or inferiority.

Finally, the mean S/E ratios calculated for unisexual and cosexual hybrids were similar to the corresponding parental sexual phenotype (i.e. 401.4 ($\pm$60.6) for dioecious hybrids *v.* 385.4 ($\pm$35.8) for *F. vesiculosus*; and 42.2 ($\pm$8.8) for
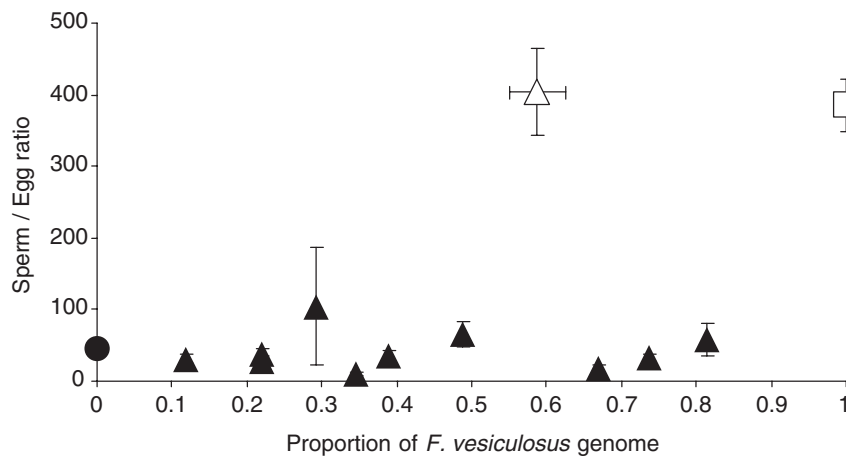
Fig. 2. Sperm/Egg (S/E) ratios of hermaphrodite hybrids (filled triangles) are represented according to genetic admixture (i.e. proportion of genome characteristic of *Fucus vesiculosus*; see Engel et al., 2005). The filled circle is the mean S/E ratio for *F. spiralis*, and the open square is the mean S/E ratio for *F. vesiculosus*. The mean S/E ratio according to the mean genetic admixture of dioecious hybrids is also presented (open triangle), with x and y standard error bars. Filled symbols: hermaphroditic individuals; open symbols: dioecious individuals.

hermaphrodite hybrids *v.* 44.3 (±6.1) for *F. spiralis*). The S/E ratio of hermaphrodite hybrids is presented in Fig. 2. The S/E ratio of hermaphrodite hybrids was not correlated with the proportion of *F. vesiculosus* genome (Spearman Rank correlation coefficient = 0.0009). Whatever the proportion of *F. vesiculosus* genome in hermaphrodite hybrids, their S/E ratio was always close to the S/E ratio of *F. spiralis*.

Discussion

Our results verify that sexual phenotype is a stable character within individuals of these two species. It was further shown that no quantitatively intermediate sexual phenotypes were present in hybrids. Together, this suggests that the genetic basis of sex determination in *Fucus* is controlled by a few genes with large effects. The second main result was that there were no differences in prezygotic fitness-related parameters (male and female gametangia densities) between putative hybrids and parental taxa.

The general absence of gender variation among conceptacles suggests the occurrence of a strong genetic component in sex determination. In brown seaweeds, a single Mendelian determinant of sex was first demonstrated in *Ectocarpus* spp. (Müller, 1967). The occurrence of sex chromosomes has been proposed in Laminariales (Evans, 1963; Fang, 1983) and in fucoids (Clayton, 1984), but has not yet been proved.

Among the 58 putative hybrids detected (see Engel *et al.*, 2005), all three sexual phenotypes were present: males (43%), females (28%) and hermaphrodites (28%). For only one individual was sexual phenotype not determined, probably due to immaturity. It has been reported (Westergaard, 1958) that when monoecious or hermaphrodite and dioecious species are crossed, it is very rare to observe all sexual phenotypes in the first generation of hybrids (F1; see Appendix); furthermore,

it is rare for all these sexual phenotypes to be fertile. Thus, it is possible that some sexual phenotypes are the result of second-generation hybrids (F2) and/or backcrosses. This implies that *F. spiralis* × *F. vesiculosus* hybrids are capable of backcrossing (suggested by Engel *et al.*, 2005), possibly due to the extremely recent divergence between *F. vesiculosus* and *F. spiralis* (Serrão *et al.*, 1999). Moreover, if we consider that the most probable crosses involve male individuals rather than hermaphrodites (see Appendix), the observed proportions of the three genders are consistent with sex determination with male heterogamety. However, these results disagree with those of Coyer *et al.* (2002) who observed only unisexual hybrids between *F. serratus* and *F. evanescens*. No simple sex determination system exists to explain this difference, except a nucleo-cytoplasmic sex determination

In this study we had no means of determining the generation of hybrids; this would require that crosses be performed under controlled conditions. However, the occurrence of backcrosses or F2 crosses is also a hypothesis that is supported by the lack of differences in fertility between hybrids and parental species. This means that hybrids are potentially as fertile as their parents. Our analyses of hybrid (prezygotic) fitness suggest that this may be greater or less than that of parental taxa, depending on region-specific effects. Indeed, it has been suggested (see Arnold, 1997) that both endogenous (e.g. disruption of co-adapted gene complexes) and environment-dependent selection on hybrids may operate. An example of the latter is the results from hybrid zones of two *Quercus* species (Williams *et al.*, 2001), in which hybrids were not necessarily less fit than their parents, especially in marginal areas. Indeed, our results show that hybrid fertility may be higher than parental fertility in Viana, the southern limit of co-occurrence of both *F. spiralis* and *F. vesiculosus* species. It would be interesting to extend these

results by investigating the relationship between relative hybrid fitness and environmental factors (e.g. those associated with geographic range).

The relative male and female fitness components in hybrids can be summarized by examining the S/E ratios for hermaphrodite and dioecious taxa. Again, for the same sexual phenotype (i.e. mating system), there were no differences in S/E between parental taxa and hybrids, while there was ten-fold difference between mating systems. These S/E ratios, obtained from cross-sections of conceptacles, were similar to those obtained by Vernet & Harper (1980) on whole conceptacles (400 and 40, respectively). It is interesting to compare these S/E ratios of *F. vesiculosus*, *F. spiralis* and hybrids with the mean Pollen/ Ovule ratios (P/O) characteristic of different breeding systems in angiosperms (Cruden, 1977). P/O ratios increase significantly with the likelihood of cross-fertilization and are, therefore, a good indicator of the breeding system. The hermaphrodite taxa, with a mean S/E ratio of 43, correspond to obligate and facultative self-fertilization, which is consistent with population genetic data revealing high heterozygote deficiencies in *F. spiralis* (Engel et al., 2005). On the other hand, dioecious taxa, with ratios of 400 correspond to the classes of facultative self-fertilization and facultative cross-fertilization. Since selfing is impossible in dioecious taxa, these ratios indicate biparental inbreeding; this hypothesis is also supported by significant heterozygote deficiencies revealed by population genetic data (Engel et al., 2005).

Interestingly, we found variation in the presence or absence of sterile paraphyses. In this study, sterile paraphyses were present in at least one conceptacle per individual in females and hermaphrodites, but were absent in males, in which all observed paraphyses bore antheridia. The occurrence of sterile paraphyses is interesting in the context of mating system evolution since they appear to correspond to relics of the antheridium-bearing paraphyses, which are present in conceptacles with male function. This is similar to land plants, where unisexual individuals of many dioecious species with cosexual relatives bear evident relics of the opposite sex (Darwin, 1877). In this context, hermaphroditism is considered as the primitive state (see for review Geber et al., 1999). Several evolutionary scenarios can explain the evolution of dioecy from cosexuality (Geber et al., 1999); one involving a minimum of two major mutations (one causing male sterility and the other female sterility), while another involves many mutations with smaller effects and a range of intermediate sexual phenotypes (Charlesworth, 2002).

The Fucaceae show a wide range of variation of mating systems among its species: hermaphrodite (most genera), monoecious (only the genus *Xiphophora*, having separate male and female conceptacles in the same individuals with occasional hermaphrodite conceptacles) and dioecious (*Ascophyllum nodosum* and some species of *Fucus*) systems are found. However, little intra-specific variation has ever been reported in the Fucaceae. For example, monoecious individuals have been reported in the dioecious species *F. ceranoides* (Hamel, 1939) although this is controversial as monoecious individuals may be result of hybridization between *F. ceranoides* and *F. spiralis* (Lein, 1984). Within the Sargassaceae, a sister group of the Fucaceae (Rousseau et al., 2001), variation for mating system between and within species is even higher. In *Turbinaria ornate*, for instance, one type of receptacle carries male or hermaphrodite conceptacles and the other type carries female conceptacles; individuals may harbour only one type of receptacle or both, and may thus be andromonoecious, female or polygamomonoecious (Stiger, 1997). The genus *Cystoseira*, which is mainly composed of hermaphrodite species, also includes some species whose individuals carry male, female and hermaphrodite conceptacles in the same receptacles (Hamel, 1939). Finally, in the genus *Sargassum*, some individuals of a monoecious species (*S. flavifolium*) harbour receptacles with male and female conceptacles whereas individuals of another species (*S. trichocarpum*) carry strictly male or female receptacles. In this context, Clayton (1984) speculated that, in the order Fucales, the ancestral states could have been hermaphrodite, as in angiosperms. In this study, the observation of sterile filaments in females and the rarity of intermediate phenotypes in *F. vesiculosus* and *F. spiralis*, even among hybrids, support the hypothesis of a simple genetic sexual determination with few major genes involved, and thus of evolution from hermaphroditism to dioecy via the gynodioecy pathway (Charlesworth, 1999, 2002).

Acknowledgements

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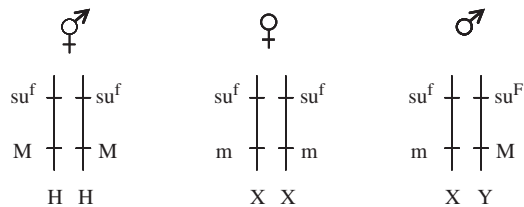
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Appendix

1. Sex determination with male heterogamety

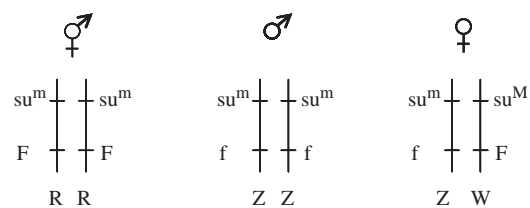Su^F: Female fertility suppressor, M: Male fertility

All possible F_1 crosses	Progeny
hermaphrodite (HH) × male (XY)*	50% hermaphrodite (HX) 50% male (HY)
female (XX) × hermaphrodite (HH)	100% hermaphrodite (HX)

All possible back-crosses	
hermaphrodite (HX) × male (XY)*	50% male (HY + XY) 25% hermaphrodite (HX) 25% female (XX)
male (HY) × hermaphrodite (HH)*	50% hermaphrodite (HH) 50% male (HY)
male (HY) × female (XX)*	50% male (XY) 50% female (HX)
hermaphrodite (HX) × female (XX)	50% hermaphrodite (HX) 50% female (XX)
hermaphrodite (HX) × hermaphrodite (HH)	100% hermaphrodite (HX + HH)

*The most probable crosses, due to the fact that male individuals produce ten times more sperm than hermaphrodite and thus that female and hermaphrodite individuals are more prone to be fertilized by a male than by a hermaphrodite. These crosses result in at least 50% of male progeny.

2. Sex determination with female heterogamety

Su^M: Male fertility suppressor, F: Female fertility

All possible F_1 crosses	Progeny
hermaphrodite (RR) × males (ZZ)*	100% hermaphrodite (RZ)
female (ZW) × hermaphrodite (RR)	50% hermaphrodite (RZ) 50% female (RW)

All possible back-crosses	
hermaphrodite (RZ) × male (ZZ)*	50% hermaphrodite (RZ) 50% male (ZZ)
female (RW) × male (ZZ)*	50% female (ZW) 50% hermaphrodite (RZ)
hermaphrodite (RZ) × female (ZW)	50% female (RW + ZW) 25% hermaphrodite (RZ) 25% male (ZZ)
female (RW) × hermaphrodite (RR)	50% hermaphrodite (RR) 50% female (RW)
hermaphrodite (RZ) × hermaphrodite (RR)	100% hermaphrodite (RZ + RR)

*The most probable crosses, due to the fact that male individuals produce ten times more sperm than herma-phrodite and thus that female and hermaphrodite individuals are more prone to be fertilized by a male than by a herma-phrodite. These crosses result in an excess of hermaphrodite progeny.