Eur. J. Phycol. (2002), **37**: 179–190. © 2002 British Phycological Society
DOI: 10.1017/S096702620200358X Printed in the United Kingdom

Non-random mating in controlled multiple-donor crosses in *Gracilaria gracilis* (Gracilariaceae, Rhodophyta)

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(*Received 20 June 2001; accepted 22 November 2001*)

Crosses were performed to identify the sources of variation in zygote production (via cystocarp production) in *Gracilaria gracilis*, a red haploid-diploid seaweed. First, because male gametes are short-lived (< 6 h), the rate of gamete encounters was evaluated in a time-course experiment. Second, the effect of water motion on gamete encounters was assessed by introducing turbulent eddies in the crossing tank and by comparing fertilization rates with and without this added turbulence. Third, variation due to individual performance was explored by performing multiple-donor crosses using 12 males and 12 females from three populations. Paternity of cystocarps produced in these crosses was determined using microsatellite markers. The results show that cystocarp yield increased with exposure time: fertilization occurred in as little as 15 min after the introduction of male branches into the crossing tank and maximum cystocarp production values were observed at 6 h. There were no significant differences in cystocarp production between the two turbulence levels. On the other hand, cystocarp production was highly influenced by male and female parental identities and to a lesser degree by an interaction between the male and female parents. The variation in cystocarp production according to male and female identity was not due to population origin as there was no difference between intra- and inter-population crosses. Thus non-random mating occurs in controlled conditions and arises from differential performance in *G. gracilis*. There was a strong deviation from equality of male performance, implicating post-adhesion events and/or male gamete production as important in generating non-random mating. Consequently, non-random mating may play a role in the evolution of mating patterns in *G. gracilis*.

Key words: fertilization, male competition, mate choice, reproductive success, spermatia, sexual selection

Introduction

Non-random mating may be attributed to incompatibility reactions, inbreeding and/or outbreeding depression, natural selection increasing gamete production and/or transport or sexual selection via male competition or female choice (Charlesworth *et al.*, 1987; Queller, 1987; Lyons *et al.*, 1989; Willson, 1990; Arnold, 1994; Tregenza & Wedell, 2000). Therefore, if non-random mating is detected, one must sort through these different possibilities to determine the evolutionary consequences of differential reproductive success. Sexual selection, 'selection arising from intra-sexual differences in mating success' (Arnold, 1994), is particularly difficult to demonstrate in free-spawning sessile organisms (e.g. marine invertebrates and seed plants) which release gametes into the environment. In free-spawning organisms male competition and/or female choice, if present, occur at the site of gamete acquisition and/or fusion but not between macroscopic individuals (Bishop & Pemberton, 1997; cf. cryptic female choice and/or sperm competition; e.g. Birkhead, 1998, 2000; Eberhard, 1998, 2000). The phenotypic traits responsible for differential mating success are therefore, at best, difficult to observe. Although it is clear that the processes involved in fertilization are under strong selection pressure, the existence, intensity and consequences of male competition for access to female gametes and/or female choice remain controversial among marine biologists (Levitan & Petersen, 1995; Serrão *et al.*, 1996; Levitan, 1998; Yund, 2000) and botanists (Charlesworth *et*

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al., 1987; Queller, 1987; Lyons *et al*., 1989; Marshall, 1998).

There is evidence for non-random mating in a natural population of a red haploid-diploid seaweed, *Gracilaria gracilis*. In a paternity analysis of cystocarps (which derive from zygotes) produced in a natural population, Engel *et al*. (1999) demonstrated that the substantial variation in male fertilization success could not be explained solely by the distance travelled by the male gamete to find a mate. Furthermore, only some of the variation between males could be attributed to the frequency of males in the immediate vicinity or to differences in thallus size (Engel *et al*., 1999). These results suggested that male competition or female choice played a role in determining the mating events.

Observed differential reproductive success may be the result of differences between individuals at any or all stages of sexual reproduction. Sexual reproduction in *G. gracilis* can be divided into three basic stages: delivery of male gametes; adhesion of male gametes and pre-fertilization; and zygote maturation (post-fertilization) (cf. Lyons *et al*., 1989; Willson, 1990, 1994). First, male gametes in red seaweeds (spermatia) are not flagellated and are passively transported in the water column until fusion with a female gamete. Second, fertilization takes place on the female plant. Male gametes adhere to the trichogyne, narrow extensions of the female gametangium (carpogonium) that protrude slightly at the surface of the female thallus (see Edelstein *et al*., 1978; Fredericq & Hommersand, 1989). Third, fertilization is followed by the formation of a cystocarp, a macroscopic hemispherical fruiting body on the female thallus within which the zygote multiplies by mitosis producing thousands of diploid spores. Thus, variation in reproductive success may have up to three basic components: male identity (intrinsic performance differences among males), female identity (intrinsic performance differences among females) and interactions between male and female identities (specific combining ability) (Lyons *et al*., 1989). However, in the paternity analysis study in *G. gracilis* (Engel *et al*., 1999), female identity effects could not be separated from distance effects so that the influence of female and/or male × female effects could not be assessed. Controlled crosses are therefore necessary for the evaluation of the relative importance of the different components of reproductive success.

Previous within- and between-population crosses of *G. gracilis* individuals from the same region showed that female identity affected the production of cystocarps but neither male identity nor the female × male interaction had any significant effects (Richerd *et al*., 1993). Thus, the strong male effect observed in paternity analysis (Engel *et al*., 1999) was absent in these crossing experiments. However, these crosses were performed using single donors, while in natural populations, female and/or male reproductive success may be affected by the presence of other males. In contrast, multiple-donor crosses should more closely simulate the competition dynamics met in the field. However, without genetic markers, testing the male effect in multiple-donor crosses is not feasible.

In this study we evaluated the sources of variation in male and female reproductive success using multiple-donor crosses. Microsatellite markers, developed for *G. gracilis* (Wattier *et al*., 1997; Luo *et al*., 1999), were used to assign paternity to cystocarps for the assessment of male success in multiple-donor crosses. Cystocarp production was then assessed for the effects of female identity, male identity and interaction of male and female identities. Parallel to parental effects, the probability of gamete encounters may depend on other factors, such as time and turbulent eddies. First, the rate of gamete encounters may vary according to exposure time. Thus, to monitor temporal variation in reproductive success, the rate of cystocarp production was evaluated in a separate time-course experiment. Second, turbulent eddies may increase the probability of contact between gametes (Sheath & Hambrook, 1990). Thus, we introduced turbulent eddies in the crossing experiment and female reproductive success was compared with and without this added turbulence.

Materials and methods

Study species

Gracilaria gracilis (Stackhouse) Steentoft, Irvine & Farnham (previously known as *G. verrucosa*) is characterized by a typical rhodophyte haploid-diploid life cycle. Gametophytic and tetrasporophytic plants are independent, isomorphic, perennial and long-lived. The gametophytic phase is dioecious. The thallus is an erect system of cylindrical branches that grow from the holdfast. Gametophytes produce gametes by mitosis and the reproductive structures (gametangia) are uniformly distributed along the thallus (Kling & Bodard, 1987) and are produced as the branches grow. Dispersal of male gametes (spermatia) is limited spatially and temporally: spermatia are non-flagellated and are viable for less than 6 h (Destombe *et al*., 1990), with fertilization generally occurring at less than 1 m from the paternal thallus (Engel *et al*., 1999). Female gametangia produce a single gamete (carpogonium), several cell layers from the surface of the thallus. Spermatia fuse with the carpogonial extension (trichogyne) and after syngamy the female gametangial apparatus develops into a cystocarp (Hommersand & Fredericq, 1990). Cystocarps reach maturity approximately 1 month after fertilization with the diploid carpospores progressively released over 6–10 weeks (Lefebvre *et al*., 1987).

Non-destructive quantification of gamete production is difficult in both males and females of the Gracilariales.

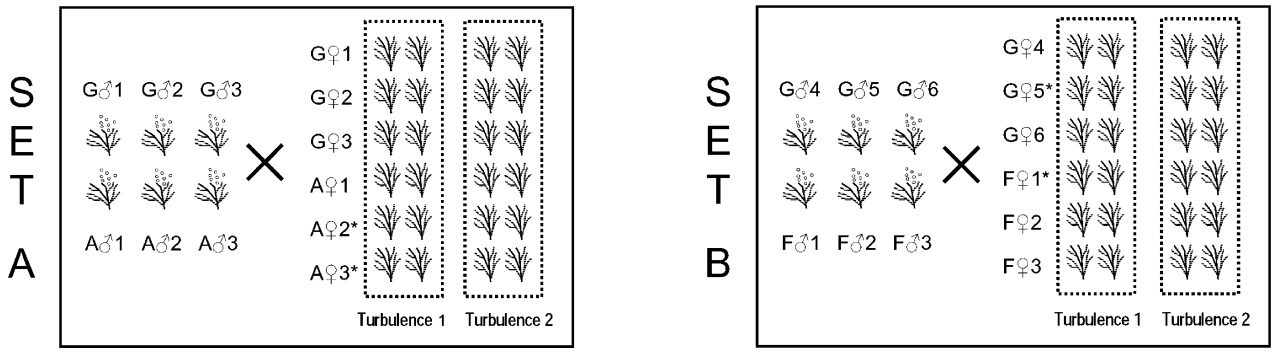

Fig. 1. Schematic diagram of the crossing design. Crosses were performed using two sets of individuals (set A and set B). Letters refer to population of origin: A, Audresselles; G, Cape Gris-Nez; F, Framzelle. All 6 males were simultaneously present in the flume with each set of females. Replicate barrettes securing the female thalli were positioned at different turbulence conditions. Males are shown releasing gametes. Female thalli were divided into several branches, or replicates, depicted in each aquarium. *Female excluded from final analyses (see Results).

Spermatia are *c.* 5 μm in diameter, immobile and lack pigments. Therefore, they can be easily confused with microorganisms and/or particles in non-sterile seawater. Trichogynes are short and difficult to observe (Edelstein *et al.*, 1978; Destombe, 1987), so that only destructive serial cross-sections of the female thallus allow the detection of carpogonia. Therefore, gamete production was indirectly controlled. Male gamete production was standardized by picking branches bearing approximately equal quantities of sexually mature new growth, determined by the observation of mature male crypts under a dissecting microscope. Similarly, the presence of female gametes was inferred on the basis of new, virgin growth and gamete production, calibrated among females using the length of new growth.

Sampling and growth culture conditions

More than 30 male and female individuals were sampled from tide pools (= populations) at three locations on the French coast off the Strait of Dover: Audresselles (A), Cape Gris-Nez (G) and Framzelle (F). Cape Gris-Nez is situated 5 km north of Audresselles and Framzelle is 500 m east of Cape Gris-Nez. Female thalli were sampled in the three pools in late March 1998. To ensure that females were virgin, female thalli were isolated and grown in open 150 l flow tanks at the Wimereux Marine Station in natural light conditions for 5 weeks. The flow tanks were heated to *c.* 16 °C to accelerate growth. Although the flow tanks were supplied with natural seawater, the risk of contamination by viable male gametes was minimal because the seawater is pumped only at high tide, allowed to decant in a reservoir and dispatched to the station at the following high tide, i.e. 12 h later, at least 6 h longer than the life-span of a spermatium. Moreover, no young or budding cystocarps were observed on new-grown thallus prior to the crossing experiments (i.e. after 5 weeks of culture). Males were sampled in the same three pools in late April, 1 week before performing the crosses. Male thalli were placed in separate flow tanks and extra care was taken not to pollute the tanks containing female thalli. Just before performing the crosses, female and male thalli were divided into several branches, or replicates, and tagged with an identifying number. Female replicates each bore at least 20 cm of new growth and any cystocarps found on older parts of the thallus were excised.

Before performing the crosses, all individuals were genotyped using seven microsatellite loci: Gv2CT (described in Wattier *et al.*, 1997) and Gg121, Gg155, Gg173, Gg182, Gg202 and Gg216 (described in Luo *et al.*, 1999). DNA extractions were carried out on three branch tips using a modified Chelex protocol (Wattier *et al.*, 1997). Polymerase chain reaction (PCR) amplifications and allele typing are described elsewhere (Wattier *et al.*, 1997; Luo *et al.*, 1999). The genotypes of the individuals used in this study are available upon request.

Crossing methods

Since the crosses were performed within a restricted time period (6 h, i.e. half of one tidal cycle) and in a large volume of seawater (see below), we assessed and enhanced the experimental conditions in two ways. First, because male gametes are short-lived (< 6 h), we evaluated the kinetics of fertilization rates in a separate time-course experiment. Second, to maximize the probability of encounters between the non-flagellated male gametes and trichogynes, we introduced turbulent eddies in the multiple-donor experiments, creating two contrasting turbulence conditions. We compared the fertilization rates with and without this added turbulence.

Crosses were performed in a closed, oval 'racetrack' flume ('track' *c.* 30 cm wide, outer circumference 4 m, inner circumference 2 m; designed by D. Davoult and D. Menu). The flume was emptied, rinsed and filled with 300 l of seawater 12 h before the crosses were performed. Water circulated in the flume by means of a wheel spanning the width of the flume and the current speed was regulated at 14 cm s^{-1}. The proximal ends of two branches of each male were taped to a PVC plate and placed 40 cm upstream from the current-generating wheel. To ensure even mixing of male gametes, a large-mesh (grid size 1 cm^2) screen was placed in front of the wheel. The proximal ends of two replicate branches of each female were secured, in random order, in a foam-lined PVC clip (barrette), *c.* 5 cm wide and 29 cm long. Barrettes were suspended from the edges of the flume in the zone opposite the wheel. Two replicate barrettes were simultaneously employed in the flume. One barrette was

placed directly behind a large-mesh screen (grid size 1 cm^2), which created a turbulence of $2{\cdot}7 \times 10^{-2}$ m^2 s^{-1}, and the other barrette was placed 80 cm behind the first, representing the base turbulence of $1{\cdot}6 \times 10^{-2}$ m^2 s^{-3}. Turbulent velocity was measured by high-frequency (100 Hz) hot-film velocimetry and the turbulent energy dissipation rate was estimated following Tennekes & Lumley (1972). The male branches were set into the flume once the barrettes were in place. Once the experiment was completed, the female branches were isolated in flow tanks and cultured for 5 weeks.

Fertilization kinetics. To assess the kinetics of fertilization, fertilization rates were compared at five successive time intervals. The males and females used in this experiment were not used in the multiple-donor crosses (see below), but came from the same pools. Three females were divided into 20 branches, providing two replicates at both turbulence levels and at each of the five time intervals. Two branches of 5 males were set in the flume at time 0. Two branches of each female and at each level of turbulence were removed from the barrettes at 15, 30, 60, 180 and 360 min after the introduction of the male branches.

Multiple-donor assessment crosses. Crosses were performed using two sets of individuals. Each set included 6 male and 6 female individuals from two populations, allowing both intra- and inter-population crosses (Fig. 1). The first set (set A) was composed of 3 males and 3 females from the G pool and 3 males and 3 females from the A pool. The second set (set B) was composed of 3 males and 3 females from the G pool and 3 males and 3 females from the F pool. Crosses were set up to facilitate paternity analyses; males used in a given set could be distinguished on the basis of only two microsatellite loci: Gv2CT and Gg173 (data not shown). Replicate female branches were necessary for the simultaneous testing of male, female *and* male × female interaction effects.

The two barrettes, each securing two replicates of the 6 females in a set, were removed 6 h after the male branches were set in the flume. Set B crosses were performed 1 day after set A crosses. A total of 48 crosses were performed (2 sets × 2 turbulence levels × 2 replicates × 6 females) (see Fig. 1).

Estimating reproductive success

Female reproductive success. After 5 weeks of culture, the number of cystocarps was counted on each replicate of each female parent. The total number of cystocarps was divided by the total length of new growth (i.e. since field collection). This measure of cystocarp production (cystocarps cm^{-1}) corresponds to (overall) female reproductive success. The number of cystocarps per unit thallus thus reflected differences between females in the number of zygotes per centimetre of initially virgin thallus.

Male reproductive success. From the females included in the final analyses (see Results), up to 21 cystocarps (mean 19·09, SE 0·11, $n = 32$) were excised from each female branch; an average of 76 cystocarps were thus analysed for each female parent. Paternity of these cystocarps was determined using the two diagnostic microsatellite loci, Gv2CT and Gg173. DNA extraction, PCR amplification and allele typing of cystocarps were performed as for branch tips, as described above.

The percentage of cystocarps sired by a male on each female branch was determined by dividing the number of cystocarps assigned to the male in question by the number of cystocarps analysed on that branch. This percentage was multiplied by the cystocarp production of the female branch, yielding cystocarps cm^{-1}. In other words, male reproductive success was weighted by the reproductive success of each female branch. This estimate is independent of the length of available virgin female thallus and it incorporates variation in female reproductive success.

Since there were six spermatia donors per branch in these crosses, there were six observations of (partial) cystocarp production for each female branch. Thus, the variable cystocarps cm^{-1} has both male and female reproductive success components, and through the implementation of a two-way orthogonal ANOVA it can be used to test simultaneously for female, male and interaction effects.

Statistical analyses

Analyses were performed using the SAS statistical package (SAS Institute, 1996). The variable cystocarps cm^{-1} was square-root transformed to homogenize variances. In all the analyses, nested ANOVAs were employed to detect differences in cystocarp production using the SAS GLM procedure. Male and female identities were nested within the set to which they belonged. The set effect was treated as a random effect. Type III sums of squares were used for tests of significance. Comparisons of means were carried out using a multiple range test (the REGWQ option in the GLM procedure) (SAS Institute, 1996).

Fertilization kinetics. We tested the effect of exposure time on overall cystocarp yield. The effects tested included length of time and turbulence levels, as well as their interaction. Both factors were declared fixed effects.

Multiple-donor assessment crosses

Turbulence conditions. To explore differences in gamete encounters in the different turbulence conditions, differences between females in total cystocarp production were tested at the two turbulence levels. The effects tested included set, female identity nested within set, turbulence level (treated as a fixed effect) and the two-way interaction between female identity and turbulence.

Variation due to individual performance. Here, we explored the effects of the male and female parent identities as well as the male × female interaction. As there were no differences between the turbulence levels (see Results), observations were pooled across turbulence levels. To investigate the magnitude of the contributions of male and female identities and their interaction, variance components were calculated using equations generated by the SAS 'random' statement.

Population structure and mating success. Finally, as there was a significant male × female interaction (see Results), we explored the association between cystocarp production of a given male–female pair and their popu-

lation of origin. We compared the reproductive output (cystocarps cm^{-1}) of males and females of the same population (intra-population pairs) with that of males and females coming from different populations (inter-population pairs). The type of pair effect was nested within sets.

Results

Fertilization kinetics

Neither turbulence level nor the interaction between exposure time and turbulence significantly affected mean cystocarp yield (results not shown; two-way ANOVA, $p > 0{\cdot}80$). We thus simplified the model by removing these terms. Cystocarp yield increased with exposure time in the flume (one-way ANOVA, $F_{4,59} = 4{\cdot}17$; $p = 0{\cdot}005$). While cystocarps were detected on branches exposed for only 15 min, the mean cystocarp yields at 15 min and 30 min were significantly lower than the mean yield at 6 h (Fig. 2). Thus, although male gametes can encounter and fuse with a trichogyne in 15 min or less, maximum cystocarp production values were observed at 6 h.

Cystocarp production

Female reproductive success. A total of 4916 cystocarps were produced in the multiple-donor crossing experiment, averaging $1{\cdot}271 \pm 0{\cdot}185$ ($x \pm$SE, $n = 48$) cystocarps per centimetre of female thallus. Some cystocarps showed arrested development. These aborted cystocarps represented only 1·6% of the cystocarps, with a mean of $0{\cdot}022 \pm 0{\cdot}004$ ($n = 48$) per centimetre of thallus. Aborted cystocarps therefore constituted a negligible source of variation and were not considered in the analyses. At least two cystocarps were produced on at least one branch of each female, indicating that all females were capable of producing cystocarps. Nevertheless, the average cystocarp production differed strongly among females (Tables 1, 2). Furthermore, two females in each set showed very low cystocarp production (Table 1, set A: A♀2 and A♀3; set B: G♀5 and F♀1). Since, on average, fewer than 10 cystocarps could be analysed per branch, these females were excluded from subsequent analyses. Differences between females remained significant even after the removal of these four females (same ANOVA model, female(set), $F_{6,16} = 23{\cdot}42$, $p < 0{\cdot}0001$); thus the extremely low reproductive success of the 4 excluded females was not wholly responsible for the female identity effect.

There were no global, significant differences between the two turbulence levels, whatever the set (Table 2). The female × turbulence interaction approached significance ($p = 0{\cdot}0612$, Table 2). However, after the exclusion of the 4 poorly receptive females, the trend was considerably weaker ($F_{6,16} = 1{\cdot}98$, $p = 0{\cdot}1284$). We therefore considered that the effect of added turbulence was negligible and obser-

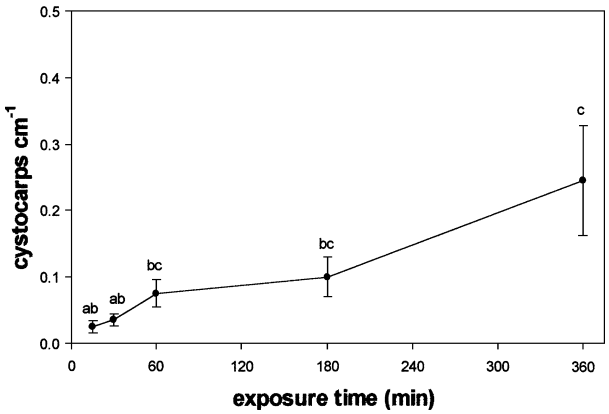

Fig. 2. Time-course of cystocarp production over a period of 6 h. Untransformed means and SE are plotted. Means with the same letter are not significantly different ($p > 0{\cdot}05$).

Table 1. Mean total number of cystocarps per centimetre of thallus for each female in both sets of multiple-donor crosses

Set A		Set B	
Female	Mean ± SE ($n = 4$)	Female	Mean ± SE ($n = 4$)
G♀1	$3{\cdot}422 \pm 0{\cdot}329^a$	G♀4	$1{\cdot}115 \pm 0{\cdot}232^b$
G♀2	$1{\cdot}985 \pm 0{\cdot}377^b$	G♀5	$0{\cdot}097 \pm 0{\cdot}027^c$
G♀3	$0{\cdot}600 \pm 0{\cdot}048^c$	G♀6	$0{\cdot}559 \pm 0{\cdot}100^b$
A♀1	$1{\cdot}832 \pm 0{\cdot}128^b$	F♀1	$0{\cdot}112 \pm 0{\cdot}035^c$
A♀2	$0{\cdot}017 \pm 0{\cdot}008^d$	F♀2	$2{\cdot}908 \pm 0{\cdot}371^a$
A♀3	$0{\cdot}012 \pm 0{\cdot}012^d$	F♀3	$2{\cdot}596 \pm 0{\cdot}462^a$

Data are raw means.
Means within a set with the same letter are not significantly different.

Table 2. Female identity and turbulence effects on total female fertilization success in multiple donor crosses: mixed-model ANOVA on total number of cystocarps per cm thallus

Source	df	SS	MS	*F*	*P*
Set	1	0·009	0·009	0·005	0·9450
Female(Set)	10	18·050	1·805	73·024	< 0·0001
Turbulence	1	0·000	0·000	0·001	0·9775
Turbulence × Set	1	0·018	0·018	0·331	0·5780
Female × Turbulence (Set)	10	0·530	0·053	2·144	0·0612
Residual	24	0·593	0·025		
Total	47	19·199			

Analysis performed on square-root transformed data.

Table 3. Mean male and female reproductive success from both sets of crosses: mean number (SE) of cystocarps per centimetre of thallus sired by a male and mean number of cystocarps borne on a female on a per-male basis

Set A					Set B				
Males ($n = 16$)		Females ($n = 24$)		Overall ($n = 96$)	Males ($n = 16$)		Females ($n = 24$)		Overall ($n = 96$)
G♂1	$0{\cdot}503^{ab}$ (0·107)	G♀1	$0{\cdot}570^{a}$ (0·122)	0·327 (0·039)	G♂4	$0{\cdot}496^{ab}$ (0·092)	G♀4	$0{\cdot}186^{b}$ (0·039)	0·299 (0·040)
G♂2	$0{\cdot}326^{bc}$ (0·056)	G♀2	$0{\cdot}330^{a}$ (0·057)		G♂5	$0{\cdot}168^{c}$ (0·036)	G♀6	$0{\cdot}093^{b}$ (0·019)	
G♂3	$0{\cdot}160^{de}$ (0·043)	G♀3	$0{\cdot}100^{b}$ (0·026)		G♂6	$0{\cdot}721^{a}$ (0·150)	F♀2	$0{\cdot}485^{a}$ (0·098)	
A♂1	$0{\cdot}227^{cd}$ (0·051)	A♀1	$0{\cdot}305^{a}$ (0·031)		F♂1	$0{\cdot}025^{d}$ (0·014)	F♀3	$0{\cdot}433^{a}$ (0·101)	
A♂2	$0{\cdot}070^{e}$ (0·023)				F♂2	$0{\cdot}309^{b}$ (0·059)			
A♂3	$0{\cdot}675^{a}$ (0·142)				F♂3	$0{\cdot}075^{d}$ (0·026)			

Data are raw means.
Means within a sex and within a set with the same letter are not significantly different ($p > 0{\cdot}05$).
'Overall' refers to the average number of cystocarps per unit thallus produced by a pair of mates.

vations were pooled over turbulence levels in subsequent analyses, giving four replicate branches for each female parent.

Paternity analyses. From the 8 females retained for subsequent analyses, 611 cystocarps were genotyped: 297 from set A females and 314 from set B females. Of these, 283 and 278 cystocarps, in sets A and B, respectively, were included in the final analyses. Cystocarps were excluded for two reasons: (1) non-amplification which precluded identification of a sire (13 and 34 cystocarps in sets A and B, respectively) or (2) identification of paternal genotypes that did not match any of the 6 potential sires (1 and 2 cystocarps in sets A and B, respectively). These unidentified genotypes indicate that the global, apparent (cross-)contamination rate was 0·5%.

Male reproductive success. All males sired at least one cystocarp on at least 3 females, indicating that all produced viable male gametes. Since there were 6 donors, if all males contributed equally to cystocarp production, each male would have sired one-sixth of the total number of cystocarps or 16·7%. However, the average proportion of cystocarps sired ranged from 2·1% to 38·3%, showing substantial variation from evenly distributed paternity (results not shown). Males sired significantly different numbers of cystocarps per centimetre of female thallus (Tables 3, 4). Further, this was true for both sets of crosses as there was no set effect (Table 4). Thus, in general, some males enjoyed higher fertilization success than others.

Male × female interaction. The significant male × female interaction confirmed that males did not fertilize the same proportion of cystocarps on all females (Table 4; Fig. 3A, B). In particular, in set A, G♂3 was a poor performer on G♀1 but sired an appreciable proportion of cystocarps on G♀2 (Fig. 3A). Likewise, G♂1 was among the best two sires on G♀1 but enjoyed only average success on A♀1 (Fig. 3A). A similar pattern was observed in set B; F♂2 was among the best sires on F♀2 but among the average sires on F♀3 (Fig. 3B). However, with the exception of G♀2 in set A, the relative rank among males changed little from one female to another (Fig. 3A, B). In general, males that had lower-than-average cystocarp production performed poorly on all females: their reproductive success did not increase on those females that had high total cystocarp production. On the other hand, cystocarp

Table 4. Male and female identity effects on cystocarp production: model II ANOVA on number of cystocarps per cm of thallus

Source	df	SS	MS	F	p	r^2 (%)
Set	1	0·102	0·102	0·069	0·7965	0*
Male(Set)	10	9·717	0·972	10·446	< 0·0001	43·4
Female(Set)	6	3·553	0·592	6·366	0·0002	16·4
Male × Female (Set)	30	2·791	0·093	2·530	< 0·0001	11·1
Residual	144	5·294	0·037			29·1
Total	191	21·456				

Analysis was performed on square-root transformed data.
r^2, percentage of total variance explained by the effects included in the model.
* Estimation of variance component was negative, attributable to sampling.

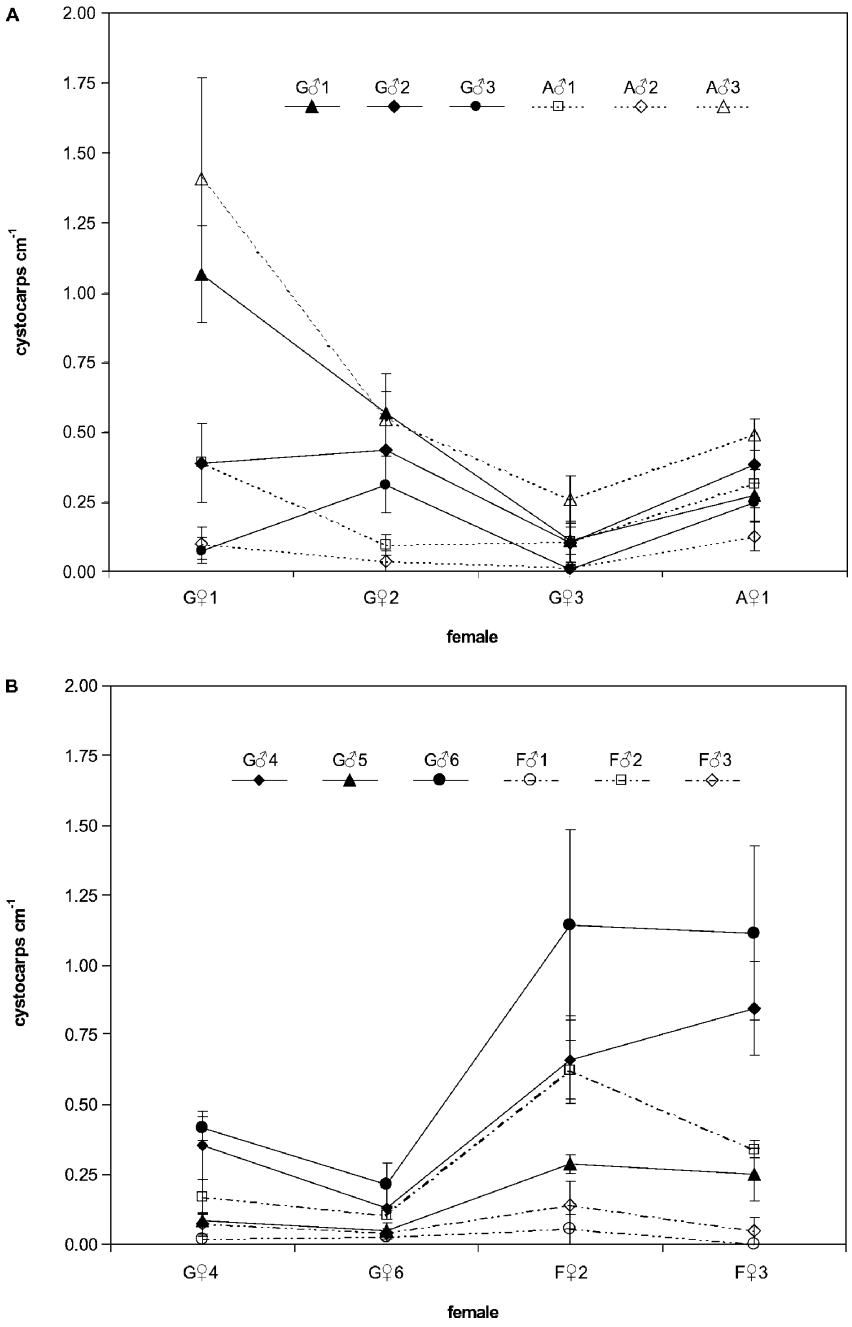

Fig. 3. Male × female interaction plots showing the average performance and standard error of each male on each female: (*A*) set A; (*B*) set B. Untransformed means and SE are plotted.

production of better-than-average males was not due to monopolization of one, particularly fertile female but to better-than-average mating success on more than one female.

Finally, the variance components of the three parental effects revealed substantial differences in the relative contribution of the male and female identities and their interaction to the overall variation in cystocarp production (Table 4). Although male and female identities both accounted for a significant proportion of the total variance in cystocarp production, male identity accounted for more than 2·5 times the variation compared with female identity (Table 4). Also, the interaction variance component was weaker than either of the parental identity components, although of the same order as the female component.

Population structure and mating success. We discerned no general trend in the mean cystocarp production according to whether the male and female pair originated from the same population or not (set A: intra-population crosses, $0{\cdot}332 \pm 0{\cdot}047$ cystocarps cm^{-1}, inter-population crosses, $0{\cdot}321 \pm 0{\cdot}062$; set B: intra-population crosses, $0{\cdot}204 \pm 0{\cdot}031$, inter-population crosses, $0{\cdot}394 \pm 0{\cdot}071$; $n = 48$). Further, there were no significant differences in either set in the cystocarp yield among inter-population pairs compared with intra-population pairs (set effect: $F_{1,2} = 0{\cdot}917$, $p = 0{\cdot}339$; pair type within set: $F_{2,188} = 2{\cdot}424$, $p = 0{\cdot}091$). This shows that the male × female interaction was not due to preferential crosses between males and females coming from the same population or from different populations: the differential reproductive output of the various

male–female pairs was not a function of population structure.

Discussion

We found significant non-random mating in *G. gracilis*. The results showed strong deviation from equality of males in multiple-donor crosses, implicating post-adhesion events and/or spermatia production as important in generating non-random mating. The fact that such differences between males were detected in spite of the relatively limited number of individuals per set of crosses suggests that the disparity of male reproductive success observed in a natural population (Engel *et al*., 1999) has a biological basis. Consequently, non-random mating may play a role in the evolution of the reproductive biology of *G. gracilis*.

Gamete encounters in G. gracilis

The crossing experiments were extremely successful even though the virgin females were exposed to males for only 6 h. Although the time-course experiment was designed only as a preliminary check of our crossing method, it showed that fertilizations occurred in as little as 15 min after the introduction of male thalli into the flume (Fig. 2). Indeed, it appears that fertilization can be rapid in red seaweeds. Other reports show that fertilization occurred in as little as 5 min in a *Palmaria* species (Mine & Tatewaki, 1994) and 10 min in *Antithamnion sparsum* (Kim *et al*., 1996). Furthermore, our experiment also showed that fertilization increased with exposure time, although not necessarily in a linear fashion. While the greatest difference was observed between 15 min and 6 h exposure times, fertilization rates did not increase significantly beyond 60 min. Similarly, while fertilization kinetics of *A. sparsum* showed that even in excess of 200 spermatia per trichogyne, 100% binding of all trichogynes was never observed, maximum fertilization levels were reached at *c*. 60 min of exposure (Kim *et al*., 1996).

Interestingly, cystocarp yield did not vary with turbulence levels (Table 2). Turbulent eddies are thought to facilitate gamete encounters (Sheath & Hambrook, 1990). However, attachment of spermatia to trichogynes may occur across a wide range of current/eddy velocities. The dynamics of spermatia dispersal in the water column and adhesion to the trichogyne may be similar to those experienced by wind-pollinated plant species. For example, varying wind velocities had no effect on pollen capture efficiency in a wind-pollinated conifer (Roussy & Kevan, 2000). While the levels of turbulence used in this study were similar to those typically found in coastal waters (Estrada & Berdalet, 1997), the turbulent conditions that we used in the crossing experiments may not have been contrasting enough to produce a differential response in cystocarp production.

Reproductive success

Female reproductive success. There was a strong female effect on overall cystocarp yield: some females bore more cystocarps per centimetre of thallus than others (Table 2). Variation in cystocarp yield in *G. gracilis* females is ubiquitous: it has been observed in natural populations of *G. gracilis* (Engel *et al*., 1999) as well as in previous single-donor crosses (Richerd *et al*., 1993). It is not surprising that there are maternal effects on cystocarp production since the development of the cystocarp mobilizes maternal resources (Hommersand & Fredericq, 1990; Guimarães *et al*., 1999).

Genetic and/or epigenetic differences may control the number of cystocarps per centimetre of thallus that can be raised by a female. Three non-mutually exclusive factors may affect cystocarp yield. First, females may differ in the number of gametes produced per centimetre of thallus. Second, phenological differences in carpogonia and/or trichogyne receptivity could cause differences in female fertilization success. Third, differences may exist in the allocation of resources needed for the development of cystocarps. However, it would be difficult at best to distinguish between gamete production and receptivity differences because it is difficult to verify the presence of carpogonia without fertilization. Furthermore, time-intensive experiments would be required for the study of the genetic basis of cystocarp yield and this kind of study is further complicated in rhodophytes by the additional diploid (tetrasporophyte) stage.

Male reproductive success. We observed a highly significant male effect, demonstrating that some males were more successful than others in producing cystocarps. There were no inherent differences between males coming from different pools: at least one male from all the sampled pools showed high performance (Table 3A). Further, all males sired a minimum of 4 cystocarps, indicating that all were capable of producing viable gametes. Thus, differences in the number of cystocarps sired by different donors were more likely to be due to adhesion and/or other pre-fertilization processes rather than an inability to fertilize carpogonia. Studies in higher plants have shown that paternity does not always reflect the relative representation in the gamete pool (Marshall & Ellstrand, 1986; Rigney *et al*., 1993; Snow, 1994; Marshall *et al*., 1996; Mitchell &

Marshall, 1998). These differences are frequently attributed to intrinsic differences in pollen tube growth and/or vigour (Snow & Spira, 1991; Delph & Havens, 1998). Analogous differences in fusion with the trichogyne and migration towards the carpogonial nucleus may exist in spermatia (Pickett-Heaps & West, 1998). However, we cannot completely exclude the possibility of inherent differences in gamete production. Although extra care was taken to ensure that roughly equal quantities of new growth were present on each replicate, actual gamete production could not be quantified in the non-sterile seawater conditions employed for this study. In addition, as suggested for females, gamete production may vary during the reproductive season due to phenological differences.

Male × female interaction. In addition to male effects, other processes are involved because differences in gamete production cannot explain the observed differences in male fertilization success across females. Male fertilization success varied across females above and beyond the female effect, suggesting that access to female gametes is not simply a 'fair raffle' (Parker, 1990), whereby paternity is determined by the proportion of total available male gametes attributable to each male. Male × female interactions have long been observed in crossing studies in plants (and more recently in animals) and are attributed to incompatibility systems, genetic complementarity, including inbreeding or outbreeding depression, or to discordant female choice (plants: Charlesworth *et al*., 1987; Waser *et al*., 1987; Waser & Price, 1989; Snow, 1994; marine invertebrate: Bishop, 1996; Bishop *et al*., 1996; Grosberg & Hart, 2000; lizard: Olsson *et al*., 1996; for a review of male × female interactions in animals see Tregenza & Wedell, 2000).

First, incompatibility seems to be of minor importance in determining the success of crosses. In haploid-diploid species, the mitotically produced gametes eliminate any intra-individual variation (barring mutation) and mate pairs consequently produce only one specific diploid combination. Thus, in red seaweeds, incompatibility reactions between the female recipient environment (trichogyne) and spermatia should be systematic across all gametes of a male individual and thus readily detectable as no 'semi-compatibility' is possible (Charlesworth *et al*., 1987; Richards, 1997). In this study, 4 females showed extremely low cystocarp yields, but it is highly unlikely that these females were incompatible with all 6 males in a set. Excluding these 4 females, only one of the remaining 48 (6♂ × 8♀) mate pairs suggested the possibility of incompatibility. Although a sufficient number of cystocarps (77 total) were analysed on F♀3, none of the cystocarps were attributed to F♂1. However, this male performed very poorly on all females, suggesting that male performance is a more likely explanation than an incompatibility reaction.

Second, the interaction among the donors and recipients did not appear to be due to genetic complementarity in this study. Genetic complementarity of male × female interactions may be attributable to the degree of genetic relatedness between pairs of individuals. For example, it has been shown that organisms sometimes discriminate against pollen from (neighbouring) relatives, thereby avoiding biparental inbreeding (plants: Waser & Price, 1989; Heywood, 1991; Snow, 1994; marine invertebrate: Bishop, 1996; Bishop *et al*., 1996; lizard: Olsson *et al*., 1996). On the other hand, reproductive success may be reduced due to the disruption of adaptive gene complexes, termed outbreeding depression (Price & Waser, 1979; Waser & Price, 1989; Waser *et al*., 2000). Although 'self'-fertilization is not possible in dioecious red algae, levels of inbreeding can be high because mating is possible among gametophytes descended from the same tetrasporophyte. In fact, one male–female pair in set A (G♀1–G♂1) shared an identical genotype, including a rare allele at the Gv2CT locus. Based on the population allele frequencies at all seven microsatellite loci, it is highly likely that these two individuals are half-sibs, that is, they share the same tetrasporophyte parent (data not shown). Cystocarp production by these putative half-sib mates was very high (Fig. 3A), indicating little or no inbreeding avoidance. Likewise, there were no negative trends among (putatively unrelated) intra-population or inter-population crossings. Therefore, both inbreeding and outbreeding depression appear to be unlikely to shape reproductive success in the pre-zygotic and early post-zygotic stages in *G. gracilis*. These results corroborate those obtained by Richerd *et al*. (1993) using within-population and between-site single-donor crosses of *G. gracilis* individuals coming from two of the locations (A and G) used in the present study. In the 1993 study, interparent geographic distance had no effect in within-population crosses, and between-site crosses (sites separated by up to 5 km) were as successful as within-site crosses (Richerd *et al*., 1993). Since these were single-donor crosses, the success of the within-population donors and the between-site donors could be altered when competitors were introduced, in the same way as when outcross pollen outcompetes self-pollen when both are simultaneously applied (Cruzan & Barrett, 1993; Snow, 1994). However, even in the presence of competitors from the same and different pools, the present study revealed the same absence of inbreeding or outbreeding avoidance.

Finally, while incompatibility and genetic complementarity appear to be unlikely sources of

male × female interactions, discordant female choice cannot be ruled out. However, while the proportion of offspring sired by some males differed across females, the discordance mainly concerned males of average performance (Fig. 3). Thus, if they exist, these female-specific 'preferences' are secondary as they did not influence the reproductive success of all males.

If male × female interactions have a physiological and/or genetic basis, these same patterns should remain even if each female is crossed separately with each suitor. Thus, to assess fully the sources of male × female interactions, single-donor crosses need to be carried out in parallel with the multiple-donor crosses, using the same set of individuals.

Evolutionary consequences of non-random mating

Sexual selection, acting on traits that may govern gamete encounters and fusion, remains a possible force influencing the mating patterns observed in this study. Two conditions must be met for sexual selection to be the driving force behind these patterns of non-random mating: first, these differences among males need to be consistent across females; and second, fitness changes resulting from selection must have a genetic basis for differences in performance to have an evolutionary response (Arnold & Wade, 1984; Charlesworth *et al*., 1987; Lyons *et al*., 1989; Marshall, 1998). The significant male × female interaction in the multiple-donor crosses was not the major cause of differences among males. In fact, the variance attributed to overall differences in the number of cystocarps sired by each male was nearly 4 times greater than that of the male × female interaction (Table 4), indicating that males differ in average reproductive success above and beyond the two-sex interaction. Poor donors were consistently poor performers across all females and better-than-average donors enjoyed high reproductive success on more than one female (Fig. 3A, B). Further, the relative ranking changed little from one female to another, with the exception of G♀2 in set A. Thus, the strong male effect may be a sign of spermatia competition and/or consistent female choice of the same male.

In plants, pollen competition may arise from interactions among individual pollen grains. Marshall *et al*. (1996) showed that pollen grains from one donor interfered with those from another donor when the grains were in contact on the style but not when on adjacent portions of the style without direct contact. In red algae there may be a similar opportunity for male–male interactions in the trichogyne (Pickett-Heaps & West, 1998; Engel *et al*., 1999). Several spermatia may adhere to a trichogyne (Hommersand & Fredericq, 1990), and nuclei from more than one male may be found in the trichogyne (Pickett-Heaps & West, 1998), but only one reaches the base of the trichogyne and fuses with the carpogonium nucleus (Fredericq & Hommersand, 1989). Alternatively, females may mediate spermatia competition through female choice. Female choice is often manifested through selective abortion, or the selective maturation of certain zygotes (Willson & Burley, 1983; Rigney, 1995; Havens & Delph, 1996). However, we observed very low abortion rates, demonstrating that female choice through selective cystocarp abortion in the early stages of post-fertilization events was not substantial in our study. Nevertheless, we cannot exclude an alternative basis of female choice, such as in the complementary receptor-carbohydrate moieties system found in Ceramiales (Kim *et al*., 1996; Kim & Kim, 1999). The putative phenotypic trait or traits governing the differences in spermatia performance and/or female choice, their genetic basis and their consequences for mating success have yet to be elucidated in *G. gracilis*.

Acknowledgements

The authors would like to thank G. Carpentier, J. Delgery, I. Meusnier, C. Monsterleet, E. Perez, M.-C. Quillet, M. Tarayre and J. Trachet for invaluable assistance in collection and culture of algal material and in the counting and dissection of the thousands of cystocarps, J.-F. Allienne for excellent technical assistance in the molecular biology laboratory, T. Caron and D. Hilde for technical assistance at the marine station, L. Seuront for turbulence measures and E. Serrão for helpful comments on a previous draft. This study was supported by funding from EC Research contract BIOGAP MAS3-CT95-0019, European Funds for Regional Development (FEDER), Nord-Pas-de-Calais Region (DYSCOP II), CNRS-IFREMER *Biodiversité Marine* programme 519501, and PhD fellowship no. 10556 from the French Ministry of Foreign Affairs to C.R.E.

References

Arnold, S.J. (1994). Is there a unifying concept of sexual selection that applies to both plants and animals? *Am. Nat*., **144**: S1–S12.

Arnold, S.J. & Wade, M.J. (1984). On the measurement of natural and sexual selection: theory. *Evolution*, **38**: 709–719.

Birkhead, T.R. (1998). Cryptic female choice: criteria for establishing female sperm choice. *Evolution*, **52**: 1212–1218.

Birkhead, T.R. (2000). Defining and demonstrating postcopulatory female choice – again. *Evolution*, **54**: 1057–1060.

Bishop, J.D.D. (1996). Female control of paternity in the internally fertilizing compound ascidian *Diplosoma listerianum*. I. Autoradiographic investigation of sperm movements in the female reproductive tract. *Proc. R. Soc. Lond. Ser. B*, **263**: 369–376.

Bishop, J.D.D., Jones, C.S. & Noble, L.R. (1996). Female control of paternity in the internally fertilizing compound ascidian

Diplosoma listerianum. II. Investigation of male mating success using RAPD markers. *Proc. R. Soc. Lond. Ser. B*, **263**: 401–407.

Bishop, J.D.D. & Pemberton, A.J. (1997). Sessile animals: attached, but promiscuous? *Trends Ecol. Evol.*, **12**: 403.

Charlesworth, D., Schemske, D.W. & Sork, V.L. (1987). The evolution of plant reproductive characters; sexual versus natural selection. In *The Evolution of Sex and Its Consequences* (Stearns, S.C., editor), 317–335. Birkhäuser, Basel.

Cruzan, M.B. & Barrett, S.C.H. (1993). Contribution of cryptic incompatibility to the mating system of *Eichhornia paniculata* (Pontederiaceae). *Evolution*, **47**: 925–934.

Delph, L.F. & Havens, K. (1998). Pollen competition in flowering plants. In *Sperm Competition and Sexual Selection* (Birkhead, T.R. & Møller, A.P., editors), 149–173. Academic Press, San Diego.

Destombe, C. (1987). Biologie des populations de l'algue rouge *Gracilaria verrucosa* Hudson (Papenfuss) (Gigartinales), intérêt et implication en aquaculture. PhD thesis. Université des Sciences et Techniques de Lille Flandres-Artois, Lille, France.

Destombe, C., Godin, J. & Remy, J.M. (1990). Viability and dissemination of spermatia of *Gracilaria verrucosa* (Gracilariales, Rhodophyta). *Hydrobiologia*, **204/205**: 219–223.

Eberhard, W.G. (1998). Female roles in sperm competition. In *Sperm Competition and Sexual Selection* (Birkhead, T.R. & Møller, A.P., editors), 91–116. Academic Press, San Diego.

Eberhard, W.G. (2000). Criteria for demonstrating postcopulatory female choice. *Evolution*, **54**: 1047–1050.

Edelstein, T., Chen, L.C.-M. & McLachan, J. (1978). Studies on *Gracilaria* (Gigartinales, Rhodophyta): reproductive structures. *J. Phycol.*, **14**: 92–100.

Engel, C., 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. Lond. Ser. B*, **266**: 1879–1886.

Estrada, M. & Berdalet, E. (1997). Phytoplankton in a turbulent world. *Sci. Mar.*, **61**: 125–140.

Fredericq, S. & Hommersand, M.H. (1989). Proposal of the Gracilariales ord nov. (Rhodophyta) based on an analysis of the reproductive development of *Gracilaria verrucosa*. *J. Phycol.*, **25**: 213–227.

Grosberg, R.K. & Hart, M.W. (2000). Mate selection and the evolution of highly polymorphic self/nonself recognition genes. *Science*, **289**: 2111–2114.

Guimarães, M., Plastino, E.M. & Oliviera, E.C. (1999). Life history, reproduction and growth of *Gracilaria domingensis* (Gracilariales, Rhodophyta) from Brazil. *Bot. Mar.*, **42**: 481–486.

Havens, K. & Delph, L.F. (1996). Differential seed maturation uncouples fertilization and siring success in *Oenothera organensis* (Onagraceae). *Heredity*, **76**: 623–632.

Heywood, J.S. (1991). Spatial analysis of genetic variation in plant populations. *Annu. Rev. Ecol. Syst.*, **23**: 335–355.

Hommersand, M.H. & Fredericq, S. (1990). Sexual reproduction and cystocarp development. In *Biology of the Red Algae* (Cole, K.M. & Sheath, R.G., editors), 305–345. Cambridge University Press, Cambridge.

Kim, G.H., Lee, I.K. & Fritz, L. (1996). Cell–cell recognition during fertilization in a red alga, *Antihamnion sparsum* (Ceramiaceae, Rhodophyta). *Plant Cell Physiol.*, **37**: 621–628.

Kim, S.-H. & Kim, G.H. (1999). Cell–cell recognition during fertilization in the red alga, *Aglaothamnion oosumiense* (Ceramiaceae, Rhodophyta). *Hydrobiologia*, **398/399**: 81–89.

Kling, R. & Bodard, M. (1987). Morphogenesis of the frond of *Gracilaria verrucosa* (Hudson) Papenfuss: the differentiation of vegetative and sexual areas. *Proc. Int. Seaweed Symp.*, **12**: 205–206.

Lefebvre, C., Destombe, C. & Godin, J. (1987). Le fonctionnement du carposporophyte de *Gracilaria verrucosa* et ses répercussions sur la stratégie de reproduction. *Cryptog. Algol.*, **8**: 113–126.

Levitan, D.R. (1998). Sperm limitation, gamete competition, and sexual selection in external fertilizers. In *Sperm Competition and Sexual Selection* (Birkhead, T.R. & Møller, A.P., editors), 175–217. Academic Press, San Diego.

Levitan, D.R. & Petersen, C. (1995). Sperm limitation in the sea. *Trends Ecol. Evol.*, **10**: 228–231.

Luo, H., Mórchen, M., Engel, C., Destombe, C., Epplen, J.T., Epplen, C., Saumitou-Laprade, P. & Valero, M. (1999). Characterisation of microsatellite markers in the red alga, *Gracilaria gracilis*. *Mol. Ecol.*, **8**: 700–702.

Lyons, E.E., Waser, N.M., Price, M.V., Antonovics, J. & Motten, A.F. (1989). Sources of variation in plant reproductive success and implication for concepts of sexual selection. *Am. Nat.*, **134**: 409–433.

Marshall, D.L. (1998). Pollen donor performance can be consistent across maternal plant in wild radish (*Raphanus sativus*, Brassicaceae): a necessary condition for the action of sexual selection. *Am. J. Bot.*, **85**: 1389–1397.

Marshall, D.L. & Ellstrand, N.C. (1986). Sexual selection in *Raphanus sativus*: experimental data on nonrandom fertilization, maternal choice, and consequences of multiple paternity. *Am. Nat.*, **127**: 446–461.

Marshall, D.L., Folsom, M.W., Hatfield, C. & Bennett, T. (1996). Does interference competition among pollen grains occur in wild radish? *Evolution*, **50**: 1842–1848.

Mine, I. & Tatewaki, M. (1994). Attachment and fusion of gametes during fertilization of *Palmaria* sp. (Rhodophyta). *J. Phycol.*, **30**: 55–66.

Mitchell, R.J. & Marshall, D.L. (1998). Nonrandom mating and sexual selection in a desert mustard: an experimental approach. *Am. J. Bot.*, **85**: 48–55.

Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelström, H. (1996). Sperm selection by females. *Nature*, **383**: 585.

Parker, G.A. (1990). Sperm competition games: raffles and roles. *Proc. R. Soc. Lond. Ser. B*, **242**: 120–126.

Pickett-Heaps, J.D. & West, J. (1998). Time-lapse video observations on sexual plasmogamy in the red alga *Bostrychia*. *Eur. J. Phycol.*, **33**: 43–56.

Price, M.V. & Waser, N.M. (1979). Pollen dispersal and outcrossing in *Delphinium nelsonii*. *Nature*, **277**: 294–297.

Queller, D.C. (1987). Sexual selection in flowering plants. In *Sexual Selection: Testing the Alternatives* (Bradbury, J.W. & Andersson, M.B., editors), 9–20. Wiley, New York.

Richards, A.J. (1997). *Plant Breeding Systems*, 2nd edition. Chapman & Hall, London.

Richerd, S., Destombe, C., Cuguen, J. & Valero, M. (1993). Variation of reproductive success in a haplo-diploid red alga, *Gracilaria verrucosa*: effects of parental identities and crossing distance. *Am. J. Bot.*, **80**: 1379–1391.

Rigney, L.P. (1995). Postfertilization causes of differential success of pollen donors in *Erythronium grandiflorum* (Liliaceae): nonrandom ovule abortion. *Am. J. Bot.*, **82**: 578–584.

Rigney, L.P., Thomson, J.D., Cruzan, M.B. & Brunet, J. (1993). Differential success of pollen donors in a self-compatible lily. *Evolution*, **47**: 915–924.

Roussy, A.-M. & Kevan, P.G. (2000). How accessible are receptive megastrobili to pollen? The example of jack pine (*Pinus banksiana*). *Am. J. Bot.*, **87**: 215–220.

SAS Institute (1996). *SAS/STAT User's Guide*. SAS Institute, Cary, NC.

Serrão, E.A., Pearson, G., Kautsky, L. & Brawley, S.H. (1996). Successful external fertilization in turbulent environments. *Proc. Natl. Acad. Sci. USA*, **93**: 5286–5290.

Sheath, R.G. & Hambrook, J.A. (1990). Freshwater ecology. In *Biology of the Red Algae* (Cole, K.M. & Sheath, R.G., editors), 423–453. Cambridge University Press, Cambridge.

Snow, A.A. (1994). Postpollination selection and male fitness in plants. *Am. Nat.*, **144**: S69–S83.

Snow, A.A. & Spira, T.P. (1991). Pollen vigor and the potential for sexual selection in plants. *Nature*, **352**: 796–797.

Tennekes, H. & Lumley, J.L. (1972). *A First Course in Turbulence*. MIT Press, Boston.

Tregenza, T. & Wedell, N. (2000). Genetic compatibility, mate choice and patterns of parentage. *Mol. Ecol.*, **9**: 1013–1027.

Waser, N., Price, M.V. & Shaw, R.G. (2000). Outbreeding depression varies among cohorts of *Ipomopsis aggregata* planted in nature. *Evolution*, **54**: 485–491.

Waser, N.M. & Price, M.V. (1989). Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness. *Evolution*, **43**: 1097–1109.

Waser, N.M., Price, M.V., Montalvo, A.M. & Gray, R.N. (1987). Female mate choice in a perennial herbaceous wildflower, *Delphinium nelsonii*. *Evol. Trends Plants*, **1**: 29–33.

Wattier, R., Dallas, J.F., Destombe, C., Saumitou-Laprade, P. & Valero, M. (1997). Single locus microsatellites in Gracilariales (Rhodophyta): high level of genetic variability within *Gracilaria gracilis* and conservation in related species. *J. Phycol.*, **33**: 868–880.

Willson, M. (1990). Sexual selection in plant and animals. *Trends Ecol. Evol.*, **5**: 210–214.

Willson, M.F. (1994). Sexual selection in plants: perspective and overview. *Am. Nat.*, **144**: S13–S39.

Willson, M.F. & Burley, N. (1983). *Mate Choice in Plants: Tactics, Mechanisms and Consequences*. Princeton University Press, Princeton, NJ.

Yund, P.O. (2000). How severe is sperm limitation in natural populations of marine free-spawners? *Trends Ecol. Evol.*, **15**: 10–13.