# 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*

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In haploid–diploid red seaweeds, the dispersal of male gametes is presumed limited due to their lack of flagella. It has been suggested that this group suffers from sperm limitation and, consequently, that fertilization is relatively inefficient. Fertilization in most floridean rhodophytes results in the formation a cystocarp, a swelling on the haploid female thallus housing the diploid zygote and its thousands of diploid daughter spores. To study the performance of non-motile male gametes in the sea, we evaluated both female and male fertilization success in a natural population of the red marine alga *Gracilaria gracilis*. Female fertilization success, estimated by cystocarp yield per unit female thallus, was evaluated with respect to the availability of male gametes. Male fertilization success, estimated by the individual contribution of different males to zygotes, was assessed by paternity analyses on 350 cystocarps produced in one reproductive season using two microsatellite loci. The results show that cystocarp yield is not sperm limited and that the large variation in male fertilization success cannot be solely explained by the distance travelled by the male gamete to find a mate. Taken together, the results suggest that, not only is fertilization efficient, but that male–male competition and/or female choice may play a role in shaping population mating patterns.

**Keywords:** fertilization success; paternity analysis; gamete dispersal; cystocarp; red alga; spermatia

## 1. INTRODUCTION

Male and female fertilization success depends on successful gamete encounters. Sperm (or pollen) limitation hampers zygote production in organisms that release their gametes into the environment, as do numerous marine organisms and land plants. Hydrodynamic models, like their aerodynamic counterparts, predict that gamete encounters are limited due to their dilution in turbulent water (Denny & Shibata 1989) or, analogously, air (Niklas 1985). In support of these models, highly variable fertilization success rates have been observed in plants (for a review, see Burd 1994) and marine organisms (for a review, see Levitan & Petersen 1995). However, many wind-pollinated land plants feature adaptive reproductive biology characteristics which maximize pollen capture (Niklas 1985). Likewise, in the sea, 80–100% of eggs can be fertilized in favourable circumstances. Examples include close proximity of individuals and/or positioning in direct current flow (e.g. in sea urchins; Pennington 1985; Levitan 1991), external cues, such as relatively calm waters, which promote synchronous spawning (e.g. in brown seaweed; Serrão *et al.* 1996) and high population densities (e.g. in a colonial ascidian; Yund & McCartney 1994; Yund 1995).

In red seaweeds, the available evidence suggests that the dispersal of spermatia (male gametes) is extremely limited, not only in space but also in time. First, spermatia lack flagella. While water movement facilitates the dispersal of all propagules, flagellated or not, without flagella spermatia cannot swim the final critical distance to a female. Second, sexual pheromones, which are instrumental in the synchronization of gamete release and/or the attraction of male gametes in many green and brown seaweeds (Brawley & Johnson 1992), are unknown in red macroalgae. Consequently, spermatia depend entirely on passive transport in the water column from the time of release to the point of fusion with a female gamete. Third, spermatia are viable for less than 6 h in *Gracilaria gracilis* (ex *Gracilaria verrucosa*) (Destombe *et al.* 1990), though data on other species are lacking.

Fertilization takes place in the female plant in floridean rhodophytes and, in most orders of this class, female gametes, once fertilized, develop into cystocarps (Hommersand & Fredericq 1990). Cystocarps are macroscopic hemispherical swellings on the surface of a female thallus, within which the zygote multiplies by mitosis, producing thousands of diploid spores.

A sperm-limitation hypothesis has been invoked to explain the evolution of the cystocarp in red seaweeds

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(Searles 1980). Searles (1980) speculated that, because male gametes depend entirely on water movement for transfer to a female gamete, syngamy should be a rare event, for which the cystocarp compensates by cloning the successful zygote. However, two studies in red macro-algae found that female fertilization success or yield of cystocarps on an individual female reaches 34–91% in the field (Sheath & Hambrook 1990; Kaczmarska & Dowe 1997), leading the authors to conclude that fertilization is relatively efficient in rhodophytes.

The number of cystocarps sired by a particular male relative to other males (or male fertilization success) may, however, be limited by access to females. In controlled crosses in *G. gracilis*, no variance in male fertilization success was detected, suggesting uniformity in spermatia quantity and/or quality among males (Richerd *et al.* 1993). However, these were single-donor, single-recipient crosses, maximizing the success of each male in the absence of others. In natural populations, where numerous males and females occur together at varying distances and densities, males may compete for fusion with the finite number of female gametes.

In this study, we investigated the consequences of passive transport of spermatia in the water column on female and male fertilization success in a natural popula-tion of *G. gracilis*. Using cystocarps as the records of fertilization of one reproductive season, we first looked for evidence of sperm limitation in female fertilization success. Then, to trace the movement of male gametes in the population, we conducted paternity analyses on cystocarps using two hypervariable microsatellite loci. The genotype of the haploid paternal parent can be readily identified in cystocarps because the maternal contribution can be established with absolute certainty by typing the haploid female thallus. We evaluated sper-matia dispersal and assessed potentially important factors in determining male fertilization success. Finally, the possibility of male–male competition and/or female choice was considered.

## 2. MATERIAL AND METHODS

### (a) *Study species*

*G. gracilis* is characterized by a typical rhodophyte haploid–diploid life cycle. The haploid and diploid generations are independent, isomorphic, perennial and long-lived. Haploid (gametophyte) and diploid (tetrasporophyte) plants coexist in populations where individuals are fixed to the substratum by a perennial holdfast bearing deciduous spaghetti-like thalli. Haploid individuals are dioecious and produce gametes by mitosis. Reproductive structures are uniformly distributed along the thallus (Kling & Bodard 1987). Although mature male crypts are readily visible under a dissecting microscope, unfertilized female gametes can only be observed on thallus sections under a light microscope. A cystocarp is fully mature approximately one month after fertilization; the diploid spores within are then progressively liberated over six to ten weeks (Lefebvre *et al.* 1987).

### (b) *Study population*

Populations of *G. gracilis* are patchily distributed in the inter-tidal zone on rocky shores. Individuals occupy rock pools and remain immersed at ebb tide. The study population was located

on the coast of the North Sea at Cape Gris-Nez (northern France, $50°44'$ N and $1°13'$ E) in the intertidal zone in an isolated pool of *ca.* $5 \text{ m}^2$ at low tide (population was included in studies by Destombe *et al.* (1989, 1990)).

### (c) *Sampling*

In August 1995, every individual within the study rock pool was mapped and sampled (figure 1). The population was composed of 64 tetrasporophytes, 37 male gametophytes and 26 female gametophytes. The length and number of thalli were recorded for each individual. In addition, for every female, cystocarps were counted on the 1 dm (i.e. 10 cm) distal extremity of three thalli. Three thallus tips of *ca.* 5 mm were excised from each individual for DNA extraction. An average of ten large cystocarps (i.e. minimum five and maximum 38; table 1) was randomly sampled and dissected from the 26 female gameto-phytes.

### (d) *DNA extraction and microsatellite genotyping*

Two microsatellite loci (Gv1CT and Gv2CT) (Wattier *et al.* 1997) were used as genetic markers in this study. For each indivi-dual and for each sampled cystocarp, DNA extractions, PCR amplifications and allele typing were performed as described in Wattier *et al.* (1997).

### (e) *Data analysis*

#### (i) *Female fertilization success*

Female fertilization success was estimated by averaging the number of cystocarps over three 1 dm thallus tips. To determine whether female fertilization success was limited by the avail-ability of male gametes, we performed a linear regression of the mean number of cystocarps per dm on the density of male thalli. The number and length of male thalli (=male size) is propor-tional to spermatia production (Kling & Bodard 1987). The availability of spermatia was thus estimated by cumulating the size of males found within an area circumscribed around each female. We examined cumulative male size in three concentric areas delimited by radius lengths corresponding to the distances at which 25, 50 and 75% of the actual fertilizations occurred.

#### (ii) *Distribution of intermate distance*

The distances between males and females in the population were calculated using the map coordinates (figure 1). The expected distribution of intermate distances is simply that of the separation distances of all male–female couples. Therefore, the effect of distance on mating events was evaluated by comparing the distribution of the distances between the identified male (as determined by paternity analysis) and female parents of each cystocarp, i.e. the actual fertilization distances, with that of the expected intermate distances.

#### (iii) *Male fertilization success*

The probability of fertilization ($\pi_{ij}$) of a gamete in female *i* by male *j* was modelled using logistic regression by fitting the proportion of cystocarps sired in female *i* by male *j* to a series of models. Model I examined the effect of separation distance ($d_{ij}$) and its exponential terms ($d_{ij}^2$ and $d_{ij}^3$):

$$\text{logit}(\pi_{ij}) = \alpha + \beta_1 d_{ij} + \beta_2 d_{ij}^2 + \beta_3 d_{ij}^3,$$

where $\alpha$ is the intercept and $\beta_k$ is the parameter of the *k*th inde-pendent variable.
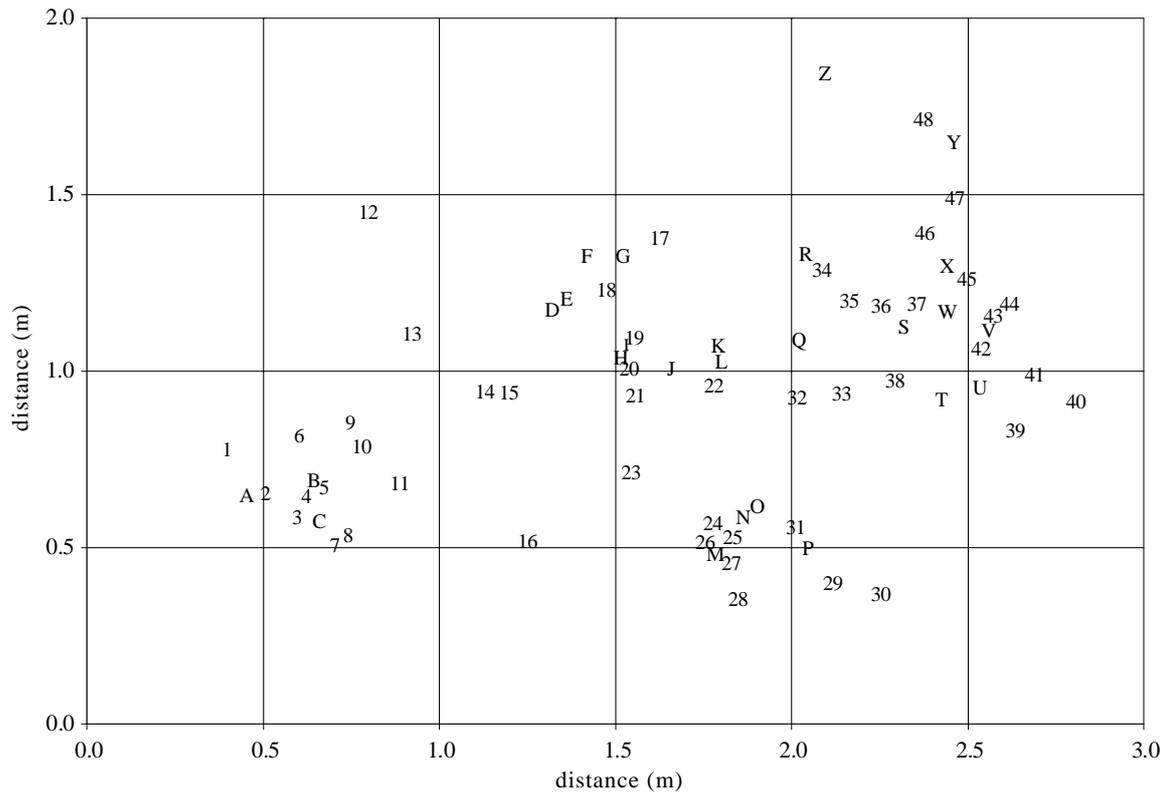
Figure 1. Map of the Cape Gris-Nez tide pool showing locations of males (numbers) and females (letters). The seaward edge is at the top of the map.
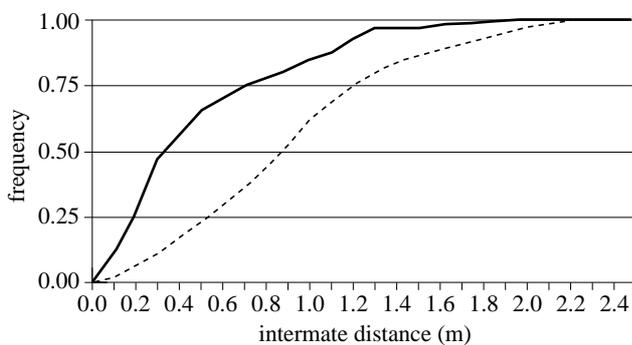


Figure 2. Cumulative frequency distributions of the expected intermate distance between females and males (dotted line) ($n = 1248$) and that of the actual distance between the assigned parents of the cystocarps studied (solid line) ($n = 229$). For the realized distribution, the first; second and third quartiles occur at 0.20, 0.33 and 0.73 m, respectively.

Model II included a male-identity categorical variable ($M_j$) and its interaction with distance, thereby exploring the differences between males:

$$\text{logit}(\pi_{ij}) = \alpha + \beta_1 d_{ij} + \beta_2 d_{ij}^2 + \beta_3 d_{ij}^3 + \beta_{4j} M_j + \beta_{5j} M_j d_{ij}.$$

The values for the quantitative variables employed to investigate differences in fertility (length and size) and competition between males (sex ratio) were different for each male, therefore, they could not be included in the same model as the qualitative male-identity term. Consequently, model III was a stepwise model replacing the male-identity term of model II with five male-specific variables:

$$\text{logit}(\pi_{ij}) = \alpha + \beta_1 d_{ij} + \beta_2 d_{ij}^2 + \beta_3 d_{ij}^3 + \sum_{k=6}^{10} \beta_k x_k + \beta_{k+5} x_k d_{ij},$$

where $x_6$ is thallus length (cm), $x_7$ is size (length and number of thalli converted into volume, cm$^3$), $x_8$ is sex ratio Q1 (proportion of males in a 0.20 cm radius), $x_9$ is sex ratio Q2 (0.33 cm radius) and $x_{10}$ is sex ratio Q3 (0.73 cm radius).

Logistic regressions were implemented with GENMODE (models I and II) or LOGISTIC procedures (model III) in the SAS statistical package (v. 6.12, SAS Institute Inc., Cary, NC, USA). The data were fitted using maximum-likelihood procedures. Males that sired no cystocarps ($n = 5$) and males that fertilized only the closest female ($n = 3$) were removed from the analysis because no unique maximum-likelihood estimate exists for either case.

## 3. RESULTS

### (a) *Female fertilization success*

On average, females bore 19.5 cystocarps (s.e. 1.1 and $n = 26$) per dm of thallus. There were significant differences between females for fertilization success (ANOVA $F_{25,51} = 2.20$, $p = 0.009$ and $n = 78$). These differences were not due to variation in male gamete availability, as we detected no significant relationships between the mean number of cystocarps per dm and the cumulative male size per unit area ($r = 0.04$ and $p = 0.82$, $r = 0.08$ and $p = 0.70$ and $r = 0.26$ and $p = 0.21$ for areas of 0.13, 0.34 and 1.67 m$^2$, respectively, with $n = 26$; see figure 2).

### (b) *Paternity analysis*

In the Cape Gris-Nez population, both microsatellite loci were highly polymorphic within the haploid (male

Table 1. *Tally of male fertilization success*

($l$, maximum thallus length; $N_c$, total number of cystocarps sired by each of the 40 genotypically unique males in the population; ambiguous, number of cystocarps for which the paternal genotype was shared by two or more males from the population; unmatched, number of cystocarps for which the paternal genotype matched none of the males in the study population; $N_f$, total number of cystocarps studied on each female.)

| female | A | B | C | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z | $N_c$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **male no. ($l$; size) cm cm³** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 (20; 6.7) | 2 | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| 2 (24; 4.0) | 4 | | | | | | 1 | | | | | | | 1 | | | | | | | | | | | | 6 |
| 3 (22; 7.5) | 4 | 2 | 1 | | | 3 | 1 | | | | | | | | | | | 1 | | | | | | | | 12 |
| 4 (24; 7.5) | | | 3 | | | | 1 | | | | | | | | | | | | | | | | | | | 4 |
| 5 (7; 0.1)[a] | | 1 | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| 6 (19; 11.6) | | | | | | | | | | 7 | | 1 | | | | 3 | | | | | | | | | | 11 |
| 7 (13; 2.7) | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |
| 8 (17; 2.7) | 3 | | 3 | | | | 1 | | | | 1 | | | | | | | | | | | | | | | 8 |
| 9 (24; 9.8) | | | | | | | | | | | 1 | | 1 | | | | | | | | | | | | | 2 |
| 10 (24; 3.4) | 1 | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| 12 (23; 5.1) | 2 | 2 | | 2 | | | | | | | | | | | 1 | | | | | | | | | | | 7 |
| 13 (7; 0.1)[a] | | | | | | | | | | | 1 | | | | | | | | | | | | | | | 1 |
| 14 (30; 4.4) | | | | | | | | | | | | | | | 1 | | | | | | | | | | | 1 |
| 15 (18; 8.1) | 1 | | | | | 1 | 2 | 1 | | | 1 | | 6 | | | 1 | | | | | | | | | | 13 |
| 16 (28; 5.6) | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |
| 17 (27; 3.9) | 2 | | | | | 4 | | 3 | 1 | 3 | 1 | | | | 1 | 6 | | | | | | | | 5 | | 26 |
| 18 (16; 3.4) | | | | | | 1 | | | | | | | | | | | | | | 1 | | | | | | 2 |
| 19 (22; 3.1) | | | | | 2 | 3 | 1 | 2 | 1 | 4 | 1 | | | | | | | | | | | | | | | 14 |
| 21 (7; 0.1)[a] | | | | 1 | | | 1 | | 1 | | 1 | 1 | | | | | | | | | | | | | | 5 |
| 22 (23; 9.3) | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |
| 23 (7; 0.1)[a] | | | | | | | | | | 1 | | | | | | | | | | | | | | | | 1 |
| 24 (25; 12.3) | | | | | | | | | | | | 2 | | | | | | | | | | | 3 | | | 5 |
| 25 (25; 12.3) | | | | 2 | | | | | | 1 | 3 | 3 | | 1 | 1 | | | | | 1 | | | | | | 12 |
| 26 (7; 0.1)[a] | | | | | | | | | | | | 2 | | | | | | | | | | | | | | 2 |
| 27 (7; 4.0) | | | | | | | | | | | 1 | 1 | | | | | | | | | | | | | | 2 |
| 29 (7; 0.1)[a] | | | | | | | | | | 1 | | | | | | | | | | | | | | | | 1 |
| 30 (7; 0.1)[a] | | | | | | | | | | | | | | 1 | 1 | | | | | | | | | | | 2 |
| 31 (16; 6.6) | | | | | | | | | | | | | | | | 1 | | 1 | | | | | | | | 2 |
| 32 (24; 16.9) | | | | | | | 1 | | 1 | | | | | | | 1 | | | | | | | | | | 3 |
| 33 (28; 16.3) | 1 | | | | | | 1 | 1 | | 3 | | | 1 | 1 | 2 | 1 | | 1 | | | | | | 1 | | 13 |
| 35 (23; 2.6) | | | | 1 | | | | 1 | | | 1 | | | | | | 7 | | 3 | | 1 | | 1 | | | 15 |
| 36 (23; 6.5) | 1 | | | | | | | | | | 1 | | | | | | | | | | | | | | | 2 |
| 37 (7; 0.1)[a] | | | | | | | | | | | 1 | | | | | | 1 | | | 1 | | | | | | 3 |
| 38 (29; 17.0) | | | | 1 | 1 | 3 | | | | | | | 1 | | 2 | | 3 | 2 | 2 | | 2 | | | | | 17 |
| 39 (11; 1.9) | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |
| 40 (16; 3.8) | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |
| 42 (14; 3.7) | | 1 | | | 1 | | | | | | | | | | | | | | | 1 | 1 | 2 | 2 | | | 8 |
| 43 (7; 0.1)[a] | | 1 | | | | | | | | | 2 | | | | | | 3 | | | 2 | 3 | 3 | 5 | | 3 | 22 |
| 47 (7; 0.1)[a] | | | | 1 | | | | | | | | | | | | | | | | 1 | | | | | | 2 |
| 48 (7; 0.1)[a] | | | | | | | | | | | | | | | | | 1 | | | | | | | | | 1 |
| ambiguous | 5 | 2 | 1 | 1 | 6 | | 1 | | | 4 | 1 | 2 | 2 | | 2 | 13 | 3 | 2 | 2 | 3 | 2 | | | 1 | 1 | 54 |
| unmatched | 12 | 1 | 2 | 2 | 1 | | | | 2 | 2 | 3 | | | 3 | | | | | 2 | | | 1 | 2 | 1 | | 34 |
| $N_f$ | 38 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 10 | 32 | 10 | 10 | 10 | 10 | 37 | 10 | 10 | 5 | 10 | 10 | 9 | 7 | 10 | 10 | 317 |

[a] Minimum length and size values were assigned to those males not observed in 1995.

and female) subpopulation. The Gv1CT locus presented 45 different alleles ranging from 130 to 524 bp while there were 12 different alleles for the Gv2CT locus (176–234 bp). The average expected exclusion probability using both loci was 0.993. For 29 males, because each possessed a distinct, unique genotype, paternity could be unequivocally determined. The eight remaining males shared genotypes: four genotypes were each shared by two males.

A paternal genotype was identified for 317 out of the 350 cystocarps analysed. Out of the 33 discarded cystocarps, one progeny array of ten showed several somatic mutations at the Gv1CT locus, rendering it impossible to distinguish the maternal from the paternal allele. Genotypes were indeterminate for the remaining 23, due either to non-amplification or to suspected masking of the paternal allele at the Gv1CT locus due to short allele dominance (Wattier *et al.* 1998). Out of the included

Table 2. *Logistic regression analyses of male fertilization success on a per-female basis: type III likelihood-ratio tests and parameter estimates for each independent variable*

(Male and male × distance parameter estimates are plotted in figure 3. See the text for a description of the independent variables. Only the results of the final, simplified model are presented; see §2 for the variables included in the full model; SR, sex ratio.)

| | model I: distance model | | | | model II: male-identity model | | | | model III: quantitative attributes model | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | $\chi^2$ | d.f. | $p$ | $\beta_x$ (s.e.) | $\chi^2$ | d.f. | $p$ | $\beta_x$ (s.e.) | $\chi^2$ | d.f. | $p$ | $\beta_x$ (s.e.) |
| intercept | — | — | — | −1.31 (0.21) | — | — | — | −0.14 (0.42) | — | — | — | −0.99 (0.37) |
| distance | 46.69 | 1 | <0.0001 | −7.78 (1.17) | 30.42 | 1 | <0.0001 | −8.93 (1.62) | 55.32 | 1 | <0.0001 | −10.30 (1.40) |
| distance$^2$ | 18.44 | 1 | <0.0001 | 6.52 (1.61) | 16.65 | 1 | <0.0001 | 8.69 (2.20) | 15.66 | 1 | <0.0001 | 6.86 (1.80) |
| distance$^3$ | 12.04 | 1 | 0.0005 | −1.91 (0.61) | 16.22 | 1 | <0.0001 | −3.05 (0.82) | 12.18 | 1 | 0.0005 | −2.22 (0.69) |
| male | — | — | — | | 89.20 | 31 | <0.0001 | — | | | | |
| male × distance | — | — | — | | 56.89 | 31 | 0.0031 | — | | | | |
| length | — | — | — | | — | — | — | — | 9.66 | 1 | 0.0019 | 0.04 (0.01) |
| size | — | — | — | | — | — | — | — | 7.12 | 1 | 0.0076 | −0.07 (0.03) |
| SR Q2 | — | — | — | | — | — | — | — | 11.39 | 1 | 0.0007 | −1.22 (0.36) |
| size × distance | — | — | — | | — | — | — | — | 12.60 | 1 | 0.0004 | 0.12 (0.04) |
| SR Q3 × distance | — | — | — | | — | — | — | — | 4.59 | 1 | 0.0321 | 2.78 (1.29) |

cystocarps, analysis of the paternal genotypes revealed that multiple paternity was common within a progeny array. On average, the ratio of males to cystocarps was 0.63 (s.e. 0.03 and $n=25$), indicating that a family of ten cystocarps had approximately six different sires (table 1). Further, 188 out of the 317 cystocarps were assigned to one out of the 29 genotypically unique males, 54 were assigned to two or more males sharing the same genotype and the remaining 75 were attributed to males that had not been observed in the study population in August 1995 (table 1). More recent sampling (data not shown) has revealed 11 additional males possessing genotypes identified in 41 cystocarps, indicating that these males were present in 1995, but not observed due to small size or early shedding of thalli. These additional males have been included in the paternity analysis. Minimum size and length values have been used for males not censused in 1995 (table 1). Such estimates are reasonable as small individuals are probably more often sanded over or inadvertently overlooked. Finally, a total of 229 cystocarps were assigned to specific males. Males sired an average of 5.7 (s.e. 1.0 and $n=40$) cystocarps (table 1).

## (c) *Distribution of intermate distance*

Figure 1 maps females and males (diploids have been omitted for clarity); the distances between potential mates ranged from 0.03 to 2.40 m. The average expected separation distance was $0.91 \pm 0.01$ m (mean ± s.e.) which contrasted dramatically with the actual separation distance of $0.49 \pm 0.03$ m. Indeed, the 50% quantiles occurred at very different distances in the actual (0.33 m) and expected (0.86 m) cumulative frequencies of intermate distance (figure 2): more than 80% of actual mate

pairs were separated by a distance of less than or equal to 0.86 m. This difference was significant ($G=46.67$, d.f. $=1$ and $p<0.0001$), indicating that distance played an important role in determining mating events.

## (d) *Variation in male fertilization success*

On a per-female basis, male fertilization success decreased as the distance to potential mates increased. There was a significant negative logistic relationship linking the separation distance (and its exponential terms) to the proportion of cystocarps sired by an individual male (model I in table 2). Further, in model II, the male-identity effect was highly significant. Thus, males varied in fertilization success even after the distance effect was taken into account (table 2). However, the force of the distance effect varied from male to male, as the interaction between distance and male identity was also highly significant (model II in table 2; figure 3).

Model III, in which the male-identity factor in model II was substituted for by a series of quantitative variables describing those males, showed that, in addition to the three distance terms, male fertilization success varied significantly with three main effect variables: thallus length, size and sex ratio Q2 (the other main effect variables were not retained in the final simplified model; table 2). First, fertilization success increases with thallus length. Second, the relationship between size and fertilization success changes with distance: at short distances fertilization success decreases with increasing size, while at long distances fertilization success increases with size. Third, an increase in the proportion of males in close vicinity (0.33 m) had a detrimental effect on fertilization success, suggesting that there was competition between males for females which was most
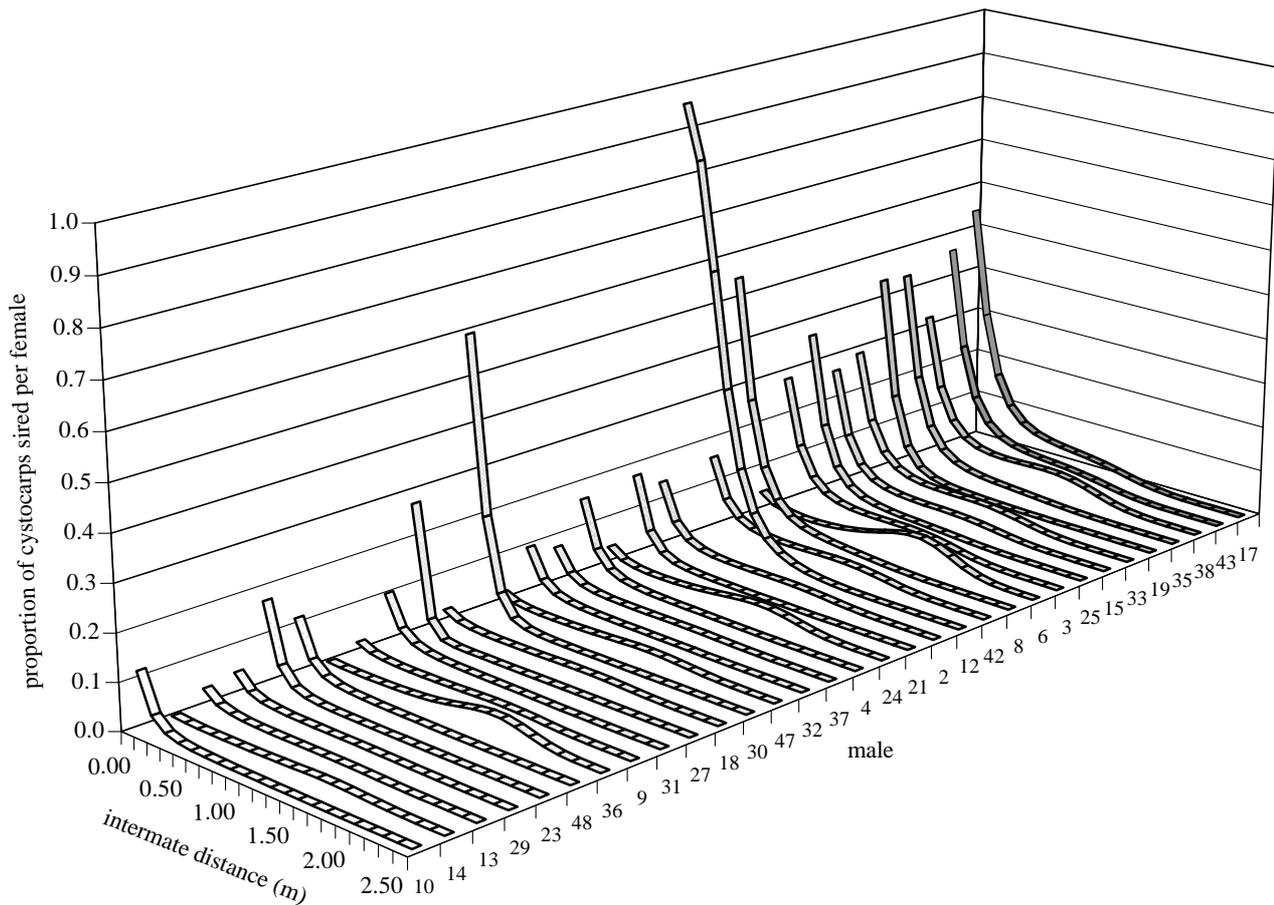
Figure 3. Logistic regression model of individual male fertilization success on a given female with respect to distance. Males are listed in increasing order of population-wide fertilization success (see $\mathcal{N}_c$ column in table 1).

intense at local distances. However, the weak interaction between sex ratio Q3 and distance seemingly contradicts this main effect.

Finally, a comparison of the models indicated that model II afforded the best fit: 45% of the variance was explained by model II, while model III explained just 27%, only marginally more than that explained by model I (22%) (table 3). Thus, the male-identity effect was only partially accounted for by thallus length, size and male competition.

## 4. DISCUSSION

In this study, cystocarps were successfully used as testimonies of fertilization events. Although only two loci were employed for the paternity analysis, their high degree of polymorphism allowed the identification of the paternal genotypes of 91% of the cystocarps, of which 72% could be traced to specific males (table 1).

As expected, reconstructing the parentage of the cysto-carps revealed a large excess of matings at short distances (figure 2). This demonstrates that near-neighbour matings are frequent and that there is a strong distance effect in fertilization success. Similar distance effects have been shown in marine invertebrates (Levitan 1991; Yund & McCartney 1994; Yund 1995; Coffroth & Lasker 1998) and pollen dispersal distances have often been reported to be leptokurtic (see, for example, Levin & Kerster 1974).

Nevertheless, 11% of the cystocarps were attributed to extra-population males because they had paternal geno-types that did not match any male in the study popula-tion (table 1). We cannot completely dismiss the possibility of mutation at one or both loci; 3% of maternal alleles showed a mutation at the GvlCT locus (data not shown). Even with this estimate, the amount of interpopulation gene flow via male gametes is far from negligible. Inter-estingly, a survey in a population 25 m away revealed males possessing some of the 'foreign' paternal genotypes (data not shown). Similarly, in land plants, paternity analyses have revealed many more long-distance, inter-population matings than expected in a wide range of species, from insect-pollinated herbaceous plants (Ellstrand & Marshall 1985; Meagher 1986) and trees (Schnabel & Hamrick 1995; Kaufman *et al.* 1998) to wind-pollinated conifers (Burczyk *et al.* 1996; Burczyk & Prat 1997).

Spermatia do not seem to be limiting in the produc-tion of zygotes in *G. gracilis*. The sperm-limitation hypothesis was previously undermined in two studies of female fertilization success in red macroalgae in which the ratio of virgin to fertilized female gametes was measured (Sheath & Hambrook 1990; Kaczmarska & Dowe 1997). These species belong to orders where female gametes, unfertilized and fertilized, can be readily enumerated by non-destructive means. This direct approach is impracticable in Gracilariales. Nevertheless,

Table 3. *Logistic regression analyses of male fertilization success on a per-female basis: analysis of deviance*

| source | model I: distance model | | | model II: male-identity model | | | model III: quantitative attributes model | | |
|---|---|---|---|---|---|---|---|---|---|
| | deviance | d.f. | $p$ | deviance | d.f. | $p$ | deviance | d.f. | $p$ |
| model | 201.03 | 3 | $<0.0001$ | 409.54 | 65 | $<0.0001$ | 248.34 | 7 | $<0.0001$ |
| residual | 711.36 | 796 | 0.9999 | 502.85 | 734 | 0.9999 | 664.05 | 791 | 0.9999 |
| total | 912.39 | 799 | — | 912.39 | 799 | — | 912.39 | 799 | — |

our study strongly suggests that cystocarp production was not correlated with the availability of male gametes. The mechanisms and timing of spermatia release which may ensure female fertilization success are unknown in red algae. In addition, the offspring of a single female were always sired by at least four different males (table 1), which conceivably reflects the variation in male fertilization success and/or female control of paternity.

In all three models, male fertilization success decreased exponentially with distance indicating a sharp decline beyond which success rates approach zero. Exponential decay in fertilization success rates was predicted by turbulent flow models (Niklas 1985; Denny & Shibata 1989). Similar negative exponential relationships have been empirically demonstrated in wind-pollinated trees (Burczyk *et al.* 1996; Burczyk & Prat 1997), an insect-pollinated plant (Devlin & Ellstrand 1990) and marine invertebrates (Pennington 1985; Grosberg 1991).

Contrary to the experimental results obtained by Richerd *et al.* (1993), male fertilization success varied greatly between males (tables 1 and 2). Male identity was highly significant in fertilization success, indicating greater differences between males than solely accounted for by the distance effect (model II in table 2). However, males did not share a single distance function (figure 3). A closer inspection of the male × distance interaction revealed that, in general, males which enjoyed high population-wide fertilization success sired more cystocarps on neighbouring females (i.e. had steeper distance slopes) than those which had lower overall fertilization success (figure 3). Since, due to the constraints of the model, it may be difficult for those males that sired only one or two cystocarps to have distance slopes as steep as males with high overall success, this interaction may be an artefact of the sampling effort. Nevertheless, males that sired an appreciable number of cystocarps (i.e. $n \geqslant 5$) showed weaker distance slopes than males with high overall success (e.g. males 2, 21 and 42; figure 3).

The three quantitative variables tested in model III account for only some (27%) of the variance in male fertilization success compared to the male-identity model (45%) (table 3). First, longer thalli seemed to ensure direct contact with female thalli and/or broader gamete diffusion clouds, enhancing fertilization. In a wind-pollinated pine, height was a significant factor in fertilization success (Burczyk *et al.* 1996). Second, the male size effect is less clear: relative representation in the gamete pool was not positively proportional to fertilization success on neighbouring females but larger males had higher success rates with distant females. This suggests that profuse reproductive effort, although detrimental in relative success with neighbouring females, may increase the number of female

partners (i.e. those at greater distances). Third, the effect of the sex ratio is ambiguous. On the one hand, high neighbouring (0.33 m radius) male:female ratios negatively affected male fertilization success, indicating that neighbouring males competed for mating opportunities. This negative effect of an increase in sex ratio has been demonstrated in the field in a dioecious perennial plant (Meagher 1991) and in laboratory manipulations of density in a colonial ascidian, *Botryllus schlosseri* (Yund 1995; Atkinson & Yund 1996). On the other hand, although weak, the sex ratio Q3 × distance interaction perhaps suggests that the sex ratio effect may be confounded with other factors, such as male size (see above), which describe relative representation in the gamete pool.

Differences between individual males may therefore involve other intrinsic factors, such as phenology and gamete quality. At the Cape Gris-Nez site, the reproductive period occurs from March to September (Destombe *et al.* 1989). The cystocarps are the result of matings over a period of four to ten weeks (i.e. during the peak reproductive period of May–July). If males differ in fertility during this period, phenology may be responsible for some of the observed variation. However, the determination of individual periods of reproductive maturity would demand repetitive, destructive sampling which would certainly interfere with the natural patterns of mating that we seek to describe.

Finally, the strong male effect (table 2) and great variance in population-wide male fertilization success (coefficient of variation 110%) indicate genetic differences in the quality and/or quantity of spermatia between males. The large variance in male fertilization success compared with that of females is a sign of sexual selection, i.e. female choice or male–male competition (Bateman's (1948) first principle). While retention of the female gamete may increase the probability of gamete encounters (Denny & Shibata 1989), there are no mechanisms or morphological features allowing male gametes to be stored or 'concentrated' in red seaweeds, as in some marine invertebrates (Grosberg 1991; Yund 1995; Bishop 1998). However, the retention and nurturing of the cystocarp by the female provides the incentive for female choice by selective abortions (Hommersand & Fredericq 1990; Richerd *et al.* 1993). Further, a recent cytological study in a red macroalga illustrated the potential for intermale competition within trichogynes, the narrow extensions of the female gamete to which male gametes attach (Pickett-Heaps & West 1998). The authors not only showed that more than one male gamete may successfully adhere to a single trichogyne but also suggested that, during the active transfer towards the base of the female gamete where fertilization occurs, the transport systems

of different gamete nuclei may interact with one another. Although we have much to learn about gamete release, gamete recognition and incompatibility systems in red algae (Brawley & Johnson 1992), several elements suggest that fertilization success in this group may be shaped by numerous selection pressures, including male competition and/or female choice.

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