

Phototaxis and the evolution of isogamy and ‘slight anisogamy’ in marine green algae: insights from laboratory observations and numerical experiments

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The evolution of anisogamy in marine algae was studied through numerical simulations of gamete mating behaviour in three dimensions, using observed traits of marine green algae as input parameters. The importance of phototaxis became apparent from the numerical experiments: all gametes with phototactic systems are favoured over those without, but this advantage is reduced with increasing tank depth or shorter search times. Phototactic gametes were advantaged over non-phototactic gametes if the water was shallower than about 30–40 mm when the time available for gamete encounter was 1000 time steps (5.55 min). If gametes of both sexes are positively phototactic, slightly anisogamous species are at a disadvantage to isogamous species, which invalidates the sperm-limitation theory as a driver for the evolution of slight anisogamy. Conflicting selection forces of search efficiency and zygote fitness may be needed. © 2004 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2004, 144, 321–327.

ADDITIONAL KEYWORDS: mating behaviour – simulation.

INTRODUCTION

Isogamous or slightly anisogamous species are taxonomically widespread in marine green algae. Gametes have specific mating types and often have a phototactic system, usually with an eyespot (see van den Hoek *et al.*, 1995). Such gametes initially show positive phototaxis prior to mating, swimming upward in the water column towards the light at the sea surface. However, their zygotes, as soon as they are formed, become negatively phototactic, and swim back down the water column towards the substrate (e.g. Bliding, 1957; Tatewaki, 1969; Melkonian, 1980; Togashi, Motomura & Ichimura, 1997). This may facilitate settlement on the intertidal substratum in photosynthetically advantageous areas, preventing the zygotes from drifting out to deep waters as they might if phototaxis remained positive.

Positively phototactic gametes, if they succeed in reaching the surface of the water, also accrue significant advantages by being able to search for potential

mates in a two-dimensional (2-D) surface rather than in 3-D space (Cox, 1983; Cox & Sethian, 1985; Togashi *et al.*, 1999). However, experimentally quantifying the advantages of such gametes is extremely difficult because visually determining the precise position in three dimensions of gamete collisions within the water column is nearly impossible.

Numerical simulations are an alternative to laboratory or field experiments, and can gain realism if specific sizes, swimming velocities and trajectories of real gametes are used as input parameters. Such gamete behaviours can be determined from video recordings of individual gamete swimming paths. Cox & Sethian (1985) used such inputs from Pommerville's films of the swimming behaviour of gametes of the fungal genus *Allomyces* to simulate gamete motion, but were limited to 2-D analysis given the 2-D plane of the film. Analytical solutions of 3-D random gamete motion are difficult to obtain, because unlike 2-D random walks, 3-D motions are non-recurrent (Hersh & Griego, 1969). Subsequent supercomputer simulations of 3-D searches (Cox, Cromar & Jarvis, 1991) resulted in the prediction that elliptically deformed rather than

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spherical objects of equivalent biomass would result in greater encounter rates in 3-D random searches, but no attempts were made to compare isogamy with anisogamy.

In our numerical experiments, we considered the effect of diffusion of gametes (inherent in 3-D random walks that are non-recurrent) through time, using input parameters from experimental and comparative data of various species of marine green algae. We also sought to elucidate any potential advantages of phototaxis and to study the mechanism of the evolution of slight anisogamy (common in marine green algal species).

MATERIAL AND METHODS

LABORATORY OBSERVATIONS OF GAMETE BEHAVIOUR

We compared our laboratory observations of a slightly anisogamous marine green alga, *Monostroma angicava* Kjellman (Togashi *et al.*, 1997, 1999) with data of other species collected from a literature survey to generate input parameters for the numerical experiments (Togashi, Miyazaki & Cox, 2002). We recorded individual swimming paths of male and female gametes of *M. angicava* under non-phototactic and phototactic conditions on videotape with an inverted microscope (Axiovert135, Zeiss), then digitized these using a commercial software package (Togashi, Motomura & Ichimura, 1998). Gametes of both sexes of *M. angicava* show positive phototaxis to white light (Togashi *et al.*, 1997) but do not respond to red (Togashi *et al.*, 1999). Thus, a non-phototactic condition was established by masking a white light with a red cut-off filter (MC-R2, Marumi). Under phototactic conditions, we videotaped horizontal gamete motion illuminated only from above by white light and vertical motion illuminated from above by red light and from the sides by white light.

NUMERICAL EXPERIMENTS

Numerical experiments were written in Java (<http://java.sun.com/products/>). Gametes are idealized as spheres, starting from a randomly distributed position on the bottom of a virtual rectangular test tank and swimming at a given speed in water. Body width of gametes is used as the diameter for determining drag-reductions to gamete velocity. When a gamete collides with the tank or water surface, angles of incidence equal those of reflection. Motion, with constant velocity, is incremental so the distance travelled by each gamete during each time interval is the same. However, at the beginning of each time interval, every gamete changes swimming direction three-dimensionally because small motile objects at low Reynolds numbers maintain straight paths for only limited time as a result of the impact of Brownian

forces (Dusenbery, 1992). Two angles are independently chosen separately for each gamete from a random sequence of integers between -30 and +30; thus any angular turn within those bounds was equally likely, but the chances of any two gametes making the same angular turns during the same time interval in both the X-Y and the Z-Y planes were small. To simulate positive phototaxis, we used absolute values of the Z-components of the swimming vector, thus ensuring steady upward motion of the gamete. Each gamete is tracked and the distances between the centres of nearby male and female gametes are measured at each step to detect collisions. All encounters are deemed to result in sexual fusion and the positions of the encounter are recorded in MySQL (<http://www.mysql.com/>). Fused gametes are then removed from the mating population. Gamete parameters and sample simulations are available at <http://members.aol.com/tandytogashi/>.

We explored four different regimes of gametes: slightly anisogamous non-phototactic, slightly anisogamous positively phototactic, isogamous non-phototactic and isogamous positively phototactic. For each regime, we varied the number of gametes released, the length of time allowed for fusion (analogous to gamete lifespan) and the test tank depth, resulting in three sets of numerical experiments. In the first set, the number of gametes released varied from 1/10 unit to 6 units, where the basic unit was 7400 male gametes and 3800 female gametes for slightly anisogamous species, and 5100 gametes of each sex for isogamous species. The trials ran for 1000 steps of 1/3 s each in a virtual test tank of 10 mm (length), 10 mm (width) and 25 mm (depth). In the second set of experiments, the tank size and gamete number were constant, but gamete life-span varied from 100 to 6000 steps of 1/3 s each. In the third set of numerical experiments, test tank depth varied from 1 mm to 10 cm for a constant number of gametes in a 1000 step trial. Each trial was repeated three times, with different random numbers used for each trial to determine the change in swimming directions of each gamete. We then calculated the mean number of zygotes formed in each trial together with a standard deviation using Stat View 5.0 software (SAS).

RESULTS

LABORATORY OBSERVATIONS OF GAMETE BEHAVIOUR

In *M. angicava*, male and female gametes are 5.90 μm (length) by 2.96 μm (width) and 7.53 μm (length) by 3.70 μm (width), respectively, yielding roughly equivalent length/width ratios. We therefore set the radii of male and female gametes in our numerical experiments at 1.48 μm (= 2.96/2) and 1.85 μm (= 3.70/2), respectively, with volumes of $4/3\pi(1.48)^3 \mu\text{m}^3$ (male)

Table 1. Swimming patterns of gametes of the slightly anisogamous marine green alga, *Monostroma angicava*. The absolute values of the change of swimming direction of gametes every 1/3 s with their ranges. Values are given as mean \pm SD

| Condition of illumination | Male | Female |
|---------------------------|---|---|
| Non-phototactic | Horizontal and vertical: $10.5 \pm 7.3^\circ$ ($n = 22$, range = 2–32°) | Horizontal and vertical: $11.3 \pm 8.9^\circ$ ($n = 21$, range = 4–37°) |
| Phototactic | Horizontal: $13.8 \pm 8.3^\circ$ ($n = 22$, range = 4–40°) Vertical: $9.8 \pm 8.3^\circ$ ($n = 23$, range = 1–38°) | Horizontal: $11.5 \pm 7.3^\circ$ ($n = 22$, range = 2–27°) Vertical: $7.2 \pm 5.7^\circ$ ($n = 22$, range = 1–25°) |

and $4/3\pi(1.85)^3 \mu\text{m}^3$ (female) for the case of slight anisogamy. We assumed the radii of the hypothetical ancestral isogamous gametes to be the average of these previous two figures: $(1.48 + 1.85)/2 = 1.67 \mu\text{m}$ with the volume of a single isogamete being $4/3\pi(1.67)^3 \mu\text{m}^3$. Our comparative data supported these assumptions on gamete size. We held total gametic biomass equal between mating types. We found that with a constant biomass allocated to gamete production we could repeatedly perform numerical experiments for a maximum of 7400 males and 3800 females owing to CPU time constraints for slightly anisogamous species (male/female = 1.95 : 1.00) and 5100 gametes of each of the two mating types for isogamous species.

In *M. angicava*, we found swimming velocities of male and female gametes to be $189 \mu\text{m s}^{-1}$ and $151 \mu\text{m s}^{-1}$, respectively. Our experiments indicated that the flagellar propulsion is equivalent for male and female gametes, so the swimming velocity of isogametes was assumed to be $168 \mu\text{m s}^{-1}$ (width \times velocity of slight anisogamete)/(width of isogamete). Male and female gametes of *M. angicava* kept their swimming directions nearly straight for about 1/3 s. Data on the changes of swimming direction of gamete every 1/3 s are shown in Table 1. Under both non-phototactic and positively phototactic conditions, the angular change in direction ranged between $\pm 30^\circ$ in both sexes. The mean angles were equivalent with no statistically significant skewing in any direction (i.e. right or left, up or down) in a Chi-squared test. We therefore set up our numerical experiments to change both the horizontal and the vertical direction of gamete motion every 1/3 s, with the angle of change being randomly chosen from $\pm 30^\circ$ against the direction of movement.

NUMERICAL EXPERIMENTS

Under the non-phototactic and positively phototactic conditions, the number of zygotes formed increased as the number of released gametes increased (Fig. 1). For species with 'slight aniso-

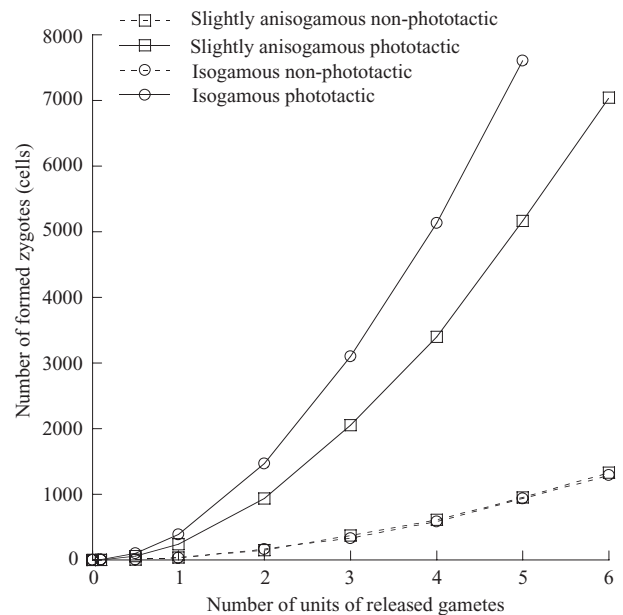


Figure 1. Number of zygotes formed according to the number of released gametes. The basic unit was 7400 male gametes and 3800 female gametes (slight anisogamy) and 5100 gametes of each of the two mating types (isogamy). [Available time: 1000 steps of 1/3 s; tank size: 10 mm (length), 10 mm (width), 25 mm (depth).] For the comparison between isogamy and slight anisogamy in phototactic gametes, the Mann–Whitney *U*-test was performed at the five-units level of gametes (six samples each) and the difference was significant at $P < 0.005$. Error bars are not shown because almost no variations were observed, and were too short to display.

gamy', the number of zygotes formed under the phototactic condition was, however, consistently about six times larger than that under the non-phototactic condition. The depth components of locations of sexual fusion are shown in Figure 2A and B. Note that with phototaxis, fusions concentrate near the water surface, whereas in the absence of phototaxis, they are scattered and many occur near the bottom. For

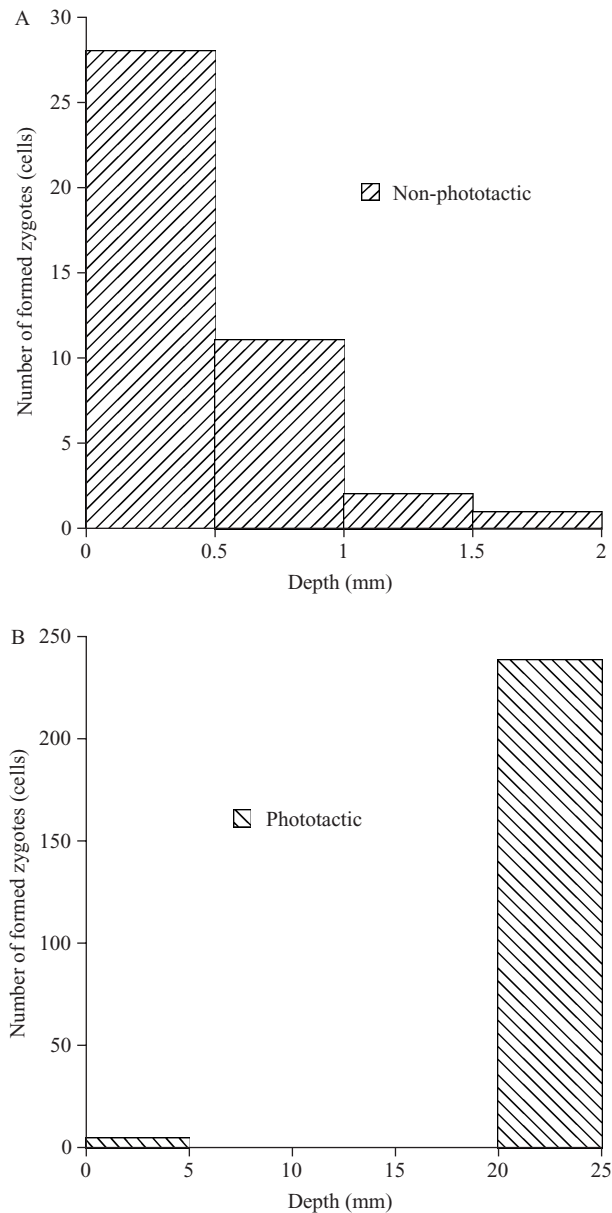


Figure 2. Depth components of locations of sexual fusion. The mating system was slightly anisogamous. (A) Non-phototactic condition; (B) positively phototactic condition. [Number of released gametes: the basic unit (see the legend to Fig. 1); available time: 1000 steps of $1/3$ s; tank size: 10 mm (length), 10 mm (width), 25 mm (depth).]

isogamous species, the number of zygotes formed under the phototactic condition was about nine times larger than that under the non-phototactic condition. The numbers of zygotes formed by isogamous and slightly anisogamous species were nearly identical under the non-phototactic conditions. By contrast, under the phototactic conditions, species with isogamy were significantly more successful in

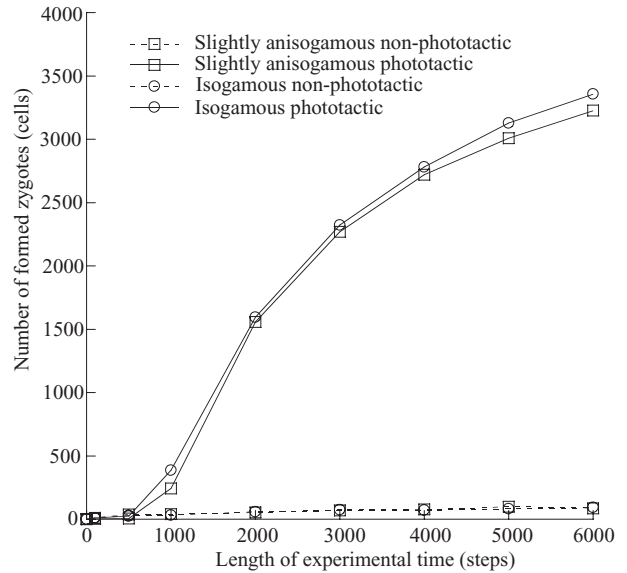


Figure 3. Number of zygotes formed according to the time available for fusion. [Number of released gametes: the basic unit (see the legend to Fig. 1); tank size: 10 mm (length), 10 mm (width), 25 mm (depth).]

producing zygotes than species with slight anisogamy (see Fig. 1).

Increasing the time available for gamete encounter and fusion among phototactic gametes surprisingly matched the plot of Koopman's equation for 2-D random search (Cox, 1983: fig. 2), whereas increasing time available for fusion had little impact on non-phototactic gametes, perhaps as a result of diffusion in three dimensions (Fig. 3). For searches lasting 4000 steps (approximately 22.2 min) the number of zygotes formed under the positively phototactic condition was nearly 40 times larger than that under the non-phototactic condition. This result held regardless of the mating system.

In our numerical experiments, shallow tanks produced exponentially higher encounter rates than deep tanks (e.g. those with depths greater than 30 mm), but again only for gametes with phototaxis (Fig. 4). However, few zygotes were formed when the depth became greater than 30 mm. For gametes released in deeper water, the majority of phototactic gametes do not appear to reach the water surface. Gametes lacking a phototactic system did not enjoy 2-D search efficiencies, even when the tank was extraordinarily shallow, confirming that increased search efficiencies in two dimensions are not merely the result of gamete concentration because at depths of 1 mm the number of zygotes formed under the positively phototactic condition was about 25 times larger than that under the non-phototactic condition. Results were little different between the two mating systems.

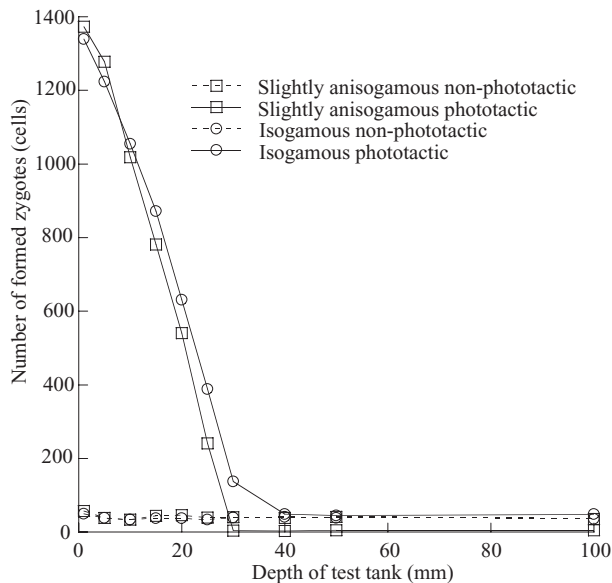


Figure 4. Number of zygotes formed according to the depth of test tank. [Number of released gametes: the basic unit (see the legend to Fig. 1); available time: 1000 steps of 1/3 s; base size of tank: 10 mm (length), 10 mm (width).]

DISCUSSION

For isogamous or slightly anisogamous species in our survey, we found little differences in gamete size, which are intermediate between male and female gamete sizes in species with marked anisogamy (Togashi *et al.*, 2002). Gamete behaviour is also generally similar across species; gametes show positive phototaxis prior to sexual fusion and negative phototaxis after zygote formation. Our idealization of gametes as spheres is slightly unrealistic (gametes of marine algal species are pear-shaped) but of little mathematical consequence because drag forces are determined by the cross-sectional radius orthogonal to the direction of travel at low Reynolds numbers according to Stokes Law (Le Méhauté, 1976; Vogel, 1981; Berg, 1993; Dusenbery, 2001).

Few experimental studies on gamete velocity and swimming pattern in marine green algae exist other than those of the slightly anisogamous species *M. angicava* (Togashi *et al.*, 1997, 1999) and the two markedly anisogamous species *Derbesia tenuissima* (De Notaris) Crouan (T. Togashi & P. A. Cox, unpubl. data) and *Bryopsis plumosa* (Hudson) C. Agardh (Togashi *et al.*, 1998). In markedly anisogamous species, male gametes, which are smaller than isogametes and slightly anisogamous male gametes, swim faster than males of *M. angicava* as predicted by the hydrodynamic considerations of Cox & Sethian (1984). Female gametes, which are larger than isogametes and slightly anisogamous female gametes, swim

slower than females of *M. angicava*. These velocities vary only slightly. At low Reynolds numbers, movement is governed by viscous forces, so inertial forces can be ignored (Purcell, 1977; Bray, 1992). Thus the force required for gamete propulsion depends solely on width of gamete (L), its velocity (v) and viscosity of the liquid (η) (Bray, 1992). Our data on size and velocity suggest that these forces are equivalent for male and female gametes, supporting an idea that, at a low Reynolds number, the inverse relationship between size and velocity is provided by Stokes Law, especially in the idealized situation of steady-state flow past a sphere (Landau & Lifshitz, 1959; Cox & Sethian, 1985).

Our assumption that a fixed amount of reproductive biomass can be subdivided into a variable number of gametes seems reasonable, given experimental data for *M. angicava* (Togashi *et al.*, 1997) and other green algae (Fritsch, 1956; Wiese, Wiese & Edwards, 1979; Tanner, 1981). There are few reports on biased sex ratio of gametophytes in natural populations of marine green algae so far. In *M. angicava* and *B. plumosa*, sex ratios of gametophytes are 1 : 1 in the field (Togashi *et al.*, 1997 for *M. angicava*; T. Togashi & P. A. Cox, unpubl. data for *B. plumosa*). In our numerical experiments, the gametic sex ratio for a slightly anisogamous species is close to that experimentally estimated for *M. angicava* (Togashi *et al.*, 1997).

In marine algal species, the maximum number of gametes released into the sea has been estimated as 9.1×10^6 cells cm^{-2} (male) and 4.1×10^6 cells cm^{-2} (female) in *M. angicava* (Togashi *et al.*, 1997). Our numerical experiments therefore might be smaller than would be realistic in nature if a maximum number of gametes were released. However, our numerical experiments predict that the number of formed zygotes would continue to increase with the number of released gametes (Fig. 1). However, even in such cases, the superiority of phototactic gametes in random searches still holds. Our study supports the idea that the chance for gametes to encounter partners and to fuse is increased in 2-D space (Cox, 1983; Cox & Sethian, 1985).

Considering that many gametes of both sexes continue to swim showing positive phototaxis for many hours (>12 h) after liberation in many slightly anisogamous and isogamous species (e.g. Miyaji, 1985; Togashi *et al.*, 1997), the advantage of phototaxis may be widely expected in nature, especially for species that release gametes in shallow water. In this regard, it is interesting to note that isogamous and slightly anisogamous marine green algae often inhabit upper or middle intertidal zones (e.g. Valiela, 1995; Dawes, 1998) and possess mechanisms for synchronous gamete release during extremely low day-time tides when phototactic swimmers (i.e. gametes and planozy-

gotes) would have the advantages suggested by our numerical experiments (Stratmann, Paputsoglu & Oertel, 1996; Togashi & Cox, 2001).

When mating efficiencies are compared between the slightly anisogamous and isogamous mating systems, as the number of released gametes is increased, a difference clearly emerges; the number of zygotes formed by phototactic isogametes is significantly larger than the number of zygotes formed by phototactic gametes with slight anisogamy under the positively phototactic condition (Fig. 1). This result suggests that the evolution of slight anisogamy from isogamy may not be explained by high encounter rates of anisogamous male and female gametes. This sheds doubt on the generality of the sperm-limitation theory (Levitán, 1996; Dusenbery, 2001). Conflicting selection forces of search efficiency and zygote fitness may be needed to explain the evolution of slight anisogamy. Cox & Sethian (1985) predicted adaptive peaks for either complete isogamy or marked anisogamy, but did not consider slight anisogamy to be evolutionarily stable. We suggest that slight anisogamy can persist if (1) phototaxis has evolved and (2) there is strong selection for large zygote size.

CONCLUSION

By combining laboratory observations with numerical experiments, we found evidence for the important role of gamete behaviour in the evolution of isogamy and slight anisogamy in marine green algae. First, positive phototaxis of gametes prior to sexual fusion can enormously increase mating efficiency by conferring the search efficiencies associated with 2-D search. Second, because the number of formed zygotes in isogamous species is significantly higher than those of slightly anisogamous species under phototactic conditions, the evolution of slight anisogamy from primitive isogamy may not be explained by high encounter rates of anisogamous male and female gametes; other considerations are required. Gamete behaviour may be a key to understanding the evolution of mating systems in marine green algae.

REFERENCES

- Berg HC.** 1993. *Random walks in biology*. Princeton: Princeton University Press.
- Bliding C.** 1957. Studies on *Rhizoclonium*. I. Life history of two species. *Botaniska Notiser* **110**: 271–275.
- Bray D.** 1992. Cell swimming. In: Bray D, ed. *Cell movements*. New York: Garland Publishing, 3–16.
- Cox PA.** 1983. Search theory, random motion, and the convergent evolution of pollen and spore morphology in aquatic plants. *American Naturalist* **121**: 9–31.
- Cox PA, Cromar S, Jarvis T.** 1991. Underwater pollination, three-dimensional search, and pollen morphology: predictions from a supercomputer analysis. In: Blackmore S, Barnes SH, eds. *Pollen and spores*. Systematics Association Special Volume no. 44. Oxford: Clarendon Press, 363–375.
- Cox PA, Sethian JA.** 1984. Search, encounter rates, and the evolution of anisogamy. *Proceedings of the National Academy of Sciences of the USA* **81**: 6078–6079.
- Cox PA, Sethian JA.** 1985. Gamete motion, search, and the evolution of anisogamy, oogamy, and chemotaxis. *American Naturalist* **125**: 74–101.
- Dawes CJ.** 1998. *Marine botany*, 2nd edn. New York: John Wiley & Sons.
- Dusenbery DB.** 1992. *Sensory ecology*. New York: W.H. Freeman.
- Dusenbery DB.** 2001. Selection for high gamete encounter rates explains the success of male and female mating types. *Journal of Theoretical Biology* **202**: 1–10.
- Fritsch FE.** 1956. *The structure and reproduction of the algae*. Cambridge: Cambridge University Press.
- Hersh R, Griego RJ.** 1969. Brownian motion and potential theory. *Scientific American* **220**: 66–74.
- van den Hoek C, Mann DG, Jahns HM.** 1995. *Algae: an introduction to phycology*. Cambridge: Cambridge University Press.
- Landau LD, Lifshitz EM.** 1959. *Fluid mechanics*. Oxford: Pergamon Press.
- Le Méhauté B.** 1976. *An introduction to hydrodynamics and water waves*. Berlin: Springer Verlag.
- Levitán DR.** 1996. Effects of gamete traits on fertilization in the sea and the evolution of sexual dimorphism. *Nature* **382**: 153–155.
- Melkonian M.** 1980. Flagellar roots, mating structure and gametic fusion in the green alga *Ulva lactuca* (Ulvales). *Journal of Cell Science* **46**: 149–169.
- Miyaji K.** 1985. Taxonomic studies on the genus *Spongomorpha* in Japan. Unpublished DPhil thesis, Hokkaido University.
- Purcell EM.** 1977. Life at low Reynolds number. *American Journal of Physics* **45**: 3–11.
- Stratmann J, Paputsoglu G, Oertel W.** 1996. Differentiation of *Ulva mutabilis* (Chlorophyta) gametangia and gamete release are controlled by extracellular inhibitors. *Journal of Phycology* **32**: 1009–1021.
- Tanner CE.** 1981. Chlorophyta: life histories. In: Lobban CS, Wynne MJ, eds. *The biology of seaweeds*. Berkeley: University of California Press, 218–247.
- Tatewaki M.** 1969. Culture studies on the life histories of some species of the genus *Monostroma*. *Scientific Papers of the Institute of Algological Research, Faculty of Science, Hokkaido University* **6**: 1–56.
- Togashi T, Cox PA.** 2001. Tidal-linked synchrony of gamete release in the marine green alga, *Monostroma angicava* Kjellman. *Journal of Experimental Marine Biology and Ecology* **264**: 117–131.
- Togashi T, Miyazaki T, Cox PA.** 2002. Sexual reproduction in marine green algae: gametic behavior and the evolution of anisogamy. In: Yoshimura J, Nakagiri N, Shields WM, eds. *Proceedings of two symposia on ecology and evolution in VIII*

- INTECOL, Seoul, Korea, 2002*. Otsu: Sangaku Publisher, 70–79.
- Togashi T, Motomura T, Ichimura T. 1997.** Production of anisogametes and gamete motility dimorphism in *Monostroma angicava*. *Sexual Plant Reproduction* **10**: 261–268.
- Togashi T, Motomura T, Ichimura T. 1998.** Gamete dimorphism in *Bryopsis plumosa*: phototaxis, gamete motility and pheromonal attraction. *Botanica Marina* **41**: 257–264.
- Togashi T, Motomura T, Ichimura T, Cox PA. 1999.** Gametic behavior in a marine green alga, *Monostroma angicava*: an effect of phototaxis on mating efficiency. *Sexual Plant Reproduction* **12**: 158–163.
- Valiela I. 1995.** *Marine ecological processes*, 2nd edn. New York: Springer.
- Vogel S. 1981.** *Life in moving fluids*. Princeton: Princeton University Press.
- Wiese L, Wiese W, Edwards DA. 1979.** Inducible anisogamy and the evolution of oogamy from isogamy. *Annals of Botany* **44**: 131–139.

