

Reproductive Strategies of Marine Green Algae: the Evolution of Slight Anisogamy and the Environmental Conditions of Habitats

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Abstract: In marine green algae, isogamous or slightly anisogamous species are taxonomically widespread. They produce positively phototactic gametes with phototactic devices including an eye-spot in both sexes. We developed a new numerical simulator of gamete behavior using C++ and pseudo-parallelization methods to elucidate potential advantages of phototaxis. Input parameters were set based on experimental data. Each gamete swimming in a virtual rectangular test tank was tracked and the distances between the centers of nearby male and female were measured at each step to detect collisions. Our results shed light on the roles of gamete behavior and the mechanisms of the evolution of anisogamy and more derived forms of sexual dimorphism. We demonstrated that not only gametes with positive phototaxis were favored over those without particularly in shallow water because they could search for potential mates on the two-dimensional water surface rather than randomly in three dimensions, but also phototactic behavior clarified the difference between isogamy and slight anisogamy. Isogamous species produced significantly more zygotes than slightly anisogamous ones only under the phototactic conditions. Our results suggested that “sperm limitation” might be resolved in the slightly anisogamous species. In marine green algae, some more markedly anisogamous species produce the smaller male gametes that have no eye-spot and swim randomly. In contrast, the larger female gametes have an eye-spot and show positive phototaxis. As a result of careful experimental observations, we discovered the first pheromonal attraction system in marine green algae. This pheromonal attraction system might have played a key role in the evolution of anisogamy in marine green algae, because it may enable markedly anisogamous species achieve 2D search efficiencies on the water surface. The mating systems appear to be tightly tuned to the environmental conditions of their habitats.

Keywords: *Anisogamy; Gamete behavior; Marine green algae; Pheromonal attraction; Phototaxis*

1. INTRODUCTION

Anisogamy with gametes of two different sizes is common to many organisms and only one universal difference between males and females [Randerson and Hurst, 2001]. This anisogamy underlies the evolution of sex differences in behavior and morphology, because it generates sexual selection whenever the number of small gametes produced by males exceeds the number necessary to fertilize the ova of a single female [Schuster and Wade, 2003]. Thus, sexual selection resulting from the variance in mate numbers of the sex producing small gametes does not exist in asexual populations.

Two main theories have been proposed to account for the evolution of anisogamy. The one is a theory in which there is disruptive selection acting on gamete size based on the two

conflicting selection forces of search efficiency and postzygotic survival [e.g. Parker et al., 1972], and the other is sperm limitation theory that considers an escape from sperm limitation as a mechanism driving anisogamy [e.g. Levitan, 1996].

In oogamous sea urchins, it has been reported that females are often sperm limited [Levitan, 1996]. On the other hand, in marine green algae, isogamous or slightly anisogamous are taxonomically widespread. Their gametes not only have specific mating types, but also have a phototactic system with an eye-spot. It has been suggested that the eye-spot evolved in the most primitive green flagellate taxa [Melkonian, 1982]. Such gametes initially show positive phototaxis prior to mating, swimming upward in the water column towards the light at the sea surface. Positively phototactic gametes may gain

significant advantages, especially in shallow water, by being able to search for potential mates in a two-dimensional surface rather than in three-dimensional space [Cox and Sethian, 1985]. There are some experiments that support this idea [Togashi et al., 1999]. So, sperm limited conditions might not be ubiquitous in these species.

In this paper, considering the effect of diffusion of gametes (inherent in 3-D random walks that are non-recurrent) through time, we sought to elucidate potential advantages of gamete phototaxis and to study the mechanism of the evolution of slight anisogamy and the environmental conditions of their habitats.

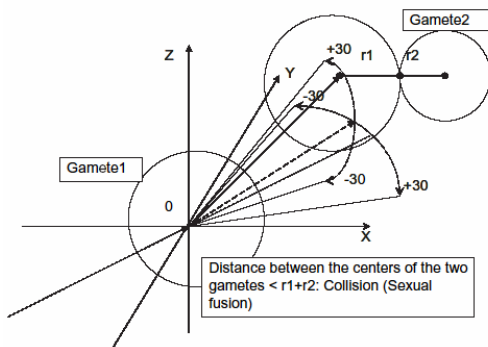


Figure 1. Gamete motion and sexual fusion.

2. NUMERICAL MODELLING OF GAMETE BEHAVIOR

2.1. Introduction

In the study of fertilization kinetics of gametes, numerical simulations using computer programming languages 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. [Although mathematical models are often the best encapsulation of ecological and evolutionary mechanisms [Wilson, 2000], they may be unsuitable to analyze isogamous or near isogamous species, because it is difficult to remove fused gametes from the mating populations through time with mathematical methods.] Such gamete behaviors can be determined from video recordings of individual gamete swimming paths. Cox and 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 two-dimensional analysis given the 2-D plane of the film. Analytical solutions of three-dimensional random gamete motion are difficult to obtain, because unlike two-

dimensional random walks, three-dimensional motions are non-recurrent. Subsequent supercomputer simulations of three-dimensional search [Cox et al., 1991] resulted in the prediction that elliptically deformed, rather than spherical objects of equivalent biomass would result in greater encounter rates in 3-D random searches, but no attempts were made to compare isogamy to anisogamy. In this paper, our simulation code is compiled from C++.

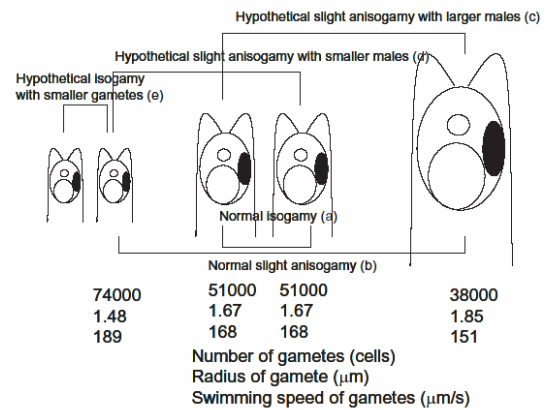


Figure 2. Experimental conditions and regimes.

2.2. Model description and input parameters

Gametes are idealized as spheres. Body width of gamete is used as the diameter. Our idealization of gametes as spheres might be slightly unrealistic (since gametes of marine algal species are pear-shaped) but would be of little mathematical consequence since drag forces are determined by the cross-sectional radius orthogonal to the direction of travel at low Reynold's numbers according to Stoke's law [Le Méhauté, 1976]. Comparative data concerning gamete traits collected by a literature survey have demonstrated that, in isogamous or slightly anisogamous species, the range of gamete size is relatively narrow, and that it is intermediate between male and female gamete size in species with marked anisogamy [Togashi et al., 2002]. Therefore, using the data of a slightly anisogamous species *Monostroma angicava* Kjellman [Togashi et al., 1997] as representatives, the radii of male and female gametes in a slightly anisogamous species are set at 1.48 μm and 1.85 μm , respectively. The radius of the isogametes is assumed to be the average of the slight anisogametes: $(1.48+1.85)/2=1.67 \mu\text{m}$.

Each gamete swims at a given speed in water, starting from a randomly distributed position on the bottom of a virtual rectangular test tank of 10 mm (length), 10 mm (width) and 25 mm (depth). Thus, the distance traveled by each gamete during each time interval is the same. Experimental

studies on gamete size and swimming speed exist in some species of marine green algae [Togashi et al., 1997; Togashi, 1998; Togashi et al., 1998]. At low Reynold's numbers relevant here, movement is governed by viscous forces [$F = 6 \pi \epsilon c r$; ϵ : viscosity of the liquid, c : swimming speed of gamete, r : radius of gamete] (see Randerson and Hurst, 2001). Experimental data suggest that these forces provided by flagellar propulsion are equivalent for male and female gametes across species, supporting our assumption that gamete size is inversely related to swimming speed.

At the beginning of each time interval (step), every gamete changes swimming direction three-dimensionally since small motile objects at low Reynold's numbers maintain straight paths for only limited time due to the impact of Brownian forces [Dusenbery, 1992]. Based on the analysis of gamete swimming paths [Togashi and Cox, in press], the step interval is set at 0.3 second, then, two angles are independently chosen separately for each gamete from a random sequence of integers between -30 and +30 to determine the changes of direction in the X-Y (horizontal) and the Y-Z (vertical) planes, respectively. When a gamete collides with the tank or water surface, angles of incidence equal those of reflection. In gametes exhibiting positive phototaxis, a vector sum of the current unit velocity vector and the normal unit vector (light direction) is taken and renormalized. Then the same random tilt and rotation matrices that the non-phototactic method uses are applied thus ensuring steady upward motion of the gamete.

The biomass allocated to produce gametes is assumed to be equal between mating types (i.e. $1.0 \times 10^5 \mu\text{m}^3$) as experimentally confirmed in some organisms including marine green algae [e.g. Togashi et al., 1997]. There are few reports on biased sex ratio of gametophytes in natural populations of marine green algae so far. So, sex ratios of gametophytes are assumed to be 1:1 (=male:female).

Each gamete is tracked and the distances between the centers of nearby male and female gametes are measured at each step to detect collisions. All encounters of sexually different gametes are deemed to result in sexual fusion. We divide the test tank into equally sized subrooms to increase the speed of calculation, but, our simulator can detect collisions even if a male and female gamete are in two different subrooms, but within "limit" distance of each other across the shared subroom face, in such a case, we should count it as a mating. Fused gametes are then removed from the mating population.

3. NUMERICAL EXPERIMENTS

3.1. Preliminary tests

As an example of the speed and precision of our simulations, we made thirty runs with populations of 10,000 male and female gametes each for 2000 time steps per run in less than 100 minutes. Because in our method we start by randomly placing the population over the bottom of the test tank, and also because the swimming motion of the gametes has random elements at each time step, we were curious about the possible run to run variations that might occur in our results.

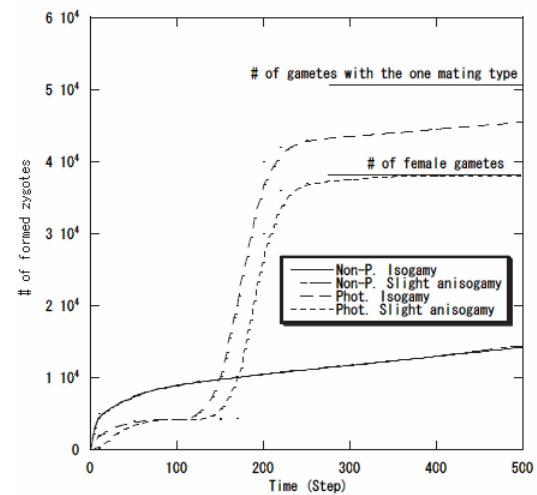


Figure 3. Mating experiments in the normal species.

3.2. Mating experiments

Our experimental conditions (i.e. gamete size, number, swimming speed) and explored experimental regimes were shown in Figure 1.

First, we performed mating experiments in the normal isogamous and slightly anisogamous species under both non-phototactic and phototactic conditions (Figure 1a and b). The numbers of gametes at the surface of water were also monitored in the isogamous species.

Secondly, in the slightly anisogamous species under the phototactic condition, we slightly increased only the size of male gametes to that of the isogametes (Figure 1c).

Thirdly, in the slightly anisogamous species under the phototactic condition, we slightly decreased only the size of female gametes to that of the isogametes (Figure 1d).

Lastly, we performed a mating experiment in a hypothetical isogamous species, in which the size of gametes of both mating types was the same as "male" gametes of the slightly anisogamous

species (Figure 1e). Therefore, in this experiment, gametes of both mating types were smaller than those of the normal isogamous species.

4. RESULTS

In our preliminary tests, using a particular set of thirty replicas with identical conditions except for different random starting points, we found that the mean number of fertilizations that had occurred by the 2000th step was 5844 with a standard error of 6.19. These results mean that we can predict the mean value of 5844 fertilizations within a 95% confidence interval of 0.2%. Because we use large numbers of gametes in our simulations, we expect similar precision in other runs.

In the normal isogamous and slightly anisogamous species (Figure 2), under the non-phototactic conditions, the numbers of formed zygotes were nearly identical and observed at a low level. So, many gametes remained unfertilized in both species. In contrast, under the phototactic conditions, they remarkably increased and the difference of the numbers of formed zygotes between the two species was clarified. As a result, species with isogamy was significantly more successful in producing zygotes than species with slight anisogamy. In the isogamous species, some unfertilized gametes remained in both mating types. However, in the slightly anisogamous species, most female gametes were soon fertilized. The numbers of gametes at the surface of water during the experiments in the isogamous species were shown in Figure 3. It appears that, only under the phototactic conditions, gametes of both sexes actually gathered just under the surface of water.

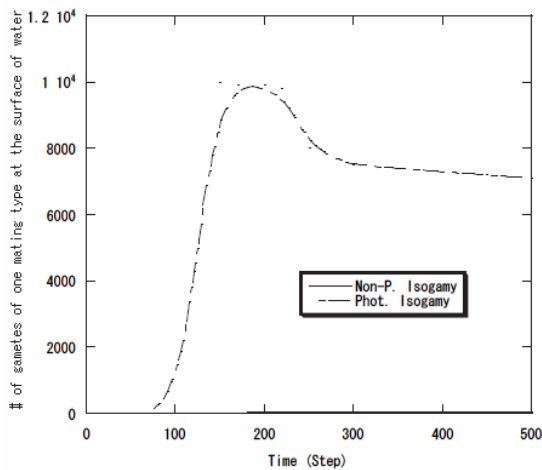


Figure 4. The number of gametes at the surface.

The numbers of formed zygotes in the hypothetical species were shown in Figure 4. In the hypothetically established slightly

anisogamous species with “larger male gametes”, some female gametes remained unfertilized. Thus, the number of zygotes formed in this species was smaller than that in the normal slightly anisogamous species.

However, in the hypothetically established slightly anisogamous species with “smaller female gametes”, most female gametes were soon fertilized. Thus, the number of formed zygotes was larger than that in the normal slightly anisogamous species.

In the hypothetically established isogamous species with “smaller gametes” of both mating types than the normal isogamous species, the number of formed zygotes was the largest of all mating experiments in this study. However, some gametes of the both mating types remained unfertilized.

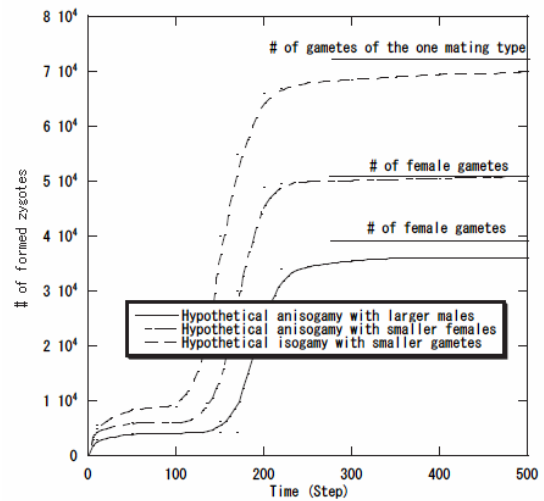


Figure 5. Mating experiments in the hypothetical species.

5. DISCUSSION

The superiority of phototactic gametes (Figure 2 and 4) may be widely expected in nature, especially for species that release gametes in shallow waters, because gametes continue to swim showing positive phototaxis for more than 12 hours in slightly anisogamous and isogamous species [e.g. Togashi et al., 1997]. These species often inhabit upper or middle intertidal zones [e.g. Dawes, 1998]. Their zygotes become negatively phototactic just after they are formed and swim back down to the water substrate [e.g. Togashi et al., 1997]. This should 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. Behaviors of such swimmers (i.e. gametes and swimming zygotes) may not be overwhelmed by sea conditions,

because they often possess mechanisms for synchronous gamete release during extremely low daytime tides under calm conditions when swimmers could make the best use of their phototaxis avoiding turbulent water movement [e.g. Togashi and Cox, 2001].

Phototaxis may actually function to introduce gametes to a two-dimensional realm (the water surface) where search efficiencies and target encounter probabilities are much higher than in three-dimensional random searches in the water column, which characterize those of non-phototactic gametes (Figure 3).

Our numerical experiments in the normal isogamous and slightly anisogamous species suggest that, although the isogamous species may be under sperm limiting conditions where the number of formed zygotes increases as more sperm is released, such sperm limitation appears to be resolved in the slightly anisogamous species, where most female gametes are fertilized and the number of zygotes depends on the number of released female gametes (Figure 2). Thus, slightly anisogamous species may be often under sperm-abundant (competitive) conditions.

We hypothetically increased only the size of male gametes in the slightly anisogamous species. It is because mating efficiency of such a species might be as high as the normal species, if slight anisogamy always resolves sperm limitation. The volume of zygotes formed in this species is larger than that in the normal species (by 15 %). Thus, if this hypothesis is true, assuming a positive relationship between zygote volume and fitness, such a species might be more advantageous than the normal species. However, our results have demonstrated that the number of zygotes formed in this hypothetical species is smaller than that in the normal species (Figure 4). This suggests that the size of male gametes has a large impact on mating efficiency. Some advantages of smaller gametes (e.g. higher speed, larger number) appear to work. This may be one reason why male gametes do not increase their size in anisogamous species in nature. Such a size should be evolutionary stable for males, because it has been suggested that it is nearly a minimal size to maintain a phototactic system [Togashi et al., 2002].

Potential advantages of larger female gametes (e.g. larger target size) may have weaker effects on mating than those of smaller male gametes, because, even if the size of female gametes is slightly decreased, most female gametes are still easily fertilized (Figure 4). In this case, the number of formed zygotes is larger than that in the normal species. However, the volume of

zygotes formed in this species is smaller than that in the normal species (by 17 %).

Advantages of small gametes do not always give satisfactory results because some gametes remained unfertilized in the hypothetical isogamous species with the smaller gametes (Figure 4). Anisogamy may be necessary to fertilize most (female) gametes. However, this hypothetical species with smaller gametes produced the largest number of zygotes in this study.

Comparing mating efficiency between the two normal species, it is greater for isogamous than for slightly anisogamous species (Figure 2). Thus, we should also note that the evolution of anisogamy from primitive isogamy may not be explained solely by high encounter rates of anisogamous male and female gametes and resultant high mating efficiency [e.g. Levitan, 1996]. Two conflicting selection forces of search efficiency and zygote fitness may be needed to explain the evolution of anisogamy in marine green algae [e.g. Parker et al., 1972].

Such a stronger anisogamy as male gametes are too small to maintain a phototactic system may not be predictable through this study without some other mechanisms to compensate the loss. Some species of marine green algae (e.g. the genus *Bryopsis*) have got over the fence by a pheromonal attraction from female gametes which retain a phototactic system [Togashi et al., 1998]. It has been considered that female characteristics to increase probability of fertilization would not have evolved without sperm limitation with the exception of egg size [Levitan, 1996]. However, this pheromonal attraction system could have developed to connect discrete gamete behaviors between sexes, and realized such a marked anisogamy even under sperm-abundant (competitive) conditions if there is strong selection for large zygote size.

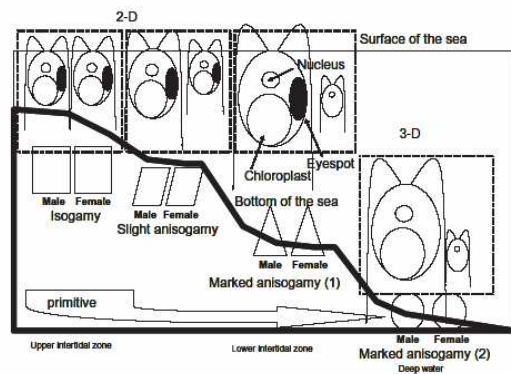


Figure 6. Mating systems and habitats in marine green algae.

Advantages of positive phototaxis may be lost in deep water and larger zygotes should be needed to develop safely in such a photosynthetically disadvantageous area. In fact, some species of the genus *Derbesia* are usually observed in deep water [Chapman et al., 1964] and produce strongly anisogamous non-phototactic male and female gametes and resultant large zygotes. Our simulations and other observations of real mating systems [Togashi et al., 2002] in marine green algae suggest that smaller zygotes might be occasionally disadvantageous, even if they had larger numbers of zygotes. In marine green algae, the mating systems appear to be tightly tuned to the environmental conditions of their habitats (see Figure 5).

6. REFERENCES

- Chapman, V.J., A.S. Edmonds and F.I. Dromgoole, *Halicystis* in New Zealand, *Nature*, 202, 414, 1964.
- Cox, P.A., S. Cromar and T. Jarvis, Underwater pollination, three-dimensional search, and pollen morphology: predictions from a supercomputer analysis, In: Blackmore, S. and S.H. Barnes, eds. *Pollen and Spores*, Systematics Association Special Volume no 44, Clarendon Press, 363-375, Oxford, 1991.
- Cox, P.A. and J.A. Sethian, Gamete motion, search, and the evolution of anisogamy, oogamy, and chemotaxis, *American Naturalist*, 125, 74-101, 1985.
- Dawes, C.J., *Marine botany*. 2nd ed., John Wiley, New York, 1998.
- Dusenbury, D.B., *Sensory ecology*, Freeman, New York, 1992.
- Le Méhauté, B., *An introduction to hydrodynamics and water waves*, Springer, Berlin, 1976.
- Levitan, D.R., Effects of gamete traits on fertilization in the sea and the evolution of sexual dimorphism, *Nature*, 382, 153-155, 1996.
- Melkonian, M., Structural and evolutionary aspects of the flagellar apparatus in green algae and land plants, *Taxon*, 31, 255-265, 1982.
- Parker, G.A., R.R. Baker and V.G.F. Smith, The origin and evolution of gamete dimorphism and the male-female phenomenon, *Journal of Theoretical Biology*, 36, 529-553, 1972.
- Randerson, J.P. and L.D. Hurst, The uncertain evolution of the sexes, *Trends in Ecology and Evolution*, 16, 571-579, 2001.
- Shuster, S.M. and M.J. Wade, *Mating Systems and Strategies*. Princeton University Press, Princeton, 2003.
- Togashi, T., Reproductive strategies, mating behaviors and the evolution of anisogamy in marine green algae, Ph.D. thesis, Hokkaido University, Sapporo, 1998.
- Togashi, T. and P.A. Cox, Tidal-linked synchrony of gamete release in the marine green alga, *Monostroma angicava* Kjellman, *Journal of Experimental Marine Biology and Ecology*, 264, 117-131, 2001.
- Togashi, T. and P.A. Cox, Phototaxis and the evolution of isogamy and “slight anisogamy” in marine green algae: insights from laboratory observations and numerical experiments, *Botanical Journal of the Linnean Society*, in press.
- Togashi, T., T. Miyazaki and P.A. Cox, Sexual reproduction in marine green algae: gametic behavior and the evolution of anisogamy, *Proceedings of Two Symposia on Ecology and Evolution in VIII INTECOL, Seoul, Korea, Aug. 2002*, Sangaku Publisher, 70-79, Ohtsu, 2002.
- Togashi, T., T. Motomura, T. Ichimura, Production of anisogametes and gamete motility dimorphism in *Monostroma angicava*, *Sexual Plant Reproduction*, 10, 261-268, 1997.
- Togashi, T., T. Motomura and T. Ichimura, Gamete dimorphism in *Bryopsis plumosa*: phototaxis, gamete motility and pheromonal attraction, *Botanica Marina*, 41, 257-264, 1998.
- Togashi, T., T. Motomura, T. Ichimura and P.A. Cox, Gametic behavior in a marine green alga, *Monostroma angicava*: an effect of phototaxis on mating efficiency, *Sexual Plant Reproduction*, 12, 158-163, 1999.
- Wilson, W. *Simulating Ecological and Evolutionary Systems in C*. Cambridge University Press, Cambridge, 2000.