Simulation Of Mating Behavior Of Gametes And The Evolution Of Anisogamy In Marine Green Algae: Isogamy, Slight Anisogamy And Marked Anisogamy

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Abstract: In marine green algae, isogamous and slightly anisogamous species produce positively phototactic gametes: phototactic devices including an eye-spot in both sexes. We numerically simulated gamete behavior in three-dimensions, and found that, all else being equal, phototactic gametes have considerable reproductive advantage over non-phototactic ones because they can search for potential mates on the water surface -- a two-dimensional plane -- rather than in three dimensions. This suggests that slight anisogamy in marine green algae has been maintained by the search efficiencies of phototactic devices in both sexes. However, in some markedly anisogamous species (e.g. the genus *Bryopsis*), the smaller male gametes have no eye-spot, swim randomly, and do not respond to light stimulus. In contrast, the larger female gametes have an eye-spot and exhibit positive phototaxis. During our study of encounter mechanisms for male and female gametes in this system, we discovered the first pheromonal attraction system in marine green algae. Adding the pheromonal system to our numerical simulations, we discovered that markedly anisogamous species can, through pheremones, achieve 2D search efficiencies on the water surface. Therefore, sexual pheromones as well as phototaxis may be a key to understand the mechanisms of the evolution of isogamy, slight anisogamy and marked anisogamy in marine green algae. Comparing mating efficiency among different mating systems, our results support the idea that the theory based on the two conflicting selection forces of search efficiency and zygote fitness was necessary to explain the evolution of anisogamy in marine green algae. The mating systems appear to be tightly tuned to the environmental conditions of their habitats.

Keywords: *Evolution of anisogamy; Marine green algae; Mating behavior; Pheromonal attraction*

1. INTRODUCTION

In marine green algae, many species are isogamous or slightly anisogamous. Their gametes not only have specific mating types, but also often have a phototactic system, usually with an eye-spot. In a phylogenetic analysis, Melkonian (1982) suggested that the eye-spot evolved in the most primitive green flagellate taxa. Such gametes initially show positive phototaxis prior to mating, swimming upward in the water column towards the light at the sea surface, but their zygotes, as soon as they are formed, immediately reverse their phototaxis into a negative condition and swim back down the water column towards the substrate (e.g. Togashi et al., 1997).

Positively phototactic gametes may gain significant advantage by being able to search for potential mates in a two-dimensional surface rather than in three-dimensional space (Cox, 1983). There are some experiments that support this idea (Togashi et al., 1999).

However, in some markedly anisogamous species of the genus *Bryopsis*, only the female gametes retain an eye-spot and show positive phototaxis prior to mating, whereas male gametes have no eye-spot and show no phototaxis. An inquiry into how the male and female gametes meet together after release from separate gametangia of dioecious gametophytes led us to the discovery of a pheromonal attraction system (Togashi et al., 1998). This pheromonal attraction system might have played a key role in the evolution of anisogamy in marine green algae, because it may enable male and female gametes of markedly anisogamous species to continue to mate in a two-dimensional surface.

In this paper, with numerical simulation of gamete behavior in three-dimensions, we examine the relative advantages of phototaxis in isogamous and slightly anisogamous species. We also sought to determine if a pheromonal attraction system is necessary for markedly anisogamous species to achieve encounters in two-dimensions. We then checked the robustness of our numerical simulations by mating efficiencies among different mating systems, in an attempt to gain insight into the evolution of isogamy, slight anisogamy and marked anisogamy in marine green algae.

2. MATERIALS AND METHODS

2.1. Description of algorithm and input parameters

Our simulations of mating behavior of gametes were carried out with our algorithm, which was programmed with the JAVA Development Kit (Sun Microsystems, see http://java.sun.com/products/). Input parameters were determined based on our own laboratory data and previous comparative data.

Gametes of both sexes were idealized as spheres in our simulations. At the very low Reynolds numbers relevant here, movement is governed by viscous forces and drag is proportional to the diameter of the sphere in accordance with Stokes law (Bray, 1992). Thus, body width was used as the diameter of gamete. In a slightly anisogamous marine green alga, *Monostroma angicava*, sizes of male and female gametes have been found to be 5.90 μm (length) X 2.96 μm (width) and 7.53 μm (length) X 3.70 μm (width), respectively (Togashi et al., 1997). So, in the case of slight anisogamy, the radii of male and female gametes were assumed to be 1.48 μm (=2.96/2) and 1.85 μm (=3.70/2), respectively. Similarly, in the case of marked anisogamy, considering the sizes of male and female gametes of *Bryopsis plumosa*, the radii of male and female gametes were assumed to be 1.18 μm (=2.36/2) and 2.415 μm (=4.83/2), respectively (Togashi et al., 1998). Based on our comparative data on gamete size (Togashi et al., 2002), we assumed the radius of the isogametes to be the average of the radii of the slight anisogametes of both sexes: (1.48+1.85)/2=1.67 μm.

The biomass allocated to produce gametes was assumed to be equal between mating types as experimentally confirmed in some organisms including marine green algae (e.g. Togashi et al., 1997). There are few reports of biased sex ratio of gametophytes in natural populations of marine green algae. So, sex ratios of gametophytes were assumed to be 1:1 (=male:female). Our previous field studies in two species, *M. angicava* (Togashi et al., 1997) and *B. plumose* (Togashi at al., 1998), support this assumption. Thus, in the case of slight anisogamy, considering that the volumes of a single gamete were set as follows: $4/3\pi(1.48)^3\ \mu m^3$ (male) and $4/3\pi(1.85)^3\ \mu m^3$ (female), we performed only one set of numerical experiments in which the number of 7400 males and 3800 females was exceeded due to CPU time constraints; all other experiments involved this number of gametes. In the case of marked anisogamy and isogamy, such values corresponded to 14700 males and 1700 females and 5100 gametes of each of the two mating types, respectively. These numbers of gametes were used as the basic unit of gametes in each experiment.

In *M. angicava*, swimming velocities of male and female gametes have been found to be 189 $\mu m\ s^{-1}$ and 151 $\mu m\ s^{-1}$, respectively (Togashi et al., 1997). Similarly, in *B. plumosa*, they have been 237 $\mu m\ s^{-1}$ (male) and 116 $\mu m\ s^{-1}$ (female), respectively (Togashi et al., 1998). We assumed these velocities to be representative of slightly and markedly anisogamous species. These experimental data indicate the viscous forces which serve as propulsion are nearly equivalent for male and female gametes, supporting an idea that, at a low Reynolds number, the inverse relationship between size and velocity is provided, especially, in the idealized situation of steady-state flow past a sphere (Cox and Sethian, 1985). So the swimming velocity of isogametes was assumed to be 168 $\mu m\ s^{-1}$ (= (width X velocity of slight anisogamete) / width of isogamete) based on Stokes law.

Based on our analyses of gamete swimming paths (Togashi and Cox, unpublished data), at the beginning of every time interval (0.3 s), every gamete changed its swimming direction three-dimensionally. [Two angles were independently chosen from a random sequence of integers between -30 and +30 and are used to determine vertical and horizontal changes of direction, respectively.] In numerical experiments simulating positive phototaxis, only the absolute values of these random integers were used for the z-components of the vector to ensure steady upward motion of the gamete. The minimum radius of the chemical halo produced by female gametes that released a sexual pheromone was assumed to be 10μm based on our microscopic observation (Togashi et al., 1998). Thus the radius of such females was seemingly increased to 10 μm, keeping the total number of female gametes unchanged.

Each gamete started from a randomly distributed position on the bottom of our virtual test tank and swam at a given speed in water as assumed above. The test tank of 10 mm (length), 10 mm (width) and 25 mm (depth) was rectangular and filled with a fluid with the viscosity of seawater. When a gamete collided with the surfaces of the test tank or the water, the angles of incidence equaled those of reflection.

To keep track of the different gametes, each gamete was tagged with its own number, and the distances between the centers of nearby male and female gametes were measured at each step of 0.3 s to detect collisions. All encounters were deemed to result in sexual fusion. Fused gametes were removed from the mating population. The positions of the fusing gametes and the tag numbers of the gametes were recorded in the data base using the data base management system MySQL (MySQL, see http://www.mysql.com/). Detailed information regarding gamete parameters as well as samples of the simulations is available at http://members.aol.com/tandytogashi/.

2.2. Numerical experiments

In this study, six sets of experimental regimes were investigated: (1) isogamous but non-phototactic gametes (I/1), (2) isogamous positively phototactic gametes (I/2), (3) slightly anisogamous but non-phototactic gametes (SA/1), (4) slightly anisogamous positively phototactic gametes (SA/2), (5) markedly anisogamous but non-phototactic gametes and (6) markedly anisogamous positively phototactic gametes. In the cases of isogamy and slight anisogamy, male and female gametes were assumed to show the same phototacic behavior without any pheromonal attraction systems. But, in the case of marked anisogamy, male gametes were always assumed to be not phototactic irrespective of illumination; in these cases pheromonal attraction systems were considered as well. Thus, (5) and (6) were divided into four subsets: (5a) non-phototactic female gametes without any pheromonal attraction systems (MA/1), (5b) non-phototactic female gametes with a pheromonal attraction system (MA/2), (6a) positively phototactic female gametes without any pheromonal attraction systems (MA/3) and (6b) positively phototactic female gametes with a pheromonal attraction system (MA/4). [The abbreviated forms in the brackets refer to the figures.] Under each condition, we performed three sets of experiments to explore the impact of varying the number of gametes released, the length of time allowed for fusion, and the test tank depth. Each trial was repeated three times because different random numbers were used for each trial to determine the change in swimming directions of each gamete.

3. RESULTS

The number of zygotes formed was calculated for various biomass units to produce gametes. We showed the results for the release of only three biomass units in Figure 1 due to space constraints, but this general result was unaltered for other numbers of biomass units. We found that phototaxis dramatically increases the number of zygotes formed in isogamous and slightly anisogamous species. Differences in mating systems had little impact in the absence of phototaxis: the numbers of zygotes formed by isogamous and slightly anisogamous species were nearly identical unless phototaxis was present, in which species with isogamy were significantly more likely to successfully generate zygotes (Mann-Whiteny U-test, $p<0.001$). In contrast, this was not true in the case of marked anisogamy under the same experimental conditions: the number of formed zygotes did not increase even under the positively phototactic condition whereas the largest number of zygotes was produced under the non-phototactic condition with a pheromonal attraction system.

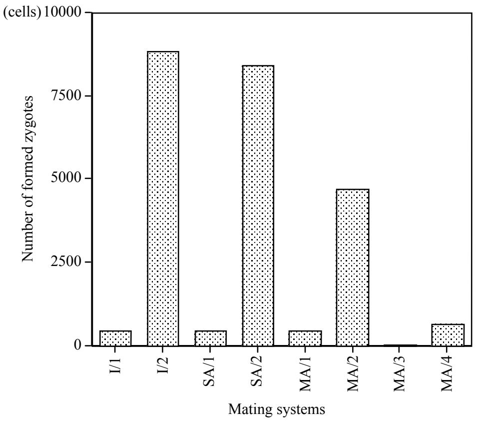

Figure 1. Mating efficiencies for isogamous (I), slightly anisogamous (SA), and markedly anisogamous (MA) gametes under conditions of 1) no phototaxis and no pheremones, 2) no phototaxis but pheremones, 3) phototaxis but no phermones, and 4) both phototaxis and pheremones.

In the cases of isogamy and slight anisogamy, the number of formed zygotes increased under the positively phototactic conditions as the time available for gamete encounter increased, although increasing the time had little impact on non-phototactic gametes. In the case of marked anisogamy, as we showed the results under the positively phototactic condition without any pheromonal attraction systems, the number of zygotes did not increase conspicuously when the time increased until 6000 steps except for the non-phototactic condition with a pheromonal attraction system (Figure 2). In such cases, the results of Figure 1 may not change as a function of time or increased search paths.

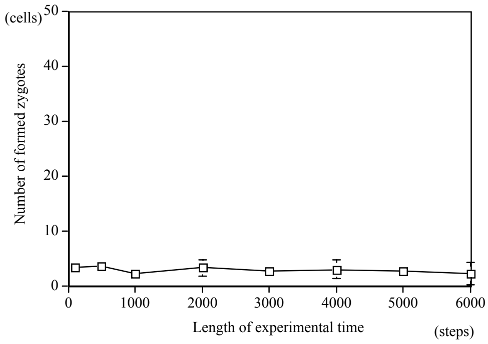

Figure 2. Number of formed zygotes according to the time available for fusion under the positively phototactic condition in the marked anisogamy without pheromonal attraction system (MA/3).

In the cases of isogamy and slight anisogamy, the impact of positive phototaxis of gametes was also clear in our numerical experiments in which we varied depth of the test tank: shallow tanks produced remarkably larger numbers of zygotes than deep tanks, but only for gametes with phototaxis (data not shown). Similar results were observed also in the case of marked anisogamy but only under the positively phototactic condition with a pheromonal attraction system (Figure 3). The depth-components of locations of sexual fusion under this condition are shown in Figure 4a with the results under the non-phototactic condition with a pheromonal attraction system (Figure 4b). Note that under the phototactic condition, zygote fusions were concentrated near the water surface, while in the absence of phototaxis, zygote fusions were scattered in the water column. High mating efficiency was observed even in deep tanks if the length of time available for gamete encounter was sufficient (Table 1). In such cases, the proportion of sexually fused female gametes often exceeded 90%.

4. DISCUSSION

We can compare the results of these numerical experiments to observations we have made of real gametes of various green algal species (Togashi et al., 2002). In isogamous and slightly anisogamous species, gametes of both sexes show positive phototaxis prior to sexual fusion. The size of isogametes is between the sizes of male and female gametes of slightly anisogamous species. In markedly anisogamous species, male gametes, which are significantly smaller than isogametes or slightly anisogamous male gametes, show no phototactic behavior and are devoid of any phototactic systems, but female gametes, which are significantly larger than isogametes or slightly anisogamous female gametes, have a phototactic system and release a sexual pheromone to attract male gametes. The possession of an eye-spot seems to be a good indicator of positive phototaxis of gametes. Mating systems of marine green algae are closely related to gametic behavior.

We therefore suggest the following evolutionary scenario: in marine green algae, male gametes may have decreased in size and lost their phototactic system, whereas females with a phototactic system increased in size and developed the ability to release a sexual pheromone, perhaps to compensate for the lack of the phototactic system in male gametes, thus leading to the evolution of anisogamy.

The adoptive advantages of such an approach are several-fold: 1) reduction of male gamete size increases the number of male gametes and swimming velocity, increasing encounter probabilities in three-dimensional random search; 2) positive phototaxis in the females allows for proximate search in two-dimensions (i.e. the water surface) while the pheromone release allows for a three dimensional target of effectively large size, thus compensating for absence of an eye-spot in the male gametes, and 3) large female size translates into a larger zygote, with presumably higher fitness.

Our numerical simulations for the isogamous and slightly anisogamous species suggest that phototaxis may actually function to introduce gametes to a two-dimensional realm (i.e. 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 1). Considering that many gametes of both sexes continue to swim showing positive phototaxis for many hours (more than 12 hours) after liberation in these species (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 notable that these algae often inhabit upper or middle intertidal zones (e.g. Dawes, 1998) and possess mechanisms for synchronous gamete release restricted to calm conditions during extremely low daytimes tides (Togashi and Cox, 2001).

Our numerical simulations for the markedly anisogamous species also suggest that even when male gametes are not phototactic and female gametes are positively phototactic, sexual fusion of gametes may efficiently occur in a two-dimensional surface if female gametes release a sexual pheromone to attract their partners to the surface (Figures 3 and 4). Many markedly anisogamous marine green algae might have introduced this system in shallow water or in conditions where their gametes have a long time available for sexual fusion (Table 1). In cases where the pheromonal gamete attraction area was increased thus dramatically increasing target size (e.g. 50μm in radius), almost all of female gametes were fertilized (data not shown) even before they reached the surface of water. Such cases may be, however, unrealistic because 1) even markedly anisogamous female gametes may not be large enough to produce such a massive amount of sexual pheromone, and 2) maintaining a concentration gradient may be difficult in turbulent water. Further, even if gametes of both sexes were not phototactic a sexual pheromone could increase mating efficiency (Figures 1 and 3). Some species of the genus *Derbesia*, which have been reported to inhabit relatively deep water (e.g. 9-12m) (Chapman et al., 1964) and whose male and female gametes are strongly anisogamous and not phototactic (Togashi, 1998), may be enjoying this advantage in deep water where turbulence may be reduced. In marine green algae, gametic behaviors seem to have a direct effect on the choice of habitats suitable for sexual fusion.

When phototaxis is introduced, mating efficiencies are greater for isogamous than for slightly anisogamous gametes (Figure 1). The proportion of sexually fused female gametes may be higher for marked anisogametes with both a pheromonal attraction and phototaxis (Table 1). However, there may be more gametes to fuse in the former systems, because the number of markedly anisogamous female gametes is remarkably smaller than the numbers of isogametes or slightly anisogamous female gametes. These results suggest that the evolution of anisogamy from primitive isogamy may not be explained by high encounter rates of anisogamous male and female gametes and resultant high mating efficiency based solely on the sperm-limitation theory (Levitan, 1996; Dusenbury, 2001). Thus, two conflicting selection forces of search efficiency and zygote fitness may be needed to explain the evolution of anisogamy in marine green algae (see Blumer, 1994). Cox and Sethian (1985) have used these conflicting forces to predict adaptive peaks for either complete isogamy or markedly anisogamy. But slight anisogamy may also evolve from isogamy if postzygotic survival is positively related to its volume. We suggest that these theories are not be mutually exclusive. As we showed using marine green algae, the theory based on the two conflicting selection forces would work in the primary stage of the evolution of anisogamy. Once after oogamy evolved, the variation of egg size might be explained by the sperm-limitation theory as suggested for sea urchin gametes.

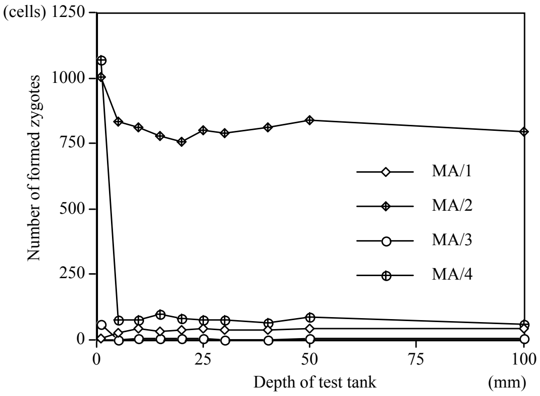

Figure 3. Effects of tank depth on mating efficiencies for markedly anisogamous (MA) gametes under conditions of 1) no phototaxis and no pheremones, 2) no phototaxis but pheremones, 3) phototaxis but no phermones, and 4) both phototaxis and pheremones.

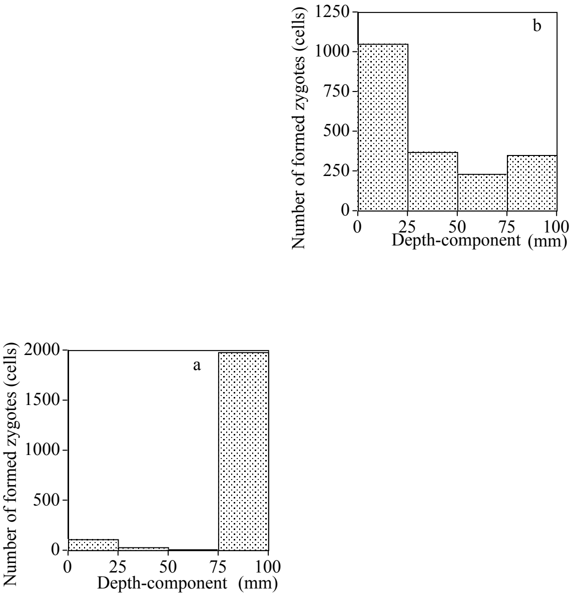

Figure 4. Tank depths of sexual fusion for

Available time (steps)	10000	20000	30000
Depth of test tank (mm)	2	5	10
Number of formed zygotes (cells)	1655	1636	1378
Percentage of sexually fused female gametes	97.4	96.2	81.1

marked anisogametes with pheromones and phototaxis (a) or no phototaxis (b).

Table 1. Additional data of mating efficiency in the marked anisogamy under the positively phototactic condition with a pheromonal attraction system.

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