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Gamete behaviours and the evolution of 'marked anisogamy': reproductive strategies and sexual dimorphism in Bryopsidales marine green algae

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ABSTRACT

Questions: In some species of markedly anisogamous marine green algae, why do male gametes alone have no phototactic device? Why do some of their partners (female gametes) have pheromonal attraction systems?

Simulation methods: Three-dimensional swimming and fertilization model with parameters based on experimental data of gametic traits (gamete sizes, swimming speeds and trajectories) and pseudo-parallel algorithms compiled for rapid calculations with large numbers of gametes.

Key assumptions: The biomass allocated to produce gametes is similar between mating types. Each spherical gamete swims three-dimensionally at a speed determined by its diameter. Swimming speed is inversely proportional to gamete size according to Stoke's law.

Results: Under conditions in which disturbance under water may be a consideration, 'markedly anisogamous species' have a greater chance of successful fertilization than species with phototactic gametes in both sexes. This is more conspicuous in species with larger female gametes or pheromonal attraction systems. However, in deeper water, species with non-phototactic gametes in both sexes generally have a numerical advantage in mating over species with phototactic gametes. Field observations and existing data on gamete size tend to support these predictions.

Keywords: anisogamy, gamete behaviour, marine green algae, pheromonal attraction, phototaxis.

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INTRODUCTION

Anisogamous organisms (i.e. those with gametes of different sizes) are common and widespread. Indeed, anisogamy appears to be an almost universal difference between males and females (Randerson and Hurst, 2001). Anisogamy underlies the evolution of sexual dimorphism in behaviour and morphology: it generates sexual selection whenever the number of male gametes exceeds the number necessary to fertilize the female gametes of a single female (Darwin, 1871).

Unlike many other organisms, isogamous or slightly anisogamous species of marine green algae are taxonomically widespread. Their gametes not only have specific mating types, but also have a phototactic system with an eye-spot. Such gametes show positive phototaxis before mating and swim towards the surface of the sea. Positively phototactic gametes may theoretically gain significant advantages in mating, especially in shallow waters, since they can search for potential mates in a two-dimensional surface rather than in three-dimensional space (Cox and Sethian, 1985). There is some experimental evidence to support this (Togashi *et al.*, 1999). Thus, an adaptive need for phototactic devices in both sexes to provide efficient mating at the surface may be responsible for the persistence of isogamy or near isogamy.

Markedly anisogamous species are observed in Bryopsidales marine green algae (e.g. *Bryopsis*, *Psuedobryopsis*, *Caurelpa*, *Halimeda*, *Udotea*, *Derbesia*, *Penicillus*, *Rhipocephalus*). Their male gametes generally do not have phototactic devices and they swim randomly in the water column, ignoring the light stimulus. Furthermore, in some species of these genera (e.g. *Bryopsis*, *Psuedobryopsis*, *Caurelpa*, *Halimeda*, *Udotea*), female gametes have a phototactic system and show positive phototaxis [see Okuda *et al.* (1979) for the genus *Psuedobryopsis*; see Togashi *et al.* (1998) for the genus *Bryopsis*; see Clifton and Clifton (1999) for the other genera]. An inquiry into how male and female gametes encounter one another in the genus *Bryopsis* led us to discover a pheromonal attraction system in marine green algae (Togashi *et al.*, 1998). Additionally, female gametes of some other species (e.g. *Udotea*, *Derbesia*, *Penicillus*, *Rhipocephalus*) have no phototactic device [see Togashi (1998) for the genus *Derbesia*; see Clifton and Clifton (1999) for the other genera].

Understanding how fertilization success is affected by varying taxis in these genera is the main aim of this study. As in other studies of the mechanisms of the evolution of anisogamy (e.g. Parker *et al.*, 1972; Cox and Sethian, 1985; Matsuda and Abrams, 1999), we focus on the size and number of zygotes formed as a function of time.

MODEL DESCRIPTION AND INPUT PARAMETERS

We study collision dynamics of gametes (fertilization) using numerical simulation. Numerical experiments are a good alternative when laboratory experiments or field observations are difficult, and they provide realistic results when simulation parameters (e.g. gamete sizes, swimming speeds and trajectories) can be based on measured experimental data. Purely analytical models also offer insights (Bulmer, 1994). Unfortunately, because we are studying such a wide range of gamete types and behaviours, it is difficult to develop an appropriate analytical model for the collision dynamics. Some previous analytical models have assumed that gametes swim randomly (Vogel *et al.*, 1982) or that swimming of gametes is negligible under natural conditions (Denny and Shibata, 1989); neither of these is applicable in our case.

In our model, we idealize gametes as spheres with diameters approximating the widths of real gametes. Drag forces are determined by the cross-sectional radius orthogonal to the direction of travel at low Reynolds numbers according to Stoke's law (Le Méhauté, 1976; Vogel, 1981; Berg, 1993; Dusenbery, 2000). Thus, the spherical gametes we use, while slightly unrealistic (since gametes of marine green algal species are pear-shaped), have swimming motions that match well with observed trajectories.

From a literature survey, we present data on the gamete size of Bryopsidales in Fig. 1. The data demonstrate that male gamete size is nearly constant, and that the range of female gamete sizes is wider. In isogamous or slightly anisogamous species, the range of gamete sizes of both sexes is relatively narrow. This range is intermediate between male and female gamete size in species with marked anisogamy (see Togashi *et al.*, 2002). We base gamete size on these data in the numerical experiments section below.

Each gamete swims at a speed determined by its diameter, starting from a randomly distributed position on the bottom of a virtual rectangular test tank. During each simulation time interval, a gamete travels in a straight line at a constant speed in the direction of its velocity vector. At the low Reynolds numbers relevant here, viscous forces govern gamete motion (e.g. $F = 6\,\pi\varepsilon cr$, where ε is the viscosity of the liquid, c is the velocity of the gamete and r is the radius of the gamete) (see Purcell, 1977; Bray, 1992; Randerson and Hurst, 2001). Experimental data suggest that the force generated by the gamete is independent of its size, so F is constant for male and female gametes across species (Togashi *et al.*, 1997, 1998; Togashi, 1998), providing support for our assumption that gamete size is inversely proportional to swimming speed.

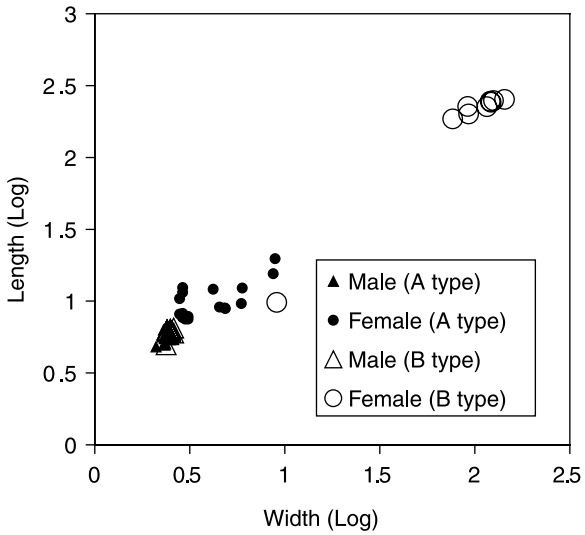

Fig. 1. Gamete sizes of Bryopsidales marine green algae. In the A type species (i.e. species of the genera *Bryopsis*, *Psuedobryopsis*, *Caurelpa*, *Halimeda*, *Udotea*), although male gametes are not phototactic, female ones are positively phototactic [see Okuda *et al.* (1979) for the genus *Psuedobryopsis*; Togashi *et al.* (1998) for the genus *Bryopsis*; Clifton and Clifton (1999) for the other genera]. In the B type species (i.e. species of the genera *Udotea*, *Derbesia*, *Penicillus*, *Rhipocephalus*), both male and female gametes are not phototactic [see Togashi (1998) for the genus *Derbesia*; Clifton and Clifton (1999) for the other genera].

At the beginning of every simulation time interval, each gamete changes swimming direction three-dimensionally, since small motile objects at low Reynolds numbers maintain straight paths for only a limited time due to the impact of Brownian forces (Dusenbery, 1992). We set the time interval to 0.25 s. Then, to determine the changes of direction in the X–Y (horizontal) and the Y–Z (vertical) planes, we automatically choose two angles for each gamete from a random interval between −30° and +30°. This time step interval and angular change method correspond well with experimental analysis of gamete swimming paths (Togashi and Cox, 2004). When a gamete collides with the sides or bottom of the tank, it is reflected back at an angle equal to the angle of incidence. In gametes exhibiting positive phototaxis, we take a vector sum of the current unit velocity vector and a vertical unit vector (light direction) and re-normalize it. Then we apply the same random tilt and rotation methods that the non-phototactic method uses, thus ensuring steady upward motion of the gametes to the upper surface. Although chemotaxis may depend on the concentration and the gradient of the chemical substance (Okubo and Grünbaum, 2001), for the sake of simplicity in our model we assume that female gametes that release a sexual pheromone seemingly increase their radius, not their overall number or swimming speed. (Algorithmic details and an animation of the resultant three-dimensional swimming motion are available at http://evol-prog.com/.)

We assume the biomass allocated to produce gametes is equal between mating types (i.e. 1.0×10^6 μm^3) as confirmed experimentally in some organisms, including marine green algae (e.g. Togashi *et al.*, 1997). There are very few reports of biased sex ratios of gametophytes in natural populations of marine green algae (see Togashi *et al.*, 1997), so we assume the sex ratios of gametophytes to be 1:1.

We track each gamete and measure the distances between the centres of nearby male and female gametes at each step to predict collisions. It is assumed that collisions of sexually different gametes result in sexual fusion and zygote formation. To speed calculation, we employ a pseudo-parallelization approach, dividing the test tank into equally sized sub-volumes so that distance calculations need only be performed among gametes in the same sub-volume. For additional accuracy, we designed our algorithm to detect collisions across adjacent sub-volume boundaries where we employ two collections of sub-volume elements. The first collection, used to hold male gametes, is composed of sub-volume elements of standard size – that is, the total volume is uniformly partitioned by the number of width, height and depth subdivisions. The entire collection of male gametes is partitioned into these primary sub-volumes according to their current position and an indexed collection (map) of the sub-volumes that contain gametes is built. The female gametes, on the other hand, are partitioned into a collection of oversized secondary sub-volume elements. There is the same number of secondary sub-volume units as standard sized ones and they have the same corresponding centre locations. Their width, height and depth, however, are larger by an amount equal to twice the time step interval × the male gamete swimming speed. This means that the oversized sub-volumes overlap slightly and that female gametes in the overlap regions are assigned to each sub-volume that shares the overlap. It has been shown that this correction method is necessary for accuracy and to make the problem of collisions near sub-volume boundaries more tractable (T. Togashi *et al.*, unpublished data). Finally, we remove fused gametes from the mating population. We compile our algorithms from C++, a suitable programming language for rapid calculations with large numbers of gametes. (Samples of our numerical experiments and microscopic photographs of real gametes are also available at http://evol-prog.com/.)

SIMULATED FERTILIZATION EXPERIMENTS

Detailed experimental conditions (i.e. gamete numbers, radii, swimming speeds, phototaxis, chemotaxis and test tank size) in each numerical experiment are summarized in Table 1. In our simulations we track the number of collisions (zygotes formed), as well as the number of gametes that reach the top surface of the water at each time step for a total time of 500 s. First, we performed numerical experiments for markedly anisogamous (M) and slightly anisogamous (S) species with normal incident light in a test tank of length 10 mm, width 10 mm and depth 20 mm. In the species with marked anisogamy, male gametes are too small to maintain phototactic devices (i.e. 1.18 μm in radius), but female gametes have them (i.e. 2.415 μm in radius). In the species with slight anisogamy, the size of male gametes is slightly larger to accommodate phototactic devices. The radii of male and female gametes in this case were 1.46 μm and 2.415 μm, respectively. Neither species had pheromonal attraction systems. Swimming speeds and numbers of released gametes were determined by the volume and speed constraints given above (see the 'Model description and input parameters' section).

Second, we performed collision experiments in the markedly and slightly anisogamous species under dark conditions (MD and SD). Third, we introduced a pheromonal attraction system in the markedly anisogamous species under both light and dark conditions (MP and MDP). Here, female gametes have their radii artificially doubled to simulate an enlarged chemotaxis volume, without changing their total number or swimming speed. Fourth, we actually doubled the size of female gametes in the markedly and slightly anisogamous species. In these species (ML and SL), the swimming speed of female gametes was thus half of that in the M and S species. The number of female gametes was about one-third of that in the M and S species. Finally, we performed mating experiments in the markedly and slightly anisogamous species under both light and dark conditions in a deeper test tank of length 10 mm, width 10 mm and depth 100 mm (M_{deep}, MD_{deep}, S_{deep} and SD_{deep}).

Table 1. Simulation parameters of each mating experiment

Females				Males				
Number	Radius (μm)	Speed ($\mu m \cdot s^{-1}$)	Phototaxis	Number	Radius (μm)	Speed ($\mu m \cdot s^{-1}$)	Phototaxis	Notes*
17 000	2.415	116.0	+	147 000	1.18	237.0	NO	M (Fig. 2), s
17 000	2.415	116.0	+	74 000	1.48	189.0	+	S (Fig. 2), s
17 000	2.415	116.0	NO	147 000	1.18	237.0	NO	MD (Fig. 3), s
17 000	2.415	116.0	NO	74 000	1.48	189.0	NO	SD (Fig. 3), s
17 000	4.83	116.0	+	147 000	1.18	237.0	NO	MP (Fig. 4), s
17 000	4.83	116.0	NO	147 000	1.18	237.0	NO	MDP (Fig. 4), s
5 115	4.83	58.0	+	147 000	1.18	237.0	NO	ML (Fig. 5), s
5 115	4.83	58.0	+	74 000	1.48	189.0	+	SL (Fig. 5), s
17 000	2.415	116.0	+	147 000	1.18	237.0	NO	M deep (Fig. 6), d
17 000	2.415	116.0	NO	147 000	1.18	237.0	NO	MD deep (Fig. 6), d
17 000	2.415	116.0	+	74 000	1.48	189.0	+	S deep (Fig. 6), d
17 000	2.415	116.0	NO	74 000	1.48	189.0	NO	SD deep (Fig. 6), d

* s = shallow tank (20 mm deep), d = deep tank (100 mm deep).

RESULTS

The results of the fertilization experiments performed for the normal markedly anisogamous (M) and hypothetical slightly anisogamous (S) species in the shallower test tank are shown in Figs. 2a and b. Under phototactic conditions, female gametes of both species arrive at the surface at about the same time (just before 200 s), since they have the same swimming speed.

For the slightly anisogamous (S) species, many faster male gametes accumulate first on the surface since they are phototactic. Then zygotes quickly begin to form as the females arrive at the surface. Almost all of the female gametes are eventually fertilized.

For the markedly anisogamous (M) species, the male gametes, being non-phototactic, diffuse slowly to the surface and are converted by the many females that have already concentrated there. Also note that some fertilizations occur below the surface (about

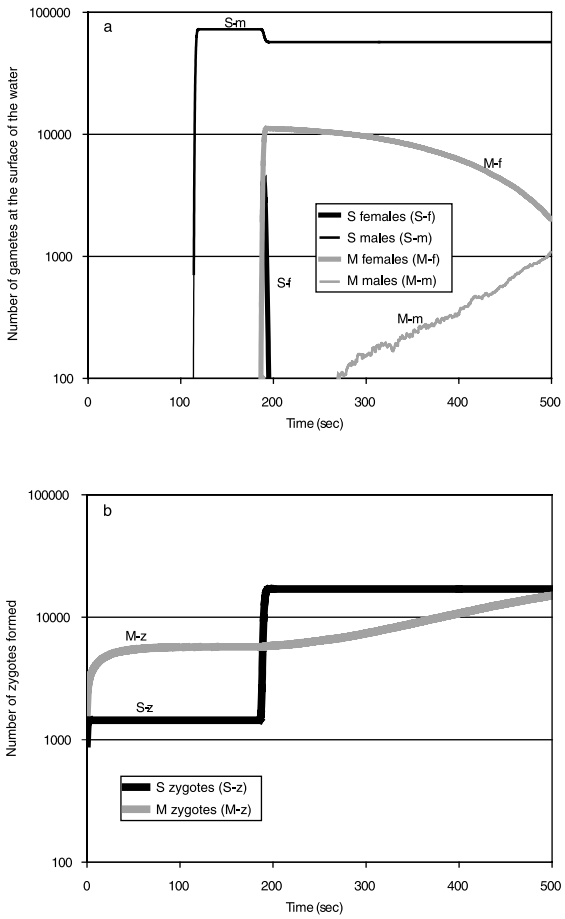

Fig. 2. Fertilization experiments with markedly anisogamous (M) and slightly anisogamous species (S) performed in a shallow test tank. We show the numbers of gametes of both sexes at the surface of the water in each species (M-f, M-m, S-f and S-m) (a), and the numbers of zygotes formed in the markedly anisogamous species (M-z) and slightly anisogamous species (S-z) (b) as a function of time. See also Table 1 for detailed experimental conditions.

one-third of the total). Thus for the first 200 s, the markedly anisogamous species has a numerical advantage in fertilization over the slightly anisogamous species. From 200 to about 600 s, the slightly anisogamous species produces more zygotes than the markedly anisogamous species. After that there is not much difference.

Under dark conditions (MD and SD), both male and female gametes propagate slowly up through the tank. As shown in Fig. 3, most of the fertilizations occur below the surface, and the number of zygotes formed increases gradually. In dark conditions, the markedly anisogamous species produces more zygotes than the slightly anisogamous species within our experimental time (500 s). The number of zygotes formed under dark conditions is also greater than under normal incident light for the markedly anisogamous species for the first 400 s.

As shown in Fig. 4, in cases where female gametes release a sexual pheromone to double their effective size, the number of zygotes formed under dark conditions (MDP) is greater

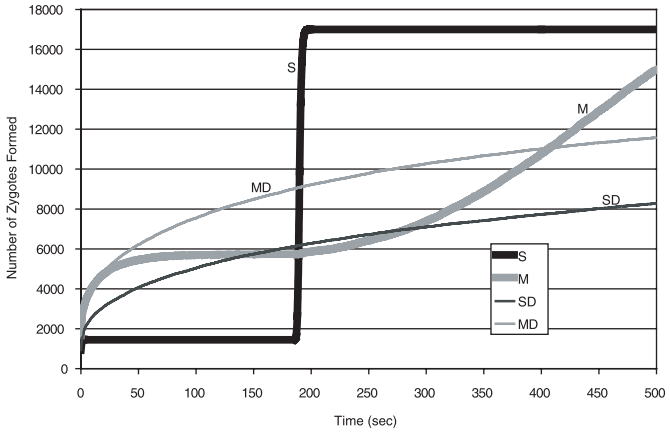

Fig. 3. Comparison of the numbers of zygotes formed in the markedly anisogamous and slightly anisogamous species under dark (MD and SD) and light (M and S) conditions. See also Table 1 for detailed experimental conditions.

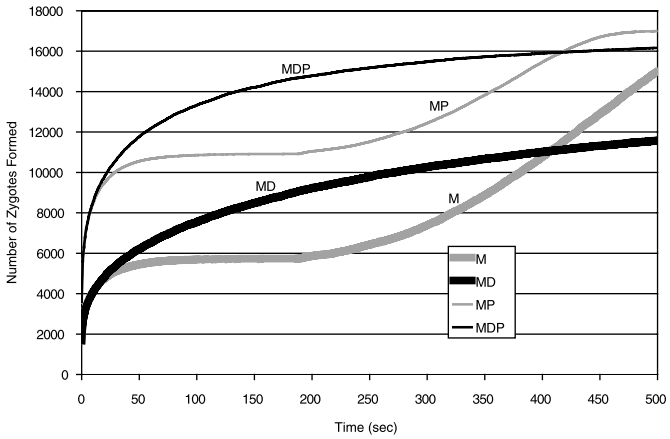

Fig. 4. Demonstration of pheromone advantages under dark (MDP) and light (MP) conditions in the markedly anisogamous species. See also Table 1 for detailed experimental conditions.

than under normal incident light (MP) for the first 400 s, similar to the experiments without pheromonal attraction systems. But, in both cases, mating efficiencies are remarkably increased.

The results for the species with larger female gametes (ML and SL) are shown in Fig. 5. They are similar to those of the markedly anisogamous and slightly anisogamous species without larger female gametes under normal incident light: the ML species has a numerical advantage in fertilization over the SL species for the first 375 s. However, for the ML species, the proportion of fertilized female gametes before this time is much greater than that for the markedly anisogamous species without larger female gametes (M).

When we release gametes in a deeper test tank (i.e. 100 mm in depth), the two species (MD_{deep} and SD_{deep}) generally show an advantage in fertilization over those in the light (M_{deep} and S_{deep}) (Fig. 6). In the dark, the male and female gametes tend to remain in closer proximity and to experience more collisions than in the light. The markedly anisogamous

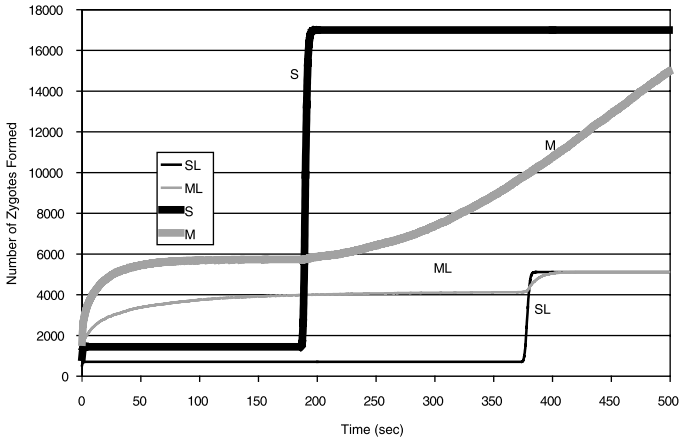

Fig. 5. Fertilization experiments with markedly anisogamous and slightly anisogamous species with larger female gametes (ML and SL). See also Table 1 for detailed experimental conditions.

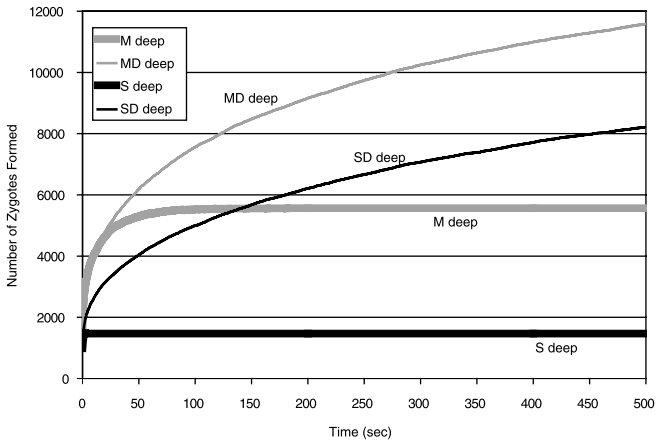

Fig. 6. Fertilization experiments with the markedly anisogamous and slightly anisogamous species performed in a deep test tank (M_{deep}, MD_{deep}, S_{deep} and SD_{deep}). See also Table 1 for detailed experimental conditions.

species in the deeper test tank under dark conditions (MD_{deep}) forms the largest number of zygotes because of the greater excess of male gametes. For the slightly anisogamous species in the deeper tank under light conditions (S_{deep}), where both gametes are phototactic, very few collisions occur in the first 500 s because the gametes are yet to have reached the surface.

DISCUSSION

Our simulation experiments suggest that under sufficient light, species that produce gametes with phototactic systems in both sexes show an advantage in mating efficiency when water depths are sufficiently shallow for gametes of both sexes to reach the surface within their life spans (S in Fig. 2 and SL in Fig. 5). Theoretical studies also support our findings that fertilization efficiency is markedly enhanced once the collision process is limited to two dimensions at the water's surface rather than in the three dimensions within the tank (Feller, 1968; Harrison *et al.*, 1984; Cox and Sethian, 1985; Okubo and Levin, 2001). Adaptation using such a fertilization strategy in shallow water may have been useful for green algae to evolve into land plants. Melkonian (1982) has suggested that the most primitive green flagellate taxa already have the eye-spot.

We also find that under light conditions, markedly anisogamous species with non-phototactic male and positively phototactic female gametes (M) are more effective in terms of fertilization than the slightly anisogamous species with phototactic gametes in both sexes (S) just after gametes are released into the water (Fig. 2). In markedly anisogamous species, there is a large abundance of male gametes swimming near the bottom, and a fair proportion of fertilizations occur below the surface. This may be why some markedly anisogamous species have successfully evolved under environmental conditions with significant water disturbance by eliminating male gamete eye-spots and decreasing their sizes.

It would appear that an even more effective strategy when there is water disturbance would be to eliminate phototaxis completely in the markedly anisogamous species as zygote formation appears always to be higher in the early time phases under dark conditions (Fig. 3). Only in cases where steady conditions persist long enough for phototactic gametes to reach the surface will they have a numerical advantage.

Some markedly anisogamous species are known to have pheromonal attraction systems (Togashi *et al.*, 1998). Our experiments show that under conditions of light or dark, the pheromonal systems have a significant advantage (MP vs. M and MDP vs. MD in Fig. 4). In the markedly anisogamous species with 'large' female gametes under normal incident light (ML), the proportion of female gametes that are fertilized in the early phases below the surface is remarkably high (~80%, Fig. 5). In these species, therefore, pheromonal attraction systems might not be that important. Thus, they would not have such systems if there are costs to producing pheromones. This might be a reason why pheromonal attraction systems are not found in all markedly anisogamous species. Incidentally, they have been reported in the genus *Bryopsis*, which has relatively small female gametes (Togashi *et al.*, 1998).

Our simulations in a shallow test tank demonstrate that fertilization in the top two-dimensional surface has significant numerical advantages over that in the three-dimensional volume of the tank (Figs. 3 and 4). Thus, in marine green algae, gametes might maintain phototactic devices in at least one sex. However, we should not expect these advantages in deep water, since gametes must pay huge costs in energy and time to reach the surface. Our simulations in a deep test tank suggest that markedly anisogamous species with non-phototactic gametes in both sexes would have the advantage in mating over species

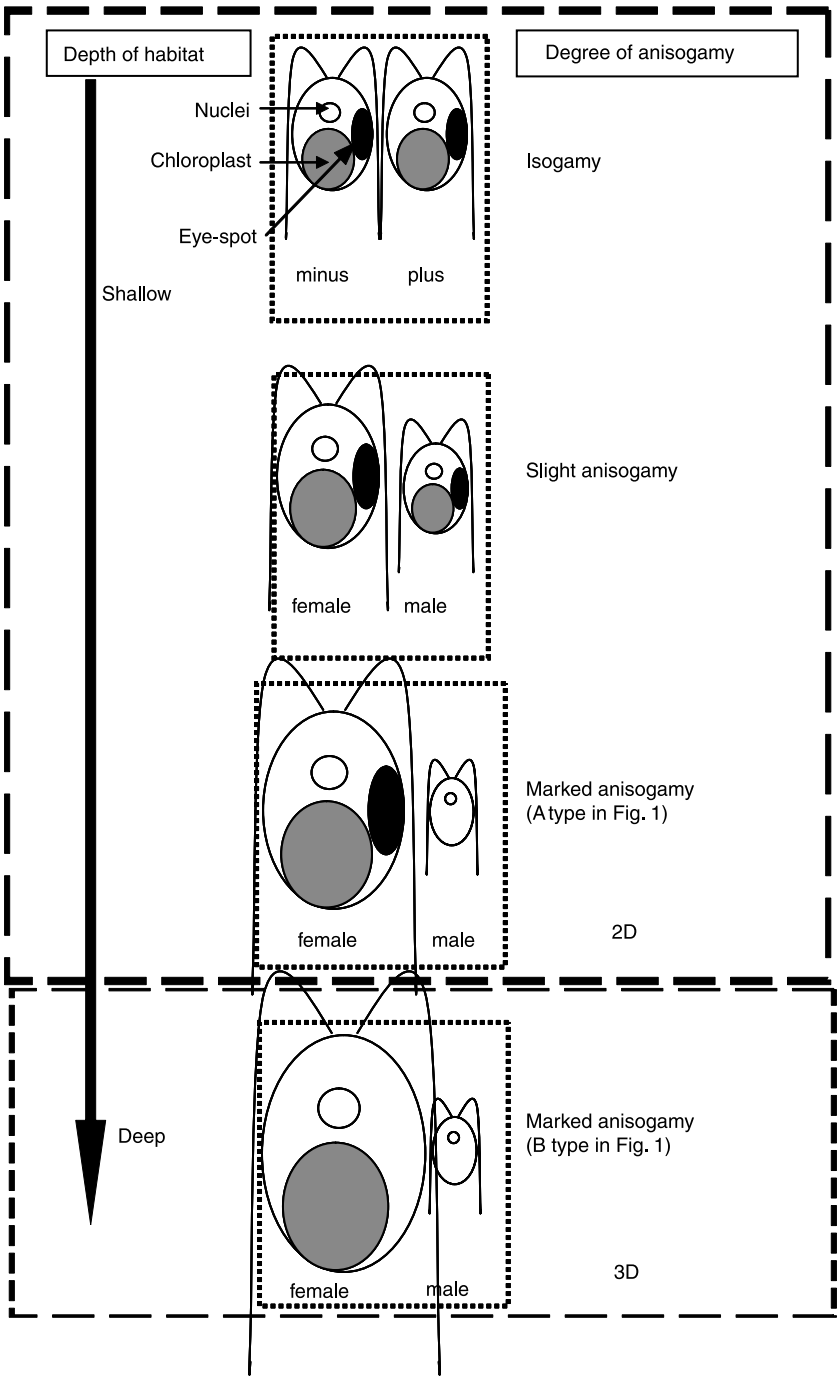

Fig. 7. Mating systems, gametic traits and the depth of water of habitats in marine green algae.

with phototactic gametes or species with slight anisogamy (Fig. 6). This is especially evident in cases where gametes of both sexes are phototactic; few of them appear to fuse below the surface.

CONCLUSIONS

Our findings are consistent with field and laboratory observations. Slightly anisogamous species with phototactic gametes in both sexes usually inhabit upper intertidal zones (e.g. Tatewaki, 1969; Abbot and Hollenberg, 1976; Yoshida, 1998). They have the ability also to release male and female gametes simultaneously during daytime low tides under calm conditions (Stratmann *et al.*, 1996; Togashi and Cox, 2001). Markedly anisogamous species with non-phototactic male and phototactic female gametes often inhabit lower intertidal zones (e.g. Abbot and Hollenberg, 1976; Yoshizaki, 1976; Yoshida, 1998), where disturbances in the water are a consideration. Such species use a light stimulus as a trigger to release gametes (Togashi *et al.*, 1998). It would be useful for them to release gametes of both sexes simultaneously at first light. Some species of the genus *Derbesia* are often observed in deep waters [e.g. 9–12 m deep (Chapman *et al.*, 1964)]. They produce markedly anisogamous non-phototactic male and female gametes (Togashi, 1998). Figure 7 presents schematic representations of the mating systems, gametic traits and the depth of water habitats. In this study, we have outlined the effects of gamete behaviour on the fertilization dynamics of marine green algae and discussed the morphological strategies that are observed for adaptation in varied environments. We are planning further numerical simulation studies of another factor, the initial gamete surface density, which also seems to play an important role in gamete dimorphism (T. Togashi *et al.*, unpublished data).

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