# Supporting Information

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### SI Text

**Zygote Formation.** The number of zygotes formed during a generation depends on the number of collisions between gametes of the opposite sex (Eq. **S1**):

$$\frac{d}{dt} z_{xy}(t) = \sigma_{xy} \cdot \left\langle v_{xy}^{rel} \right\rangle \cdot g_x(t) \cdot g_y(t), \qquad \textbf{[S1]}$$

where $z_{xy}(t)$ is the number of zygotes formed from collisions between two gametes with different mating types, $x$ and $y$ (more details in ref. 1). Gametes are spherical. The collision cross-section is (Eq. **S2**)

$$\sigma_{xy} = \pi \cdot \left(r_x + r_y\right)^2, \qquad \textbf{[S2]}$$

with $r_x$ and $r_y$ being the radii of two gametes of opposite sex, respectively. The average relative velocity is given by (Eq. **S3**)

$$\left\langle v_{xy}^{rel} \right\rangle = \sqrt{\langle v_x \rangle^2 + \langle v_y \rangle^2} = \left(\frac{W}{r_x \cdot r_y}\right) \cdot \sqrt{r_x^2 + r_y^2}, \qquad \textbf{[S3]}$$

where $\vec{v_x}$ and $\vec{v_x}$ are random and uncorrelated (i.e., $\langle \vec{v_x} \cdot \vec{v_x} \rangle = 0$). The average velocity of gametes is inversely proportional to their radius (i.e., $\langle v \rangle = W/r$, where $W$ is a constant). Finally, $g_x(t)$ and $g_y(t)$ are the time $(t)$ dependent concentrations of the two gametes, respectively. The initial gamete density is given by (Eq. **S4**)

$$g_x(0) = n_x \cdot N_x, \qquad \textbf{[S4]}$$

where the number of gametes produced per gametophyte during gametogenesis of $x$ is $n_x$, and the number of gametophytes of $x$ per unit volume is $N_x$.

**Mutant Invasion.** Here, we examine the ability of an invading mutant variety $a$ to compete with an original variety $b$ in the fertilization of $c$ gametes. Note that $a$ and $b$ are of the same mating type, and therefore, only $a$–$c$ and $b$–$c$ zygotes are formed. When $a$ is a mutant of $c$ fertilizing $b$ gametes, the treatment is exactly the same. Therefore, the results in Figs. S2, S3, and S4 should be symmetrical when centering the line ($b$ division = $c$ division).

According to Eq. **S1**, the rate of zygote formation is defined as (Eq. **S5**)

$$\begin{aligned} \frac{d}{dt} z_{ac}(t) &= \sigma_{ac} \cdot \left\langle v_{ac}^{rel} \right\rangle \cdot g_a(t) \cdot g_c(t) \\ \frac{d}{dt} z_{bc}(t) &= \sigma_{bc} \cdot \left\langle v_{bc}^{rel} \right\rangle \cdot g_b(t) \cdot g_c(t) \end{aligned} \qquad \textbf{[S5]}$$

with gamete decay and the initial conditions (Eq. **S6**)

$$\begin{aligned} g_a(t) &= g_a(0) - z_{ac}(t) \\ g_b(t) &= g_b(0) - z_{bc}(t) \\ g_c(t) &= g_c(0) - z_{ac}(t) - z_{bc}(t) \end{aligned} \qquad \textbf{[S6]}$$

and (Eq. **S7**)

$$\begin{aligned} z_{ac}(0) &= 0 \\ z_{bc}(0) &= 0. \end{aligned} \qquad \textbf{[S7]}$$

If we adjust the time scale with the substitution (Eq. **S8**)

$$T = g_c(0) \cdot \sigma_{bc} \cdot \left\langle v_{bc}^{rel} \right\rangle \cdot t \qquad \textbf{[S8]}$$

and introduce the relative collision rate function (Eq. **S9**)

$$K = \frac{\sigma_{ac} \cdot \left\langle v_{ac}^{rel} \right\rangle}{\sigma_{bc} \cdot \left\langle v_{bc}^{rel} \right\rangle}, \qquad \textbf{[S9]}$$

we can rewrite Eq. **S5** in nondimensional form as (Eq. **S10**)

$$\begin{aligned} \frac{d}{dT}\left(\frac{z_{ac}(T)}{g_a(0)}\right) &= K \cdot \left(1 - \frac{z_{ac}(T)}{g_a(0)}\right) \cdot \left(1 - C_a \cdot \frac{z_{ac}(T)}{g_a(0)} - C_b \cdot \frac{z_{bc}(T)}{g_b(0)}\right) \\ \frac{d}{dT}\left(\frac{z_{bc}(T)}{g_b(0)}\right) &= \left(1 - \frac{z_{bc}(T)}{g_b(0)}\right) \cdot \left(1 - C_a \cdot \frac{z_{ac}(T)}{g_a(0)} - C_b \cdot \frac{z_{bc}(T)}{g_b(0)}\right) \end{aligned} \qquad \textbf{[S10]}$$

with (Eq. **S11**)

$$C_a = \frac{g_a(0)}{g_c(0)} \qquad C_b = \frac{g_b(0)}{g_c(0)}. \qquad \textbf{[S11]}$$

Solving the coupled set of differential equations (Eq. **S10**) must be done numerically, but these equations determine the complete time-dependent behavior of all zygote formation and gamete depletion using only three dimensionless quantities: $K$, $C_a$, and $C_b$. In Fig. S2, we show the result of numerical integration of Eq. **S10** with typical values of these quantities. Notice, in Fig. S2*A*, that, although the density of $b$ gametes is 20× the density of the $a$ gametes, the $a$ gametes compete more successfully for the $c$ gametes, because $K > 1$. However, in Fig. S2*B*, the situation has reversed, and now, $b$ is more competitive, because $K > 1$. The relationship between gamete size and swimming velocity is only used to determine $K$. Thus, even if we used another relationship, the rest of our analysis would remain unchanged.

**Survival of Zygotes.** Survival in a population is described by a logistic function that obeys a differential equation such as (Eq. **S12**)

$$\frac{d}{dt} S(M,t) = -M \cdot S(M,t) \cdot [1 - S(M,t)]. \qquad \textbf{[S12]}$$

The probability, $S$, that a zygote will survive to become a breeding adult has a sigmoidal shape, because as shown by Eq. **S12**, the rate of change approaches zero as $S$ approaches either zero or one. Here, $M \geq 0$ is the specific or individual zygote mortality rate determined by the particular environment. The solution of Eq. **S12** is given by (Eq. **S13**)

$$S(M,t) = \frac{1}{2} \cdot \left[1 + \tanh\left(\frac{C - M \cdot t}{2}\right)\right], \qquad \textbf{[S13]}$$

where $C$ is a constant determined by the environment. The specific or individual zygote mortality rate is assumed as $M = \alpha - \beta \cdot V_z \geq 0$, where $V_z$ is the provisioning of resources of a zygote. We take the provisioning to be proportional to $kV_m + V_f, (0 < k < 1)$, where $V_m$ and $V_f$ are the male and female gamete volumes, respectively.

The volume of an $x$ gamete is given by (Eq. **S14**)

$$V_x = \frac{4\pi}{3} \cdot r_x^3 = \frac{G}{n_x}, \qquad \textbf{[S14]}$$

where the total gamete volume of the gametophytes is $G$ (constant). The allocation to each sex is equal. We consider that cell division during gemetogenesis cannot go on ad infinitum. Realistically, there must be a minimum gamete volume, $V_g = \frac{G}{n_g}$, below which a gamete is not viable. We also introduce the notion that there must be a minimum level of provisioning (expressed in volumetric terms), $V_{\min} = \frac{2G}{n_p}$, below which a zygote cannot survive. With these definitions, we introduce the provisioning volume (Eq. **S15**)

$$V_z = \begin{cases} kV_m + V_f, & kV_m + V_f \geq V_{\min} \\ 0, & \text{otherwise} \end{cases}. \qquad \textbf{[S15]}$$

The probability, $S$, after the time $t^*$ between generations then takes the form (Eq. **S16**)

$$S\left(\frac{V_z}{V_h}\right) = \frac{1}{2} \cdot \left\{1 + \tanh\left[2\gamma \cdot \left(\frac{V_z}{V_h} - 1\right)\right]\right\}, \qquad \textbf{[S16]}$$

where $V_h = \frac{\alpha \cdot t^* - C}{\beta \cdot t^*} = \frac{2 \cdot G}{n_h}$ is the zygote volume for which the survival probability is 50%, and $\gamma = \frac{\alpha \cdot t^* - C}{4}$ is the slope of the survival rate curve at the 50% point ($V_z = V_h$). Fig. S3*A* shows a plot of Eq. **S16** for several values of $\gamma$. Both $V_h$ and $\gamma$ are parameters determined by the environment. The time $t^*$ is considered the end of the current generation.

We will also consider another function relating the provisioning of a zygote to the probability, $S$, which has a shape similar to Eq. **S16** (Eq. **S17**):

$$S\left(\frac{V_{xy}}{V_h}\right) = 1 - \exp\left[-\phi \cdot \left(\frac{V_{xy}}{V_h}\right)^2\right], \qquad \textbf{[S17]}$$

where $\phi$ is an environmental parameter. This function, which is also sigmoidal in shape and where survival probability reaches a plateau as zygote size increases, is shown in Fig. S3*B*.

**Evolutionary Dynamics.** We define a successful invasion by $a$ to be when the proportion of $a$ gametophytes between $a$ and $b$ in a succeeding generation is larger than the proportion in the current generation. In the current generation, this proportion is $\frac{N_a}{N_a + N_b}$, where $N_x$ is defined as the initial gametophyte density of type $x$. Note that $N_c = N_a + N_b$. In the next generation, we can estimate the proportion of $a$ by $\frac{S(V_{ac}) \cdot z_{ac}(\hat{t})}{S(V_{ac}) \cdot z_{ac}(\hat{t}) + S(V_{bc}) \cdot z_{bc}(\hat{t})}$, where $S(V_{xy}) \cdot z_{xy}(\hat{t})$ is the expected density of $x$–$y$ zygotes that survive into the succeeding generation. Using these proportions, we define the invasion ratio (2), $R_a(\hat{t})$, to measure the invasion success of $a$ by (Eq. **S18**)

$$R_a(\hat{t}) = \left(\frac{N_a + N_b}{N_a}\right) \cdot \left(\frac{S(V_{ac}) \cdot z_{ac}(\hat{t})}{S(V_{ac}) \cdot z_{ac}(\hat{t}) + S(V_{bc}) \cdot z_{bc}(\hat{t})}\right). \qquad \textbf{[S18]}$$

Here, when $R_a \leq 1$, we can say that $b$–$c$ is stable against invasion by $a$. Conversely, when $R_a > 1$, we say that $a$ invades successfully. Numerical values of $R_a$ may be computed using Eqs. **S4**, **S10**, **S11**, and **S16** using only the five independent dimensionless parameters $\gamma$, $\left(\frac{N_a}{N_b}\right)$, $\left(\frac{n_a}{n_h}\right)$, $\left(\frac{n_b}{n_h}\right)$, and $\left(\frac{n_c}{n_h}\right)$. The number of gametes per gametophyte and the number of cell divisions during gametogenesis, $m_x$, are easily related by (Eq. **S19**)

$$n_x = 2^{m_x}. \qquad \textbf{[S19]}$$

As one example of an invasion study, we examine the evolutionary trajectories of gamete size decreasing the zygote volume for which the survival probability is 50% with the zygote survival function (Eq. **S16**) and another zygote survival function (Eq. **S17**).

We also consider the effects of the minimum provisioning of a zygote (Fig. S4 *A*–*E*) and the minimum gamete volume (Fig. S4*F*). They explicitly affect the evolutionary trajectories of gamete size. If the minimum provisioning of a zygote is large (Fig. S4 *A* and *B*), stable isogamous solutions are different from the solutions shown in Fig. 2*A*, even under the same environmental condition. Both the minimum provisioning of a zygote and the minimum gamete volume represent barriers to the possible evolutionary trajectory: as they become smaller, the allowed area of the trajectory becomes larger, and vice versa (Fig. S4). Considering that we observe the two types of isogamy (i.e., small and large) in marine green algae (Fig. S1*A* and Table S1), both the minimum provisioning of a zygote and the minimum gamete volume may be sufficiently small.

**Generation Tracking.** We test the stability of several simple combinations of gamete types and their resultant zygotes over a large number of generations when small numbers of mutant gametes invade a WT gamete population. Again, the mutations involved are for genes that control the number of mitotic divisions in one of the respective gametophytes by +1 or −1. We track each of the possible variations of mutants and WTs to determine which invasions successfully produce zygote sizes. At the end of the generation, each surviving zygote (sporophyte) produces (through meiosis) two zoospores that germinate into two gametophytes with mating type plus and minus, respectively. We initialize each generation with gametes derived from such gametophytes that survive the previous generation.

For instance, some results of our numerical experiments in cases with the two mating types are shown in Fig. S5. We plot the relative zygote concentrations over 50 generations and generally observe that particular mutant zygote sizes completely disappear (Fig. S5*A*) or completely overtake and dominate other zygote sizes (Fig. S5*B*). In contrast, in cases without mating types, we observe cases where several zygote sizes persist and coexist at constant relative concentrations. It is because a state of equilibrium of fitness is possible between mutants and WTs if they have no mating type. However, this state is not the case in the mating type simulations on which we mainly focused in this study. These results are consistent with the predictions derived from a population genetic approach (3).

1. Togashi T, Cox PA, Bartelt JL (2007) Underwater fertilization dynamics of marine green algae. *Math Biosci* 209:205–221.
2. Togashi T, et al. (2009) Evolution of gamete size in primitive taxa without mating types. *Popul Ecol* 51:83–88.
3. Charlesworth B (1978) The population genetics of anisogamy. *J Theor Biol* 73: 347–357.

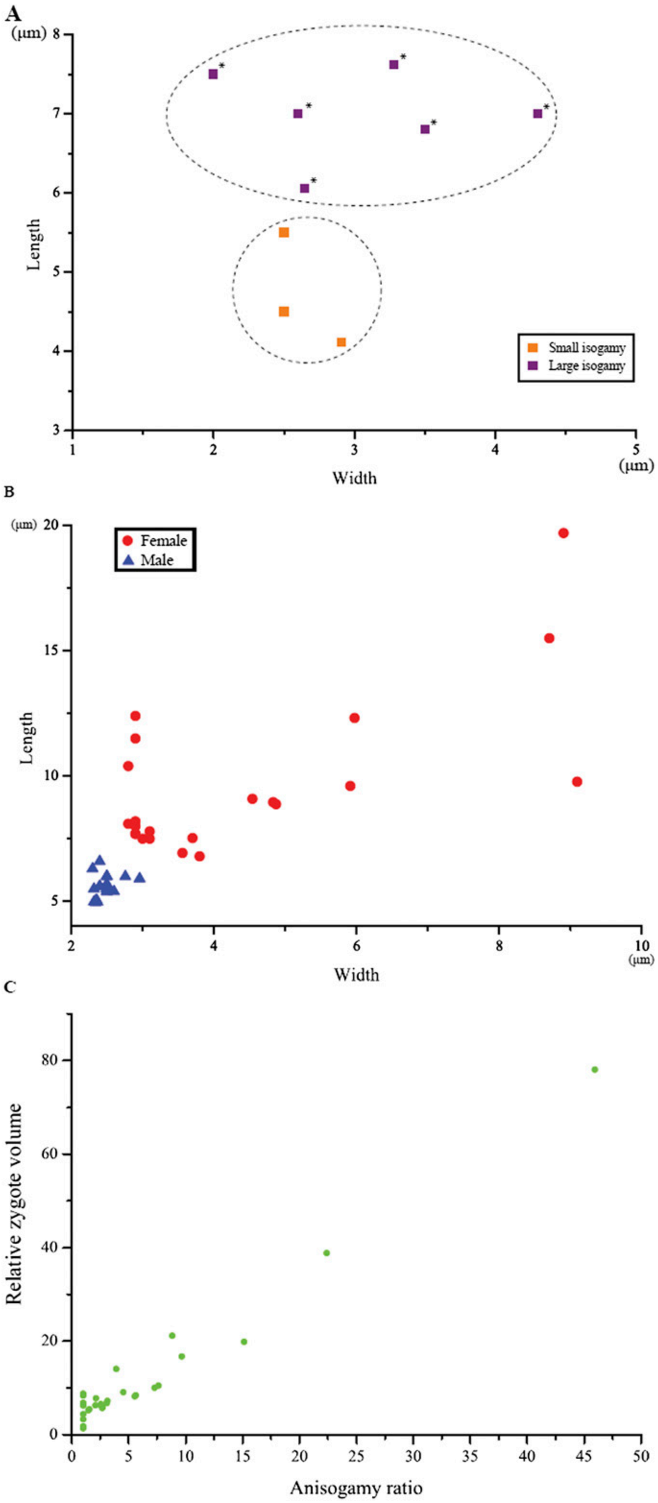

**Fig. S1.** Gamete and zygote sizes of Ulvophyceae. (*A*) Gamete size of isogamous species. *With an eyespot. (*B*) Gamete size of anisogamous species. (*C*) Zygote size and the anisogamy ratio. References are in Table S1.

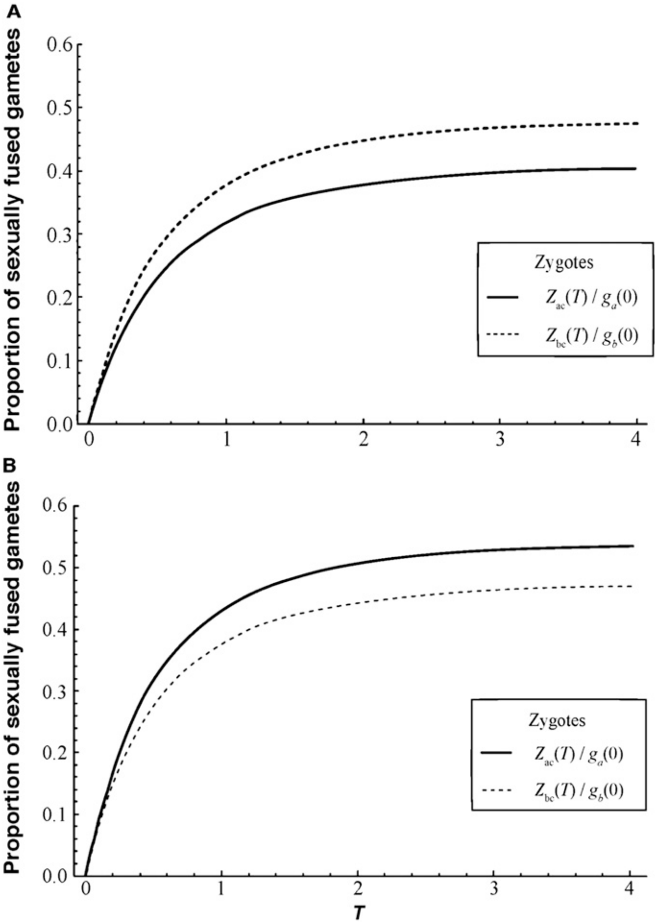

**Fig. S2.** Normalized zygote formation over time. $C_a = g_a(0)/g_c(0) = 0.1$ and $C_b = g_b(0)/g_c(0) = 2.0$ with (*A*) $K = 0.8$ and (*B*) $K = 1.2$. *K* is the relative collision rate function.

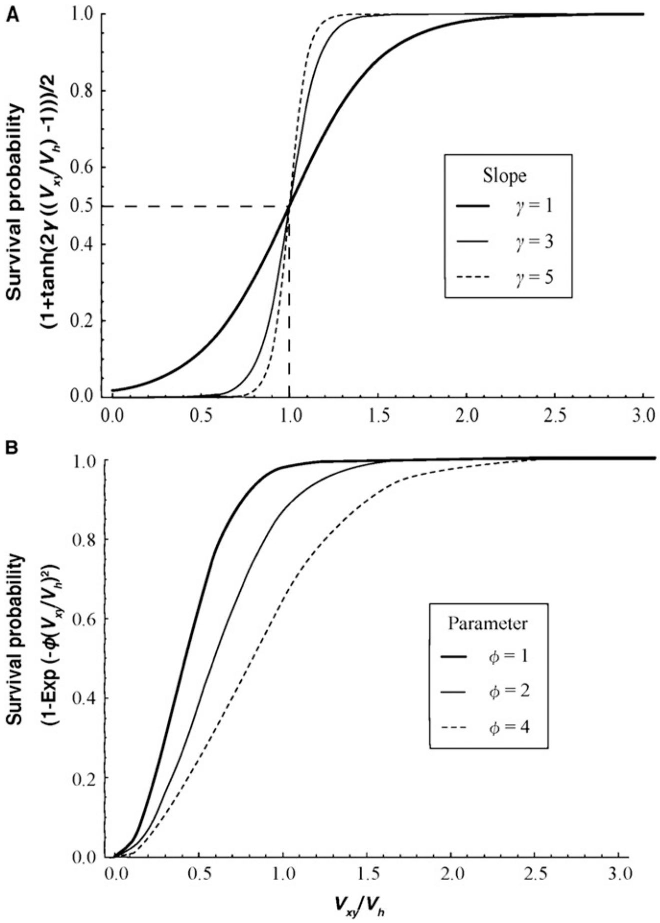

**Fig. S3.** The zygote survival probability functions after the time $t^*$ between generations. $V_{xy} = V_z \cdot V_h = \frac{2 \cdot G}{n_h}$ is the zygote volume for which the survival probability is 50%. (*A*) $S\left(\frac{V_{xy}}{V_h}\right) = \frac{1}{2} \cdot \left\{1 + \tanh\left[2\gamma \cdot \left(\frac{V_{xy}}{V_h} - 1\right)\right]\right\} \cdot \gamma = \frac{\beta \cdot t^* - C}{4}$ is the slope of the survival rate curve at the 50% point. Both $V_h$ and $\gamma$ are environmental parameters. (*B*) $S\left(\frac{V_{xy}}{V_h}\right) = 1 - \exp\left[-\phi \cdot \left(\frac{V_{xy}}{V_h}\right)^2\right] \cdot \phi$ is an environmental parameter.

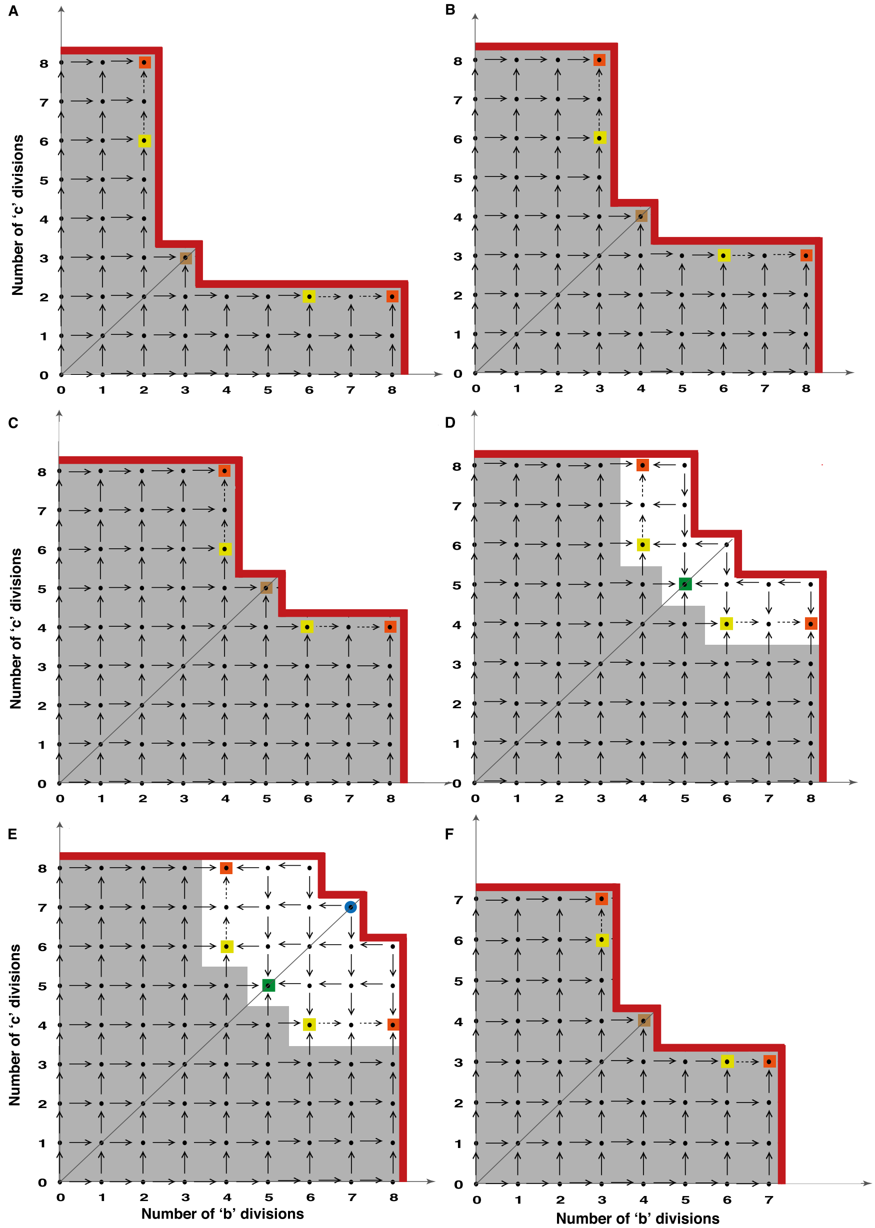

**Fig. S4.** The minimum provisioning of a zygote, the minimum gamete volume, and the evolutionary trajectories of gamete size. The zygote survival probability function is Eq. **S16**. $N = 0.001$, $k = 1.0$, $\gamma = 3.0$, $n_h = 38.0$. (*A*) $n_p = 10$, $n_g = 260$. (*B*) $n_p = 20$, $n_g = 260$. (*C*) $n_p = 40$, $n_g = 260$. (*D*) $n_p = 80$, $n_g = 260$. (*E*) $n_p = 160$, $n_g = 260$. (*F*) $n_p = 20$, $n_g = 250$. $n_g$, the number of gametes to produce the minimum volume in an individual gametophyte; $n_p$, the number of gametes to produce one-half of the volume of the minimum provisioning of a zygote in an individual gametophyte. Fig. 2 explains the other parameters, arrows, and squares.

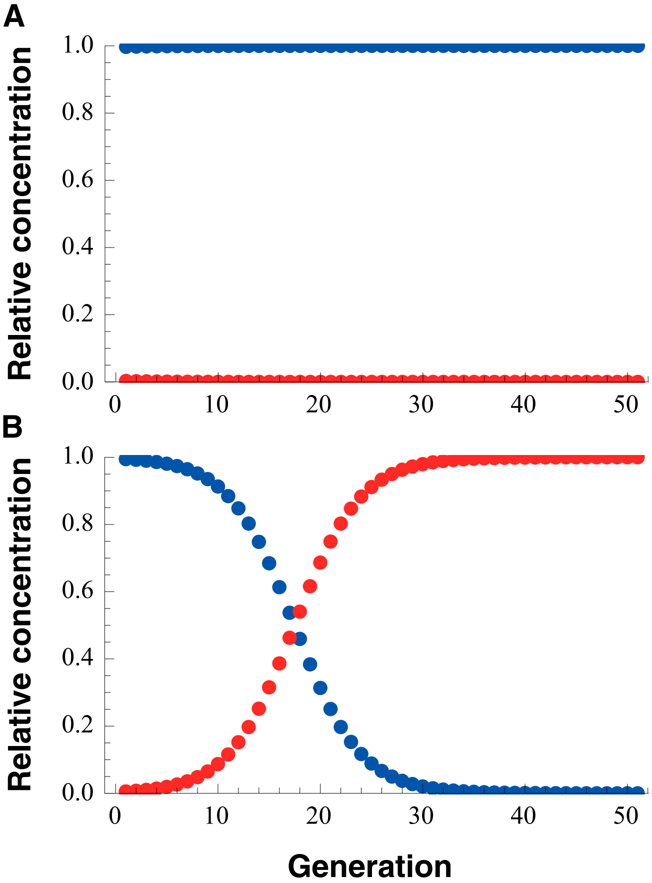

**Fig. S5.** The plot of the 50-generation zygote survival tracks with two mating types. Blue, $z_{bc}$; red, $z_{ac}$. (*A*) $V_c/V_h = 0.1$, $V_b/V_c = 2$, $V_a/V_c = 4$. (*B*) $V_c/V_h = 0.5$, $V_b/V_c = 2$, $V_a/V_c = 4$. The other conditions for the simulations were the same as in Fig. 2*A*.

## Table S1. Mating systems of Ulvophyceae (Chlorophyta)

| Taxa | Anisogamy ratio* (mating system†) | Phototactic devices (eye spot)‡ | | Reference |
|---|---|---|---|---|
| | | Male§ | Female¶ | |
| Bryopsidales | | | | |
| *B. maxima* | 5.54 (MA) | – | + | 2 |
| *B. muscosa* | 15.14 (MA) | – | + | 3 |
| *B. plumosa* | 5.62 (MA) | – | + | 2 |
| *B. triploramosa* | 7.27 (MA) | – | + | 4 |
| *C. cupressoides* | 9.66 (MA) | – | + | 5 |
| *C. mexicana* | 8.83 (MA) | – | + | 5 |
| *C. racemosa* | 2.13 (MA) | – | + | 5 |
| *C. serrulata* | 3.08 (MA) | – | + | 5 |
| *C. sertularioides* | 3.14 (MA) | – | – | 5 |
| *Derbesia tenuissma* | 7.62 (MA) | – | – | 3 |
| *H. discoidea* | 2.59 (MA) | – | + | 5 |
| *H. goreaui* | 2.56 (MA) | – | + | 5 |
| *H. incrassata* | 22.38 (MA) | – | + | 5 |
| *H. monile* | 2.60 (MA) | – | + | 5 |
| *H. opuntia* | 2.68 (MA) | – | + | 5 |
| *H. simulans* | 45.95 (MA) | – | + | 5 |
| *H. tuna* | 2.68 (MA) | – | + | 5 |
| *Udotea flabellum* | 3.91 (MA) | – | + | 5 |
| Dasycladales | | | | |
| *Acetabularia caliculus* | 1.00 (SI) | – | – | 2 |
| Siphonocladales | | | | |
| *Cladophora opaca* | 1.00 (LI) | + | + | 6 |
| Ulotricales | | | | |
| *M. angicava* | 2.08 (SA) | + | + | 2 |
| *M. latissimum* | 1.00 (LI) | + | + | 2 |
| *Spongomorpha heterocladia* | 1.00 (LI) | + | + | 2 |
| Ulvales | | | | |
| *Blidingia minima* | 1.00 (SI) | – | – | 2** |
| *Capsosiphon groenlandicus* | 1.00 (LI) | + | + | 2 |
| *E. compressa* | 1.46 (SA) | + | + | 2 |
| *E. linza* | 1.00 (LI) | + | + | 2 |
| *Kornmania leptderma* | 1.00 (SI) | – | – | 2 |
| *Ulva pertusa* | 1.55 (SA) | + | + | 2 |
| *Ulvaria obscura* | 1.00 (LI) | + | + | 2 |

*B.*, *Bryopsis*; *C.*, *Caulerpa*; *E.*, *Enteromorpha*; *H.*, *Halimeda*; *M.*, *Monostroma*.

*Anisogamy ratio ($A$) = ovum (female gamete) cell mass/sperm (male gamete) cell mass (1). Cell mass was relatively estimated as $(\text{gamete length})^3$ assuming that gametes are similar in shape.

†LI, large isogamy ($A = 1.00$); MA, marked anisogamy ($2.10 < A$); SA, slight anisogamy ($1.00 < A \leq 2.10$); SI, small isogamy ($A = 1.00$).

‡+, presence; –, absence.

§One mating type in cases of isogamy.

¶The other mating type in cases of isogamy.

**Median.

1. Parker GA (1982) Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. *J Theor Biol* 96:281–294.
2. Togashi T, Miyazaki T, Cox PA (2002) Sexual reproduction in marine green algae: gametic behavior and the evolution of anisogamy. *Proceedings of Two Symposia on Ecology and Evolution in VIII INTECOL*, eds Yoshimura J, Nakagiri N, Shields WM (Sangaku, Otsu), pp 70–79.
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