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Evolution of the Two Sexes under Internal Fertilization and Alternative Evolutionary Pathways

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ABSTRACT: Transition from isogamy to anisogamy, hence males and females, leads to sexual selection, sexual conflict, sexual dimorphism, and sex roles. Gamete dynamics theory links biophysics of gamete limitation, gamete competition, and resource requirements for zygote survival and assumes broadcast spawning. It makes testable predictions, but most comparative tests use volvocine algae, which feature internal fertilization. We broaden this theory by comparing broadcast-spawning predictions with two plausible internal-fertilization scenarios: gamete casting/brooding (one mating type retains gametes internally, the other broadcasts them) and packet casting/brooding (one type retains gametes internally, the other broadcasts packets containing gametes, which are released for fertilization). Models show that predictions are remarkably robust to these radical changes, yielding (1) isogamy under low gamete limitation, low gamete competition, and similar required resources for gametes and zygotes, (2) anisogamy when gamete competition and/or limitation are higher and when zygotes require more resources than gametes, as is likely as multicellularity develops, (3) a positive correlation between multicellular complexity and anisogamy ratio, and (4) under gamete competition, only brooders becoming female. Thus, gamete dynamics theory represents a potent rationale for isogamy/anisogamy and makes similar testable predictions for broadcast spawners and internal fertilizers, regardless of whether anisogamy or internal fertilization evolved first.

Keywords: female, male, isogamy, anisogamy, volvocine algae, sperm packets.

Introduction

Anisogamy has evolved several times in evolutionary history (Kirk 2006; Lehtonen and Parker 2014; da Silva 2018; Hanschen et al. 2018), and conditions may have been different each time. Ubiquitous across life forms, its evolution represents one of the most fundamental problems in evolutionary biology. It generates the two sexes, males and females, and sexual selection and sexual conflict develop from it (Darwin 1871; Bateman 1948; Parker et al. 1972; Togashi and Cox 2011; Parker 2014; Lehtonen et al. 2016; Hanschen et al. 2018).

The volvocine green algae are classically the group used to study both the evolution of multicellularity (e.g., Kirk 2005; Herron and Michod 2008) and transitions from isogamy to anisogamy and oogamy (e.g., Knowlton 1974; Bell 1982; Nozaki 1996; da Silva 2018; da Silva and Drysdale 2018; Hanschen et al. 2018). They offer rich diversity in organismal complexity (from unicellular to complex multicellular) and gamete morphology (from isogamy to oogamy). Anisogamy has developed at least twice from isogamy (see Kirk 2006; da Silva 2018; Hanschen et al. 2018), and its most advanced form, oogamy, seems to have arisen independently either three (Hanschen et al. 2018, using maximum likelihood and Bayesian statistical models) or four (da Silva 2018) times. Unicellular forms are most often isogamous with two mating types, and recent studies show that anisogamy tends to evolve in multicellular forms (da Silva 2018; Hanschen et al. 2018).

Two types of solution (reviewed by Lessells et al. 2009) have been proposed for the evolution of anisogamy from isogamy. The first relies on the biophysics of the processes of syngamy and zygote survival: there is a limited energy budget for gamete production, and zygote viability relates positively to zygote size, creating disruptive selection between gamete size and number. The second relies on the outcomes of intracellular conflicts resulting from syngamy. It is the first set of models (hereafter "gamete dynamics," or "GD," models) that we develop here.

Gamete dynamics solutions began with Kalmus (1932; see also Kalmus and Smith 1960; Scudo 1967), who proposed that with a fixed size requirement for the zygote, anisogamy may result in a higher number of successful fertilizations in a population than isogamy, because of the large number of microgametes available in the fertilization pool. Though initially set in a group selection context, gamete limitation can nevertheless generate anisogamy via individual-level selection (Iyer

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and Roughgarden 2008). An alternative individual-level selection theory based on GD assumptions is that of gamete competition (Parker et al. 1972): anisogamy arises from isogamy by disruptive selection favoring individuals producing large gametes (which survive well as zygotes) and those producing small gametes (which gain a larger share of fertilizations). Gamete competition models have received considerable attention (reviewed in Parker 2011, and in the same context Matsuda and Abrams 1999 discuss why isogamy is relatively rare). Recently, gamete limitation and gamete competition were combined into a single, unifying GD model (Lehtonen and Kokko 2011), showing that both selective forces can generate anisogamy, either separately or in unison. Gamete competition is likely to be the stronger selective force unless there are very few competitors present (Lehtonen and Parker 2014; Parker and Lehtonen 2014).

Isogamous unicellular organisms typically produce gametes of two mating types, and most GD models assume linkage between the mating-type locus and the gamete size locus; if linkage is initially loose, selection subsequently favors closer linkage (Charlesworth 1978). Evidence for linkage has been examined in volvocine algae (reviewed in Nozaki 2014). However, anisogamy can evolve without mating types (Parker et al. 1972; Bell 1978; Maire et al. 2001), though disassortative fusion between large and small gametes is expected to evolve later (Parker 1978).

Gamete dynamics theory predicts a positive correlation between anisogamy ratio (female/male gamete size) and multicellular organismal complexity (Parker et al. 1972; Bulmer and Parker 2002; visualized in fig. 4 of Lehtonen and Kokko 2011). The first empirical evidence came from volvocine algae (Knowlton 1974), and several more extensive empirical tests have also been supportive (e.g., Bell 1982; Randerson and Hurst 2001; da Silva 2018; da Silva and Drysdale 2018; Hanschen et al. 2018). This correlation has been the most important empirically testable prediction of GD theory, and the volvocine algae have been the most important empirical study system for such comparative tests (see below).

External fertilization is widely assumed to be ancestral to internal fertilization (e.g., Levitan 1998, 2010; Parker 2011; Parker et al. 1972) because of its ubiquity in marine organisms (Giese and Kanatani 1987; Wray 1995). Gamete dynamics theory generally assumes broadcast external fertilization, which raises the question of whether the same predictions would be upheld under internal fertilization. While the stability of anisogamy has been examined under subsequently evolved internal fertilization (Parker 1982, 2011), Lessells et al. (2009) point out that a full theory for evolution of anisogamy under internal fertilization does not exist. So (1) whether anisogamy could initially evolve under internal fertilization and (2) whether the predicted correlation between anisogamy ratio and multicellular complexity holds under internal fertilization, even if anisogamy evolved under external fertilization, have not been modeled. Correlation 2 has been tested mainly with volvocine algae, which typically release sperm in discrete packets and have internal fertilization in their more advanced, anisogamous colonial forms (fig. 1), further increasing the mismatch with broadcast-spawning assumptions, which has been argued to put empirical tests of GD theory at risk (Randerson and Hurst 2001). That the most extensive tests involve organisms whose biology partly fails to match GD assumptions (e.g., Knowlton 1974; Randerson and Hurst 2001; da Silva 2018; Hanschen et al. 2018) implies that the theory should be generalized to cover these features, regardless of the order in which they arose in evolutionary history.

Figure 1 illustrates schematically three modes of reproduction in volvocine algae. Early coloniality is reflected by an ancestor resembling *Gonium* (see Hanschen et al. 2016), with typically only 4–16 cells per colony, and both mating types broadcast motile isogametes (see fig. 1*A*; e.g., *Gonium quadratum*; Nozaki 1993). More advanced colonial forms show increases in colony cell numbers: the most complex is *Volvox*, where colonies can be monoecious (hermaphroditic) or dioecious (gonochoristic; having separate sexes), varying from around 500 to 60,000 *Chlamydomonas*-like cells (Sharma 1986). In complex colonial forms, the transition from isogamy to anisogamy has been accompanied by the formation of "sperm packets" (Nozaki 1996). Almost all have oogamy and internal fertilization: eggs are retained in the colony, but sperm are released as a group (packet) arising from cell divisions of a single gametangium. For example, in the dioecious *Volvox carteri*, each nonmotile egg is formed in a separate flask-shaped sac with an external opening to the outside of the female colony. Groups of biflagellate male gametes are released from the male colony in hollow spheres that release free-swimming sperm when close to or in contact with a female colony bearing eggs. Fertilization is internal, within the egg sac. A hypothetical *Volvox*-like ancestor with few cells in the colony is shown in figure 1*C*. The 16–32-cell *Colemanosphaera* may form a "missing link" in the transition to internal fertilization (Nozaki et al. 2014): sperm packets are released from the male colony, and sperm swim from their packets only when close to a female colony. However, motile female gametes are then released from the female colony, so that syngamy is external. A hypothetical *Colemanosphaera*-like ancestor is shown in figure 1*B*.

While *Colemanosphaera* suggests that anisogamy might have first evolved by external fertilization following the sequence in figure 1 from *A* to *B* to *C*, an origin of anisogamy under internal fertilization cannot be fully ruled out. Hanschen et al. (2018) found evidence that internal fertilization evolved from external fertilization in volvocines, and similarly anisogamy predated internal fertilization in the *Euvolvox* group. However, anisogamy and internal fertilization evolved along the same branch in the *Eudorina* group, which leaves the possibility that internal fertilization evolved first. But how might internal fertilization precede anisogamy? In an isogamous ancestor, any mutation linked to either the plus or the minus mating-type locus resulting in retention of gametes within the colony would affect only that mating type. So as coloniality evolved, such a single mutation preventing gametes from leaving the colony would effectively mark the onset of internal fertilization.

We simply do not know how GD theory operates under this potential ancestral condition. For example, in broadcast-spawning models, there is potential for competition between gametes released by both mating types, the only constraint being that syngamy occurs only between plus and minus gametes. Under internal fertilization, intercolony competition between gametes cannot easily occur in the gamete-retaining mating type, which may alter whether and under what conditions isogamy and anisogamy can evolve as alternative states.

In summary, GD theory has progressed (table 1) from gamete limitation models based on group selection (Kalmus 1932; Scudo 1967), to individual-level selection models based on gamete competition (Parker et al. 1972), to a model that encompasses both gamete competition and limitation (Lehtonen and Kokko 2011), but all assume external fertilization. Parker (1982) examined the stability of anisogamy under internal fertilization but not an origin or evolutionary dynamics (table 1). Our aim here is therefore to examine the robustness of GD theory under alternative evolutionary sequences of events, particularly involving forms of internal fertilization. Specifically, we aim to (1) confirm whether GD theory can still explain anisogamy, had internal fertilization preceded it, (2) confirm whether the empirically testable correlations predicted by GD theory remain unaltered for primitive internally fertilizing organisms, regardless of whether internal fertilization or anisogamy evolved first, and (3) examine the effect of "sperm packets" under internal fertilization (the typ-

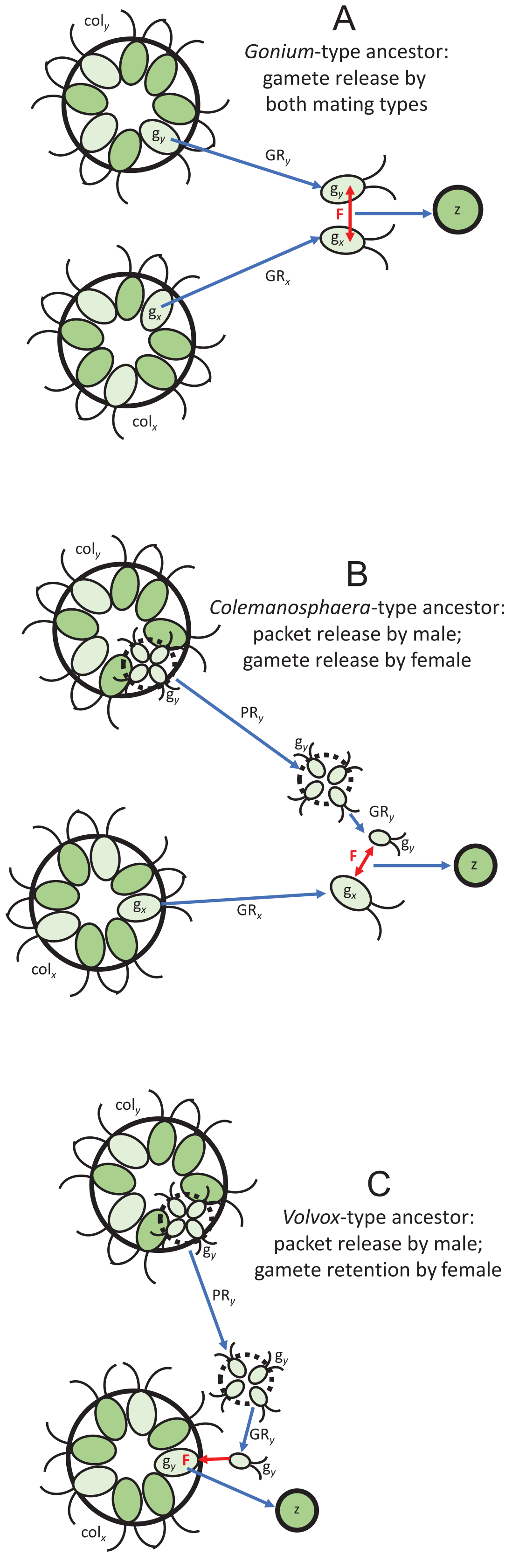


Figure 1: Schematic representation of fertilization modes in multicellular volvocine algae. To conform to our model notation, mating types are signified by subscripts x and y (replacing plus and minus, which are typically used in the literature). *A*, In a *Gonium*-type ancestor, isogametes are released externally by both mating types, so that syngamy and zygote formation are external. *B*, In a *Colemanosphaera*-type ancestor, sperm packets are released from the male colony, and free-swimming sperm dissociate from the sperm packet only in the proximity of a female colony. Motile female gametes are then released from the gelatinous matrix of the female colony, so that syngamy and zygote formation are external. *C*, In a *Volvox*-type ancestor, nonmotile gametes (ova) are retained by the x mating type producing the larger gametes (females), and packets are released by the y mating type producing the smaller gametes (males). Sperm are released from the sperm packets close to the female colony, which they enter so that syngamy and zygote formation are internal. The zygote is later released. col$_x$, col$_y$ = colonies; g$_x$, g$_y$ = gametes; GR$_x$, GR$_y$ = gamete release from colonies or packets; PR$_y$ = packet release from y mating-type colony; F = fusion between gametes (syngamy); z = zygote.

Table 1: Historic developments of anisogamy evolution models relevant to this article

Model	Individual-level selection	Gamete limitation	Gamete competition	External fertilization	Internal fertilization	Gamete packets
Kalmus 1932		X		X		
Parker et al. 1972	X		X	X		
Parker 1982[a]	X		X	X	X	
Iyer and Roughgarden 2008	X	X		X		
Lehtonen and Kokko 2011	X	X	X	X		
This study	X	X	X	X	X	X

[a] Stability analysis only.

ical reproductive mode of colonial volvocine algae). Addressing these omissions is timely, given recent interest in the causes and consequences of anisogamy from conceptual and theoretical (Schärer et al. 2012; Ah-King 2013; Kokko et al. 2013; Lehtonen and Parker 2014; Parker 2014; Lehtonen et al. 2016), as well comparative (e.g., Janicke et al. 2016; da Silva 2018; Hanschen et al. 2018), perspectives. We show that GD predictions remain robust and find a fundamental new prediction—the evolutionary interaction between internal fertilization and anisogamy (whichever came first) means that when internal fertilization evolves, the noncompetitive sex (i.e., the sex within which fertilization occurs) becomes female.

Models

We investigate three alternative evolutionary scenarios of increasing fertilization complexity. For convenience, we use terms typically associated with marine invertebrates (e.g., Bishop and Pemberton 2006; Henshaw et al. 2014; fig. 2).

Model 1: "Broadcaster" (fig. 2, scenario 1). In broadcast spawning, both mating types release free gametes into the aqueous external medium (resembling *Gonium*; fig. 1*A*). Fertilization occurs randomly between gametes of the two mating types, following the assumption of previous GD models with external fertilization.

Model 2: "Gamete caster/brooder" (fig. 2, scenario 2). One mating type ("brooder") retains its gametes, and the other ("gamete caster") releases free gametes (i.e., not in packets) that swim to the brooder colonies, resulting in internal fertilization within the brooder colonies. This can be seen as representing sperm casting (sensu Bishop and Pemberton 2006).

Model 3: "Packet caster/brooder" (fig. 2, scenario 3). Both mating types produce gamete packets (gametangia). Brooders retain the packets within their colonies to produce gametes; packet-caster colonies release their packets, which swim to the brooder colonies and only then release their gametes to fertilize the gametes of the opposite mating type retained in brooder colonies (resembling *Volvox*; fig. 1*C*).

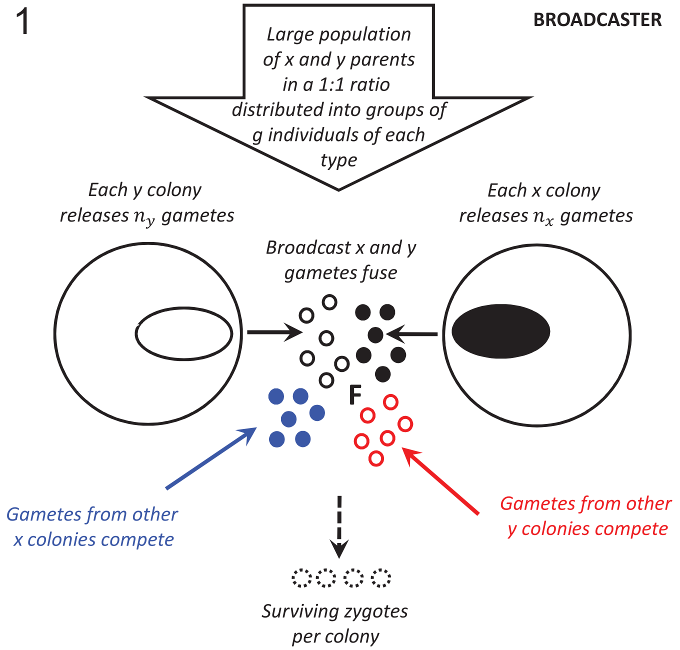
Though cases 2 and 3 appear biologically very different, under simple parallel assumptions, the two converge. Thus, if gamete-caster/brooder colonies are evenly structured into groups, each containing g individuals of each mating type, and packet-caster/brooder colonies exist in a large unstructured population and each packet caster releases k packets that are well mixed and evenly distributed among the x colonies, we show in the supplemental material (available online) that cases 2 and 3 are mathematically identical when $g = k$.

Our models of these scenarios all assume a very large population of individuals of two mating types, x and y. The broadcaster analysis partly replicates the results of Lehtonen and Kokko (2011), which combines previous models of gamete competition or gamete limitation by incorporating both components simultaneously. The population of colonies is structured into groups each consisting of equal numbers of parents, $g_x = g_y = g$. Both x and y types broadcast their gametes, implying that the results should be symmetric. Here, however, gametes are released in a single burst (this model) versus continuous spawning (Lehtonen and Kokko 2011), and we assume a fixed minimum gamete size (this model) versus size-dependent gamete mortality (Lehtonen and Kokko 2011). Modifying Lehtonen and Kokko's model in this way enables us to establish benchmark GD predictions against which we can compare results for the two brooder models.

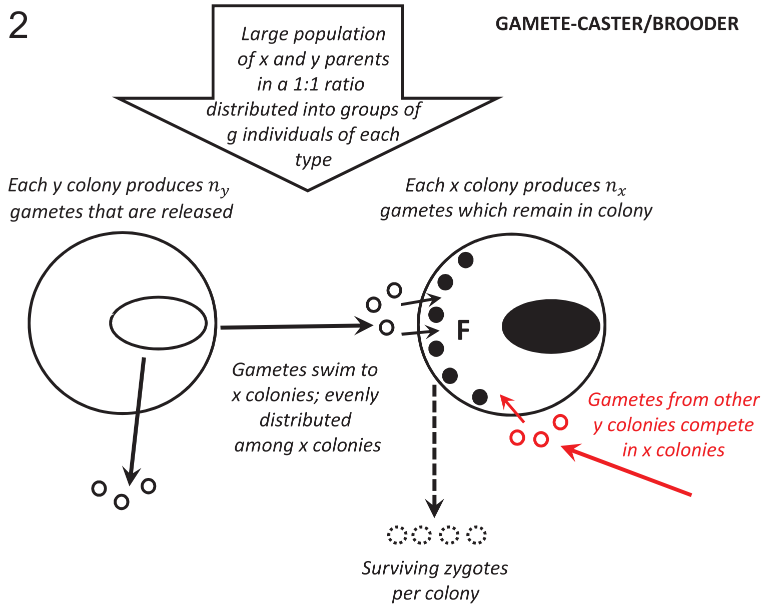
The gamete-caster/brooder analysis is a modified version of the broadcaster approach, with one difference: fertilization is internal in the mating type that retains its gametes (arbitrarily chosen to be x), while the other mating type (y) casts its gametes into the water, where they face direct gamete competition from gametes of other y individuals. Importantly, we make the reasonable assumption that brooders do not face gamete competition from other brooders. This follows logically if all brooders retain their own gametes internally, which effectively prevents any competition between x gametes, while competition between y gametes can readily occur because of broadcasting by y parents.

In the packet-caster/brooder model, all individuals produce their gametes in a fixed number k of packets, but only y parents release their packets, which are assumed to be well mixed and evenly distributed among x colonies. In each mating type, packets are divided into gametes, the sizes of which may vary by mutation. The y packets release their gametes once they reach x colonies and compete for fertilizations;

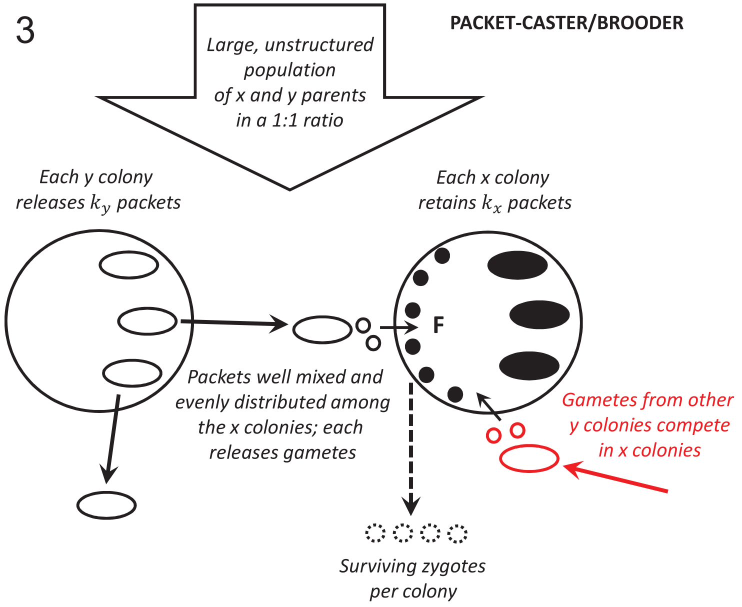
1


BROADCASTER
Large population of x and y parents in a 1:1 ratio distributed into groups of g individuals of each type
Each y colony releases n_y gametes
Each x colony releases n_x gametes
Broadcast x and y gametes fuse
F
Gametes from other x colonies compete
Gametes from other y colonies compete
Surviving zygotes per colony

2


GAMETE-CASTER/BROODER
Large population of x and y parents in a 1:1 ratio distributed into groups of g individuals of each type
Each y colony produces n_y gametes that are released
Each x colony produces n_x gametes which remain in colony
Gametes swim to x colonies; evenly distributed among x colonies
F
Gametes from other y colonies compete in x colonies
Surviving zygotes per colony

3


PACKET-CASTER/BROODER
Large, unstructured population of x and y parents in a 1:1 ratio
Each y colony releases k_y packets
Each x colony retains k_x packets
Packets well mixed and evenly distributed among the x colonies; each releases gametes
F
Gametes from other y colonies compete in x colonies
Surviving zygotes per colony

competition is restricted to gametes from the set of packets around each female colony. The population is not divided into groups: division of gametes into packets has an effect similar to group structure in the previous scenarios. We show this mathematically in the supplemental material (sec. 1.4); a brief intuitive explanation is that a y individual that casts k packets can distribute its gametes over a maximum of k x individuals, analogous to a gamete caster that casts its gametes in a group that contains g x colonies.

Following previous GD models, we make two important assumptions in all analyses (see table 2 for notation). First, there is a limited reproductive budget, which results in a size-number trade-off during gamete production. Each parent produces gametes by dividing its fixed reproductive resource M into gametes of size m, resulting in $n_x = M/m_x$ and $n_y = M/m_y$ gametes per individual of each mating type. In the packet-caster/brooder model, each packet therefore contains M/km_x or M/km_y gametes. Second, survival of zygotes increases (at least over some range) with their size. So when an x and a y gamete fuse to form a zygote, the survival probability of the resulting zygote is described by a function $s(m_x + m_y)$, in which we use parameter β to vary the resource required for a successful zygote and apply the commonly used Vance-Levitan zygote survival function (Vance 1973; Levitan 2000). We do not model gamete mortality separately and instead set a minimum gamete size (α) below which gametes are inviable (as in Maynard Smith 1978, 1982; Bulmer and Parker 2002, model d).

Fertilization success is modeled with an equation derived by Togashi et al. (2007; see also Lehtonen 2015 and, in this work, table 2 and supplemental material, sec. 1.1). The function calculates average fertilization success (F) in a given fertilization environment (e.g., inside a brooder colony or in a local spawning group) as a function of the total gamete number of each type and a parameter that controls overall fertilization efficiency ("aptitude for fusion"; Scudo 1967). Gamete competition can be envisioned in two equivalent ways. If the focal individual of mating type i produces n_i gametes and there are a total of N_i gametes of the type i (including those of the focal) competing for fertilizations in the same fertilization environment, then the focal individual will on average gain a fraction n_i/N_i of the F successful fertilizations. Equivalently, given that F of the N_i gametes are successfully fertilized, the probability of fertilization for a randomly picked i gamete is F/N_i. A focal individual producing n_i gametes will then gain a total of $(n_i/N_i)F = n_i(F/N_i)$ fertilizations, whichever way we choose to look at the situation.

Combining these components, the fitness of a gamete caster is of the form $(n_i/N_i)Fs$, the product of gamete competition, fertilization success, and zygote survival, as outlined in Parker and Lehtonen (2014) and Lehtonen (2015). Brooders do not face gamete competition (see above for a justification), and their fitness is of the form Fs. Gamete competition (n_i/N_i) and gamete limitation (F) both select for increased gamete numbers, while zygote survival (s) may drive increased gamete size. The same functional forms apply to the packet-caster model, where the relevant gamete numbers (n_i and N_i) depend on packet numbers (see supplemental material, sec. 1.4).

We investigate all three strategies, using an analytical approach incorporating aspects of game theory and adaptive dynamics (Maynard Smith 1982; Parker and Maynard Smith 1990; Dieckmann and Law 1996; Lehtonen 2018). Evolutionary trajectories and end points are found by examining fates of mutants in either the x or y mating type that deviate from the resident gamete size strategy, that is, by playing $\hat{m}_x \neq m_x$ or $\hat{m}_y \neq m_y$ (see table 2 for notation). Mutations have small effects on gamete size. Apart from the strategy differences, we use similar parameter values and model assumptions for all three alternatives to enable a direct comparison between internal and external fertilization.

Full details of the models are given in the supplemental material, where we also undertake further investigations to validate our conclusions, using (1) an "invasion grid" approach similar to that of Togashi et al. (2012) and (2) a simulation approach with stochastic mutations. Biologically, the main differences are that in the analytical approach gamete size is treated as a continuous trait with very small mutational steps, while the other two approaches incorporate an arguably more realistic mutation model where a mutation changes the number of cell divisions in gametogenesis, thus allowing for very large changes in gamete sizes in a single mutation. The analytical and invasion grid approaches result in a determin-

Figure 2: Summary of the biological scenarios investigated. Colonies are of two mating types, x and y, in a large population in a 1 : 1 ratio, and each colony has a fixed amount of resource (M) for gamete production. *1*, Broadcaster. Colonies are in spawning groups, each containing g colonies of each mating type. Colonies of both types divide reproductive resources into gametes (n_y from mating type y and n_x from mating type x), which are released into the external medium, where syngamy takes place. Fertilization (F) is external. *2*, Gamete caster/brooder. Colonies are in spawning groups, each containing g colonies of each mating type; both types divide reproductive resources into gametes. Mating type y colonies release n_y gametes into the external medium; mating type x colonies retain n_x gametes internally. The y gametes from different parents are evenly distributed among the x colonies. Gametes from y colonies enter x colonies, where syngamy takes place. Fertilization (F) is internal. *3*, Packet caster/brooder. The population is large and not structured into groups. Colonies of both types divide reproductive resources into smaller packets (equivalent to gametangia). Mating type x colonies retain their gamete packets internally. Mating type y colonies release packets into the external medium. The y packets release their gametes adjacent to x colonies. They then enter x colonies, where syngamy takes place. The y packets are assumed to be evenly distributed between female colonies so that each x colony receives k packets (assuming that each y colony produces k packets, the sex ratio is even, and all y packets find an x colony; we relax the assumption of evenly distributed packets and all packets finding a colony in figure 5 and figs. SI1–SI4 in the supplemental material, available online). Fertilization (F) is internal.

Table 2: Notation and parameters

Notation	Name of parameter, variable, or equation
x, y	Mating types x and y; in the gamete-caster/brooder and packet-caster/brooder scenarios, type x is the brooder and y is the caster
m_x, m_y, $\hat{m}_x$, $\hat{m}_y$	Gamete sizes of x residents, y residents, x mutants, and y mutants, respectively
M	Total resource available for gamete production per adult
n_x, n_y, $\hat{n}_x$, $\hat{n}_y$	Number of gametes produced by each x resident, y resident, x mutant, and y mutant, respectively
N_x, N_y	Total number of x- and y-type gametes in a fertilization environment (i.e., in a spawning group or in a brooder colony), including the contributions from all resident and mutant individuals to that environment
φ	Aptitude for fusion/gamete collision rate parameter
$F = F(\varphi, N_x, N_y) = N_x N_y \frac{e^{\varphi Nx} - e^{\varphi Ny}}{N_x e^{\varphi Nx} - N_y e^{\varphi Ny}}$	Average number of successful fertilizations as a function of total gamete numbers of each type in a fertilization environment, and a parameter for fertilization efficiency[a]
k	Number of gamete packets per individual in the packet-caster/brooder scenario
g	Group size in the broadcaster and gamete-caster/brooder scenarios
β	Parameter that changes the resource requirements of the zygote
$s = s(\beta, m_x, m_y) = e^{-\beta/(m_x + m_y)}$	Survival probability of a zygote as a function of parental gamete sizes and a zygote resource requirement parameter
α	Minimum gamete size

[a] See supplementary information, section 1.1, available online, for the special case $N_x = N_y$.

istic depiction of all possible evolutionary trajectories, while the simulation generates random examples of such trajectories, with stochastic mutations.

All approaches (three biological scenarios, using analytical, invasion grid, or simulation methods) are based on similar biological principles, with three major causal determinants of fitness: (1) overall fertilization success (which allows for selection driven by gamete limitation), (2) competition between gametes for fertilizations, and (3) survival of the resulting zygote. However, for gamete-caster/brooder and packet-caster/brooder scenarios, the second of these affects only casters: brooders do not face direct competition from gametes of other individuals, because each individual retains its gametes internally and separately from the gametes of other brooders (similar to sperm competition in, e.g., mammals). The gamete-caster (broadcast-spawning) scenario, however, is completely symmetrical, and any component of selection can potentially affect both mating types.

Results

Size and Complexity

The relations between zygote survival s and zygote size $m_x + m_y$ and between $s(m_x + m_y)$ and minimum gamete size are critical in determining whether anisogamy evolves (Parker et al. 1972) and what anisogamy ratio results, a central testable prediction of GD theory (Matsuda and Abrams 1999; Bulmer and Parker 2002; Lehtonen and Kokko 2011). Figure 3 shows the result of increasing the disparity between the minimum gamete size (α) and the zygote size by increasing parameter β, for broadcaster (row 1) and the two brooder models (row 2/3; results are identical for the gamete-caster/brooder and packet-caster/brooder models—see supplemental material, sec. 1.4); the larger β, the more resource is required by the zygote for survival and the larger zygote size must be relative to minimum gamete size. Gamete competition in figure 3 is quite strong ($g = k = 8$), so that anisogamy evolves (cols. B, C) unless the ratio β/α is low (col. A), as found by Bulmer and Parker (2002). When anisogamy evolves, the main effect of increasing β is to increase the anisogamy ratio. Note that only one of the alternative equilibria in row 1 can evolve, depending on the starting conditions; the isogamous equilibrium in columns B and C is only locally stable and becomes increasingly vulnerable to replacement by anisogamy as β increases.

The main difference between row 1 (external fertilization) and row 2/3 (internal fertilization) is that when there is gamete competition, when anisogamy evolves the type that retains gametes and has no gamete competition (the brooder) becomes female, while the type that broadcasts its gametes (the caster) and experiences gamete competition becomes male. A second result is that internal fertilization further destabilizes isogamy; in row 2/3 of columns B and C, the isogamous equilibria are lost.

In the supplemental material, we show (using invasion grid and simulation approaches) that the packet-caster/brooder conclusions shown here for mutations of small effect remain robust for mutations of large effect—that is, for mutations that change the number of cell divisions—and hence have an exponential effect on gamete size (powers of 2). We also confirm that the results hold if some packets never find a female colony (see supplemental material, figs. SI1–SI3).

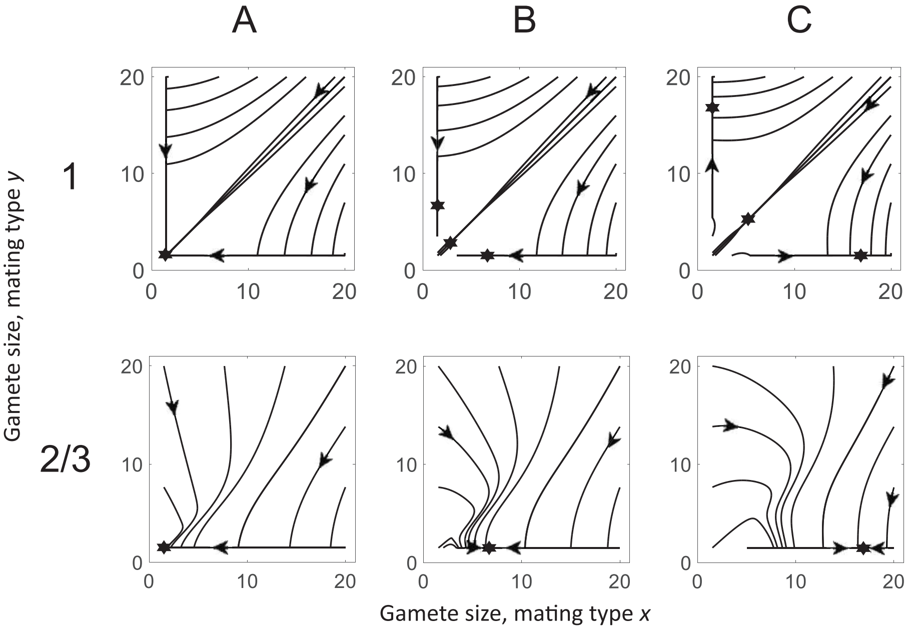


Figure 3: Effect of increasing zygote resource requirements in internal-fertilization models under gamete competition. Row 1 is for the broadcaster model and row 2/3 for the gamete-caster/brooder and packet-caster/brooder models. Stable equilibria are indicated with stars; only one of the alternative equilibria in row 1 can evolve, depending on the starting conditions, and the isogamous equilibrium is only locally stable. As with previous models, anisogamy evolves as the resource requirements of the zygote increase (likely linked to an increase in organismal size and complexity; parameter β in the models). Zygote resource requirements (through parameter β) increase through columns A–C, with $\beta = 4$ in column A, $\beta = 10$ in column B, and $\beta = 20$ in column C. Other parameters: $\alpha = 1.5$, $\varphi = 0.1$, $g = k = 8$, and $M = 1{,}000$. Note that under internal fertilization (row 2/3), the gamete-retaining type (x) becomes female and the gamete-casting type (y) becomes male.

These results confirm that radical changes in the assumptions about the nature of the fertilization process do not affect previous conclusions of GD theory for the evolution of anisogamy. Internal fertilization and/or release of gametes in packets do not in themselves cause issues for testing anisogamy predictions, regardless of which evolved first. If internal fertilization evolved before anisogamy (with or without packets), we can imagine going from left to right in row 2/3. If anisogamy evolved first, we can imagine going from left to right in row 1, then down to row 2/3. The resulting stable anisogamous equilibrium is similar on each row, except that with the constraint of internal fertilization, only the competing sex (y) can evolve to become males. Therefore, the results in figure 3 demonstrate that, aside from this asymmetry, internal fertilization or sperm packets in, for example, volvocine algae do not compromise the validity of comparative tests.

Gamete Limitation and Competition

In figure 3, the main driver of increased gamete numbers is gamete competition. Previous models have shown that under broadcast spawning, anisogamy can be generated by either gamete limitation or gamete competition (see Lehtonen and Kokko 2011), though when both forces operate, even if gamete limitation is very severe, gamete competition tends to be the stronger selective force, provided that the average number of competitors is one or more (Parker and Lehtonen 2014). Figure 4 confirms that under all fertilization scenarios investigated, anisogamy can arise by gamete limitation alone (col. A) or by gamete competition (cols. C, D). Results in column A are gamete limited, in the sense that fertilization is very inefficient ($\varphi = 0.01$; gametes are very bad at finding each other even when they are confined within a colony) and gamete competition is absent. In this case (col. A), there are two alternative anisogamous equilibria (indicated with stars); that is, either mating type can become female. Note that this result applies even under internal fertilization (row 2/3). Either type can, at least in principle, equally well improve fertilization prospects by increasing gamete numbers, while the other type provides most of the resources for the zygote. The isogamous equilibrium in column A (indicated by a small filled circle) is not convergence stable (Eshel et al. 1997). It can be approached only along the diagonal line, with even the slightest deviation resulting in a divergence to anisogamy, and in this sense it is an artifact of the symmetry of the analytical model.

In column B, gamete limitation is low ($\varphi = 0.2$) and gamete competition again absent. Isogamy now has a wide range

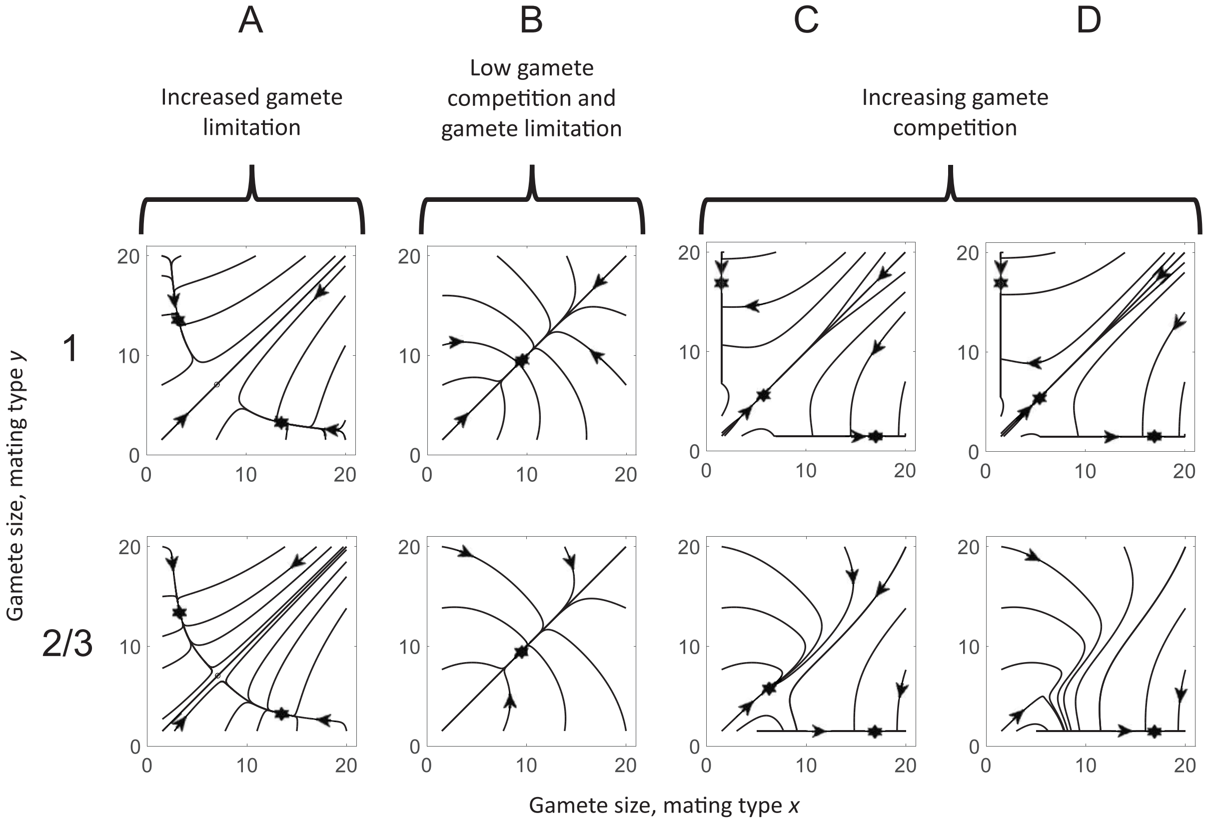


Figure 4: Effects of gamete limitation (col. A, where selection for anisogamy is driven by gamete limitation) and increasing gamete competition (cols. C, D, where selection is mainly driven by gamete competition). Row 1 is for the broadcaster model and row 2/3 for the gamete-caster/brooder and packet-caster/brooder models. Stable equilibria are indicated with stars; only one of the alternative equilibria in row 1 can evolve, depending on the starting conditions, and the isogamous equilibrium is only locally stable. Anisogamy can arise by gamete limitation (col. A) or gamete competition (cols. C, D). Group size/number of packets: $g = k = 1$ (cols. A, B), $g = k = 4$ (col. C), $g = k = 8$ (col. D). Gamete collision parameter $\varphi = 0.01$ in column A and 0.2 in columns B–D. Other parameters: $\beta = 20$, $\alpha = 1.5$, and $M = 1{,}000$.

of stability under the parameters stated in the figure 4 legend. As gamete competition is increased to $k = 4$ (col. C), there are three alternative local equilibria (one isogamous and two anisogamous) in the external-fertilization model (row 1), but the isogamous equilibrium has only a narrow basin of attraction. In the brooder models (col. C, row 2/3), the "male brooding" alternative equilibrium is lost. The isogamous equilibrium disappears from brooder models when gamete competition is increased further (col. D).

Summary of Main Results: Effects of Internal Fertilization and Gamete Packets on GD Theory

The major differences between the results for the model of broadcast spawning (figs. 3, 4, row 1; see also Lehtonen and Kokko 2011) and those for the brooder models of internal fertilization (figs. 3, 4, rows 2/3) are that (1) the former show symmetric dynamics on either side of the line of gamete size equality ($m_x = m_y$), while the latter are symmetric only in the complete absence of gamete competition (row 2/3, cols. A, B in fig. 4); (2) when anisogamy is driven by gamete competition in the brooder models (row 2/3 of col. C in figs. 3, 4 and of col. D in fig. 4), only the competing sex (y) can evolve small gametes (become male) and the noncompeting sex (x) large gametes (become female); and (3) internal fertilization acts to destabilize locally stable isogamous equilibria. In next section, we show that the isogamy destabilization result (3) is further increased by random (and hence uneven) distribution of packets among x colonies. Again, apart from the asymmetry that only brooders become female under gamete competition, predictions of GD theory remain unchanged under internal fertilization, with or without sperm packets. Casters become male because they are the only mating type that faces gamete competition from other casters and can hence profit from producing a larger number of (smaller) gametes. We have also shown that the effect of gamete packets is similar to the effect of colony grouping: both can restrict the extent of gamete competition if g or k is very small but in general do not prevent the evolution or maintenance of anisogamy or change central theoretical predictions.

Robustness of the Main Results

In our extensions of GD theory we have aimed to examine the effects of two biological features of typical model organisms: internal fertilization and gamete packets. To isolate the effects of these features, we have kept the models otherwise relatively simple. However, we separately examined robustness to a selection of alterations. Here we present two important alterations to the packet-caster models in rows 2/3 of figures 3 and 4: random distribution of gamete packets among females (as opposed to the even distribution of packets used in the main results) and mutations of large effect in gamete size. The former is inevitable in the real world, and the latter is a well-known feature of gamete size adjustment in many algae: many algal species in nature vary gamete size by varying the number of cell divisions (e.g., Togashi et al. 2015; Horinouchi and Togashi 2018), which implies a large change in gamete size per division.

A reanalysis of figure 3 (which investigates the effect of multicellular complexity, parameter β) under these two additional assumptions shows little departure from the original results; under the assumption of large mutations and stochasticity, the equilibria appear at the value that is closest to those of the analytical model with small mutations (see supplemental material, fig. SI1).

However, figure 5 (which investigates the effect of gamete competition and limitation) shows some interesting differences from figure 4. Most notably, including stochasticity in the distribution of packets per female increases the effect of gamete competition: moving from left to right along row 1 of figure 5, anisogamous equilibria appear earlier and isogamous equilibria disappear earlier, compared to row 2/3 of figure 4. The reason for this is that introducing variance in the number of packets per female (and hence in the number of competitor packets a focal packet faces) gives a higher ef-

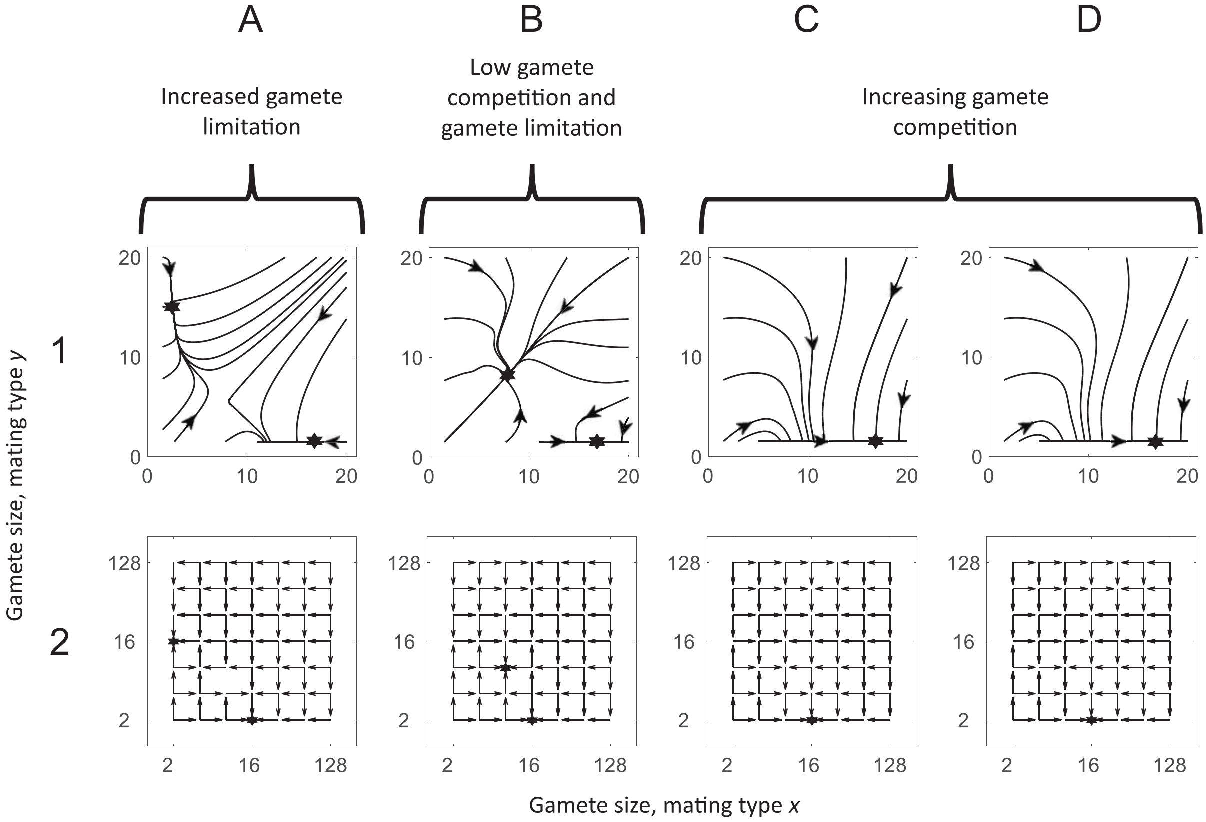


Figure 5: Robustness of packet-caster/brooder results of figure 4 for gamete limitation and gamete competition. In row 1, we increase biological reality by adding a Poisson distribution of *y* gamete packets among *x* colonies. In row 2, we retain this effect and add the feature that mutations have large effect by changing the number of cell divisions (using the invasion grid approach; see supplemental material, sec. 2.3, available online). Stable equilibria are indicated with stars. Results can be compared with figure 4, row 2/3, which lacks these additional effects. Adding the Poisson distribution increases gamete competition; anisogamous equilibria appear earlier, and isogamous equilibria disappear earlier (see text). Parameter values are as in figure 4. Axes in row 2 are logarithmic.

fective level of gamete competition. When packets are Poisson distributed among females, the packets (seemingly paradoxically) experience, on average, an environment with a total of $k + 1$ packets, despite the mean number of packets per female being k (for an explanation, see supplemental material, sec. 3). Thus, a random distribution of packets increases the effective level of competition and makes anisogamous equilibria appear (and isogamous equilibria disappear) under seemingly lower levels of competition, compared to a simplified scenario with fixed group sizes.

In row 2 of figure 5, stochastic packet distribution is retained, and we further add the potential for mutations of large effect in gamete size, using an invasion grid approach (supplemental material, sec. 2.3). Reassuringly, the results remain qualitatively unchanged. The equilibria appear at the value that is closest to that given by the analytical model with small mutations (cf. row 2 to row 1 in fig. 5—note that the axes in row 2 are on a logarithmic scale for clarity because mutations change gamete size in powers of 2). An analysis with the same assumptions is repeated with a simulation approach in supplemental material, figure SI4. This confirms the invasion grid result with random mutations of large effect.

Discussion

The Models and Volvocine Biology

Although we have used terms that typically refer to multicellular organisms (e.g., colony, brooder), the models are compatible with the biology of unicellular organisms. Unicellular algae typically produce small gametes by extra cell divisions (Fritsch 1948; Smith 1955). In many unicellular volvocines, the entire parent cell divides itself into gametes, fitting the assumption of a limited and fixed resource budget that is then split into smaller gametes. Release of motile gametes by both mating types of isogamous unicellular species likely conforms well to broadcast-spawning models. An origin of oogamy starting from isogamy with nonmotile, functionally noncompetitive x gametes and motile, competitive y gametes is also potentially compatible with our models. If a mutation in an x unicell suppresses gamete motility, this effectively becomes a brooder. If such a mutation spreads throughout the population, the resulting scenario would correspond to the brooder models.

Our analyses focus on gamete competition and gamete limitation. Both occur in multicellular broadcast-spawning marine organisms (e.g., Yund 2000; Levitan 2018), though sperm limitation is often low or insignificant in many sperm casters (Phillippi et al. 2004; Bishop and Pemberton 2006). The ecology of fertilization in many marine macroalgae has been studied and shows variable levels of sperm limitation, but much less is known about unicellular algae (Brawley and Johnson 1992). Most research on volvocines is conducted in the lab with microscopes, so that conditions surrounding fertilization in nature are poorly understood.

Volvocine densities can be high, and there is evidence in many species of colony or sperm packet clumping and sex-inducer pheromones (Frenkel et al. 2014). While our models cover some of these factors, our aim is not to reproduce the biology of any individual species but to gain general insight into how internal fertilization and/or sperm packets in themselves may affect predictions of GD theory. Colony clumping before syngamy occurs in both isogamous unicellular forms (e.g., *Carteria palmata*; Suda et al. 2005) and simple isogamous colonial forms (e.g., *Pandorina morum*; Coleman 1959) and resembles our broadcast-spawning (gamete-caster) model, where the number of colonies in a spawning group (i.e., a "clump" of colonies) can be adjusted by changing the group size parameter g. In more advanced colonial forms, after release, sperm packets dissociate as free-swimming sperm only when a packet approaches a colony bearing ova; the sperm then swim directly into colonial matrix to fuse with female gametes (e.g., *Eudorina elegans*; Smith 1955; Nozaki 1983). There is again evidence of high densities and reproductive synchrony. For example, in *Volvox*, colony clustering (Drescher et al. 2009), coupled with powerful species-specific sex-inducing pheromones (Hallmann 2003), makes sperm competition likely among sperm packets from different colonies. Variation in the number of competing y gamete packets per x colony is modeled in the packet-caster/brooder model (parameter k). To prevent our models from becoming prohibitively complex, we do not incorporate colony clumping simultaneously with sperm packet production: clumping is modeled only in the broadcaster and gamete-caster/brooder models. Furthermore, as a first approximation, the effects of clumping (g) and packets (k) are identical (see supplemental material, secs. 1.3, 1.4).

Many additional effects (see Lessells et al. 2009) are not included in our models, for example, effect of gamete size on collisions between gametes ("target theory"; e.g., Levitan 1993; Cox and Sethian 1985; Dusenbery 2000), phototaxis and pheromonal attraction (e.g., Dusenbery 2011; Togashi and Bartelt 2011), and gamete size-speed relationships (Seed and Tomkins 2018). Polyspermy is lethal and reported in some algae but is generally prevented by various polyspermy blocks, unless these fail (Brawley and Johnson 1992). Some volvocines retain the zygote for some time before its release, possibly providing opportunity for simple forms of parental care (Randerson and Hurst 2001). This could alter the relationship between zygote size and zygote survival (function s; table 2), an integral component of GD models. Published photographs show no apparent increase in zygote size between immature and fully mature zygotes (e.g., *Pleodorina japonica* [Nozaki et al. 1989], *Pleodorina sphaerica* [Nozaki et al. 2017], *Volvox carteri* f. *kawasakiensis* [Nozaki 1988], *Volvox capensis* [Nozaki et al. 2015]), suggesting no significant maternal provisioning after fertilization. However, parental care could come in the form of reduced mortality risk in the relatively protected internal colony environment. A comprehensive GD theory could incorporate all these aspects, but at the cost of rapidly expanding complexity—our aim has been to examine core aspects of previous GD models under alternative plausible ancestral scenarios and transitions while maintaining manageable complexity.

Colemanosphaera, the "missing link" (fig. 1*B*) between broadcast spawning (fig. 1*A*) and packet casting (fig. 1*C*; Nozaki 2014), releases sperm in packets, but (unusually for packet-releasing species) also releases its ova. We have not explicitly modeled this fertilization mode, but, depending on the events surrounding fertilization, it can be interpreted with our present approaches. On the one hand, suppose that the population is structured into groups of g colonies of each mating type and that all gametes from all packets of both mating types in a group were released and randomly mixed before fertilization. Both mating types then face gamete competition, and predictions would match the broadcaster model 1. On the other hand, suppose that the population of y colonies is large and releases packets that become well mixed and evenly dispersed among the x colonies, which in turn retain their gametes on the colony surface. Predictions then match the packet-caster/brooder model 3 because competition between x-type gametes is prevented. Observations support sperm release from packets around or on the female colony, after which the female gametes are released and remain close to the colony, where they are fertilized (Nozaki 2014). Hence, the fertilization process probably approximates the packet-caster/brooder model. Modeling intermediate forms of dispersion between these two limits may be useful, since they may illuminate the shift from the symmetric alternative equilibria in row 1 of figures 3 and 4 to the "only female brooding" asymmetric equilibrium in row 2/3. While we have explicitly modeled only the end points of such a continuum, it seems unlikely that any major departures for GD predictions would be found.

Robustness of GD Theory

We have shown that GD theory offers a very robust, general solution for the isogamy/anisogamy dichotomy and the evolution of two sexes. While previous GD models have assumed broadcast spawning, changing the fertilization conditions quite radically to ancestral or subsequently evolved forms of internal fertilization does not change qualitatively the evolution or stability of the GD isogamous/anisogamous equilibria. In other words, the ancestral condition (internal or external fertilization, packets or no packets), as well as the order of these transitions in evolutionary time, makes little difference to the testable predictions of the theory, making it appropriate to use groups such as the volvocine algae (which feature different combinations of these conditions) in comparative studies. It is also interesting to compare our results to some of those in Parker (1982), which showed that the stability of anisogamy decreases as sperm competition decreases (as is likely under internal fertilization) and increases with the anisogamy ratio (as is likely under multicellularity). Our analysis supports that conclusion: as group size or packet numbers increase, anisogamy becomes more stable. However, our analysis shows that at the same level of gamete competition (here group/packet size), internal fertilization tends to destabilize isogamy.

Another new result of this study is that when anisogamy is driven by gamete competition, the symmetry of alternative equilibria across the two mating types is lost under our internal-fertilization gamete competition scenarios, where the noncompeting mating type (which retains its gametes) becomes female and the competing mating type (which broadcasts its gametes) becomes male. So if anisogamy arose under or evolved after internal fertilization and if gamete competition was the major selective force, we should expect the noncompetitive sex (here brooders) always to be female. This parallels the finding of Henshaw et al. (2014) for marine invertebrates that "egg casting" (where females broadcast eggs but males retain sperm) lacks evolutionary stability and is apparently not found in such taxa in nature. We can therefore explain this "universal sexual asymmetry" (Henshaw et al. 2014) from GD theory, under conditions where anisogamy arises from isogamy under internal-fertilization scenarios, driven by gamete competition.

However, we note one instance in which internal fertilization can generate anisogamous symmetry such that the noncompeting sex (here x) can become male, violating the conclusion that only females can become brooders. It occurs only in the total absence of gamete competition, that is, when there is just one individual of each type per group (in the gamete-caster/brooder scenario) or one gametangium or reproductive packet per parental colony (in the packet-caster/brooder scenario), combined with strong gamete limitation. This makes isogamy unstable: the sexual-asymmetry rule then breaks down, and either x or y can become male or female (fig. 4, col. A). Whether such a scenario has ever occurred in ancestral isogamous states remains uncertain. It is analogous to the case for broadcasting spawning for no gamete competition and strong gamete limitation, which generates stable anisogamy (fig. 4, col. A, row 1; lower row of fig. 5 in Lehtonen and Kokko 2011). As gamete limitation is reduced (i.e., fertilization is more efficient; fig. 4, col. B), an isogamous equilibrium is generated, and at intermediate levels of gamete competition, alternative isogamous and anisogamous equilibria can appear (fig. 4, col. C).

The robustness of the unified GD theory (encompassing gamete competition and gamete limitation) as an explanation for anisogamy and the fact that it is based on simple, arguably indisputable biophysical assumptions relating to consequences of splitting fixed resources into gametes and the need for resources for successful zygote growth and survival make it a highly potent selective rationale for the evolution of isogamy/anisogamy, and hence the two sexes, and thus one that cannot be disregarded. Further, empirical support for the theory has been found through the link with the evolution of multicellularity (reviewed in Parker 2011; Togashi and Bartelt 2011; see also recent comparative studies by da Silva 2018, da Silva and Drysdale 2018, and Hanschen et al. 2018).

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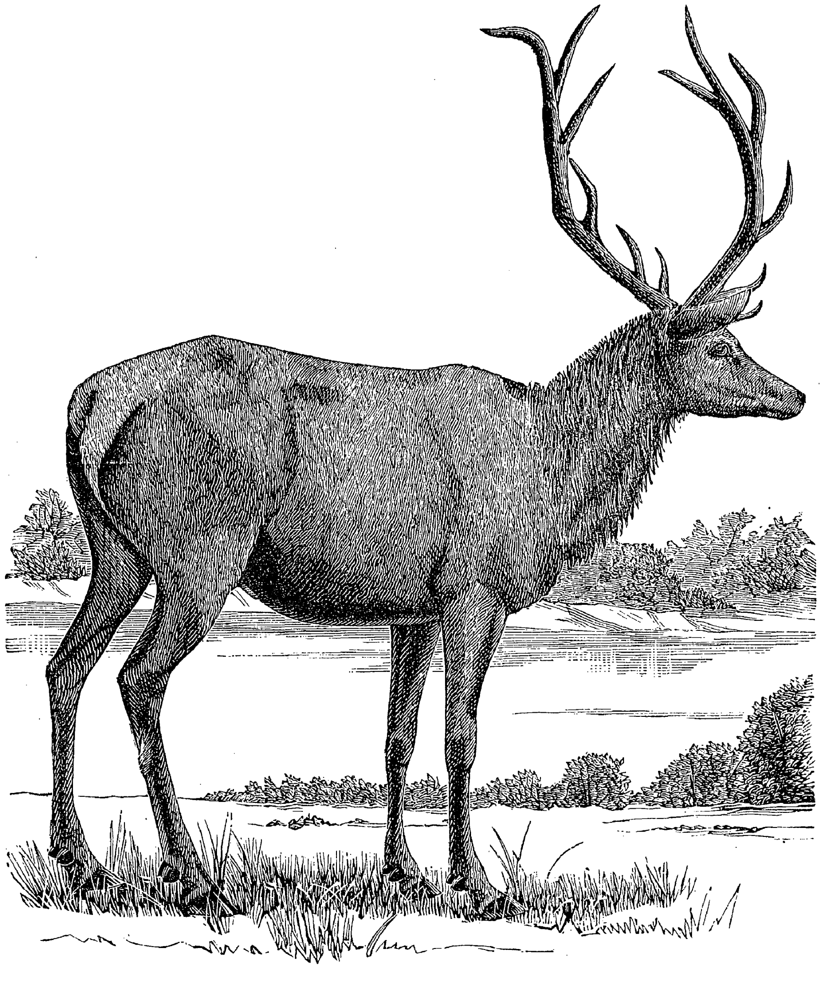

"Persons are frequently puzzled by the reverse use of the terms 'moose' and 'elk.' The author makes it perfectly clear that the American *moose* is the analogue of the palmate-horned animal called 'elk' in Europe; and that the American *elk* is the analogue of the stage or red deer of Europe." Figured: "Male Elk or Wapiti of America." From the review of Caton's *Deer of America* (*The American Naturalist*, 1877, 11:354–358).