



The origination events of gametic sexual reproduction and anisogamy

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Abstract

The evolution of gametic sex (meiosis and fertilization) and subsequent transition from isogamy (fusion between two equal-sized gametes) to anisogamy (dimorphism into eggs and sperm, namely, females and males) is one of the largest enigmas of evolutionary biology. Meiosis entails genome-dilution cost and anisogamy entails male-production cost. Despite much progress has been made for the maintenance mechanisms of sex, its origination events under such “twofold cost of sex” are still unsolved. Here, we posit two hypothetical scenarios as follows: the “Seesaw Effect” hypothesizes that automictic selfing between isogametes effectively purged deleterious mutations from an organism’s lineage and simultaneously fixed the sex-controlling allele and all other loci (no genome-dilution cost raised). The high relatedness among homoeologous cell colonies led to multicellularization. The “inflated isogamy” hypothesizes that multicellularity increased the reproductive investment of both mates, resulting in excessively large isogametes. This redundancy induced cheating of one sex (evolving to male) to reduce gamete size. However, the other sex (evolving to female) allowed this cheat because her cost did not change. Therefore, anisogamy originated as a kind of commensalism but turned into beneficial for females because it solved the gamete limitation problem inherent to isogamy. Thus, smooth transition to anisogamy had been attained.

Keywords Evolution of sex · Anisogamy · Deleterious mutation · Kondrashov effect · Automixis

Introduction

Sex is costly

Sex is the mixing of genetic information to create an offspring. In most eukaryote, both diploid parents produce haploid gametes via meiosis and fuse two gametes into a diploid zygote. Although this gametic sexual reproduction dominates in biological world (Williams 1975), its evolution has been considered as a paradox: first, diploid parents transfer only the half of their genomes to offspring compared with asexual reproduction. This inefficiency is called “cost of meiosis” (Williams 1975; Maynard-Smith 1978, 1982; Maynard Smith and Szathmáry 1995; Lessells et al. 2009; Togashi and Cox 2011) or “genome dilution cost” (Williams

1975; Maynard-Smith 1978, 1982). To compensate for this cost, sexual organisms have to leave two times many offspring as asexual rivals but this is difficult in most species.

Second, in most multicellular organisms, females produce large eggs and males fertilize them by their many tiny sperm. This gametic dimorphism is called anisogamy (Williams 1975; Maynard-Smith 1978). In anisogamy, males do not lay eggs, and thus population growth rate becomes half compared with asexual competitor. This unproductivity of anisogamy is called “male production cost” (Maynard-Smith 1978; Lessells et al. 2009; Lehtonen et al. 2012; Kobayashi and Hasegawa 2016). Considering that there are insects and reptiles that reproduce without males (thelytokous parthenogenesis; Butlin 2002; Normark 2003; Simon et al. 2003; Schwander and Crespi 2009), why females make sons is a mystery. In general, these costs are well known as “the twofold cost of sex” (Maynard-Smith 1978; Lessells et al. 2009; Lehtonen et al. 2012; Kobayashi and Hasegawa 2016).

Advantages of sex

Various hypotheses have been proposed for the origin and maintenance of sexual reproduction (Fisher 1930;

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Muller 1932, 1964; Parker et al. 1972; Bell 1978; Parker 1978; Hamilton 1980; Kondrashov 1988, 1993; Hamilton et al. 1990; Hurst 1990; Matsuda and Abrams 1999; Agrawal 2001, 2006; Otto and Lenormand 2002; Otto 2009; Lehtonen et al. 2012, 2016; de Vienne et al. 2013; Kawatsu 2013; Lumley et al. 2015; Kobayashi and Hasegawa 2016). These hypotheses can be classified into two categories: (1) genetic diversity in offspring generated by genome mixture, which enables rapid evolution in response to environmental changes (Fisher–Muller effect: Fisher 1930; Muller 1932), especially in the arms race with parasites (Red Queen hypothesis: Hamilton 1980; Hamilton et al. 1990; Morran et al. 2011). Today, the Red Queen hypothesis has been much tested and its validity is confirmed (Lively 1987, 1996; Howard and Lively 1994; Koskella and Lively 2007; Jokela et al. 2009; King et al. 2009; Morran et al. 2011; Gibson et al. 2017). Category (2) is that sex can purge deleterious mutations (*dms*) from their genetic line. Recombination during meiosis creates gametes that contain more and less *dms*, and consequently offspring derived from the former is killed (Kondrashov effect: Kondrashov 1988). In asexual reproduction, *dms* will continue to accumulate and eventually all individuals will exceed the lethal threshold (represented as *dmt*, the threshold beyond which individuals die) and become extinct (Muller 1964; Kondrashov 1988). This “Muller’s ratchet” has sometimes been rejected as a kind of group selection (Hamilton 1980; Hamilton et al. 1990; Lessells et al. 2009) but it can be adapted to current gene-based or individual-based evolutionary theories by reinterpreting it as a way to purge *dms* from their own genetic lineage. Today, category (2) hypotheses also have a plenty of empirical evidences (Andersson and Hughes 1996; Moran 1996; Elena and Lenski 1997; Whitlock and Bourguet 2000; Zeyl et al. 2001).

Genome size and sex

Across organisms, the mutation rate/nucleotide/genome copy is known to be ca. $10^{-8\sim-9}$ (Muller 1950). Accordingly, the number of newly occurring *dms* per generation increases with the total length of functional (e.g., protein-coding) genes in a genome. The total gene length in which 1 *dm* or more occurs per generation necessarily rotates Muller’s ratchet. We refer to this critical gene length (ca. $10^{8\sim9}$ bp) as the “Kondrashov threshold” (Kondrashov 1988). This predicts that organisms with functional regions larger than the Kondrashov threshold cannot survive without sex because all individuals bear at least one new mutation in every generation, and thus, they will inevitably exceed the lethal threshold *dmt*. This also means that an asexual organism with a short functional region is not destined for extinction via Muller’s ratchet because mutation does not hit all individuals in every generation and the individuals bearing

no new mutations remain and reproduce in a population. Figure 1 and Table 1 show that the presence or absence of sex is obviously correlated with the length of protein-coding regions in the total genome of an individual. When a total gene length exceeds the Kondrashov threshold (ca. 1 *dm*/genome/generation; red borderline in Fig. 1), organisms exhibit obligate sex, eukaryotes appear and gametic reproduction (meiosis and fertilization) started. Therefore, the origin of gametic reproduction would relate to the Kondrashov effect to purge large number of *dms* at once. Hereafter, we focus the mutation purging function of sex.

At the origin, gametic sexual reproduction would have been performed by fusion between two equal sized gametes (isogamy). At this phase, both parents equally shared the resource investment to a zygote, in other words, males did not yet exist. Thus, isogamy entailed no cost of male production. Only genome-dilution cost due to meiosis was imposed. At the subsequent evolution of anisogamy, the male production cost had raised. Our questions are follows: how had the first isogamous organism overcome the genome dilution cost and how could anisogamy evolve from isogamy despite the male production cost?

In this paper, we propose two hypotheses: “seesaw effect”, to explain the origin of gametic sex without the genome dilution and “inflated isogamy”, to explain the smooth transition to anisogamy.

Hypothesis 1: the “seesaw effect” fixed the sex-controlling locus

Definitions

The “seesaw effect” is a mechanism to purge large number of *dms* at once by an automictic selfing between clonal gametes.

An evolutionary scenario of gametic reproduction

Asymmetrical distribution of deleterious mutation between genomes Consider the Kondrashov effect on quantitative traits in diploid unicellular organisms with multiple pairs of chromosomes including a sufficiently long coding region to rotate Muller’s ratchet (Fig. 2). We assume that the increase in *dms* in diploid genomes is harmless until *dmt*, but when *dms* reaches *dmt* + 1, the organisms will die (Kondrashov 1988). This is favorable condition for asexual organisms because their reproductive rate does not decrease until *dmt* + 1. Thus, we sought to identify the mechanism that benefits sex even under such harsh conditions. We also assume that a mutant allele (S) regulates meiosis and fertilization. Here, sex is considered a single-locus trait for simplicity, but even if multiple loci are involved, the result is qualitatively the same. The S allele is inevitably heterozy-

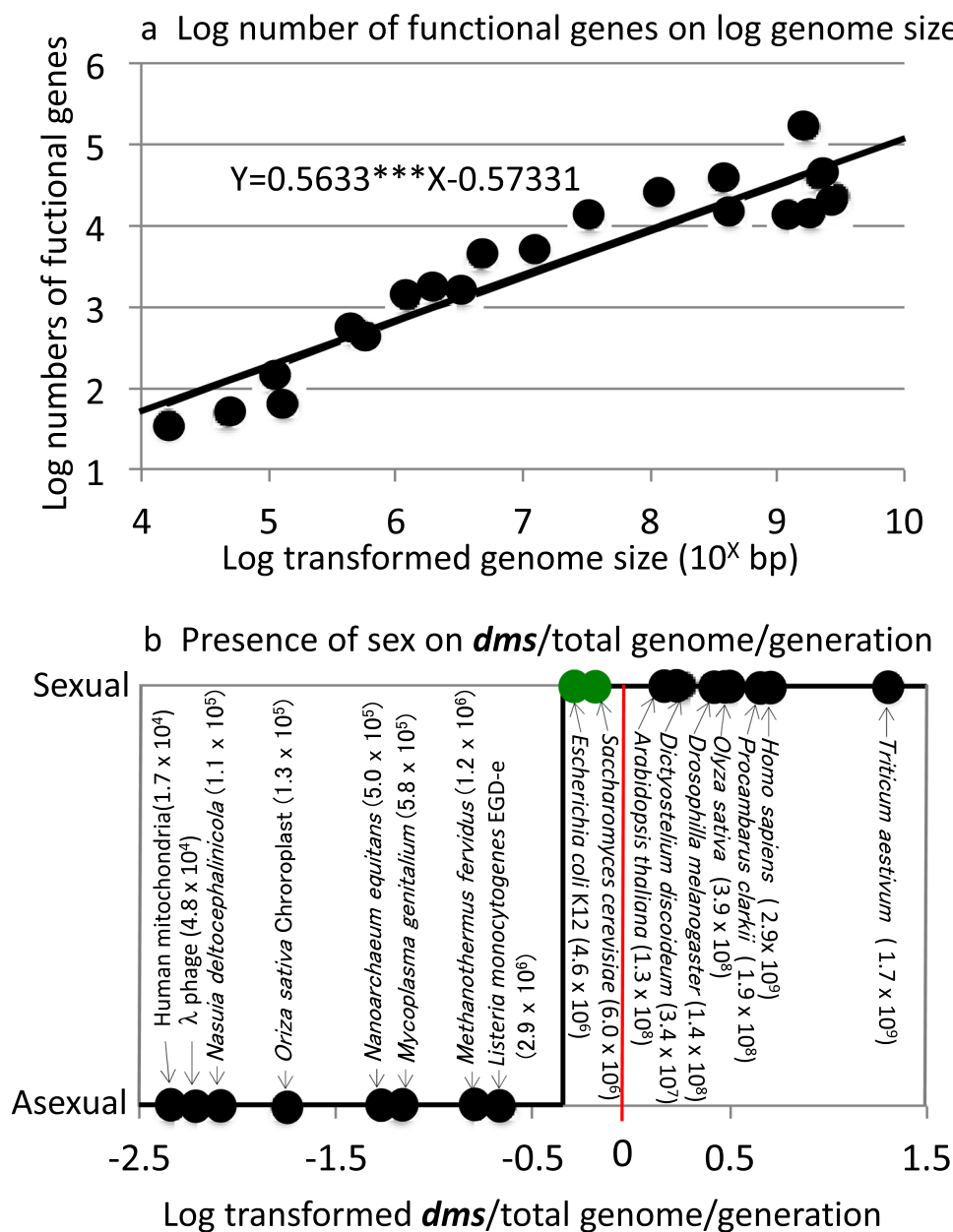


Fig. 1 **a** The relationship between genome size and the number of protein-coding genes in the genomes of 21 organisms (Table 1). The number of protein-coding genes is positively correlated with genome size. **b** The relationship between the number of deleterious mutations per generation (Δdms ; rate of increase in *dms*) and the existence of sex in the 21 organisms included in **a**. The Δdms of each organism was estimated under the following assumptions: (1) the point mutation rate was 10^{-8} per nucleotide across taxa, (2) the average length of a protein-coding gene was 15,080 bp=5027 codons*, and (3) every point mutation was deleterious. The x-axis shows the log-transformed Δdms estimated for each organism. In general, the longer the protein-coding region in the species is, the more deleterious mutations will occur in that region per generation (greater Δdms). On the log-scaled x-axis, 0 corresponds to the Kondrashov threshold required to rotate Muller's ratchet ($\Delta dms=1$, i.e., 1 *dms*/whole genome/genera-

tion). Note that all organisms with a value larger than this threshold exhibit obligate sex. The two green circles represent *Escherichia coli* and budding yeast. They seem to be located in the transition area, in which species with genomes (10^6 orders) smaller than the threshold exhibit facultative sex. We estimated Δdms in both species via computer simulations. Under a mutation rate following a binomial distribution with 6.0×10^6 (*Saccharomyces*) and 4.6×10^6 (*E. coli* K12 strain) trials and an occurrence probability of 10^{-8} , the probabilities that two or more mutations will occur in a generation are 0.00193 and 0.00103 in 100,000 iterations, respectively. This means that mutation simultaneously kills two daughter cells (i.e., extinction) once every 1000 binary fissions (once every 333 h) in these species. Thus, these species need to periodically discard *dms* via sex. *Value estimated using human data (*Human Molecular Genetics* by T. Strachan, A. Read, 2010).

Table 1 Genome size, the number of genes and the existence of sex in various organisms

	Genome size (bp)	No. protein genes	<i>dms</i> /generation	Sexual/Asexual	GenBank accession no
Human mitochondria	16,569	37	0.00468686	Asexual	NC_012920
λ phage	48,000	50	0.00633360	Asexual	NC_001416.1
<i>Oryza sativa</i> chloroplast	130,000	65	0.00823368	Asexual	MG252500
<i>Nanoarchaeum equitans</i>	500,000	536	0.05915582	Asexual	AACL01000000
<i>Mycoplasma genitalium</i>	580,073	467	0.08613696	Asexual	L43967
<i>Methanothermus fervidus</i>	1,200,000	1283	0.16252018	Asexual	CP002278
<i>Listeria monocytogenes</i> EGD-e	2,944,528	2926	0.37064227	Asexual	AL591824
<i>Escherichia coli</i> K12	1,639,221	4337	0.54937646	Sexual	NC_00913
<i>Saccharomyces cerevisiae</i>	12,495,682	5770	0.87011600	Sexual	U18795, U18779, U18530, U18778, U18796, U18813, U18814, U18839, U18916, U18917 and U18922
<i>Arabidopsis thaliana</i>	115,409,949	25,498	6.45976531	Sexual	GCA_000001735.1
<i>Dictyostelium discoideum</i>	338,000,000	12,500	3.16680000	Sexual	AAFI00000000
<i>Drosophila melanogaster</i>	122,653,977	13,472	3.41305037	Sexual	AE002566-AE003403
<i>Oryza sativa</i>	390,000,000	32,000	8.10700800	Sexual	PRJNA234782, PRJNA448171
<i>Procambarus clarkii</i>	2,600,000,000	40,000	10.13376000	Sexual	AY151515-AY151525, DQ919058
<i>Homo sapiens</i>	30,000,000,000	26,626	6.74553734	Sexual	GCA_013364845.1
<i>Triticum aestivum</i>	170,000,000,000	207,002	52.44271469	Sexual	GCA_902810685.1

gous for the asexual allele (N) at emergence (Fig. 2a) but this SN mother cell could attempt sexual reproduction (i.e., S was dominant over N).

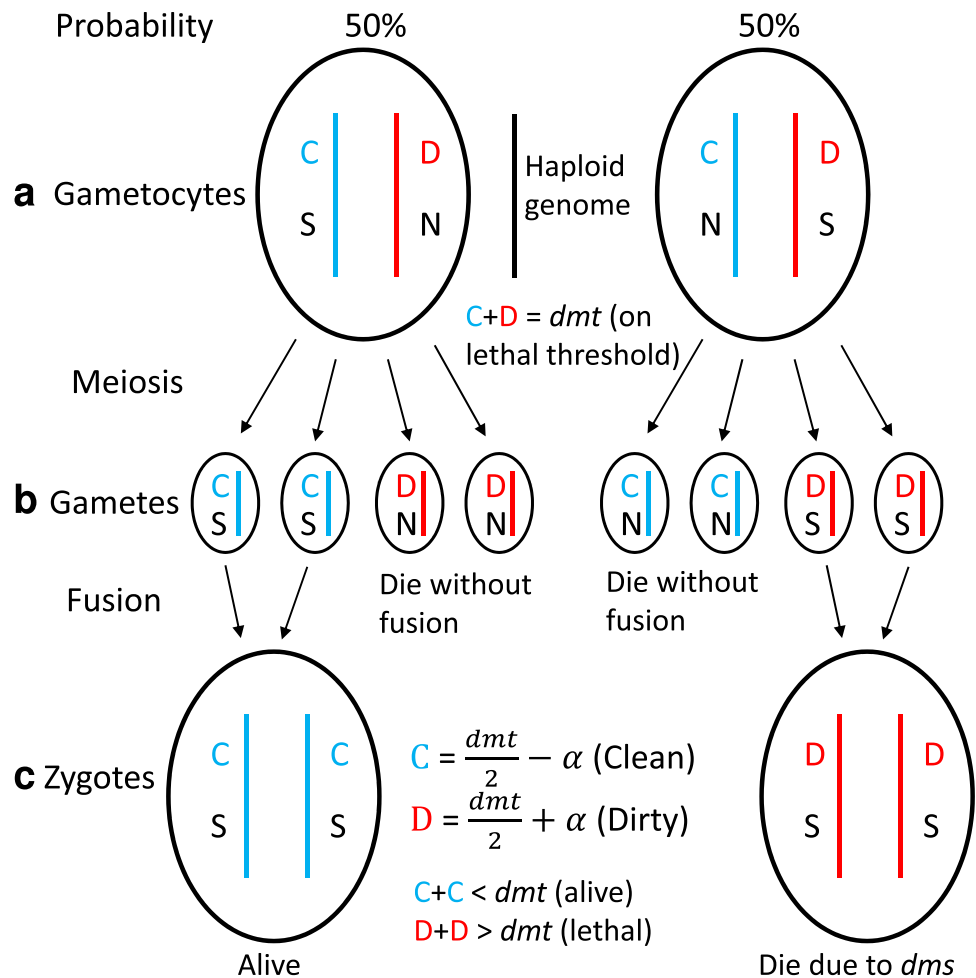
The total *dms* across all chromosomes reached *dmt* (Fig. 2a). If this organism remained asexual, it would go extinct within a finite number of generations. We assumed that the initial form of meiosis was likely primitive and simpler than its present form and sex initially lacked inner-arm recombination between nonsister chromatids in the tetraploid phase. During genome duplication to produce a tetraploid genome, the sex allele was also duplicated. In addition, a few *dms* were expected to occur according to the Kondrashov threshold probability (1 *dm*/diploid genome/generation on average). In the diploid gametocyte (Fig. 2a), the two genomes showed different *dms* because mutations had occurred independently on each chromosome. In the first meiotic division, a pair of chromosomes (2*n*) in this hypothetical first sexual individual were duplicated (4*n*) and were randomly distributed into four gametes (Mendel's law of independence; Fig. 2b). During this process, the *dms* were expected to be asymmetrically distributed between gametes. If two gametes were to show more than half of *dmt* ($\frac{dmt}{2} + \alpha$; referred to as "dirty" or "D"), the other would necessarily show less than half of *dmt* ($\frac{dmt}{2} - \alpha$; referred to as "clean" or "C"), where α is a positive value representing the deviation from equal division of *dms* ($\alpha \gg 1$). Which gametocyte (C or D) mutation of the sex allele (S) occurred was determined by chance (probability 0.5; Fig. 2a). Via meiotic division, the four sets of genomes were separately allocated to four haploid gametes (C, C, D and D; Fig. 2b).

The two Cs were clones, as were the two Ds, except for a few new mutations, which were ignorable because $\alpha \gg 1$.

Automixis between clonal gametes

Because there was no mate for the first sexual individual, the first fusion of gametes must have occurred via selfing between two of its own isogametes, producing a diploid zygote (Fig. 2c). Meiosis created 2 possible combinations of gametes: (1) 2 CS and 2 DN (Fig. 2b left) or (2) 2 CN and 2 DS (Fig. 2b right). Because only the gametes bearing the S allele can fuse, the CS + CS and DS + DS fusions were possible (Fig. 2c). This corresponds to present-day terminal fusion automixis (Engelstadter 2017). In case (1), CS + CS resulted in a viable zygote because the total *dms* were less than *dmt* ($dmt - 2\alpha < dmt$), while 2 DN gametes could not fuse and died (left side of Fig. 2). In case (2), 2 CN gametes also died, and DS + DS resulted in an inviable zygote (the summed *dms* = $dmt + 2\alpha > dmt$; right side of Fig. 2). This process cannot always produce viable zygotes (mortality rate = 50%) but at least prevents the fusion of C + D, which returns to the lethal maternal genomes (C + D + 1 > *dmt*; mortality rate = 100%; + 1 means an additional mutation). Consequently, selfing between 2 CS gametes automatically led to the evolution of gametic reproduction. The genetic load of the lineage of the first sexual individual was reduced from $dmt + 1$ to $dmt - 2\alpha + 1$ ($\alpha \gg 1$). Meiosis divides the critical diploid genomes of a mother cell ($dmt + 1$) into

Fig. 2 Schematic illustration of the advantage of the first sexual individual resulting from the seesaw effect. Possible combinations of the sex allele (S) and non-sex allele (N) entering the clean genome (C) or dirty genome (D) are shown. S (dominant over N) controls meiosis and fusion. The first automictic selfing event is successful with a 50% probability. See text



clean and dirty gametes. We refer to this process as the seesaw effect (i.e., if one side goes down, then the other goes up). Subsequent automixis combines the duplicated clean gametes into a viable zygote (Fig. 2).

Importantly, when CS + CS occurs, S is fixed in the first reproduction event, and all the subsequent offspring bear the sex allele at the locus as homozygotes. This selfing simultaneously makes all loci homozygous except for loci with new mutations. Therefore, the first genetic reproduction event erases the genome-dilution cost not only at the sex locus but also at other loci (meiosis cannot dilute the fixed loci). In the first generation, the increasing rate (Darwinian fitness) of the sexual genotype is 1 [a mother cell produces a viable zygote (CS + CS)], but it increases to 2 in the following generations because all 4 homoelogenous isogametes are CS and result in 2 viable zygotes. Therefore, the sexual genotype increases twice every generation until *dms* return to *dmt* (by additional mutations) and soon takes over the asexual gene pool, in which most individuals reach *dmt* via Muller’s ratchet and will go extinct within a finite amount of time.

The number of sexual individuals increases rapidly in a population and they show not only selfing but also allogamy between isogametes from different individuals. This increased genetic variation in the population. New mutations that independently occur in the different individuals and the subsequent evolution of inner-arm recombination recreates the *dms* asymmetry (α) between genomes; thus, the seesaw effect further reduces *dms*. Although we described the seesaw effect as the process in unicellular organisms, it works also under multicellularity because meiosis is the process that started from a single cell (gametocyte) even in multicellular organisms.

Hypothesis 2: evolution of anisogamy via “inflated isogamy”

Definitions

The “inflated isogamy” is an intermediate step during the anisogamy evolution that allows a gradual increase of reproductive cost for females.

An evolutionary scenario of anisogamy

Why is “smart isogamy” so rare? There is a consensus that the first gametic reproduction event would have been isogamous, after which anisogamy evolved from isogamy (Parker et al. 1972; Maynard-Smith 1978; Togashi and Cox 2011; Lehtonen et al. 2012, 2016). Today, isogamy is observed in the limited unicellular organisms such as budding yeast (Greig and Leu 2009), *Chlamydomonas* (Harris 2009), and Pinnacles diatoms (Edlund and Stoermer 1997; Koester et al. 2007), while in complex multicellular organisms such as vertebrates, anisogamy is widespread. Because the sex allele fixation by seesaw effect erased genome-dilution cost and male production did not yet occur, isogamy is free from the twofold cost. Nevertheless, why is isogamy so rare (Matsuda and Abrams 1999)? Notwithstanding the transition from isogamy to anisogamy must have been hard because it bears male production cost, why was isogamy evolutionarily unstable?

Figure 3 shows the scheme of economy in each reproductive mode. Consider that a single parent invests $2R$ of resource (= mother cell size) to reproduction and minimal viable or developable size of offspring (daughter cell or zygote) is R . A unicellular asexual organism (size R) grows into size $2R$, then split into two size R daughter cells by mitosis binary fission (Fig. 3a). In isogamy (Fig. 3b), a size $2R$ matured mother cell split into four size $0.5R$ isogametes; isogamy ($0.5R + 0.5R$) resulted in one size R zygote, which grew into a size $2R$ mature cell and then reproduced again. Hereafter, we refer to this mode of sex as smart isogamy. “Smart” means that both sexes pay the minimal requirement (R) for embryo survival by splitting the bill. This is the basic economy of unicellular life. Parker et al. (1972, the famous PBS model) demonstrated the rapid transition from isogamy to anisogamy based on sperm competition argument, but they presupposed the existence of large variance in gamete size from sperm to eggs in a population. However, under unicellularity, the economy of smart isogamy would have been at a minimal viable level. Because there were no redundant resources, it must have been difficult to increase gamete size to the size of large eggs as supposed in the PBS model. If one sex had not produced an excessively large gamete (egg) in advance, the other sex, producing a small gamete (sperm), would not have evolved.

Multicellularity leads to anisogamy via “inflated isogamy”

Some theoretical works have suggested that multicellularity promotes the evolution of anisogamy from isogamy (Bulmer and Parker 2002; Lehtonen and Kokko 2010; Hanschen et al. 2018; Lehtonen and Parker 2019). Here, we propose a scenario of the evolutionary pathway to anisogamy. If

unicellular organisms participate in gametic reproduction, they must perform external fertilization because the internal space does not exist in their unicellular body. Although external fertilization requires many gametes to ensure fertilization because of the limited gamete-encounter rate, unicellular organisms cannot increase the number of gametes to more than four because a mother cell splits into four isogametes at meiosis. Thus, a higher degree of anisogamy (numerous sperm per egg) would require multicellularity. To achieve higher organic differentiation and further adaptation, organisms would also require the evolution of multicellularity.

In the early stage of outcrossing in a homoeologous sib colony, many highly related cells are the descendants of a single ancestor (relatedness ≈ 1) and aggregate together, as observed in *Volvox*. These cells might adhere to each other and evolve into a multicellular organism with differentiation between the soma and germ lines via kin-selected mutualism. Multicellularity enables greater resource storage than unicellularity due to both increased cell numbers and resource redundancy resulting from increased energy generation. Under this resource redundancy, we assume that the gametocyte sizes of both parents would increase to, for example, $4R$ (twice larger than smart isogamy), and that the resulting size R isogametes would then fuse into size $2R$ zygotes (Figs. 3c, 4). This would be a valuable investment because it would significantly increase offspring survival and would eventually induce the evolution of more complex organisms. Under such “inflated isogamy”, the size R gametes bears a twofold cost increase relative to smart isogamy (size $0.5R$ gametes), but this cost is evenly shouldered by both mating partners (i.e., no inequity between sexes). In other words, it is not the male production cost.

Male evolution as commensalism

Importantly, multicellularity consisting of highly related cells could increase the number of gametocytes, which enabled the production of more than four gametes. Inflated isogamy would have triggered one parent (becoming male) to cheat its mate (becoming female) by producing many small gametes (e.g., eight size $0.5R$ sperm from two size $2R$ spermatocytes) to fertilize more eggs from multiple mates because the female had already contributed the R resources necessary for embryo development. Consequently, the first example of anisogamy (gamete size; R in females, $0.5R$ in males) originated without a significant reduction in offspring survival because zygote size ($1.5R$) was still larger than the minimal viable size (R) (see Figs. 3d, 4).

However, this male cheating would have been tolerable for females because their investment (R) would not have changed from that under inflated isogamy. Thus, females would have suffered no additional costs, and only males

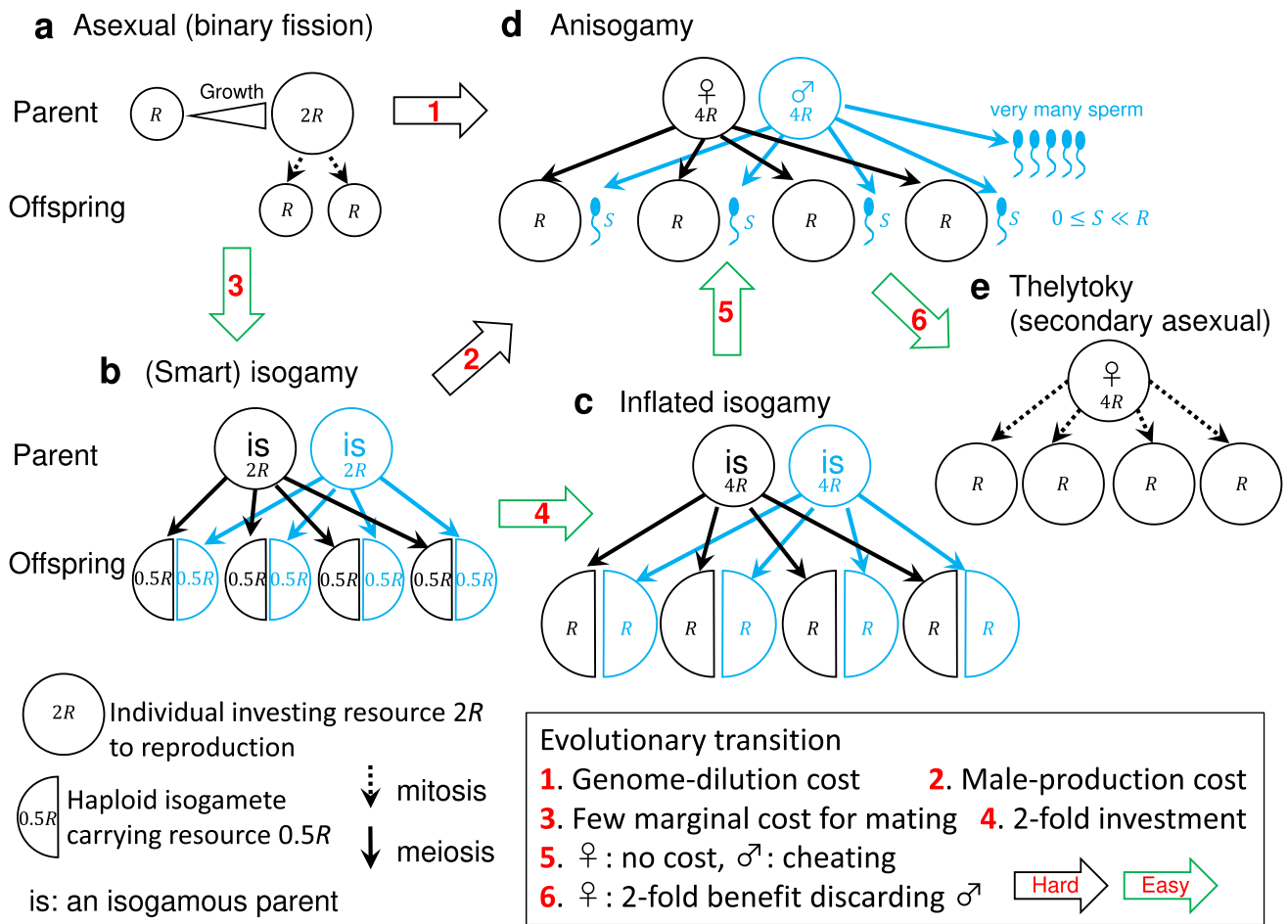


Fig. 3 Evolutionary pathways between asexual reproduction (a), smart isogamy (b), inflated isogamy (c), anisogamy (d) and thelytoky (e). The transitions 1, 2 and 4 are costly but 3, 5 and 6 are relatively

easy. The processes a and b presuppose unicellular organisms but c–e need multicellularity (greater amount of resources). See text

would have profited from this cheating (i.e., a kind of commensalism). Hence, some mutualistic coevolution between sexes could most likely have begun. The production of many tiny sperm increase the dispersal range and egg fertilization rate (Fig. 5), and provide genetic diversity benefits such as Red Queen. Outbreeding across different sib groups also creates new genetic variations for greater evolvability. Therefore, male cheating could provide a large benefit to females, and a smooth transition from isogamy to anisogamy would therefore be achieved.

Anisogamy increases the egg fertilization rate

An anisogamy-specific benefit for females is fertilization assurance. The greater the number and the smaller the size of the sperm males produce, the higher the egg fertilization rate will be. We examined this in simulations (Fig. 5a–c), specifically evaluating whether the first anisogamous mutant invades an isogamous population (see

“Appendix” for details). Even when the fertilization space was minimal (1×2 lattices), meaning that the wide-range dispersal of gametes was unnecessary, the probability that 4 sperm would fertilize one egg over 100 generations without failure was 0% (Fig. 5a). When the number of eggs was fixed at 4, the probabilities of 100-generation persistence in situations involving 4 sperm (equivalent to isogamy) or 8 sperm (minimum degree of anisogamy) were 7% and 78%, respectively (Fig. 5b). This means that to ensure the fertilization of 1 egg, at least 5 sperm are needed, and 8 sperm are required to ensure the fertilization of 4 eggs. To produce more than 4 sperm, more than one spermatoocyte (again, multicellularity) is necessary. The benefit of fertilization assurance would lead to the evolution of a very large number of sperm, as observed in species with external fertilization, such as corals and seaweeds. Therefore, isogamy could not be sustained in large fertilization spaces; instead, a higher degree of anisogamy must have been selected (Fig. 5c).

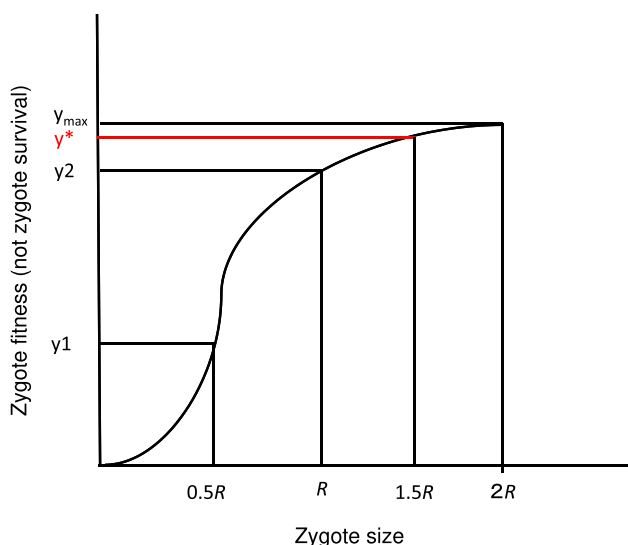


Fig. 4 The relationship between zygote size and zygote fitness (not zygote survival). In smart isogamy, two gametes (size $0.5R$) fuse into a zygote (size R). The unfused gamete cannot live (fitness y_1) because of insufficient resources and haploidy, but the zygote of size R shows a sufficient level of fitness (y_2). In inflated isogamy after multicellularization, the surplus resources abruptly increase gamete size to R , and the resulting size $2R$ zygote enables the development of a more complex body and greater adaptation. Zygote fitness is maximized (y_{\max}) at size $2R$, but this size is wastefully larger than the sufficient size ($1.5R$) in the early stage of multicellularity. Before the two sexes gradually cooperatively reduce gamete size from R to $0.75R$ to increase the number of offspring, one sex (male) rapidly downsizes its own gamete to $0.5R$ (male cheating led to anisogamy). However, the other sex (female) allows this situation because zygote fitness (y^*) is still sufficient (the difference between y_{\max} and y^* is not significant), and female investment (R) does not differ from that under inflated isogamy. In other words, the optimal gamete size under sexual cooperation is $0.75R$, but sexual conflict leads to gamete dimorphism (R in egg and $0.5R$ in sperm). See text

Counteradaptation of females

The increased resource redundancy resulting from multicellularity enables an increase in egg numbers while maintaining egg size in females. The number of oocytes could increase, and the oocytes could then undergo division into one large egg and three small polar bodies via two sequential cell divisions, as observed in present-day meiosis. Egg size has been optimized via size-number trade-offs (Parker et al. 1972; Maynard-Smith 1982). The mass production of large eggs directly increases female fitness (i.e., the number of surviving offspring). Females have since evolved to supply all zygote resources without depending on males, while males have shown a continuous increase in sperm number with decreasing sperm size (Fig. 3d). This situation led to the basic sex roles of females and males and further to various forms of sexual selection and sexual conflict. Thus, sexual dimorphism escalated further, and the presently observed dominance of anisogamy inevitably occurred.

In some phylogenies such as those of insects, fishes and reptiles, females have evolved to discard the high male production cost by developing thelytoky as a secondary mode of asexual reproduction (Fig. 3e). Because of this “twofold benefit” (increase in genome transfer from n to $2n$), thelytoky has repeatedly evolved and flourished transiently but cannot be sustained over a longer period due to dm accumulation (Butlin 2002; Normark 2003; Simon et al. 2003; Schwander and Crespi 2009).

Discussion

The first gametic sex and anisogamy

Many hypotheses successfully explain the maintenance mechanisms of sex but the mechanisms favoring the first individual bearing a sex mutation are still unclear. Likewise, the scenario enabling a smooth transition from isogamy to anisogamy imposing the twofold cost is not fully understood. We have explained these issues according to the seesaw effect and inflated isogamy. The seesaw effect of automictic selfing does not require another sexual individual at the origin of gametic sex. Mutations independently occurring in two genomes of the sexual individual (dms in diploidy) are unevenly divided into gametes ($\frac{dms}{2} + \alpha$ and $\frac{dms}{2} - \alpha$: α is a positive value representing the deviation from the equal division of dms). This is the only necessary condition, which is satisfied in most cases ($\alpha \gg 1$).

Previous studies have considered automixis only in the sense of negative consequences, such as the loss of heterozygosity and inbreeding depression, and could not explain why automixis has been sustained across diverse taxa from yeast to insects and reptiles (Matsuura et al. 2004, 2009; Engelstadter 2017). This study shows that the seesaw effect achieved by automixis reduces deleterious genes. If thelytokous species usually produce clonal offspring without meiosis but periodically perform automictic selfing, they may purge the dms accumulated during asexual generations via the seesaw effect. If this is the case, the seesaw effect may be the key mechanism preventing these species from going extinct. Instead, in such reproductive modes, limited genetic diversity restricts adaptability to changing environments, but some thelytokous species could persist in specific niches (mostly as relic species such as Komodo dragons; Watts et al. 2006). Thus, automixis may have a positive function in certain situations.

Three cautions regarding the cost of sex

Here, we note three cautions that should be considered when arguing the cost of sex. First, meiosis does not necessarily always enforce the genome-dilution cost in sexual

organisms. In fact, the authors of some previous studies (e.g., Dawkins 1976; Lehtonen et al. 2012) have argued that assortative mating can instantly fix the sex allele in a descendant lineage, and thereafter, the genome-dilution cost disappears because mating occurs only between the individuals bearing the sex allele. However, the first sexual individual could not find a mate. In contrast in our scenario, fusion between CS (clean and sexual) gametes in the form of automictic selfing is a mechanical necessity. Mate searching and discriminating, which are required in assortative mating are all unnecessary in our case.

Importantly, automictic selfing also fixes all other alleles in the genome at the time of the first reproduction event. Thereafter, the twofold cost disappears for entire genome, where the interests of all genes coincide. Moreover, because the evolutionary interests of all the homozygous alleles at all the loci in all individuals coincided within the offspring population (clonal colony), multicellularization as the necessary step toward anisogamy evolution would occur smoothly.

Instead, genetic diversity that is necessary for further evolution is lost by selfing. Newly occurring mutations and subsequent outcrossing (initially within the sib colony and later between non-kin individuals) would create heterozygosity at all loci in offspring populations and revive the twofold cost. The offspring of the NN genotype at the sex-controlling locus, which would be generated by selfing or sib mating between SN mutants, would return to asexuality and avoid the cost of sex, but this lineage is ultimately destined to go extinct again based on Muller's ratchet. Thus, stabilizing selection would retain the SS genotype (i.e., sexuality), but sex would enforce the genome-dilution cost at the other heterozygotic loci, leading to intragenomic conflict. However, this conflict would result in the victory of the sex locus because the secondary asexuals soon go extinct and the other loci must require genetic diversity for further evolution, even if they incur a twofold cost. Therefore, all loci would finally reach a point of compromise leading to coexistence under sexuality.

Second, we should not confound investments with costs. Investments should be increased if they are beneficial, but costs should always be reduced. As life evolved from simple unicellular organisms to complex multicellular organisms, the construction cost increased enormously, but this may have been a high-return investment. This considerable investment in the body absorbed the mere twofold cost of eggs. Considering mammals that supply nutrition to their offspring via placentation and lactation, the sex difference in gamete size is no longer problematic. This study explains the evolution of anisogamy by assuming the occurrence of inflated isogamy (an intermediate step between smart isogamy and anisogamy; Fig. 4). Inflated isogamy is a necessary step because if one sex shows a reduced investment under smart isogamy, only the embryo

(size $\ll R$) will die (male cheating is impossible; Fig. 4). Then, male–female coevolution would continue in either a synergistic or antagonistic (sexual conflict; Lessells et al. 2009) manner, leading to the present-day diversity of the reproductive system.

Third, we should not overlook the notion that complex multicellular organisms require anisogamy irrespective of its cost. When diploid multicellular organisms reproduce sexually, they have to produce haploid germ cells that represent their genetic information because it is impossible to fuse each of the billions of cells in a differentiated body with those of another individual, and somatic cells have lost the totipotency that is necessary for the organic differentiation of embryos. Multicellularization allows more advanced adaptations due to the division of labor among differentiated (but genetically identical) cells within individuals. However, relatedness among cells in a multicellular individual will necessarily decrease due to independent mutations occurring during differentiation. Gametic sexual reproduction, namely, restarting from a single stem cell, can solve specific problems of multicellularization at the same time: (1) it resets the relatedness in an individual body to 1, (2) it recovers totipotency, and (3) it purges deleterious genes from genetic lineages. Another reason why isogamy does not exist in the multicellular organisms larger than plankton is that they require large amounts of resources (large zygotes) for ontogeny, but fusion between two very large cells (two eggs) seems physically impossible. Among external fertilizers, a large amount of cytoplasm disturbs fusion, in addition to decreasing mobility (mate searching cost; Parker et al. 1972; Lehtonen et al. 2012). Among internal fertilizers, if a large nutritious egg is sent into the mate's reproductive tract, it will be consumed by the mate (sexual conflict; Lessells et al. 2009). Therefore, gametic sex is possible only as isogamy between micro-sized gametes or as fusion between large eggs and small sperm that can penetrate the egg cytoplasm. All these factors would force anisogamy on higher organisms.

Conclusion

Many hypotheses have been proposed to explain the evolution of sex and anisogamy, but what happened at the beginning of these process has remained unclear. Our scenario suggests that gametic reproduction could have originated as automictic selfing via the seesaw effect without the genome-dilution cost and that subsequent multicellularization would promote the evolution from isogamy to anisogamy by bypassing the male production cost (via inflated isogamy). Although our hypotheses are still speculative and are supported by little evidence, we

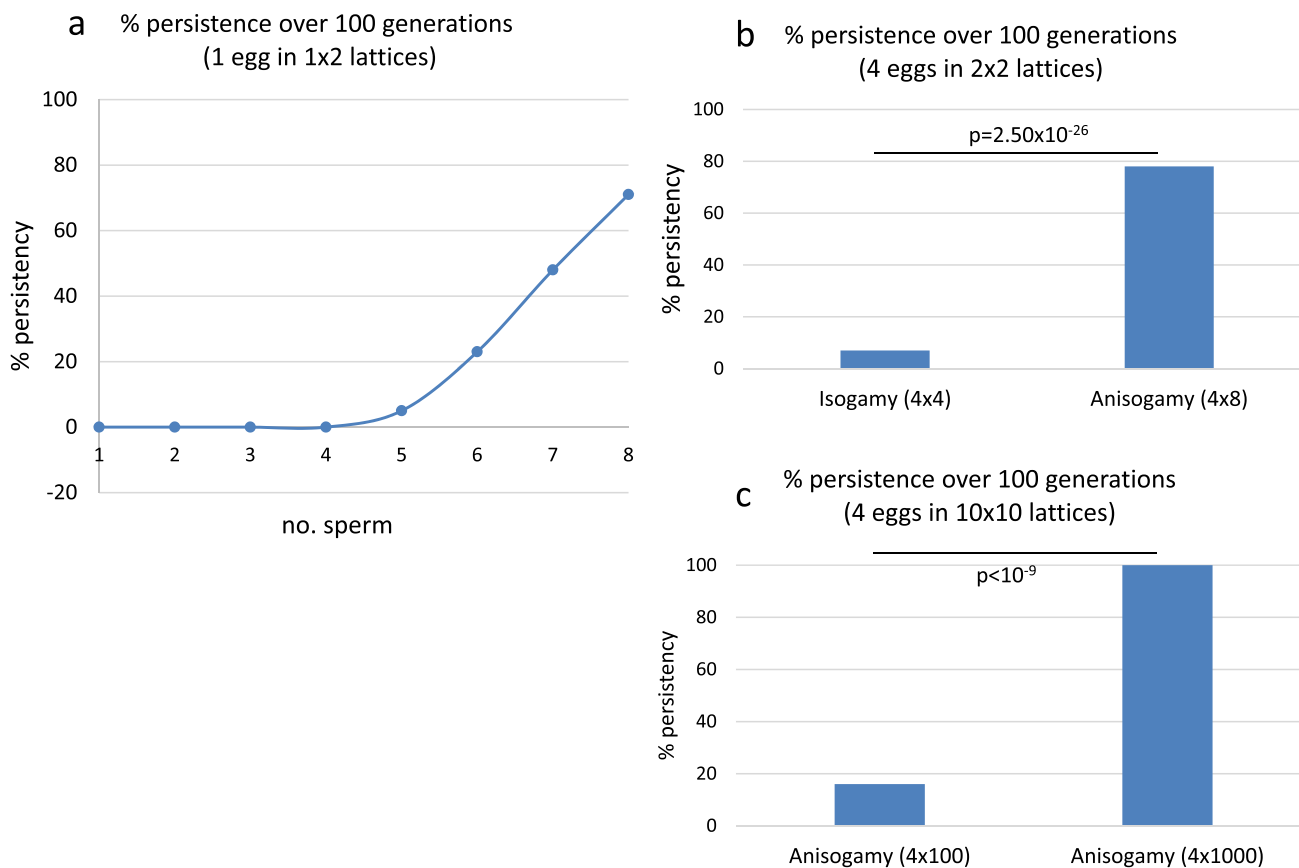


Fig. 5 Simulations of the “sperm limitation” problem. Anisogamy reduces unfertilized eggs (by reducing mate search or encounter costs inherent to isogamy). In every generation, a fixed number of eggs and sperm randomly enter a small lattice (size 1×2 cells and 2×2 cells). If eggs and sperm exist in the same cell, fertilization occurs and the female lineage continues to the next generation. The probability that an egg’s genetic line would continue for 100 generations was estimated. **a**. The relationship between the degree of anisogamy (1 egg to 1–8 sperm) and 100-generation persistency in a fertilization space with minimal structure (1×2 lattices) (100 iterations). Five or more

sperm are needed to increase the persistence rate to more than 5%. **b**. Comparison of the 100-generation persistence between isogamy (4 isogametes \times 4 isogametes) and anisogamy (4 eggs \times 8 sperm) in 2×2 lattices (100 iterations). To fertilize at least one of 4 gametes of the focal parent across 100 generations, the partner’s 4 isogametes are insufficient, but 8 sperm are successful. The difference is highly significant (Fisher’s exact probability test). **c**. Comparison between anisogamy (4 eggs \times 100 sperm) and anisogamy (4 eggs \times 1000 sperm) in 10×10 lattices (100 iterations). A large fertilization space requires an enormous number of sperm

hope that this framework will stimulate many theoretical and empirical studies in this field. Five questions are particularly important: (1) whether automixis was the initial form of gametic fusion, (2) whether automixis significantly reduces deleterious genes in the genome (i.e., the seesaw effect), (3) whether thelytokous species usually produce apomictic eggs but sometimes or periodically perform automictic selfing, (4) whether multicellularity promoted anisogamy (Knowlton 1974; Hanschen et al. 2018), and (5) whether the increase in isogamete size (i.e., inflated isogamy) occurred prior to the evolution of anisogamy. Question (2) can be tested in model organisms such as budding yeast, and question (3) is also testable in organisms such as insects and reptiles, while the other questions will require historical, comparative and phylogenetic analyses.

Appendix

Methods

Computer simulation of anisogamy and the fertilization rate (Fig. 5)

The relationship between the fertilization space and the degree of anisogamy and its effect on the persistence of a genetic line across 100 generations was examined via the following simulation. The program was written in Mathematica (Wolfram language) for Windows ver. 12.2 (Wolfram Research).

1. Imagine an isogamous species with external fertilization in a marine environment as an ancestral state of ani-

sogamy evolution. These organisms would produce the minimum number of isogametes and fertilization would occur within a short range. Unicellular organisms can produce a maximum of only 4 gametes, but multicellularization enables anisogamy with the production of more and smaller gametes (i.e., sperm). The movement of eggs and sperm simply depends on the water flow. For simplicity, we focused on the effect of gamete numbers (not mobility); thus, the results are conservative estimations ignoring sperm mobility. (In isogamy, the words egg and sperm refer to heterosexual isogametes.)

2. First, we considered a minimal fertilization space. A single unfertilized egg was placed randomly in a cell of a 1×2 lattice space. Various numbers (1–8) of sperm were also scattered randomly over the lattice space. Fertilization occurred only in the cells containing both an egg and sperm. The fertilized egg developed into an adult female. The female produced 1 egg for the next generation. The number of eggs and sperm in each generation was fixed because we wanted to confirm the independent effect of the degree of anisogamy (no. sperm/no. eggs = 1 in isogamy and 2–8 in anisogamy) on the persistence of a single mother's lineage. If the number of eggs and sperm increases in a small space, the fertilization rate is soon saturated at 100% (making it impossible to detect differences).
3. If the egg remained unfertilized, the female lineage went extinct. Based on 100 trials, we calculated the probability that the focal female lineage would be sustained for 100 generations (% persistence) and examined the relationship of this value and the degree of anisogamy.
4. Next, in a 2×2 lattice space, we compared the 100-generation persistence between isogamy (4 isogametes \times 4 isogametes) and anisogamy (4 eggs \times 8 sperm). To evaluate the effects of a large fertilization space, 2 anisogamous cases (4 eggs \times 100 sperm and 4 eggs \times 1000 sperm) in 10×10 lattices were compared.

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Data archiving The codes of the simulations will be provided as an Electronic Supplementary Material.

Declarations

Conflict of interest The authors declare no potential conflict of interest. We followed all ethics guidelines of the journal.

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