Nuclear selection is effectively policed by mating restrictions of the dikaryotic life cycle of mushroom-forming fungi.

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Abstract: Altruistic social interactions generally evolve between genetically related individuals or other replicators, whereas sexual interactions usually occur among unrelated individuals. This tension between social and sexual interactions is resolved by policing mechanisms enforcing cooperation among genetically unrelated entities. For example, most organisms with two haploid genomes are diploid, both genomes encapsulated inside a single nuclear envelope. A fascinating exception to this are Basidiomycete fungi, where the two haploid genomes remain separate. Uniquely, the haploid nuclei of the dikaryon can fertilize subsequent gametes encountered, the presumed benefit of this lifecycle. The implications for the balance of selection within and among individuals are largely unexplored. We modelled the implications of a fitness tradeoff at the level of the haploid nucleus versus the level of the fungal individual. We show that the most important policing mechanism is prohibition of fusion between dikaryons, which can otherwise select for detrimental levels of nuclear mating fitness. An additional policing mechanism revealed by our model is linkage between loci with fitness consequences. Our results show that benefits of di-mon matings must be paired with policing mechanisms to avoid uncontrolled selection at the level of the nuclei. Furthermore, we discuss evolutionary implications of recent claims of nuclear exchange in related fungal groups.

Keywords: Basidiomycete, Dikaryon, Di-mon mating, Modelling, Cooperation

Introduction:

Kin-selection theory shows that altruistic interactions between individuals, or between other replicating entities, can evolve between genetically related replicators (Hamilton, 1964). Examples are altruistic interactions between full sisters in social insects, cooperation between copies of mitochondrial DNA within eukaryotic cells, and cooperation between clonal cells of multicellular individuals. In sharp contrast to social interactions, sexual interactions typically are avoided among genetically related and promoted among genetically unrelated individuals. This difference in preferred relatedness creates a tension between sexual and social interactions. This is illustrated in parental conflict over resource allocation to offspring. In species where females can carry embryos from multiple males, paternal genomes compete to extract nutrients from a common uterus/ovary (Haig, 1993). The competition between genetic elements, in this case paternal alleles from different embryos, inevitably leads to conflict over limited resources from the mother. Any paternal competitive trait expressed in the embryo provides the selective ground for modifiers of maternal origin to repress these competitive traits (Rice & Holland, 1997), which can be resolved by mechanisms such as genomic imprinting.

Most sexually reproducing organisms have such mechanisms to police competition among the unrelated genetic elements or to re-establish high genetic relatedness upon sex. For example, social insects typically are monogamous, meaning that social interactions occur among full siblings (Boomsma, 2009). Clonal relatedness among organelles is maintained by uniparental transmission and a bottleneck during sexual reproduction (Cosmides & Tooby, 1981). In multicellular organisms, the genomes from two parents are typically united in the single diploid nucleus of the fertilized egg, and thus co-transmitted through mitotic divisions to form a multicellular offspring with diploid cells that are genetically identical barring *de novo* mutations (Buss, 1987). To reduce conflict between the two gametic genomes in diploids plasmogamy is immediately followed by karyogamy, aligning the fates of the two genomes until the next meiosis. Tension is reduced between the unrelated haploid genomes because the benefits of a competitive allele from one genome are shared equally with the other genome. However, Basidiomycetes, the fungal clade containing mushrooms, rusts and smuts, provide an exception to this general phenomenon.

Basidiomycetes use delayed karyogamy to increase mating opportunities. In these fungi the fusion of gametic plasma membranes, plasmogamy, occurs between two multicellular gametes (monokaryons), but nuclear fusion, karyogamy, is delayed until immediately prior to meiosis. The mated organism with two separated haploid nuclei, termed a dikaryon, is genetically similar to a diploid by having two copies of the nuclear genome per cell, although gene regulation may be fundamentally different due to the compartmental genomes (Banuett, 2015; Schuurs et al., 1998). The dikaryotic state differs as the separate haploid nuclei retain the ability to fertilize further monokaryons (reviewed in Raper, 1966). Since the fertilization of a monokaryon is not associated with resource

investment in the resulting new dikaryon, the dikaryotic lifecycle corresponds to the retention of the male role (Aanen et al., 2004). This unique form of mating between a dikaryon and monokaryon is known as di-mon mating, often called Buller mating (Buller, 1934). Di-mon mating, clearly impossible for a diploid organism, has been thought to be the main benefit of the dikaryotic lifestyle (Raper, 1966 p.264). The exchange of nuclei between dikaryons is thought to be prevented in nature since fusion of two dikaryons triggers non-self recognition followed by apoptosis, quickly killing the fused cell (Aylmore & Todd, 1986). Since the nuclei in the dikaryon can participate in further matings, they can increase the resources available to that genotype, while remaining with the original partner in the original territory. However, the benefits of this delayed karyogamy presumably carry a cost due to persistent tension in social interactions between the unrelated nuclei.

These di-mon matings present a competitive arena for the paired nuclei. Due to the high number of mating types in Basidiomycetes, generally both nuclei of a dikaryon are capable of fertilizing an unrelated monokaryon. This results in competition between the nuclei, the dynamics of which are poorly understood. Initially both nuclei appear to invade hyphae of the monokaryon, but ultimately only one of the two dikaryotic nuclear genotypes fertilizes the entire monokaryon (J. B. Anderson & Kohn, 2007). Competitive success within the dikaryon could be based on variation in rates of nuclear division, migration or percentage of hyphae colonized (J. B. Anderson & Kohn, 2007; Raper, 1966; Vreeburg et al., 2016). Evidence of variation for mating success has been found in natural systems, where replicate di-mon matings consistently result with the same fertilizing nucleus (Ellingboe & Raper, 1962, 1962; Nieuwenhuis et al., 2011; Nogami et al., 2002). When background genetic variation was reduced by backcrossing, mating success between nuclei was shown to be transitive, with a hierarchy of mating competitiveness (Nogami et al., 2002). Given that intraspecific variation in mating success exists, the strength of selection for mating success is likely based on the historical frequency of di-mon matings. If a species has common di-mon matings, stronger selection for mating success should reduce the variation in mating success. However, since mating success is difficult to quantify, the magnitude of this variation remains unknown.

In fungi, two common measures of fitness are vegetative (mycelial) growth, and spore production (Pringle & Taylor, 2002). While these undoubtedly capture only a fraction of actual fitness variation between individuals, fitness components related to aspects like enzymatic variation have not yet been found (Allison et al., 2018). For these sessile organisms, vegetative growth is required to explore nearby resources, and spore production is needed for dispersal to new sites. It has been thought that fungal vegetative growth and spore production may be under a life history trade-off (Schoustra & Punzalan, 2012; Bastiaans, Debets, & Aanen, 2016; but see J. L. Anderson, Nieuwenhuis, & Johannesson, 2019). Such a tradeoff assumes that high vegetative growth to explore resources comes at the expense of either rapid or abundant spore production (Gilchrist et al., 2006; Pringle & Taylor, 2002).

To explore the dynamics of this dikaryotic lifecycle, we assumed a three-way tradeoff between growth, spore production, and mating success. We simulated the outcome for three lifecycles: diploid, standard dikaryon (with di-mon matings), and open dikaryon (a hypothetical life cycle without non-self recognition between dikaryons). While the open dikaryon is not known from nature, we explore potential consequences of such a dikaryon to understand this apparent absence. Ultimately our simulations aimed to answer the question: What is the consequence of the dikaryotic life cycle, compared to a diploid, for the levels at which selection occurs? This unique lifecycle allows for additional matings through di-mon matings, but presumably comes with the cost of selection at the level of the nucleus (James, 2015). Our results show that the standard dikaryon benefits from increased mating opportunities but pays the price of selection at the level of the nucleus reducing dikaryon fitness. However, this cost is greatly reduced compared to when exchange between dikaryons is allowed. Our simulations also show additional mechanisms to reduce the cost from parasitism, primarily through linkage.

Materials and Methods:

The model

The arena: The "substrate" on which the simulated mycelia live is two-dimensional, represented by a square lattice each site supporting a single mycelium, or section thereof. The lattice takes a toroidal topology with the first and the last site of each row/column being neighbours, so that the lattice has no edges. At time 0 the lattice is inoculated with a random initial pattern of spores from a pool of *m* different mating types. Each inoculated site contains the monokaryotic mycelium sprouted from a single spore.

Nuclear fitness: The nuclear fitness of each spore (and the corresponding monokaryon and dikaryon) consists of three heritable (genetically encoded) components:

1) vegetative fitness w_v which determines the rate G at which the mycelium expands to neighboring empty sites: $G = g \cdot w_{\mathrm{v}}$, where g is the basic rate of mycelial growth.;
2) reproductive fitness w_r which determines the rate R at which the mycelium produces spores: $R = r \cdot w_{\mathrm{r}}$, where r is the basic rate of spore production.;
3) mating fitness w_m that determines the propensity of the nucleus to become part of a dikaryon upon mating.

These three fitness components are traded off so that any one of them can increase only at the expense of the other two in a linear fashion: $w_v + w_r + w_m = 1.0$.

Non-linear fitness: We also explored non-linear trade-offs should constrain the combinations of possible w_v, w_r and w_m of a certain nucleus in a way such that any actual combination remains below the trade-off surface defined by $1 = (w_v^{\beta} + w_r^{\beta} + w_m^{\beta})$. Results of the non-linear fitness are shown in Supplemental Figure 3. In our model c = 1 in all cases.

Dikaryotic fitness: The vegetative and the reproductive fitness components (W_v and W_r) of dikaryons depend on the nuclear fitness components (w_v and w_r) of the two nuclei it harbors and on the dominance interaction, Θ. For example, dikaryotic mycelia grow into neighbouring sites at a rate $W_{\mathrm{v}} = g\left[w_{\mathrm{v},max} \cdot \Theta + w_{\mathrm{v},min} \cdot (1 - \Theta)\right]$, where g is the basic mycelial growth rate, $w_{\mathrm{v},max}$ is the vegetative fitness of the fitter nucleus and Θ specifies its phenotypic dominance over the less fit nucleus (of nuclear vegetative fitness $w_{\mathrm{v},min}$). Θ= 1.0 is absolute dominance, Θ= 0.0 means absolute recessivity. Reproductive fitness (i.e., the spore production rate) of the dikaryon is calculated the same way.

Mating: In all scenarios, monokaryotic mycelia of different mating types coming into spatial contact (i.e., those on neighboring sites) will sexually fuse and produce dikaryons if they are of different mating types. In case of more possible mates, the focal ("female") monokaryon chooses one of the compatible nuclei from the neighboring mycelia, with the chance that a given "male" nucleus is chosen depending on its mating success w_m. The three mating scenarios ("diploid": mon-mon; "standard dikaryon": di-mon; open dikaryon: di-di) differ in the number of nuclei competing for becoming one of the two actual nuclei taking over the fused mycelia. In mon-mon matings only the nuclei from monokaryons surrounding a focal receiving monokaryon; in di-mon matings the two nuclei of each dikaryon compete as well as surrounding monokaryons , and in the di-di scenario two of the four nuclei win, with the chances depending on w_m. The winning pair of nuclei spread all over the spatially connected parts of the affected male and female mycelia, transforming them into the same dikaryon.

Mutation: Spore production occurs with the sexual fusion of the two nuclei of the dikaryon, preceded by mutations during the dikaryotic state, and followed by meiosis. Mutations affect the nuclear fitness components (w_v, w_r and w_m), such that mutant fitnesses (fitness w_v', w_r' and w_m') are drawn from Gaussian ditributions with standard deviation σcentered on the parental values and scaled back to satisfy $w_v' + w_r' + w_m' = 1.0$.

Recombination: Recombination during meiosis is also interpreted on the phenotype level. We assume that the loci affecting a fitness component are equally linked in the genome with linkage parameter 0.0 $\leq \lambda \leq$ 1.0. A value λ = 0.0 means no linkage, equivalent to a state with many loci throughout the genome, resulting in all spores from a dikaryon having equal fitness components as all of them take the average of the parental fitnesses. Conversely, λ = 1.0 represents complete linkage, with all variation residing in a single locus, with half of the spores taking the phenotype (fitness) of one parent nucleus and the other half inheriting the other parent's corresponding fitness. Mutation precedes meiosis so spores produced from separate sites of a dikaryon, or from the same site over time, will differ from each other by mutations. Mating type is inherited from one of the parent nuclei and is itself linked to the fitnesses based on λ.

Updating algorithm: One generation of the simulations consists of *N* elementary updating steps; *N* is the number of sites in the lattice. An updating step starts with choosing a site at random, which if occupied dies with probability *d*. If the site is instead empty, the mycelia on the neighboring eight sites compete to occupy it, chances to win depending on their vegetative fitness. If the focal site is occupied and survives, it may engage in mating in the "female" role, provided that it is capable of mating with at least one of its neighbors. Mating type, mating scenario compatibility and mating success w_m of the potential "male" partners determines the actual outcome of mating, resulting in a dikaryon in accordance with the rules detailed in the *Mating* section above. If the focal site contains a dikaryotic mycelium, it may produce spores which are locally dispersed onto sites within the dispersal radius *R* of

the parental dikaryon, which germinate to monokaryotic mycelia in the next generation. One spore per site may survive on each empty site that has received spores; the survivor is chosen at random. Identical nucleotypes instantly fuse somatically upon spatial contact.

The rules and the algorithm of the simulations are summarized in Figure 1. Psuedocode for the simulation can be found in Supplemental Text 1.

Competitions: To compare the competitive advantage of dikaryotic male function (the standard dikaryon scenario), the simulation was modified to represent an allele conferring dikaryotic male function (DMF) linked to the mating loci, having 15 mating types with this allele, and 15 without. Homokaryons of either type were reproductively compatible, and the case of heterozygotes was evalued for the DMF allele being recessive, dominant, or co-dominant where only the nucleus with the DMF allele could participate in di-mon matings. Due to algorithmic complexity, we did not attempt a similar competition for dikaryotic female function (the open dikaryon scenario).

Parameter settings: Due to the number of parameters, we could not assess all possible combinations. Preliminary results showed that simulations with grid sizes of less than 150 squares had stochastic outcomes (data not shown). To combat this, results shown here were performed with a grid size of 300x300, except data from Figure 5 and 6 where due to computational resources, smaller grid sizes of 200x200 were used instead. Except where otherwise specificed the base parameters were: g = 0.1; r = 1; d = 0.3; gs = 300; σ=0.01; and simulations were run for 1000 iterations.

[Figure 1 here]

Results:

[Figure 2 here]

Genetic assumptions have a strong influence on equilibrium fitness distributions.

Simulations run under different scenarios (linked/unlinked, recessive/co-dominant/dominant) showed a strong influence of these assumptions on the resulting nuclear fitnesses (Fig. 2). The diploid, incapable of di-mon or di-di matings, resulted in stable populations over all tested genetic parameters. When fitness traits are unlinked, the nuclei cluster near the middle of the fitness space. If instead fitness traits are linked the nuclei cluster near a single optimum maximizing spore production. When the phenotypes are fully dominant ($\Theta = 1.0$) with linked fitness traits it results in one set of nuclei optimizing spore production and the others balancing vegetative growth and mating fitness, particularly with high growth rates (Fig Suppl. 2). Although the diploid does not take part in di-mon matings, competition during monokaryon matings continues to make use of some level of mating fitness. There is no obvious difference between the diploid simulations under the high spore (A) or low spore (B) state.

When dikaryotic male function is allowed through di-mon matings, the standard dikaryotic state found in nature, there is a strong drive for mating fitness. When recombination is allowed, in the unlinked scenarios, the nuclei exclusively optimize mating fitness. However, when recombination is restricted (i.e. when fitnesses are linked), a clear polymorphism emerges with one set of nuclei strongly optimizing mating fitness, and the other optimizing spore production. Changing the dominance coefficient of the vegetative growth and spore production phenotypes had quantitative effects on the proportion of the nuclei optimizing mating fitness (Figure 2 pie charts).

When di-di matings are allowed, the open dikaryon situation, the increased selection for mating fitness caused most scenarios to collapse. In scenarios with low spore production (Figure 2B), the open dikaryon population crashes, regardless of linkage or dominance. When spore production is increased (Figure 2A), the populations survive as less spore production fitness is required, but all nuclei optimize mating fitness. When phenotypes are recessive and fitnesses are linked, even this increased level of spore production is not sufficient to maintain the population.

To assess the stability of these assumptions, we performed the same simulations with two modifications to the global parameters, either increased basal growth rate, g, or increased mutational width, σ. Increaed basal growth rate allowed the open dikaryon to maintain stable populations (Figure Supplemental 1). Increased mutation width allowed the diploid unlinked scenario to optimize fitness around high spore production instead of a balance between the fitness componenets, as well as allowing more open dikaryon scenarios to survive.

[Figure 3 here]

Reduced recombination partitions mating fitness in the standard dikaryon

As the initial simulation showed clear differences between the linked and unlinked states, we more systematically investigated the influence of recombination. Linkage in these simulations refers to how the spores produced reflect the parental nuclei, prior to the post-meiotic mutational step. When linkage is 0, the fitness of spores produced is mid-point between the parents, and when linkage is 1 the spores have identical fitnesses to one of the parental nuclei. In effect, low linkage values reflect a situation with many loci involved, while high linkage reflects a single responsible locus. To visualize the effect of linkage, population size dynamics are shown in Figure 3A, and average population fitness proportions are shown in Figure 3B. As population size results from the balance of growth and the random death process, this size can be considered an outcome of average fitness at the level of the mycelium (Gilchrist et al., 2006). Figure 3A shows an element of dikaryon fitness, while 3B shows the fitness components of the individual nuclei.

In the diploid situation, increasing linkage led to an increase in population size. Figure 3B shows that this increase in population size was co-incident with an increase in the average spore production fitness, and a roughly symmetrical decrease in vegetative growth and mating fitness. For the standard dikaryon the lowest linkages value of 0.00 resulted in population collapse (again note Figure 3B only shows the first 500 generations), and linkage values of 0.75 and above resulted in stable populations, with increased linkage leading to larger populations. In the open dikaryon no amount of linkage was sufficient to prevent population collapse, and Figure 3B shows the similar rapid and irreversible increase in mating fitness across all linkages.

In the standard dikaryon, with increasing linkage there is a separation between the fitnesses of the nuclei that were the original monokaryon (the female role) versus those of the incoming mating nucleus (the male role). With high linkage, the male role is still occupied with nuclei that are specialized for mating fitness, but the female role is filled with nuclei that still retain appreciable amounts of vegetative and spore production fitness. Interestingly, in the standard dikaryon the highest linkages values of 0.99 and 1.00 show an increase in mating fitness in the female role as well, but this is purged by approximately generation 75.

[Figure 4 here]

Dikaryotic male function increases in frequency in direct competition.

Although our simulations aimed to address the consequences of three different life cycles for the balance between levels of selection, and not to study the evolution of transitions between them, we wanted to assess whether the dikaryotic state was competitive under the imposed fitness tradeoff. To this end, we

competed mixtures with varying starting proportions of nuclei with and without Dikaryotic Male Function (DMF). We simulated DMF as a dominant, co-dominant (each nucleus acting independently), or recessive trait. As seen in Figure 4, in most cases when DMF is allowed, it increases in frequency (rows 1 & 3), but with differences between the two nuclear position. When fitnesses are recombined in offspring (unlinked), the DMF allele frequency increases, particularly in the male nucleus. Importantly, in the unlinked scenario this increase in DMF frequency comes at the cost of total population size, which drops to approximately 1/3 of when DMF is not allowed. When fitnesses are not recombined in the offspring (fully linked situation), the DMF type still increases in the male nucleus (except when DMF is recessive), but the population size remains similar to when DMF is not allowed. Interestingly, in the scenario with full linkage, the initial increase in population size is halted due to increasing parasitic nuclei in the female role, but this is purged with the first 250 generations regardless of the starting proportion. This purging of parasites in the female role is less effective in the recessive case, presumably because the recessiveness hides the allele from selection at low proportions. Without dikaryotic male function (right column) there is no emergence of parasitic nuclei, and the proportion of DMF nuclei is consistent over time. As the decreased population size with increased mating fitness is predicated on an assumed fitness trade-off, we tested the effects of a non-linear tradeoff (Fig. S3). In all cases the DMF allele increased in frequency, and the effect of the non-linearity only affected the rate of increase and the resulting population size.

[Figure 5 here]

<u>Costs and benefits of dikaryotic lifecycle</u>

Next, we tested the effect of dikaryons on monokaryotic proportion of the population, the proportion of unmated individuals, under different environmental stabilities, by varying the death rate and growth rate. When these two rates are equal a given space has the same probability of dying as it does of being grown into again. When the death rate is higher than growth rate, then a larger proportion of cells will be available to be colonized by spores. As can be seen in Figure 5A, the populations were smaller with increasing death rate (y-axis) in all three types of dikaryons, and low growth rate combined with high death rate led to population collapses (white square in Figure 5A). The open dikaryon was susceptible to population crashes across a larger range of parameter values. In general, the diploid had the largest population size, the standard dikaryon intermediate, and the open dikaryon the smallest. Looking at the mating success, the proportion dikaryotic, in almost all scenarios the open dikaryon is 100% dikaryotic, while the diploid has a much higher percentage of monokaryons (Figure 5B). As it is difficult to visually compare the three scenarios based on color intensity, the overall averages of surviving populations are plotted in Figure 5C. This shows that indeed the open dikaryon has the highest proportion of dikaryons, but the lowest population size, and the opposite for the

diploid. The standard dikaryon is intermediate between the other two scenarios. Notably, there seems to be a relatively linear relationship between population size and proportion dikaryon in the three scenarios.

[Figure 6 here]

Factors that reduce spread of genotypes detrimental to the dikaryon

We investigated potential factors that in the standard dikaryon may influence the proportion of nuclei maximizing their mating fitness, which is detrimental to the dikaryon due to the imposed fitness tradeoff. We varied four factors: the number of mating types, total potential spore production, the basal growth rate, and the phenotypic dominance (Figure 6). As linkage is apparently an important factor for the levels of mating fitness, we first calculated the levels of mating fitness across a range of linkage values. The results showed difference below linkage values of 0.75 (Figure 6A). As the linkage increases the phenotypes are more unevenly distributed among nuclei, with increasing linkage resulting in increasing unevenness between nuclei. The results in Figure 6A are consistent with results from Figure 3B, but note that Figure 6A is presented per nuclei, while population averages are shown in Figure 3B. From these linkage values, we selected; 0.95 which showed almost all nuclei having >90% mating fitness, 0.99 which showed nuclei with a range of mating fitnesses, and 1.00 where a smaller proportion of nuclei optimized mating fitness over 90% (Figure 6B).

As individuals with the same mating type cannot mate, reduction of the number of mating types could be expected to reduce selection for mating fitness, as more spaces would be occupied by incompatible mates thus leaving fewer compatible mates for a given focal female. However, we saw little effect of this, and the only difference noticeable was a slight decrease of mating fitness with only two mating types when linkage was 0.99 or 1.00, but a slight increase in mating fitness when linkage was 0.95. Similarly, increasing spore production had a mild affect, increased parasitism when linkage was 0.95.

Increasing the growth rate with lower linkage (0.95) also decreased parasitism generally, particularly with values of g greater than 0.5. Particularly with the highest linkage values this decrease was more quantitative, with a similar number of nuclei being selected for mating fitness but instead resulting in less specialization. Increasing the dominance, especially above 0.5, resulted in increasing proportion of mating fitness specialists.

Discussion

While the mechanistic origin of the dikaryotic lifecycle is perhaps best left to cell biologists, it remains unclear how evolutionary forces act upon this unique life history. Here, we assess the impact of the dikaryotic lifecycle on the different levels of selection. The benefits of the dikaryon for increased mating opportunities seem obvious but the costs are perhaps less clear. As the nuclei within a dikaryon have low genetic relatedness, theory suggests that competition between them for additional mating partners inevitably leads to genomic conflict. Our results show increased selection at the level of the nucleus in the dikaryon compared to a diploid, despite our imposed fitness tradeoff at the organismal level (Figs. 2 & 3). Importantly the dikaryotic state results in an increased proportion of dikaryons in the population compared to a diploid (Fig. 4), due to the increased mating opportunities. As monokaryons generally have no reproductive output in Basidiomycetes, the resources they control (e.g. section of a tree stump, or ectomycorrhizal tree roots) represent an opportunity for nuclei from dikaryotic mycelial neighbors. Our results from Figure 5 show that the balance between the costs and benefits of a dikaryotic lifecycle are strongly influenced by the environment, in the sense of persistence and density of monokaryons.

A key assumption in our model is the tradeoff between the three fitness components. While we do not specify the underlying nature of the tradeoff, our assumption is consistent with antagonistic pleiotropy of the genes underlying the three fitness components. This may seem a strong assumption. However even without antagonistic pleiotropy, tradeoffs are likely (Stearns et al., 1984; Matthewson & Weisberg, 2009) particularly if selection occurs at multiple levels (Booth, 2014; Lewontin, 1970). If selection is predominately at the level of the nuclei, then selection will automatically be relaxed for the fitness components of the dikaryon, allowing accumulation of mutations deleterious for dikaryon fitness, resulting in a similar tradeoff. Modifying our trade-off with non-linearity resulted in population decreases proportional to the non-linearity (Figure Supplement 3). This shows that the fitness trade-off affects the resulting population size, but nuclear selection for mating success occurs regardless of deleterious effects. Importantly, nuclei capable of dikaryotic male function are selected for regardless of the linearity of the trade-off. Even when the fitness is strongly non-linear ($\beta=2$ in Figure Supplement 3), a strong fitness cost of mating fitness, the DMF allele has increased fitness.

A clear result of our simulations is the strong drive for mating fitness in the standard and open dikaryon. Under all tested scenarios and parameters the selection for mating fitness resulted in the majority of nuclei specializing in mating fitness. Depending on the parameters, this often resulted in population collapse. The selection benefit of di-mon mating was particularly visible when individual nuclei with and without dikaryotic male function (DMF) are competed directly (Figure 4). The DMF allele spreads throughout the population, even though it comes with the trade-off, regardless of the shape of the trade-off (Supplemental Figure 3). This is because the resident monokaryons provide a

415 reliable route for gene copy increase. The marginal decrease in fitness from vegetative growth/spore
416 production is outweighed by the benefit of nuclear migration. This drive for mating fitness is even
417 stronger in the open dikaryon, leading to population collapse under a wider range of parameter
418 settings than the standard dikaryon.
419
420 Our simulations indicate that reduced recombination limits the deleterious effects of nuclear
421 selection. The standard dikaryon, the natural state in most Basidiomycetes, was not sustainable with
422 low spore production, but increasing linkage reduces the effect of the trade-off (Fig. 2B, Fig. 3). Low
423 linkage in our model is equivalent to competitive loci spread throughout the genome. Considering only
424 fitness for a particular trait, free recombination between a nucleus with competitive alleles at multiple
425 loci and a nucleus without those competitive alleles would result in gametes competitive at half of
426 those loci. With increasing linkage, the nuclei produced from meiosis become increasing differentiated
427 into two types, producing either competitive or non-competitive gametes. Additionally, the details of
428 our model equate the amount of linkage to the proportion of competitive loci located within the
429 mating type determining locus. In general, Basidiomycete mating type loci are non-recombining and
430 have multiple genes, mainly transcription factors and pheromone/receptor pairs. These loci typically
431 span 50-150Kb (Brown & Casselton, 2001), but are greatly expanded in some lineages (Branco et al.,
432 2017). Of potential interest, backcrossing of wild isolates have shown that variation in mating success
433 potentially resides within the mating locus (Nogami et al., 2002). One testable hypothesis from our
434 model is that variation between strains in mating success will be correlated with decreased growth
435 and/or reproduction. Such "parasitic" mating types could be stable in a population since the tradeoffs,
436 between reduced growth and increased mating success, would be linked.
437
438 While it is often assumed that in fungi the mycelial individual (either monokaryon or dikaryon)
439 is the unit of selection, in multinucleate fungi this is not the only level (Lewontin, 1970). Our model
440 shows an example of how selection at the level of the nucleus, from competition between unrelated
441 nuclei for mating, could be detrimental to the level of the dikaryon. Nuclear selection has been shown
442 to act rapidly in hyphal fungi, where cheater nuclei can be selected in experimental evolution
443 (Bastiaans et al., 2016), but also in natural isolates of a dikaryotic ascomycete, *Neurospora tetrasperma*
444 (Meunier et al., 2017). While *N. tetrasperma* is not a basidiomycete, and thus presumably does not
445 engage in di-mon matings, selection at the level of the nucleus still has outcomes detrimental to the
446 dikaryon. An important factor in the dikaryotic fitness considerations is the level of phenotypic
447 dominance, which can mask deleterious mutations. The prevalence of dominant phenotypes in
448 Basidiomycetes is currently unknown, although recent work has found little support for dominant-
449 recessive relationships (Clergeot et al., 2019; Hiscox et al., 2010; Nobre et al., 2014). The presence of
450 the clamp connection found in most, but not all, Basidiomycete species, may provide a mechanism to
451 limit nuclear selection. These hyphal outgrowths, expressed in the dikaryon due to mating locus genes,
452 police most nuclear migration and prevent internuclear competition inside a dikaryon during

vegetative growth. However di-mon matings, and the accompanying selection, are not regulated by these clamp connections.

The selection of mating fitness in di-mon matings is a topic that has been discussed previously (James, 2015; Murata et al., 2005; Raper, 1966). The ideas of nuclear selection were not initially connected with fitness tradeoffs, instead predicting continued selection for fitter phenotypes (Raper, 1966). A recent reappraisal of intranuclear competition phrased it as the "selfish nucleus model" (James, 2015). Such selfish nuclei could result from tradeoffs between nuclear mating fitness and growth/sporulation at either the monokaryon or dikaryon stage, a levels of selection problem (Buss, 1987). Our results, open dikaryons resulting in detrimental levels of nuclear selection, are consistent with the intuition of James et al., who postulated the occurrence of these open dikaryons *"provided the negative effects of nuclear competition are not also increased"* (James, 2015). We expect selection at the level of the nucleus to inevitably come at some cost to the dikaryon, either because of antagonistic pleiotropy or due to mutation accumulation if selection at the mycelium level has reduced importance.

Nuclear exchange between dikaryons, di-di matings in our simulations, has been reported in both natural (Johannesson & Stenlid, 2004) and laboratory conditions (James et al., 2009). However, we argue this is likely not equivalent to an open dikaryon. Nuclear exchange between dikaryons is mainly documented in *Heterobasidion,* a common Basidiomycete study system, but one in which dikaryons have abundant monokaryotic hyphal sections (Johannesson & Stenlid, 2004). Presumably the nuclear exchange between dikaryons occurs through monokaryotic hyphal intermediates. This inclusion of monokaryotic hyphal intermediates makes this more similar to either di-mon or mon-mon mating, depending on how the monokaryotic sections are fertilized. Crucially, there is a qualitative difference between di-di and di-mon matings through monokaryotic intermediates, as novel dikaryons from the latter will be somatically incompatible with the two established dikaryotic progenitors, having no spatial access to further resources (Johannesson & Stenlid, 2004). Thus, the apparent di-di matings found in *Heterobasidion* would not experience the same evolutionary forces as if true di-di matings were allowed, due to the physical restriction of the novel dikaryons..

Our simulations aimed to understand mushroom-forming Basidiomycetes, but our interpretations conflict with relevant literature from rust fungi. Rust fungi, widely distributed obligate biotrophic plant pathogens, are another subphylum of Basidiomycota which also have a dikaryotic stage. Research on these pathogens has implicated the exchange of nuclei between dikaryons, "somatic hybridization", in the production of novel pathogenic rust races (Nelson et al., 1955). While initial evidence was based on isozymes (Burdon et al., 1981), recent genomic evidence has shown that variation between pathogenic races cannot be explained only by meiosis, with one nucleus shared between races (Li et al., 2019; Wu et al., 2019). This has been interpreted as exchange between

dikaryotic isolates (Fig. 7 of Li et al., 2019), what we call the open dikaryon. It is difficult to reconcile the concept of somatic hybridization with our results showing that open dikaryons are highly susceptible to nuclear selection (Fig 3). One possibility is that somatic hybridization is exceedingly rare, as a single somatic hybridization event could produce a rust race able to grow on otherwise resistant hosts. Another potential reconciliation would be monokaryotic intermediate hyphae, similar to that in *Heterobasidion* (Hansen et al., 1993; Johannesson & Stenlid, 2004), protecting the resources of the original dikaryons and thus limiting parasitism.

Additionally, the idea of nuclear selection is likely relevant to fungi other than Basidiomycetes. Recent evidence has shown that the abundant Arbuscular Mycorrhizal Fungi from the phylum Mucoromycota also exist in a dikaryotic state (Ropars et al., 2016). Field isolates appear to be either monokaryotic or dikaryotic (the number of nuclei per compartment can vary greatly so we here use mono/dikaryotic to refer to the number of classes of nuclei), although mating events have not yet been directly observed. While hyphal fusion occurs in this lineage (Croll et al., 2009), the potential for di-mon, or even di-di matings, remains unknown.

The open dikaryon of our model corresponds to the “unit mycelium” concept formulated to explain observations of cell fusions in cultured material (Buller, 1934). This “unit mycelium” concept was the working paradigm for many mycologists over many decades, with its assumption of cooperation between genetically unrelated individuals. However accumulating evidence from natural isolates, showing that neighboring dikaryotic mycelia were distinct, led to the influential synthesis by Rayner of the “individualistic mycelium” (Rayner, 1991). This model of distinct genets separated by allorecognition responses was consistent with the finding of somatic incompatibility between dikaryons, where fusions with non-self individuals lead to cell death, not cooperation (Booth, 2014). This is the model that mycologists generally use now, where dikaryons maintain their individuality and nuclei are only exchanged through monokaryotic intermediates. The “individualistic mycelium” is not only consistent with experimental evidence but also compatible with evolutionary theory, which would predict cooperation between non-related dikaryons to be exploited almost immediately (Czárán et al., 2014). The exchange of nuclei between dikaryons is predicted by our model to lead to extremely high levels of nuclear mating fitness, predicted to be detrimental for the individual.

The persistent dikaryotic state has been retained for over 400 million years (Chang et al., 2015), yet few extant species are diploid. The presence of rare diploid Basidiomycete species indicates that the transition to diploidy is possible. Yet, the retained dikaryon indicates something about the balance of the costs and benefits. Presumably the costs of selection at the nuclear level are outweighed by the benefit of di-mon matings, indicating that di-mon matings are a common occurrence. While it is extremely difficult to obtain data on the prevalence of di-mon matings, it is likely much more common than is generally assumed (Nieuwenhuis et al., 2013). Given the strong drive for mating fitness that a

dikaryon allows, for this state to persist the benefit must be larger than the cost. Without a mechanism to police the interactions between the unrelated nuclei, competition within a dikaryon will lead to its demise.

Conclusion:

Potential benefits of a dikaryon stage have been a subject of discussion for some time. The presence of the dikaryotic state emphasizes the level of selection on the individual nuclei, something that is often overlooked in evolutionary discussions of fungi. The persistent association between unrelated haploid nuclei will invariably select for mating success, to the detriment of the individual. Our results show the potential costs for mycelium-level fitness components of selection at the level of nuclei for mating, and how those can be mitigated/reduced. First, restricting fertilization by dikaryons to monokaryons reduces the level of parasitism, compared to completely free exchange between dikaryons. Second, restricting competitive loci to regions with reduced recombination may effectively reduce the effect of potential tradeoffs. Using the results of our model, we hypothesize that alleles conferring mating competitiveness may be linked closely to the mating type. We also hypothesize that recent examples of dikaryon nuclear exchange are due to monokaryotic intermediates, or result from extremely rare events. Our results show that conflict between the unrelated haploid nuclei in a dikaryon can be severe, and there must exist mechanisms to police it.

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Figure 1: Lifecycle of Basidiomycetes and schematic of model. A) Lifecycle of dikaryon showing the three different mating possibilities (mon-mon, di-mon, and di-di), as well as the separation between mating and karyogamy. B) Shows some rules of the algorithm and three types of mating, and which matings are found with each of the three lifecycles. C) Example of the actions taken during one generation of the simulation. Note that in this diagram matings are shown as deterministic based on mating fitness, but in the simulations the outcome of competition is probabilistic.

Figure 2: Equilibrium fitness distributions under different linkage values and dominance coefficients for the three dikaryon scenarios with A) high spore production or B) low spore production. Points represent individual nuclei remaining on the grid at end of simulation. Note that there is significant overlap of points, thus the amount of black is not proportional to population size. Shading of pie charts show percentage of nuclei with >66% mating fitness (w_m; region covered by bar). Simulations with no surviving population have no dots and associated pie charts have a red cross.

Figure 3: A) Population size with varying amounts of recombination across the three life strategies, initialized with 60000 squares occupied. Dotted vertical lines indicate region shown in B. B) Average proportion of fitness in nuclei per generation for the first 500 generations. Decreasing linkage values are shown on separate rows. The different fitness components are indicated by color: Red (top) is mating fitness, w_m, yellow (middle) is spore production, w_r; and green (bottom) is vegetative growth, w_v. White areas in Open Dikaryon are from dead populations. For each linkage value, the average fitnesses are calculated separately for nucleus 1 (the nucleus of the original monokaryon) and nucleus 2 (the fertilizing nucleus) Note that increased non-mating fitness in the open dikaryon with linkage of 0.99 is due to extremely small population sizes.

Figure 4: Results of competition between nuclei with and without DMF function. Proportion of DMF nuclei and proportion of parasitic nuclei (>66% mating fitness) are shown for the female nucleus (nucleus 1) in the top two rows, while the male nucleus (nucleus 2) is shown below. Note that the population begins as completely homokaryotic, thus the Nucleus 2 position is empty for generations 0. The bottom row shows the total population size. Results of different initial proportions of DMF:non-DMF nuclei are shown with different line styles.

Figure 3: Population size and mating success with differing environmental stability. A) Population size after 500 generations under varying death and growth rate. Intensity of black is proportional to population size. B) Mating success of simulations from panel A), but intensity of black refers to proportion of occupied cells that are dikaryotic. Orange values indicate simulations had no surviving cells. C) Grand mean of simulations used for A) and B) but grouped by scenario showing the relative differences in mating success and population size between the three scenarios.

Figure 6: A) Effect of linkage on mating fitness in the standard dikaryon. B) Mating fitness distributions for selected linkage values. C) Effect of parameters on the proportion of parasitic nuclei in the standard dikaryon scenario. Rows show results under linkage values of 0.95, 0.99, and 1.00. Columns from left to right show results of modifying the number of mating types, spore production, growth rate, and

dominance. Shading is proportional to mating fitness, calculated in steps of 10% across 4 replicates. White area represents nuclei with < 10% mating fitness.

Supplemental Figure 1: Overview of simulation outcomes using higher mutation width of 0.05 instead of 0.01 used in other simulations. Note that in the unlinked diploid sceanrio the nuclei do not cluster near the middle, but instead optimize spore production similar to the linked scenario.

Supplemental Figure 2: Overview of simulation outcomes using higher basal growth rate of 0.5 instead of 0.1 used in other simulations. Note that this increased growth rate allows the open dikaryon to survive, and increases the vegetative growth fitness of both diploid and standard dikaryon scenarios.

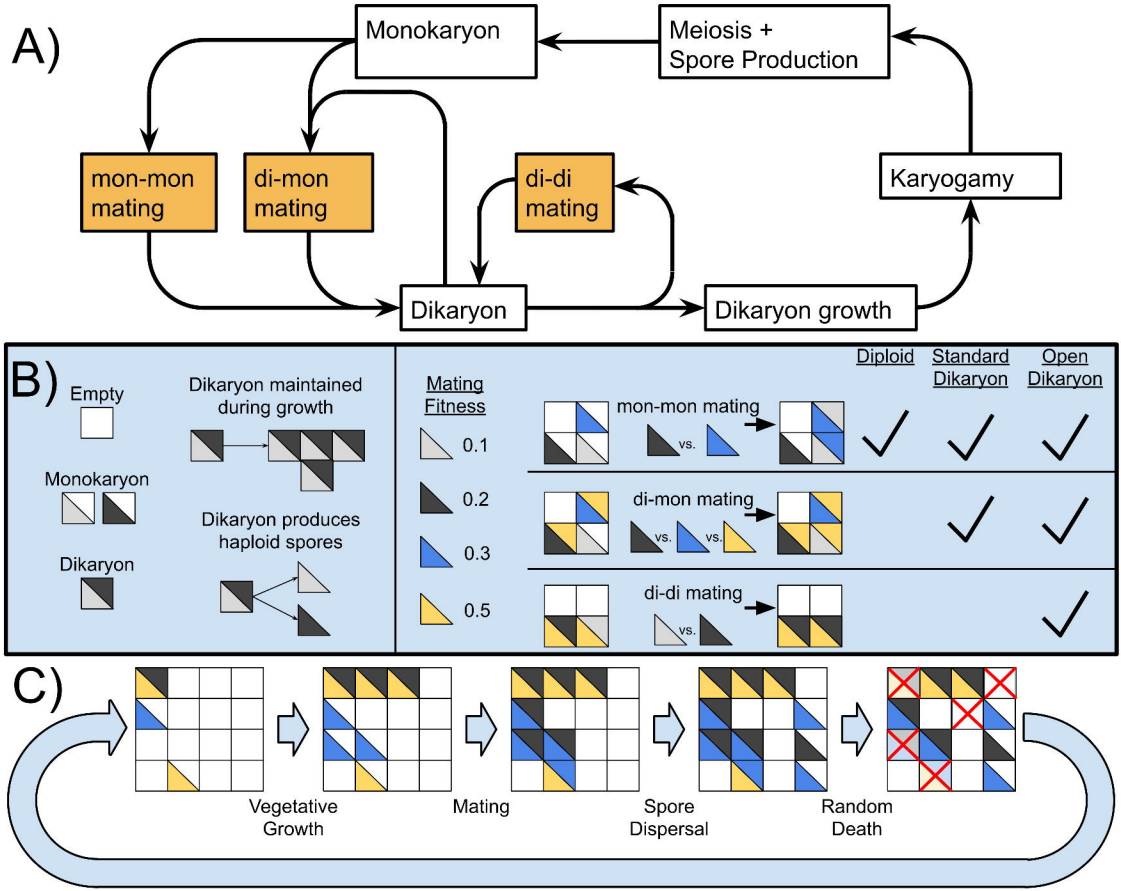
Supplemental Figure 3: Linearity of the fitness trade-off has limited effect on mating selection
Here is the figure for the non-linear fitness. It shows that in the co-dominant state, where DMF spreads fastest, the DMF allele spreads through the population regardless of trade-off shape. The beta value has some effect on the strength of selection, so with beta of 2 (the long dash line) in the unlinked scenario the population size is smallest since fitness costs are highest. So when unlinked the selection against the allele slows the spread of parasites, but not of the DMF allele itself, and it takes longest for the parasites to spread in the female role (nucleus 1).

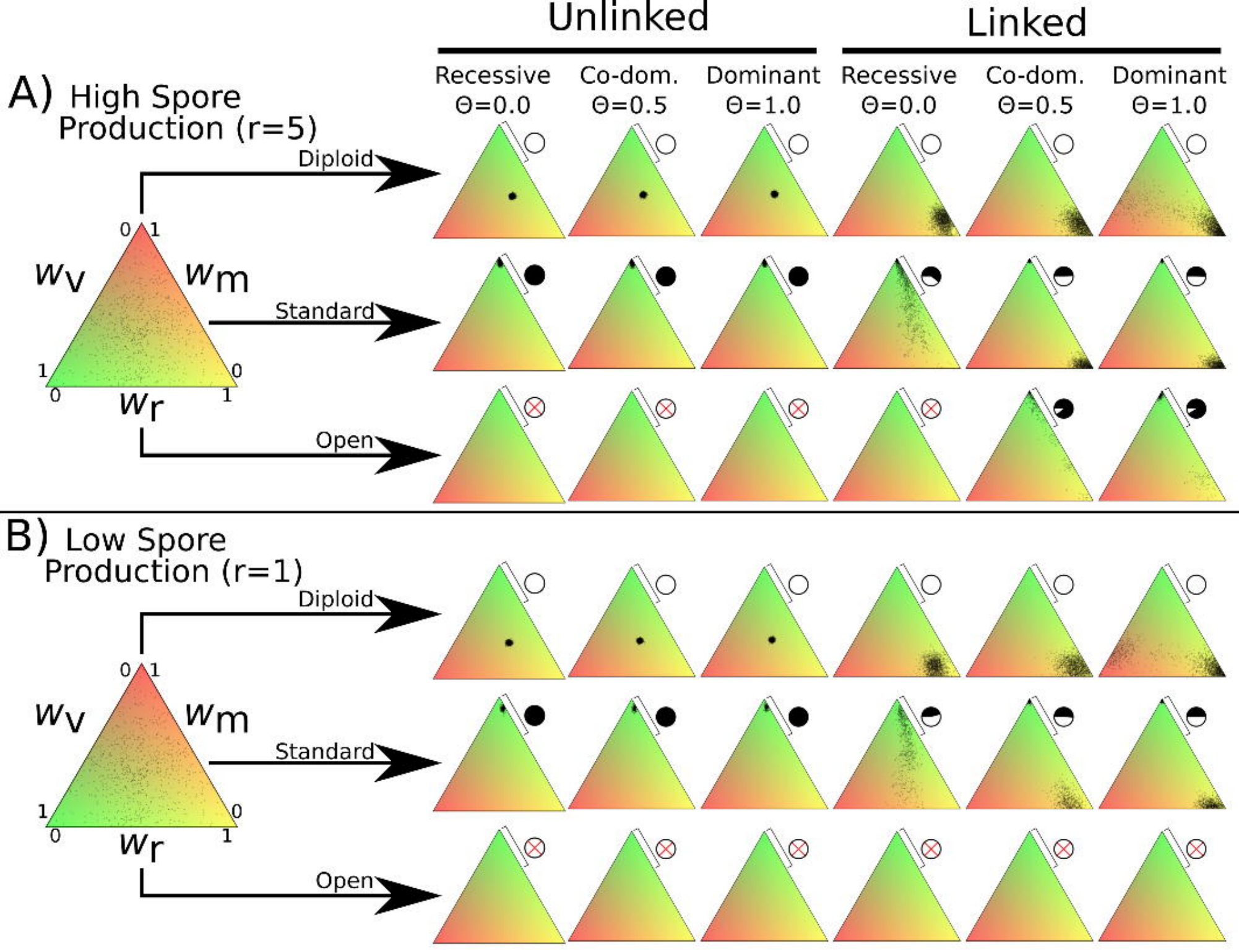
Supplemental File 1: Psuedocode for the simulation algorithm.

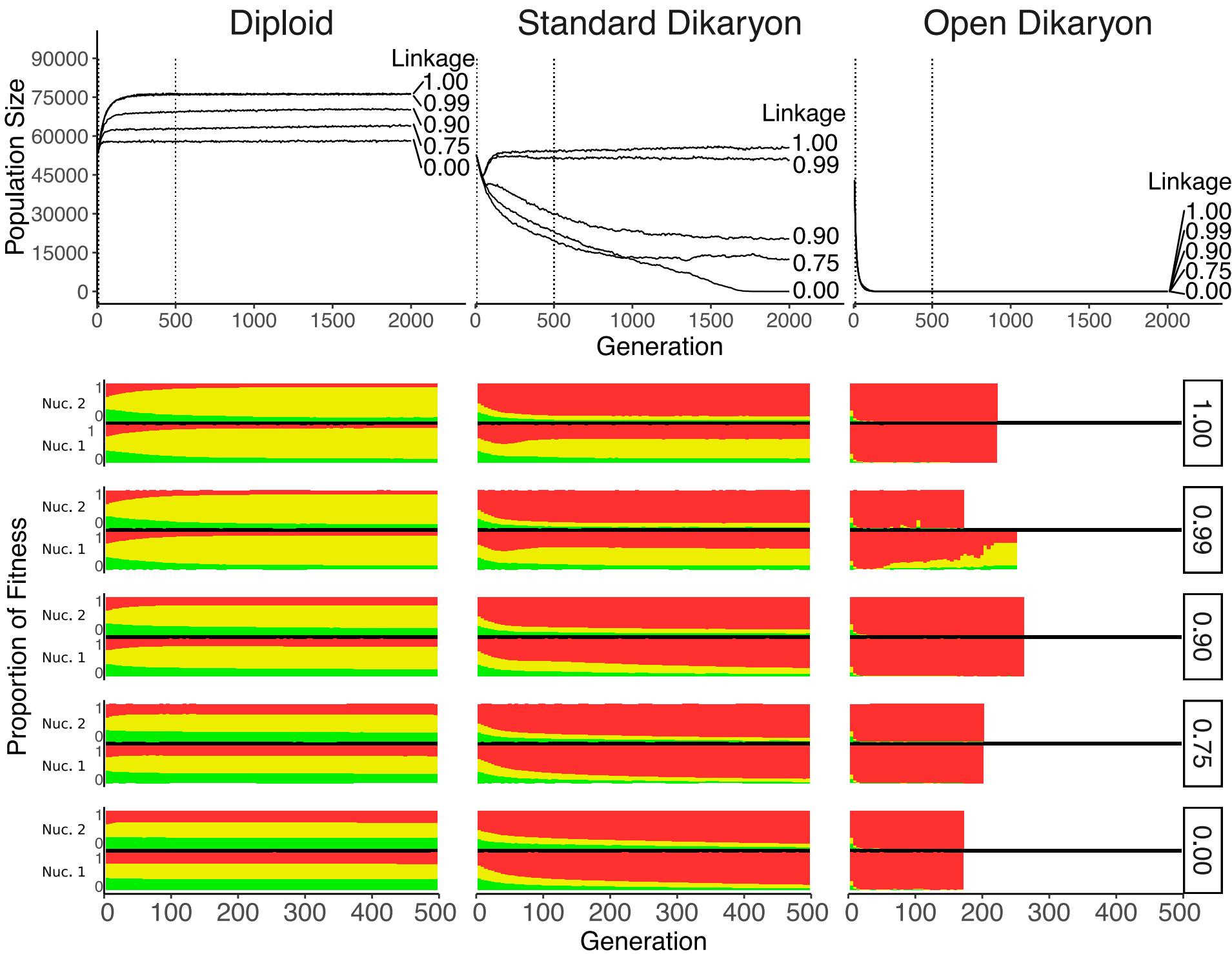
Supplemental File 2: Animation of the first 100 generations of the diploid life cycle under standard conditions. Dots indicate individual nuclei. Bars indicate population size, and “% maters” which is the proportion of nuclei with >66% of fitness allocated to mating fitness, similar to that shown in Figure 2.

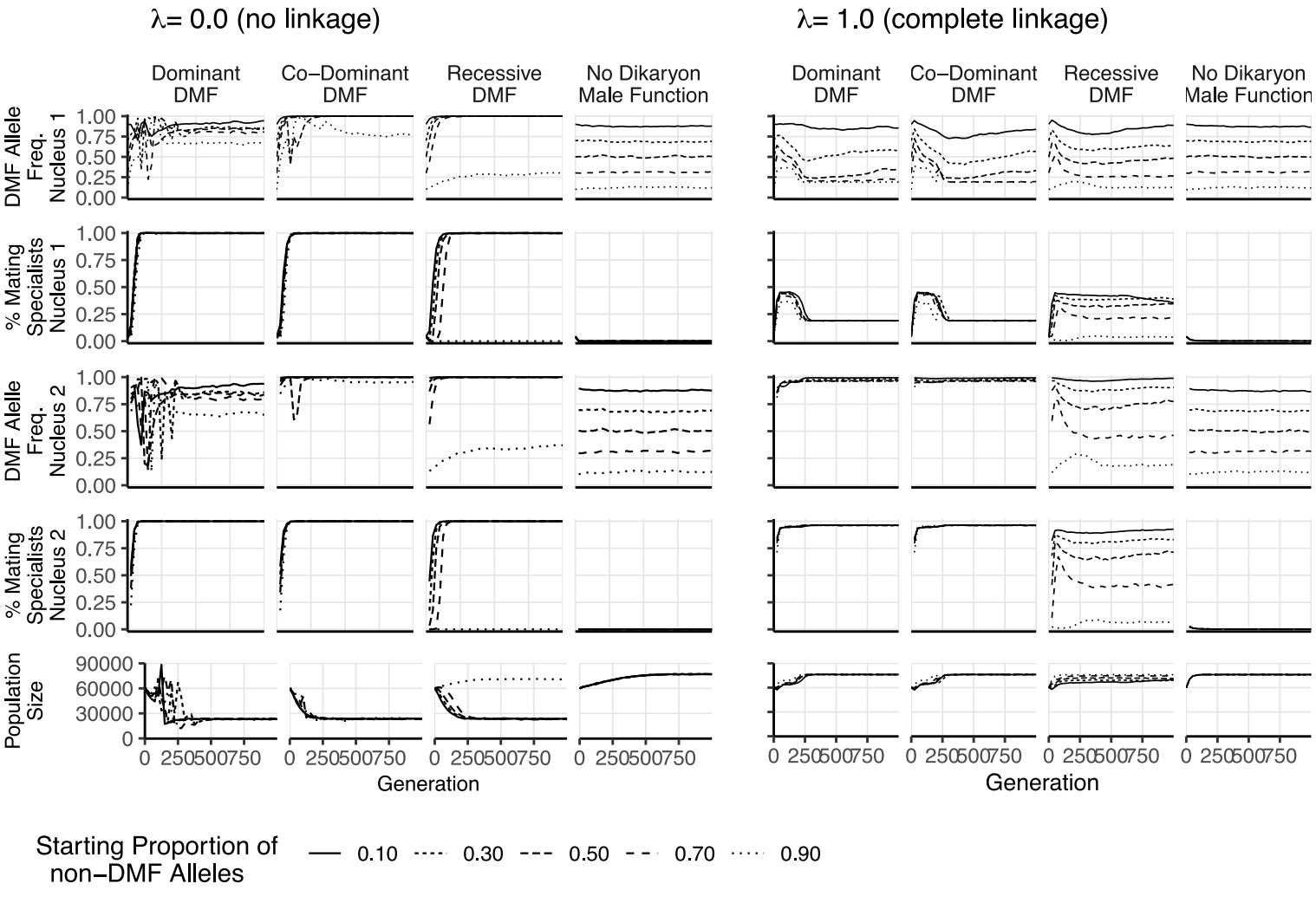
Supplemental File 3: Animation similar to Supplemental File 2, except of the standard dikaryon life cycle.

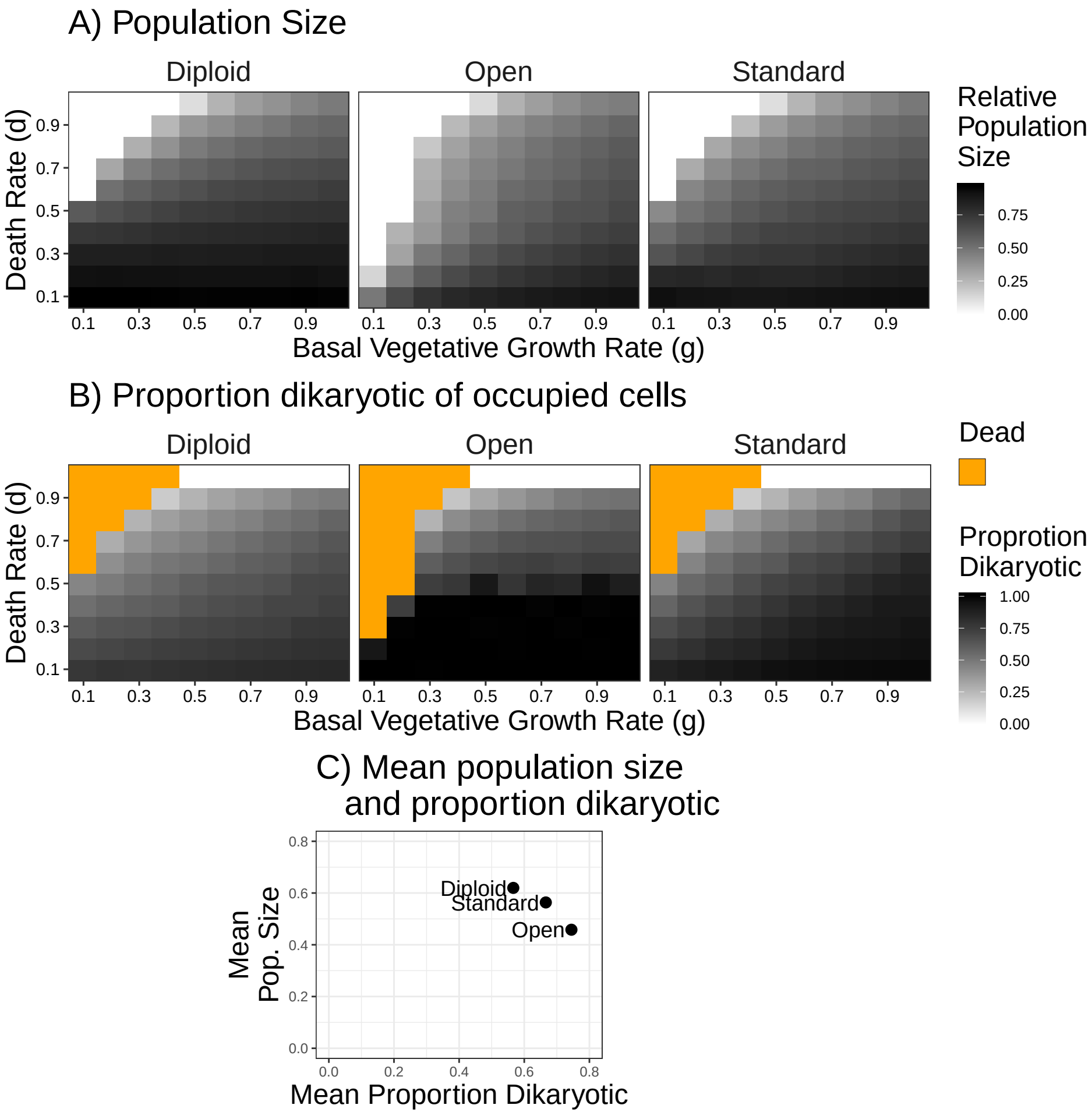
Supplemental File 4: Animation similar to Supplemental File 2, except this time with with open dikaryon life cycle.

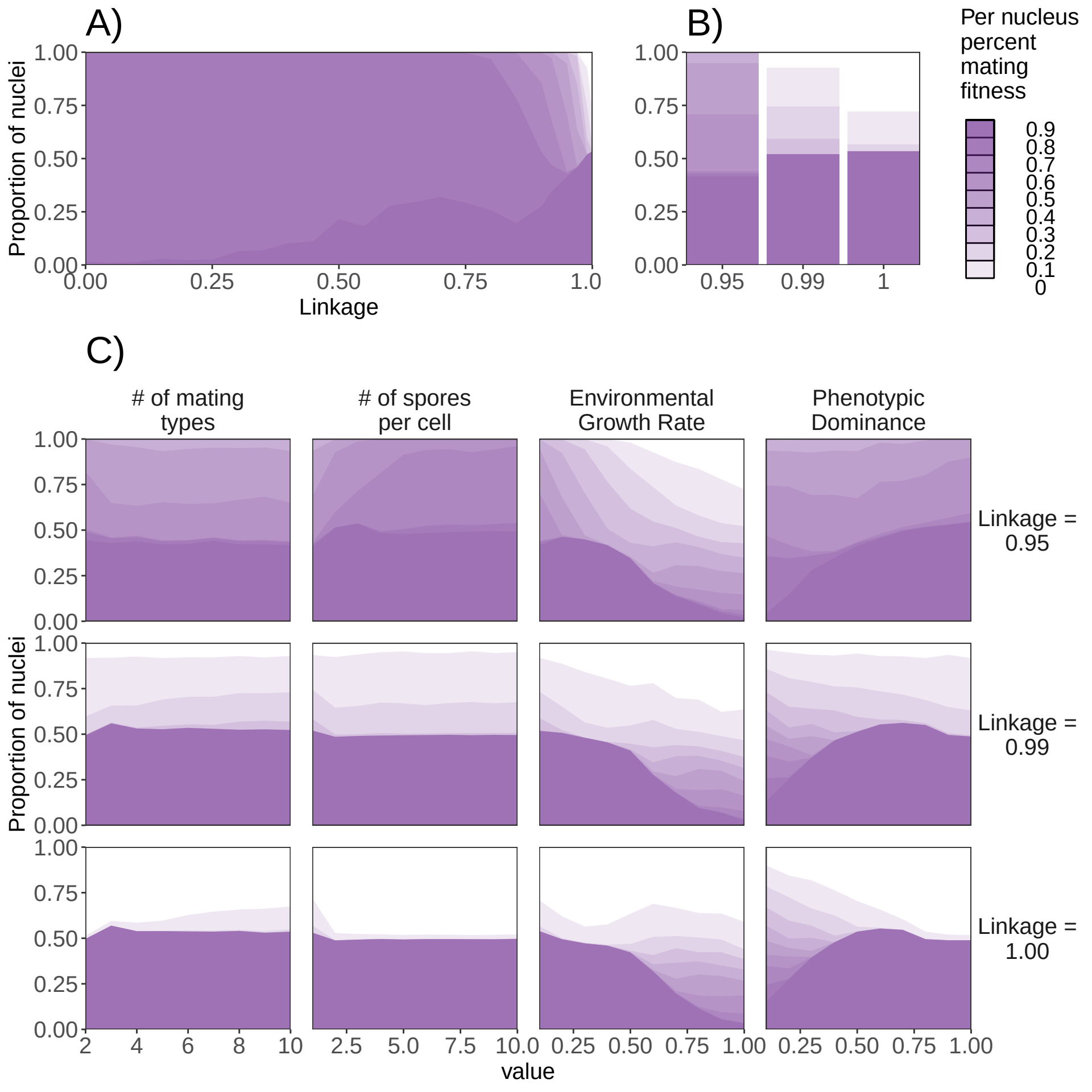

A)
Monokaryon
Meiosis +
Spore Production
mon-mon
mating
di-mon
mating
di-di
mating
Karyogamy
Dikaryon
Dikaryon growth
B)
Empty
Monokaryon
Dikaryon
Dikaryon maintained
during growth
Dikaryon produces
haploid spores
Mating
Fitness
0.1
0.2
0.3
0.5
mon-mon mating
vs.
di-mon mating
vs.
vs.
di-di mating
vs.
Diploid
Standard
Dikaryon
Open
Dikaryon
C)
Vegetative
Growth
Mating
Spore
Dispersal
Random
Death

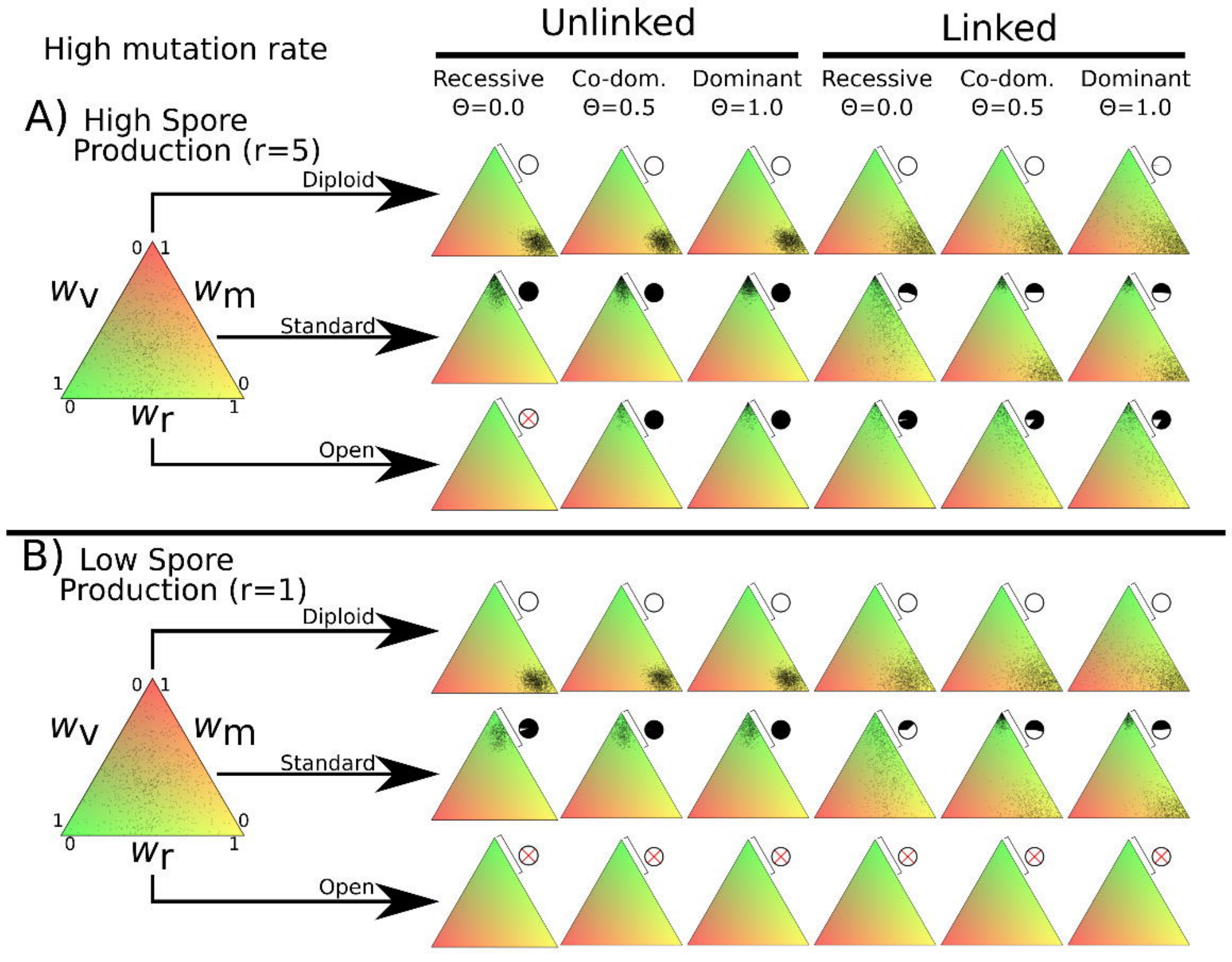

Unlinked
Linked
Recessive Θ=0.0
Co-dom. Θ=0.5
Dominant Θ=1.0
Recessive Θ=0.0
Co-dom. Θ=0.5
Dominant Θ=1.0
A) High Spore Production (r=5)
Diploid
Standard
Open
wv
wm
wr
0
1
B) Low Spore Production (r=1)
Diploid
Standard
Open
wv
wm
wr

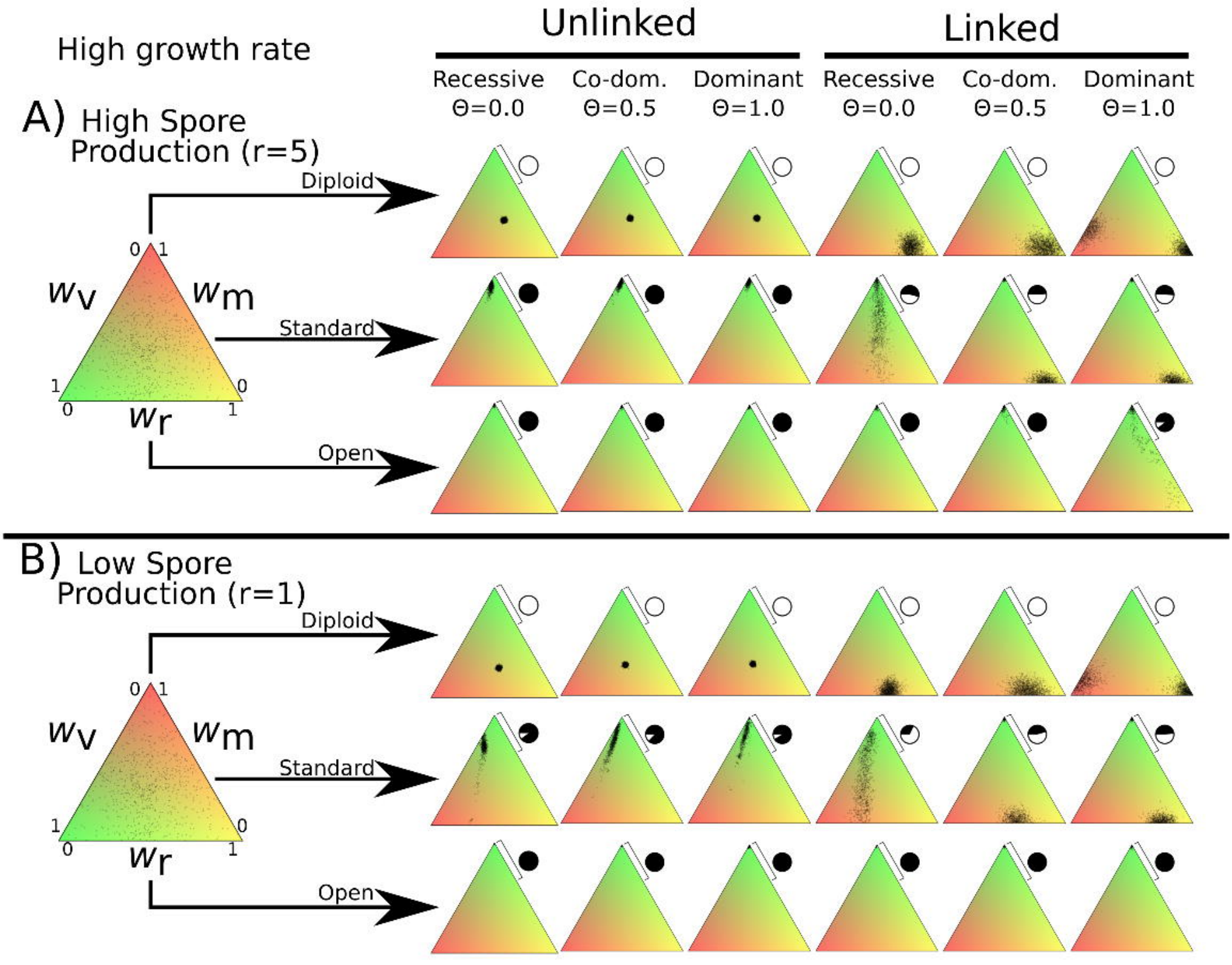

Diploid
Standard Dikaryon
Open Dikaryon
Population Size
90000
75000
60000
45000
30000
15000
0
Linkage
1.00
0.99
0.90
0.75
0.00
0
500
1000
1500
2000
Generation
Proportion of Fitness
Nuc. 2
Nuc. 1
1
0
100
200
300
400
500

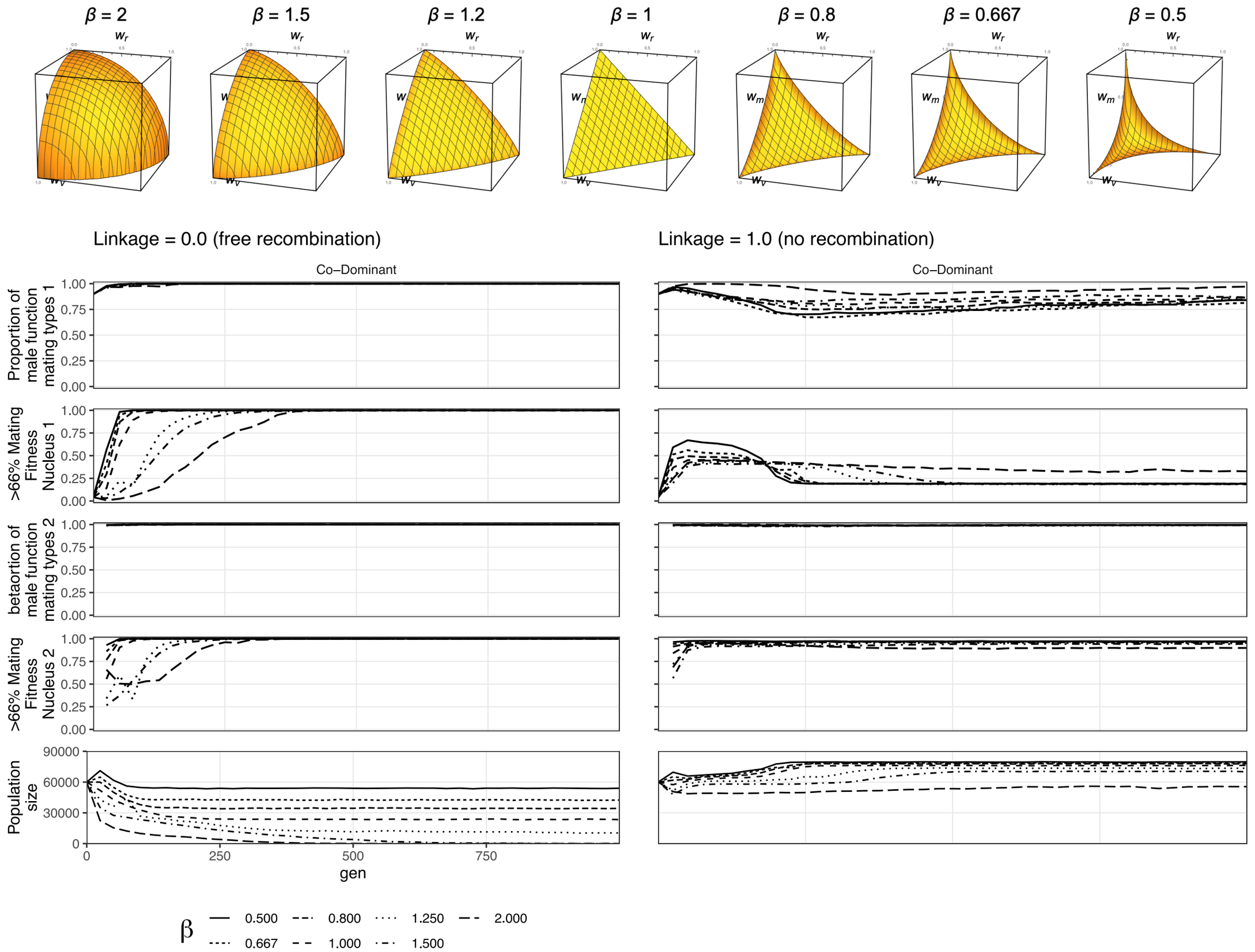

λ= 0.0 (no linkage)
λ= 1.0 (complete linkage)
Dominant DMF
Co−Dominant DMF
Recessive DMF
No Dikaryon Male Function
DMF Allele Freq. Nucleus 1
% Mating Specialists Nucleus 1
DMF Alelle Freq. Nucleus 2
% Mating Specialists Nucleus 2
Population Size
1.00
0.75
0.50
0.25
0.00
90000
60000
30000
0
0 250500750
Generation
Starting Proportion of non−DMF Alleles
0.10
0.30
0.50
0.70
0.90


A) Population Size
Diploid
Open
Standard
Death Rate (d)
0.9
0.7
0.5
0.3
0.1
0.1
0.3
0.5
0.7
0.9
Basal Vegetative Growth Rate (g)
Relative Population Size
0.75
0.50
0.25
0.00
B) Proportion dikaryotic of occupied cells
Dead
Proprotion Dikaryotic
1.00
C) Mean population size and proportion dikaryotic
Mean Pop. Size
Mean Proportion Dikaryotic
Diploid
Standard
Open
0.0
0.2
0.4
0.6
0.8


A)
B)
C)
Proportion of nuclei
Linkage
Per nucleus percent mating fitness
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
# of mating types
# of spores per cell
Environmental Growth Rate
Phenotypic Dominance
Linkage = 0.95
Linkage = 0.99
Linkage = 1.00
value


High mutation rate
Unlinked
Linked
Recessive Θ=0.0
Co-dom. Θ=0.5
Dominant Θ=1.0
Recessive Θ=0.0
Co-dom. Θ=0.5
Dominant Θ=1.0
A) High Spore Production (r=5)
Diploid
Standard
Open
wv
wm
wr
0
1
B) Low Spore Production (r=1)
Diploid
Standard
Open
wv
wm
wr
0
1


High growth rate
Unlinked
Linked
Recessive Θ=0.0
Co-dom. Θ=0.5
Dominant Θ=1.0
Recessive Θ=0.0
Co-dom. Θ=0.5
Dominant Θ=1.0
A) High Spore Production (r=5)
Diploid
Standard
Open
wv
wm
wr
0
1
B) Low Spore Production (r=1)
Diploid
Standard
Open
wv
wm
wr


β = 2
β = 1.5
β = 1.2
β = 1
β = 0.8
β = 0.667
β = 0.5
Linkage = 0.0 (free recombination)
Linkage = 1.0 (no recombination)
Co−Dominant
Co−Dominant
Proportion of male function mating types 1
>66% Mating Fitness Nucleus 1
betaortion of male function mating types 2
>66% Mating Fitness Nucleus 2
Population size
gen
β
0.500
0.667
0.800
1.000
1.250
1.500
2.000