

**Dikaryons, diploids, and evolution**

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The regular association of unfused, haploid, gametic-type nuclei within the dikaryon is a striking outcome of evolution that is unique to the fungi. Many fungi with normally dikaryotic mycelia can also exist as diploids (12, 32, 44, 55, 59). What are the genetic and evolutionary implications of dikaryosis and diploidy? Remarkably, the majority of the genetic inferences about dikaryons, diploids, and their interactions reviewed by John Raper in Genetics of Sexuality in Higher Fungi in 1966 (66) remain essentially unchanged to the present. While the genetics of dikaryons and diploids have been established for more than 40 years, the evolutionary implications are only now becoming accessible through experiments. The purpose of this review is to examine the immediate and long-term consequences of dikaryosis, with comparison to diploidy. In keeping with the theme of Raper's book, this review focuses on fungi with prolonged dikaryotic phases, mainly the Hymenomycetes (nomenclature of higher taxa follows the Tree of Life Project, <http://tolweb.org/Fungi>). The four interrelated conclusions of this review are that: i) the known dynamics of nuclear migration and dikaryon formation suggest that mating in nature is asymmetric for male and female function, ii) the transmission of nuclear and mitochondrial genomes follow different rules, iii)

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dikaryons produce recombinant genotypes without fruiting,  
and iv) dikaryons and diploids carry different expectations  
for evolution.

**THE DIKARYON: MALES, FEMALES, NUCLEAR MIGRATION, AND  
ASYMMETRY IN MATING**

*The dikaryon in the Ascomycota and Basidiomycota.* In most eukaryotes, including plants and animals, plasmogamy and karyogamy occur in rapid succession to produce a diploid zygote nucleus. In the Ascomycota and Basidiomycota, plasmogamy and karyogamy are separated in time and the dikaryon occupies the extended period after plasmogamy and before karyogamy. Within the cells of the dikaryotic mycelium, a pair of haploid nuclei of the original, gametic genotypes physically associate with one another and divide synchronously through an indeterminate number of cell cycles. In most species of Basidiomycota, the dikaryon constitutes the extended vegetative phase, while in the Ascomycota, the dikaryon is usually restricted to the ascogenous system within fruit bodies. In the Basidiomycota, the dikaryon may or may not be associated with clamp connections and, in the Ascomycota, the dikaryon in the ascogenous system of the fruit body may or may not be accompanied by croziers, which are likely homologous to

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clamp connections. While the dikaryons of the Ascomycota and Basidiomycota appear to be fundamentally similar in their coordinated nuclear division, the dikaryon of the Basidiomycota has been studied in more genetic detail because it is free-living and more amenable to experimentation.

In the Hymenomycetes, there is considerable variation on the theme of dikaryosis. For example, in *Schizophyllum commune* and *Coprinus cinereus*, the cells of dikaryotic mycelia are regularly binucleate with clamp connections. In *Heterobasidion annosum* the cells are highly multinucleate and clamp connections are present only on some of the hyphae (13, 47), while in *Agaricus bisporus* the cells are also highly multinucleate, but with no clamp connections (65). Regardless of these morphological variations, the intricate association of two haploid, nuclei of gametic genotypes remains constant.

Within the dikaryon there is molecular communication between the paired nuclei. The distance between nuclei has major consequences for gene expression (73, 87), especially for the production of hydrophobins, secreted proteins that have a major effect on whether hyphae grow into the air or remain immersed in a hydrated substrate or host. These consequences are most likely mediated by the local

deployment of *B* mating-type pheromone receptors on the cell surface nearest the nucleus within which they are encoded, as well as by the extent of binding with pheromones of the compatible *B* mating type produced by the other nucleus of the pair (17, 73, 87). There is coordination between the *A* and *B* mating type genes, but many of the details of this crosstalk, and the regulation of downstream genes, have yet to be clarified (9).

***Mating and development.*** Monokaryons stand ready to interact with a potential mate. In tetrapolar species, a dikaryon results whenever both the *A* and *B* mating types are compatible and their respective developmental pathways are both activated. Hyphal anastomosis within and between confronted mycelia of the same species is constitutive and not regulated by mating type. Recognition of sexual compatibility occurs only after hyphal fusion. In monokaryons, G-protein-coupled pheromone receptors encoded by the *B* mating-type genes are activated by binding with a peptide pheromone from a compatible *B* mating type (9). G-protein-coupled pheromone receptors are of central importance in the early stages of mating in all higher fungi examined to date and proteins in this family have a

variety of important signaling functions in all eukaryotes (48).

When the *B* mating types are compatible, a monokaryon receives an incoming nucleus that divides and migrates through the existing hyphae. The long-range migration of nuclei is guided by microtubule tracks (68). The motive force for nuclear movement is most likely by dynein motor proteins, which are associated with the spindle pole body so that nuclei are pulled toward the minus ends of microtubules. This migration, along with dissolution of septa as migrating nuclei pass through, is controlled by the *B* mating types, but the exact nature of that control is unknown.

After migrating nuclei reach the hyphal tips, nuclear pairing and hook-cell formation are controlled by the *A* mating type genes, which encode pairs of homeodomain-proteins that activate transcription within the dikaryon when the proteins of a heterodimeric pair are derived from different mating types. Interestingly, fusion of the hook cell with the sub-apical peg that develops on the sub-apical cell is again controlled by the *B* mating type genes; this is the cell fusion in the Basidiomycota that most resembles cell fusion in *Saccharomyces* (6), which is similarly guided by the interaction between mating-type

pheromones and receptors. Just before fusion, the hook cell and the sub-apical cell are uninucleate and of opposite mating type. In the dikaryotic hypha of *C. cinereus* the positions of the two gametic type nuclei switch regularly in the apical cell (37). This phenomenon, which may be a general feature of dikaryons, has also been observed in a dikaryon from a diploid-by-haploid mating of *Cryptococcus neoformans* (52).

**Male and female functions.** In both the Basidiomycota and the Ascomycota, a mycelium may act simultaneously as male and as female. In the Ascomycota, female gametangia are well differentiated within fruit bodies and the males may be conidia, microconidia, or even a vegetative hypha. In the Hymenomycetes, there are also distinct male and female roles in fertilization, but there are no morphologically differentiated gametes or gametangia. The acceptance of fertilizing nuclei that migrate through existing mycelium can be regarded as a female role. Only the cytoplasm of those portions of monokaryons acting as females is transmitted to the new dikaryon. In contrast, the male contributes little or no cytoplasm to the dikaryon. On the male side, the source of the fertilizing nucleus in the Hymenomycetes can be a germinating spore, another haploid monokaryon, a dikaryon, or even a diploid

of the same species. The details of how exactly an "extra" nucleus of a dikaryon becomes available for fertilizing a monokaryon are not known.

***How mating actually happens; asymmetry in nature.*** The avidity of single nuclei in monokaryons for associations with nuclei of compatible mating types is convincingly illustrated by "spore trapping." To sample fertilizing nuclear genotypes, a Petri dish with a haploid monokaryon is opened and placed outdoors for one or more days (2). In many species, such as *Pleurotus* species (86), and *S. commune* (39), the resident mycelium is almost invariably dikaryotized. Although the actual fertilization event in spore trapping, or anywhere else in nature, has never been witnessed directly, the simplest inference is that spores readily settle out from the air column, germinate, and then fuse with the resident monokaryon. Even monokaryotic mycelia placed outside of the normal geographic range of the species can be fertilized, illustrating the ability of the vast numbers of fungal spores to disperse and potentially find an appropriate mate (33). A reasonable hypothesis is that most mating in nature is also asymmetric; spores germinate to form small monokaryotic colonies, which are soon fertilized by spores. If this hypothesis holds true, then the effective size of the two



mates in nature is usually unequal, in contrast to the majority of experimental matings where the size of the two mates is made equal.

**TRANSMISSION OF NUCLEAR AND MITOCHONDRIAL GENOMES FOLLOWS DIFFERENT RULES**

*Nuclei move rapidly in matings; mitochondria do not.*

Where two equally sized monokaryotic mycelia are allowed to mate, each mycelium usually acts simultaneously as male and female, both donating and accepting incoming nuclei that subsequently migrate and proliferate (Figure 1).

Mitochondria do not accompany the rapid and long-range movement of nuclei (7, 34, 73, 78). Symmetric pairings of haploid monokaryons typical of laboratory matings therefore result in a mycelium of uniform dikaryotic genotype that is mosaic for mitochondrial type. In the zone where the two mycelia meet, there is limited cytoplasmic contact. Here, the often tubular mitochondria may physically mix, and anastomose with one another (8). The mitochondrial genomes recombine and produce non-parental genotypes when mitochondrial DNAs (mtDNAs) of different descent are in close proximity (70). After cytoplasmic mixing, the different mtDNA genotypes rapidly sort to pure types in the growing dikaryon. The sorting of mtDNAs when heteroplasmic

cells proliferate is not always random. In *C. neoformans*, mtDNA transmission from newly mated cells is uniparental and from the *MATa* parent (90).

In nature, the population structure of at least one species, *Armillaria gallica*, is highly recombined for mtDNA (70). Although the actual rate of recombination per-nucleotide interval per generation may be very low, this apparently has been enough over time to erase most linkage disequilibrium in the mtDNA genome that may have existed in the past. For nuclear genomes, the population structure of basidiomycetes within large geographical areas approximates panmixia (38, 71), reflecting the high levels of outbreeding expected from sexual incompatibility systems and from the known dispersal ability of basidiospores (71). Since the extent of cytoplasmic mixing in matings is small, most of the resulting dikaryon has only one or the other parental mtDNA type from the beginning. Because matings in nature carry a small probability that the region of cytoplasmic mixing will be the one to proliferate and become fixed in the mycelium, a minority of resulting vegetative individuals will have a recombinant mtDNA. In addition to the homologous recombination in mtDNA, small selfish elements of the genome, such as the omega element in yeast (28, 30), may also be spread unilaterally; these

elements have been described in the Ascomycota, and may well also exist in the Basidiomycota.

The fundamental nature of mitochondrial genetics in fungi is more akin to population genetics of bacteria and viruses than conventional Mendelian genetics. MtDNAs exist as moderately sized populations, on the order of magnitude of  $10^2$  molecules per cell. Multiple rounds of exchange in heteroplasmic cells may occur and the individual exchange events may be reciprocal or non-reciprocal. Unfortunately, there is as yet no means for isolating the immediate products of any mtDNA exchange event in any fungus, especially in filamentous fungi.

***Are individuals mtDNA mosaics?*** Although laboratory matings of monokaryotic mycelia of different mtDNA types establish a dikaryon or diploid that is mosaic for mtDNA type, more than one mtDNA type has not been detected within naturally existing individuals (71), even where mitochondrial mosaics of the same species have been demonstrated in the laboratory (Figure 2). If the sizes of the mating individuals are strongly asymmetric in nature, as when a small monokaryotic colony is fertilized by a spore, then one or the other parental mtDNA type is more likely to predominate and the other type is either lost or

not detected. Only the mtDNA of the monokaryon that acted as a female is transmitted.

***Slow movement of mitochondria in matings.*** Although mitochondria do not migrate rapidly (i.e. faster than the mycelial growth rate) along with nuclei in compatible matings of monokaryons, mitochondria may well move more slowly and gradually displace resident mitochondrial types (25). These outcomes are analogous to suppressive mtDNA types in *Saccharomyces cerevisiae* where one mtDNA type may displace another over several rounds of budding due to a replicative advantage (18, 24).

***What's good for a mitochondrial genome can be bad for the nuclear genome.*** That nuclear and mitochondrial inheritance are uncoupled, and that mated mycelia may be mosaic for mtDNA type raises the possibility of genomic conflict: that the two original mtDNA types are in direct competition at the level of the entire dikaryon from the mating (1). With genomic conflict, enhanced transmission of one mtDNA type is always at the expense of the other. Further, enhanced transmission of one mtDNA type is at the expense of the fitness of the whole dikaryon; the individual mtDNA benefits, but the dikaryon suffers. The reason for the conflict is that the transmission of a mitochondrial genome is tied only to the female function,

while the transmission of nuclear genes is tied to both the male and the female functions. If, for example, a mtDNA carries total male sterility, then the reproductive output of the entire mated colony may be greatly reduced because the unfertilized part of the colony will not make any contribution to the gene pool.

Such genomic conflict has been proposed by Aanen et al. (1) as an explanation for unilateral migration seen in many pairings of monokaryons from the wild. The model of Aanen et al. includes male-sterile and male-fertile mtDNA types, plus another set of nuclear determinants specifying either resistance or susceptibility to the cytoplasmic male sterility. In a resistant monokaryon, mtDNA-based male sterility is nullified and nuclear migration occurs regardless of whether the opposing mate is male sterile or male fertile. In a susceptible monokaryon, nuclear migration occurs only from male-fertile, and not from male sterile, mates. This model explains to a remarkable extent the patterns of bilateral migration, unilateral migration, and no migration among mated monokaryons in the *Hebeloma crustuliniforme* complex (1).

**Possible mechanisms of male sterility.** A possible mechanism for the male sterility is that mtDNA mutations somehow either block the mating pheromone receptors of the

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opposing mate or the production of the pheromones of the male sterile strain. Alteration of the timing or efficiency of fruiting could also alter the proportions of spores with the two mtDNA types. Regardless of the mechanism of male sterility, an important part of the model is strong selection on the nuclear genome for resistance mutations that nullify male sterility (1).

At first glance, this model of genomic conflict leading to male sterility would not seem to be applicable to fungi like *S. commune*, in which the distribution of strains defective in nuclear migration appears to be different from that in the *H. crustuliniforme* complex. In *S. commune* many monokaryons from the wild are able to donate, but not to receive, nuclei regardless of their partner (66). Therefore, these strains are highly male-fertile, but completely female-sterile. This is not surprising as the female function is in general more complex and presents a larger mutational target than the male function. The female function is therefore more easily lost through deleterious mutation than the male function (51). (For an excellent treatment of loss of female function in Ascomycota, see ref. no. 53). Before the cytoplasmic male sterility model is discounted, however, a more detailed analysis of the quantitative

aspects male function is needed even in fungi such as *S. commune*. Whether or not male sterility appears may depend on population history and whether selection has had enough time to act so that male sterility and the corresponding nuclear resistance type reach detectible frequencies.

One factor working against the spread of male sterility would be if all mating in nature is highly asymmetric to begin with; this would be the case whenever established monokaryons acting as the female are fertilized by spores acting as the male. Here male sterility would block all gene transmission, a dead end for that spore. Alternatively, if mating in nature occurs predominantly via the Buller phenomenon, then male sterility would block the ability of the dikaryon to fertilize monokaryons. Here there would be no benefit to the mtDNA, but there would be a cost to the nuclear genome of the dikaryon. Cytoplasmic male sterility would not evolve under these conditions.

#### **DIKARYONS PRODUCE RECOMBINANT GENOTYPES WITHOUT FRUTING**

##### ***Escape from dikaryons and reassociation of nuclear types.***

The association between the paired nuclei in the dikaryon is not absolute. Single nuclei may escape this association either through the production of uninucleate mitospores

(oidia) as in *Coprinus cinereus* or through sectors of mycelium that contain only one genotype of haploid nucleus. In *Heterobasidion annosum* the association of paired nuclei is not universal in the mycelium and monokaryotic sectors are common. Even in species where the dikaryotic association covers every cell, single nuclear genotypes might escape as monokaryons, especially when the nuclei are far apart. This raises the possibility that nuclei from different dikaryons can form new associations (42, 84). *H. annosum* commonly occurs on cut stump surfaces where many dikaryons may encounter one another. Here, new dikaryons are commonly formed by reassociation of nuclei formerly in different dikaryons (40). There are limits on this process as each existing dikaryon remains highly resistant to modification by invading nuclei. How the multitude of dikaryotic genotypes on a substrate sorts out may depend on chance or on the competitive fitness of the individual dikaryons. Once a substrate is colonized, the prevailing dikaryons are thought to function as physiologically distinct individuals due to somatic incompatibility (89), rather than as a genetic mosaic functioning as a cooperative network (69).

**The Buller phenomenon.** Dikaryons are well-known for their ability to contribute a fertilizing nucleus to a



monokaryon. This behavior was first described by A. H. R. Buller (10, 11), coined in the literature by Quintanilha (62) as the "Buller phenomenon," and comprehensively reviewed by Raper (66). In essence, all matings are a manifestation of the Buller phenomenon. When nuclear migration is in progress in a mating of monokaryons, part of the colony is dikaryotic and part of the colony remains purely monokaryotic, until it too is eventually dikaryotized after nuclear migration is complete. In this transitional stage, the colony actually represents a di-mon mating.

In tetrapolar species, "di-mon" matings can be classified according to the mating types of the dikaryon and monokaryon as compatible or incompatible depending on whether the dikaryon has at least one resident nuclear type that is fully compatible with the monokaryon (Table 1). In any di-mon interaction, there are three possibilities: First, the two nuclei of the dikaryon may simply replace the resident nucleus of the monokaryon (66). This can happen in either compatible or incompatible di-mon interactions. Second, one of the two nuclei of the dikaryon may dikaryotize the monokaryon; this is only observed in compatible di-mon interactions. Lastly, a recombinant nucleus may dikaryotize the monokaryon. This

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is the most common outcome in incompatible di-mon interactions, but it can also occur in compatible interactions. Even where both nuclei of the dikaryon are compatible with that of the monokaryon (16, 42, 56) somatic recombination for mating types may still occur; because these recombinants, also compatible with the monokaryon, are not necessary for fertilization, Papazian aptly described this an "...apparently useless occurrence.."

**Nuclear selection in di-mon matings.** In compatible di-mon interactions when both nuclei of the dikaryon are compatible with that of the monokaryon one nucleus of the dikaryon may dikaryotize the monokaryon more frequently than the other (15, 20, 22, 41, 42). These observations of nuclear selection include more than one species and within species, strains that were either genetically heterogeneous or inbred to be isogenic for all but mating type genes. Not surprisingly, these studies come to different conclusions as to the basis for selection. For compatible, highly isogenic di-mon matings of *S. commune*, nuclear selection is associated with the *B* mating types, with a subsidiary influence by the *A* mating types (22). The basis for this selection is not at the level of nuclei entering or migrating within the monokaryon. Both nuclear types of the dikaryon enter the monokaryon and migrate with

approximately equal efficiency, but one dikaryotizes more frequently than the other. This inter-nuclear selection based on the *B* mating types may share a common mechanism with another phenomenon described more recently by Carlene Raper (64); when certain dikaryons of *S. commune* are de-dikaryotized, one nuclear type is recovered more frequently than the others and there is a linear hierarchy of biased recovery based on the *B* mating types.

In non-isogenic strains, especially those from the wild, other factors may be involved in nuclear selection in compatible di-mon matings. For example among genetically heterogeneous strains of *Coprinus*, simple relatedness by descent is the main determining factor, with the less related nuclear genotypes favored by selection over the more related (41, 42). Mating types are not implicated in nuclear selection here, presumably because of modifiers of mating type activity that might be expected in a highly heterogeneous genetic background. While nuclear selection is well documented in the laboratory, there is no evidence for or against nuclear selection in di-mon matings in nature.

***Analysis of somatic recombinants in di-mon matings.***

Much of the investigation of somatic recombination has focused on incompatible di-mon matings because recombinants

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with mating types compatible with that of the monokaryon can be readily selected (Table 1). Thus even extremely rare recombination events embedded within a dikaryotic mycelium can be recovered. After the recombinant type is captured in the new dikaryon, any other markers can be assayed.

Early studies of somatic recombination were hampered by the difficulty of genotyping the fertilizing nucleus in di-mon matings from the resulting dikaryon. If the new dikaryon from an incompatible di-mon mating is merely fruited, the mating types of the spores are the same whether somatic recombination occurred or not (Table 2). Quintanilha's method (63) addressed this problem by pairing the dikaryotic product of incompatible di-mon matings with tester monokaryons of compatible mating types designed to separate and distinguish the mating types of the nuclei in this dikaryotic product (Table 2). The new dikaryons from these tester di-mon matings were then fruited. Scoring the segregating mating types from these fruit bodies allowed the mating type of the original fertilizing nucleus in the first di-mon mating to be deduced. This was an effective, but very laborious, process allowing analysis of only a limited number of recombination events in di-mon matings.

In Papazian's method (56) the dikaryons from fertilized monokaryons of di-mon matings were paired with tester monokaryons and the resulting patterns of full compatibility versus incompatibility are morphologically distinguishable (Table 3). Further, the testers used in Papazian's method can carry auxotrophies to deduce whether or not the nucleus being assayed also contains a non complementing auxotrophy (19, 21).

Although not yet used widely in studies of somatic recombination, it is now easy to separate and genotype the component haploid nuclei of any dikaryon by protoplast formation and regeneration (3, 88); the direct recovery of haploids avoids the complication of the dominance or recessiveness of the markers and simplifies the genetic analysis. Of all three methods of analyzing di-mon matings, however, Papazian's method requires the least labor and therefore may permit genotyping of the largest numbers potential recombinants in di-mon matings.

***Patterns of somatic recombination in di-mon matings.***

What is the nature of recombination in di-mon matings? Does it resemble parasexuality, meiotic recombination, or isolated transfer of specific elements between genomes? The surprising answer is all of the above, depending on the kind of interaction and the species. In *Coprinus*, somatic

recombination appears to be essentially parasexual with reduction via intermediate stages of aneuploidy and little recombination among genes within chromosomes (61, 82, 83). In *S. commune* recombination in incompatible di-mon matings is entirely different, with two classes of recombinants that include intra-chromosomal recombinants, i.e., those that appear to be derived from a meiotic-like process (Figure 3) and those that appear to involve only the specific transfer of mating types between nuclei, and not other genes (19, 23). The meiotic-like recombinants show reassortment of genes on different chromosomes and numerous recombinants within chromosomes (Figure 3). Here the recombinant mating types fertilizing the monokaryon have a variety of alleles from either of the two nuclear types of the original dikaryon. All of these recombinants in di-mon matings arise in the absence of fruit bodies and basidia.

In contrast to the meiotic like recombinants, specific-factor transfer involves only the mating type genes of the dikaryon; all of the other non-selected nuclear markers, including even the *pab* gene located between the  $A\alpha$  and  $A\beta$  loci are from the monokaryon of the di-mon mating (19). Specific-factor transfer must therefore involve all three types of nuclei in the di-mon mating (19, 23). This would be difficult to explain by conventional

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crossing over, because several nuclear fusions would be required, along with double crossovers bracketing each mating-type region transferred. Although the actual mechanism of specific-factor transfer remains unknown, the process may be general when the selection on certain genes is strong. For example, a very similar situation occurs in *Cryphonectria parasitica* in which some heterokaryotic strains from the wild are capable of selfing because they are heteroallelic for mating types, but not for any other loci (54). These mating-type heterokaryons could have arisen from somatic exchange between strains with different multilocus genotypes between which only a mating type is somehow transferred into a new nuclear background. Like the specific-factor transfer described by Ellingboe (19), the mating-type heterokaryons in *C. parasitica* cannot easily be explained by conventional crossing over. Loci responsible for the unusual hyperplastic growth phenotype in *S. commune* termed "mound" appear to be subject to a process similar to specific-factor transfer, but not involving mating types (49, 50).

**Somatic recombination in lone dikaryons.** Although studied mostly in di-mon matings, somatic recombination is not limited to di-mon matings, but also occurs in isolated dikaryons from which the nuclear components are recovered

after a short period of growth, either with or without selection for recombinant types. There are indications that the somatic recombination in isolated dikaryons of *S. commune* is also meiotic-like (27, 58)

***Somatic recombination in dikaryons: state of the field.*** Despite the many early reports of somatic recombination, no unified explanation of somatic recombination in dikaryons has since emerged. There are several impediments to a complete understanding. First, somatic recombination is not associated with a specific developmental stage. In contrast, conventional meiosis occurs in well-defined basidia from which basidiospores containing the immediate meiotic products can be isolated. This makes it possible to calculate recombination frequencies and, with analysis of whole tetrads, to determine whether any individual recombination event is reciprocal or non reciprocal. In somatic recombination, the exchange events are buried within the dikaryotic mycelium, and the fate of any individual recombinant, extinction or proliferation, is uncertain. Whether any or all of the cellular machinery associated with meiosis participates in this process is an open question. Further, after a recombination event, multiple rounds of division may occur and the recombinant genotypes may proliferate to



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different degrees, so that their frequency of recovery may not reflect the frequency of recombination. Yet another complication is whether the monokaryon merely selects for a nucleus of compatible mating type after somatic recombination has occurred, or actually induces somatic recombination.

Several conditions, duly noted by Raper (66) are needed for accurately characterizing somatic recombination further both in di-mon matings and in isolated dikaryons. First, larger numbers of recombinants from independent events are needed, and, second, a densely marked genetic map is essential for a full evaluation of somatic recombination. Does somatic recombination fit the same mapping function relating recombination frequency to true map distance as in meiosis? Are the somatic recombination events reciprocal? The background genotype is also likely to be a major factor (66). Isogenic strains cannot be expected to show exactly the same correspondence between recombination frequency and physical distance as outbred strains. Modifiers of recombination frequency are well-known in *S. commune* (45, 72, 79-81).

For specific-factor transfer, the nucleotide sequences surrounding mating types may provide clues about the process. For example, specific recombination hotspots

flanking the mating-type locus in *Cryptococcus neoformans* have been identified (36). These hotspots show negative crossover inference; more double crossovers occur than are expected on the basis of the single-crossover frequencies, exactly what would be expected for the transfer of mating types, and not other genes, to new haploid backgrounds. In *S. commune*, the distribution of short nucleotide repeats and identity islands appears to play a role in determining the rate of recombination in the region between  $B\alpha$  and  $B\beta$  (26); whether or not sequence elements on the other sides of the *B* mating-type genes could promote mating type transfer in conjunction with the elements between  $B\alpha$  and  $B\beta$  is an open question. In further characterizing specific factor transfer, large numbers of di-mon pairings should be followed because this process is detected in some pairings, but not in others (21).

**Evolutionary significance of somatic recombination; is there any?** The diversity of somatic recombination processes in dikaryons, parasexual-like, meiotic-like and specific-factor transfer, although interesting in their own right, are of unknown evolutionary significance. Although it is tempting to hypothesize that somatic recombination systems might somehow be adaptive at the levels of individuals or populations, it is also possible that

somatic recombination has little or no effect on fitness, and a similarly negligible effect the evolution of populations. The vast majority of somatic recombinants may simply be lost from mycelia, just as most mutant alleles are lost from most populations soon after they arise. In short-lived dikaryotic individuals, it is difficult to envision how somatic recombinants could rise to a sufficiently high frequency to confer an adaptive benefit or deficit. The survivability and ability of somatic recombinants to spread within a dikaryotic mycelium has not been studied.

***Long-term changes in Dikaryons.*** If dikaryons have the capacity for internal genetic exchange, how do they behave as a population of cells over a long time? Dikaryons offer the advantage in evolutionary studies that the individual nuclear types can be recovered intact, most easily by protoplast formation and regeneration. This is not possible with diploids because of the genetic shuffling that occurs with any kind of reduction division, meiotic or non-meiotic. In experimentally evolved dikaryotic lineages, the fitness of the paired nuclear types can also be measured to examine how dikaryons change over time with vegetative growth. From evolved dikaryons, the nuclear components can be paired with nuclei of other histories to

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measure their fitness at the level of the dikaryon or they can be measured alone as monokaryons. The time at which a mutation arose can be approximated after the fact by sampling the evolving lineages retained in a culture archive.

Only one such study examining long-term evolution in dikaryons has appeared. Clark & Anderson (14) evolved dikaryons in replicated populations all from a common ancestor with a uniform cytoplasm over 13,000 generations, defined as the time required for a hyphal tip cell to divide (ca. 90 min. for *S. commune* on minimal medium). Selection was for high growth rate, which was considered as a measure of fitness. (In nature fitness is more complicated than merely capturing resources through vegetative growth for eventual fruiting. Fruiting timing and efficiency, basidiospore production and germination, and mating efficiency are also important.) In addition to the replicate dikaryons, the original haploid components with the common cytoplasm were also evolved with replication.

Among the haploids, there was no overall change in growth rate, which was about twice that of dikaryons in the beginning of the experiment. Among the dikaryons, there were sharp increases in growth rates. Several dominant

mutations for higher growth rate were identified. These mutations did not increase frequency of cell division, but rather increased the length of the cell compartments. The distance between nuclei also increased and the colony margin changed from irregularly lobate to smooth, as in monokaryons. Along with the change in colony morphology, the production of a self inhibitor (43) responsible for the slow growth and lobate colony margin was lost. In the wild-type dikaryons, the self inhibitor is expressed when the mycelium is grown in light, but not in darkness. In the dark, even the wild type is fast growing with a smooth colony margin, exactly like the mutant dikaryons in the light or the dark. At the end of the evolution experiment, the growth rate of the mutant dikaryons nearly matched that of the monokaryons. Although gene expression has not been monitored in these evolved dikaryons growing under light, we speculate that it will be more "monokaryon-like" than dikaryon-like.

What might explain the relative lack of change in the growth rate of the monokaryons? In filamentous fungi, the effective population sizes are undoubtedly much smaller than those of planktonic unicells. This is because the different growing points of a mycelium are all related to a recent common ancestor cell by a short path of descent. In

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contrast, different cells drawn from a planktonic cell culture are on average much more distantly related by descent and the overall mutational diversity is higher, at least until a selective sweep homogenizes the populations. The dominant mutations for increased growth rate in the dikaryons would have to occur at a very high frequency for a response to selection in the dikaryotic lineages, which must be of small effective population size.

Detrimental mutations also accumulated over the course of the evolution experiment. Two different recessive lethal mutations were detected as the inability to recover one of the two nuclear types from dikaryons beyond a specific time in the experiment. Interestingly, one of these also exerted a dominant deleterious effect on dikaryotic growth in pairings of nuclei from all of the histories except the one with which it evolved. A compensatory mutation restoring growth occurred in the other nucleus co-evolving in the dikaryon; the nuclear types that evolved together in this dikaryon were fitter when paired together than with any other nuclear types. The original recessive lethal mutation and the corresponding compensatory mutation were traced to particular times in the experimental lineages. This kind of compensation could be the basis for a co-adaptive process between the haploid

genomes of an asexually evolving dikaryon in which changes in one nucleus set the selective environment for change in the other nucleus in a continuing reciprocal process. The compensatory event here represented but one observation. More observations are needed before the generality of co-adaptation in dikaryons can be tested.

Finally, the evolved dikaryons were tested for somatic recombination by separating and genotyping each nuclear type. Of 25 single nucleotide polymorphisms there were eight events of reciprocal, and two events of non-reciprocal transfer between the nuclei of six of 12 dikaryons, with 8 of the 25 loci affected. The recombination process was decidedly not meiotic-like as a majority of the 25 markers were not reshuffled and most events were separated in time and occurred in different populations. Further, no recombinant mating types were detected during the evolution experiment, but none would necessarily be expected as the experiment was not like an incompatible di-mon mating where selection for recombinant mating types is strong. The process most resembles specific-factor transfer except that no selection on any of the 25 loci is expected. It is possible that many segregants in dikaryons cultured for long periods of time,

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as those seen over the short term, may not proliferate and persist.

One limitation of the study by Clark and Anderson (14) is that dikaryons of *S. commune* may not grow for 13,000 generations in the wild and are likely more short lived. But many dikaryons of other species in the wild do persist for long periods of time such as fairy rings, for example of *Marasmius oreades*. These represent naturally-occurring evolution experiments in that the mycelium grows over a long period of time from a common ancestor in a physically obvious growth pattern.

#### **EXPECTATIONS FOR DIPLOIDS AND DIKARYONS IN EVOLUTION**

By the time of publication of Raper's book in 1966, many of the fundamental genetic properties of dikaryons, including their exquisite control of nuclear migration, nuclear pairing, and formation of clamp connections by the mating type genes, their ability to contribute a fertilizing nucleus to a haploid monokaryon on contact and their capacity for somatic recombination had been well worked out. About the evolutionary origin and maintenance of dikaryosis, one central question remains even now. Is the dikaryon maintained because of some selective advantage, or is the dikaryon merely an evolutionary holdover?



There are clues about the relative merits of dikaryosis and diploidy. What follows is a mixture of established fact and pure speculation. Many normally dikaryotic basidiomycetes have the capacity for diploidy (12, 32, 44, 55, 59). Although the total genetic complement of a dikaryon may be identical to that of its corresponding diploid state, the patterns of gene expression (5) and the phenotypic expression are different - for example, while dikaryons may have clamp connections, diploids of *S. commune*, *C. cinereus*, and *Armillaria* species do not. (Note that diploids of *Cryptococcus neoformans* have hook cells, see ref. 74) Diploids can be selected from compatible or incompatible confrontations of monokaryons as epigenetic states that are stable enough to persist for at least some time (12, 55, 59). Outside of the Hymenomycetes, diploids of the distantly related *Microbotyium violaceum* and *Ustilago maydis* can easily be selected and maintained in culture. In the Hymenomycetes, the best example of a genetically based diploid in a normally dikaryotic fungus is the dominant mutation *dik-* in *S. commune* that causes dikaryons to become stably diploid after a short period of growth (29, 44, 67). Essentially the *dik-* mutation confers a life cycle typical of many *Armillaria* species on *S. commune*.

***Life-cycle variation for diploidy and dikaryosis.***

Species that produce dikaryons also vary in their propensity for forming diploids (Figure 5). In most species of *Armillaria*, dikaryons are produced in matings, but these quickly become stably diploid through nuclear fusion (32, 46, 85). Also, in most species of *Armillaria*, the sub-basidial cells and basidia are again dikaryotic, a baffling observation given the uniformly diploid vegetative condition. In *A. tabescens* (31) and *A. gallica* (60) there is strong evidence for a pre-meiotic reduction mechanism in fruit body tissues; this is yet another potential recombination system outside of conventional meiosis awaiting full characterization with large numbers of recombinants evaluated in the context a densely marked genetic map.

In *Armillaria* there is variation in the proportion of time spent as diploids and dikaryons. In *A. tabescens*, the dikaryotic phase is longer than in *A. ostoyae* or *A. gallica*. In *A. mellea* there is no dikaryon either after mating or in the sub-basidial cells of fruit bodies; diploids form during mating and persist through the production of basidia in fruit bodies.

***Evolutionary merits of diploidy and dikaryosis.*** With their capacity for diploidy, why has dikaryosis,

predominated in the basidiomycetes? One possibility is that different conditions favor different ploidy states. A possible advantage of diploidy for extremely long-lived individuals inhabiting stable environments might be a lower mutation rate, enhanced genetic stability, and reduced need for the phenotypic flexibility afforded by dikaryosis (35). Within a diploid, an intact DNA template is always available for repair, but in the haploid nuclei of a dikaryon an intact template is available only after DNA replication in the G2 phase of the cell cycle. Another possibility in species with long-lived diploids is that the mutation rate may have been driven low by natural selection. In two long-lived diploid individuals of *A. gallica*, no mutations have been detected despite a sampling and sequencing regimen that would have detected point mutations occurring at less than  $10^9$  per generation.

Given the potential for somatic recombination, diploids would have yet another bias toward stability. Genetic exchange in diploids does not create new combinations of alleles within nuclei, but in dikaryons new combinations of alleles arise with exchange (Figure 6). This could be important among genes whose interactive control relationships extend only within nuclei and not for

genes whose products can interact, or complement at the cellular level (such as auxotrophies).

Another difference between diploids and dikaryons is that in tetrapolar species dikaryons mate readily with only two of the four possible sibling mating types, whereas diploids can mate readily with all four sibling mating types (4). The mating types in diploids are effectively co-dominant. Whether or not this is of evolutionarily significance depends on the frequency with which the Buller phenomenon and its diploid counterpart occur in nature. But even if di-mon mating is predominates in natural populations of Hymenomyces, very little mating advantage would accrue to diploids because there are so many compatible mating types available; both dikaryons and diploids are capable of mating efficiently in populations. At the post-zygotic level, however, the reproductive output from dikaryon-monokaryon matings may well be higher than that of diploid-monokaryon matings in which the potential for irregular patterns of genetic segregation and lower meiospore viability is high. Here, the evolutionary advantage may well go to the dikaryon.

In addition to the genetic differences between diploids and dikaryons their different phenotypic responses may confer advantages or disadvantages depending on

conditions. Nuclear spacing and associated variation in gene expression are inherent to dikaryons, but not to diploids. Dikaryons are therefore expected to be capable of supporting a greater range of phenotypes in response to environmental variation than diploids. With an enhanced range of phenotypes, dikaryons might be more adept than diploids in coping with heterogeneous environments.

### **CONCLUSION**

While all of the above explanations for the advantages and disadvantages of dikaryosis and diploidy are plausible, none have been definitively tested. Neither have the evolutionary roles of nuclear mitochondrial genomic conflict or somatic recombination been clarified. In the next phase, the strongest inferences about dikaryons and evolution will come from a combination of molecular biology, genomics, and evolutionary analysis, both retrospective and experimental. It may now be possible to make fair comparisons of dikaryotic cell populations with and without opportunities for nuclear-mitochondrial genomic conflict, somatic recombination, and diploidy and to compare the evolutionary outcomes.

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Table 1. Classification of di-mon matings.

	Dikaryon	Monokaryon	
Di-mon interaction	mating types	mating type	
Compatible (Legitimate <sup>a</sup> )	<i>A1B1 + A2B2</i>	X	<i>A1B1</i>
Compatible (Legitimate <sup>a</sup> )	<i>A3B3 + A2B2</i>	X	<i>A1B1</i>
Incompatible (Illegitimate <sup>a</sup> )	<i>A1B2 + A2B1</i>	X	<i>A1B1</i>

<sup>a</sup>Terminology of A. H. R. Buller

Table 2. Genotyping nuclei in incompatible di-mon matings of the form  $(A1B1 + A2B2) \times A1B2$ ; Quintinilha's test (63)

		Dikaryon	
New dikaryon from di-mon mating	X Tester	from di-mon mating with tester	<i>B</i> mating types expected in offspring
Expectation for nuclear replacement			
$(A1B1 + A2\mathbf{B2})^a$	X $A1B3$	$A2\mathbf{B2} + A1B3$	<b><i>B2</i></b> , <i>B3</i>
	X $A2B3$	$A1\mathbf{B1} + A2B3$	<b><i>B1</i></b> , <i>B3</i>
Expectation for somatic recombination			
$(A1\mathbf{B2} + A2B1)^a$	X $A1B3$	$A2\mathbf{B1} + A1B3$	<b><i>B1</i></b> , <i>B3</i>
	X $A2B3$	$A1\mathbf{B2} + A2B3$	<b><i>B2</i></b> , <i>B3</i>

<sup>a</sup>Both dikaryons, either from nuclear replacement or somatic recombination, fruit to produce the same mating types among the offspring:  $A1B1$ ,  $A2B2$ ,  $A1B2$ ,  $A2B1$ .

Note: for each di-mon cross with a tester, the diagnostic ***B*** mating type is in **bold type**.

Table 3. Genotyping nuclei in incompatible di-mon matings of the form (A1B1 + A2B2) X A1B2; Papazian's test (56).


New dikaryon from di-mon mating	Tester monokaryons			
	A1B1	A1B2	A2B1	A2B2
a Nuclear replacement (A1B1 + A2B2)	C	I	I	C
b. Somatic recombination (A2B1 + A1B2)	I	C	C	I

C = compatible; the homokaryon is quickly and uniformly dikaryotized. This can be readily seen in *S. commune* as a change in colony morphology. I = incompatible; the monokaryon is not uniformly dikaryotized, but does show a "flat" reaction consistent with migration of the nucleus with a different B mating type gene, but with the same A mating type. Sectors of dikaryotic growth are commonly seen - somatic recombination generates a nucleus fully compatible with the monokaryon; this happens only after a delay.

Glossary of terms

**Buller phenomnom**            The process in which a monokaryon is fertilized by a dikaryon in a "di-mon" mating.

**cell**            The hyphal compartments bounded by septa. Since in most cases the cytoplasm is continuous between hyphal compartments, the hypha is sometimes considered to be acellular because the cytoplasm is continuous throughout.

**hook cell**            A developing clamp connection, arising from the base of the apical cell, but not fused with the subapical cell. 

**female**            The capacity of a monokaryon to accept a fertilizing nucleus of compatible mating type, which migrates throughout its resident mycelium, resulting in a dikaryon with the cytoplasm of the resident monokaryon.

**male**            The capacity of a monokaryon to donate a fertilizing nucleus in a mating; the cytoplasm of the nuclear donor is not transferred to the opposing monokaryon.

**parasexual recombination**            Chromosomal reassortment is common, but crossing over is rare. Alleles of loci on different chromosomes are shuffled; alleles of loci on the same chromosomes remain parental with rare exception.

**meiotic-like recombination**            Both chromosomal reassortment and crossing over are common. Alleles of loci

on different chromosomes are shuffled, as are alleles of loci on the same chromosomes.

**Somatic recombination**                      Creation of non parental combinations of alleles in a mycelium without fruit-body formation.

**specific-factor transfer**                      One or more genes under strong selection move between nuclei and into another genetic background without carryover of any additional genes.

**tetrapolar**                                      Two factors specify mating type such that the meiotic offspring of a dikaryon include four mating types compatible in two pairs: A1B1 and A2B2; and A1B2 and A2B1; synonym **bifactorial**. (Species with only one such factor are **bipolar**; synonym **unifactorial**.)

**monokaryon**                                      A haploid mycelium derived from a single basidiospore. Many monokaryons have mainly uninucleate cells, but some also have cells with variable numbers of nuclei. See also the definition of Papazian (57). Diploids may also have predominantly unicleate cells and are also technically monokaryons. (**Homokaryon** is the term for a mycelium with only a single genotype of nucleus, regardless of the numbers of nuclei per cell.)

**dikaryon**                                      A mycelium within which each cell contains paired, synchronously dividing nuclei, one of each of the

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original gametic genotypes. See also the definition of Papazian (57). (**Heterokaryon** is the term for a mycelium with more than one genotype of nucleus, regardless of the numbers of nuclei per cell.)

#### **FIGURE LEGENDS**

Figure 1. Mating of haploid strains of *Armillaria gallica*. Two mates were inoculated alone on either side; the pairing appears in the middle. The dotted line indicates the region of initial contact between mycelia, where fusion of hyphae from the different mates occurs. Nuclear migration proceeded bi-directionally, with each mate functioning both as donor (male) and recipient of nuclei (female). While the nuclei migrate rapidly (i.e., on the order of ten fold faster than the mycelial growth rate), the mitochondria do not and the final mated colony is mosaic for parental mtDNA types. Heteroplasmy for mtDNA is restricted to those cells resulting from fusion of hyphae of the two mates near the center (dashed line). The rhizomorphs (arrow) carry the mtDNA type from the area of the mated colony from which they arose. In *A. gallica*, the initial dikaryon established after nuclear migration becomes diploid, but the dynamics of nuclear migration and mitochondrial inheritance are otherwise typical of Homobasidiomycetes. Note that the colony morphology of the diploid is different



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from that of the mates; it has less aerial mycelium.

Figure 2. Unpublished spatial map of genetic individuals of *Armillaria gallica* in a mixed hardwood forest of Michigan. The rectangle (130 m in length) in the middle is a clear-cut site that was replanted with red pine and was the subject of intensive sampling of *Armillaria* in individuals (75-77). The dots represent collection points and the lines encircle collections with identical multilocus genotypes. Each individual has a unique mtDNA type, such that the samples of each individual have only a single mtDNA. No mosaicism for mtDNA that may have been present in the initial mating has been detected in these or any other individuals of *A. gallica* from which multiple collections were made (67, 68).

Figure 3. Somatic recombination in an incompatible di-mon mating of *Schizophyllum commune*. Graphic representation of the data of Ellingboe (21). The dikaryon genotype is at the top. The homokaryon is below the dikaryon. The two haploid genomes of the dikaryon are marked as light / dark gray. Linkage between markers is indicated by a solid line; breaks in lines indicate that markers are not linked. The nuclei fertilizing the monokaryon were genotyped by the

method of Papazian. Recombinants include numerous examples of unlinked and linked loci, as would be expected in meiosis. Interestingly, specific-factor transfer was not observed in this di-mon mating; recombination was exclusively meiotic-like.

Figure 4. Reciprocal and non reciprocal genetic exchange between nuclei during long-term growth. Two of 25 SNP markers detected by Southern hybridization of amplified DNA of marker loci with allele-specific oligonucleotide probes (14). The original paired haploid nuclei carried different alleles for each of the 25 marker loci. Asterisks show reciprocal genetic exchange; circles show an non reciprocal exchange.

Figure 5. Generalized basidiomycete life cycles. Diploid nuclei in black; haploid nuclei in gray. a, typical of most homobasidiomycetes with dikaryotic vegetative phases. b, typical of *Armillaria mellea*. No dikaryotic stage has been observed and matings produce only diploids, which carry through the vegetative phase and into the basidia. c, typical of most *Armillaria* species including *A. ostoyae*, *A. gallica*, and *A. tabescens*. A dikaryon forms in matings but the duration of the dikaryon phase is variable. The nuclei

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fuse, leading to a persistent diploid. During fruiting there is a pre-meiotic reduction with dikaryons appearing in the pre-basidial cells. Finally, the nuclei of the dikaryon fuse to form a diploid that immediately undergoes meiosis. *Dik-* strains of *S. commune* form dikaryons that become diploid as in Fig. 5c; the events during fruiting and up to meiosis have not been characterized for *dik-* strains. Modified with the permission of Kari Korhonen, who created the original version (see ref. 31).

Figure 6. Genetic exchange in diploids and dikaryons affecting two loci of unknown linkage relationship. Both reciprocal and nonreciprocal exchange produce new combinations of alleles within the nuclei of the dikaryon, but not in the diploid.

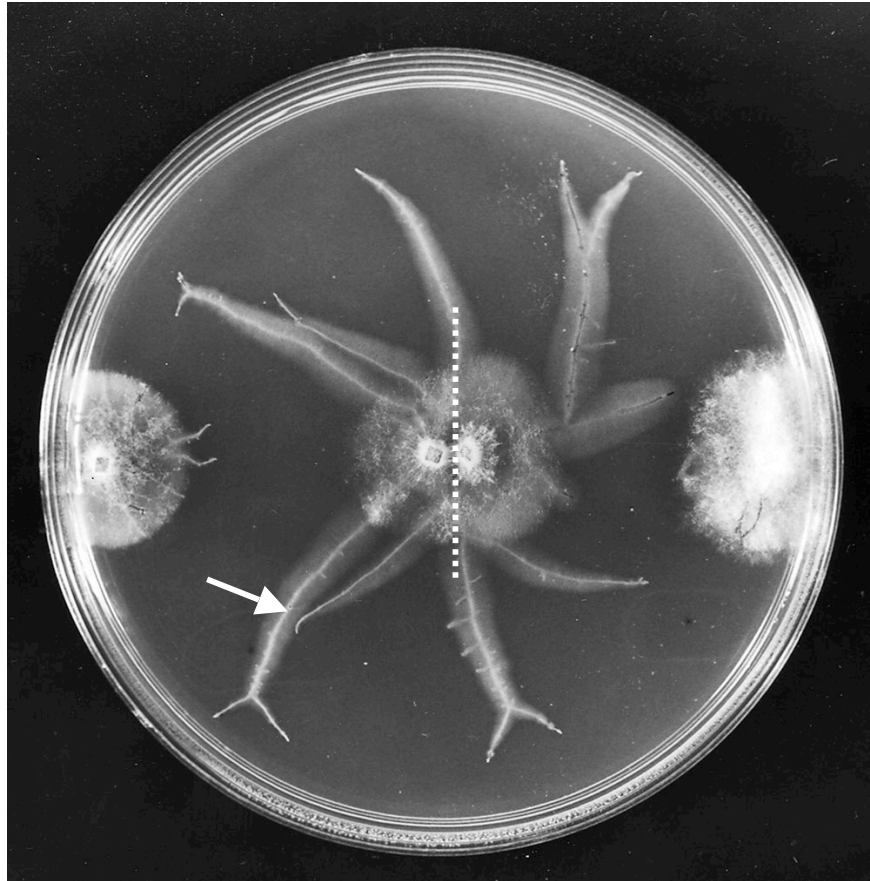


Figure 1 (draft version)

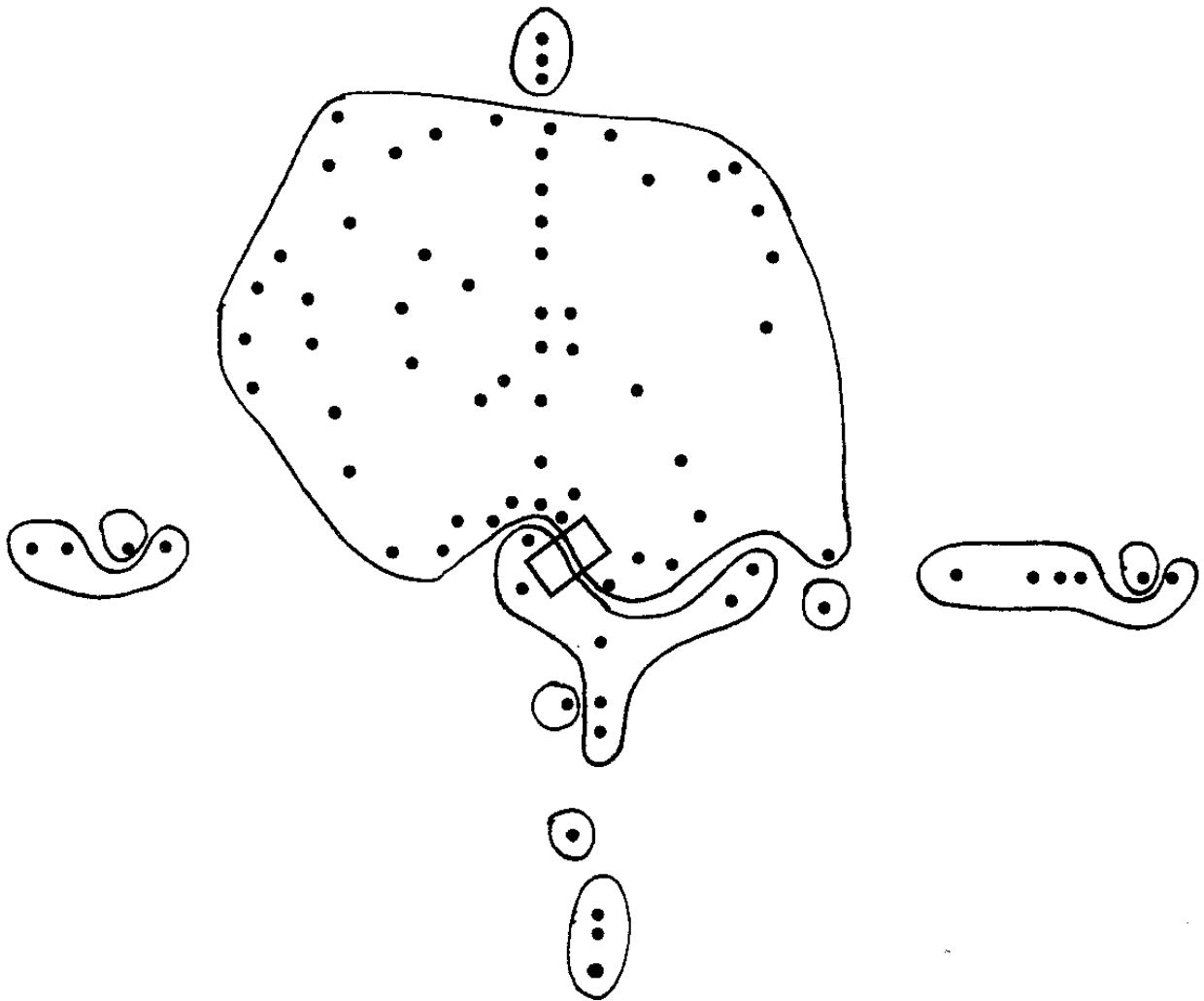
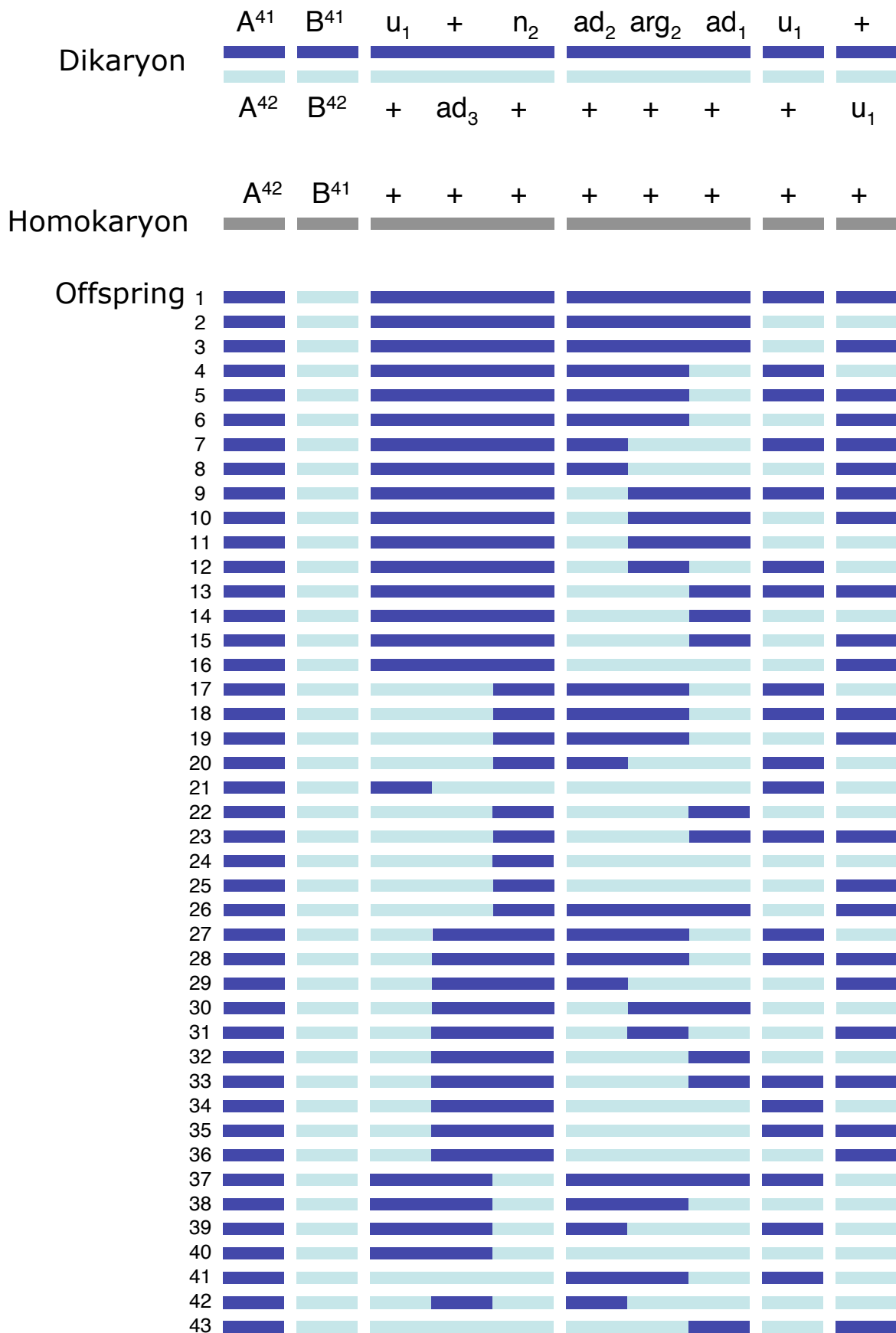


Figure 2 (draft version)



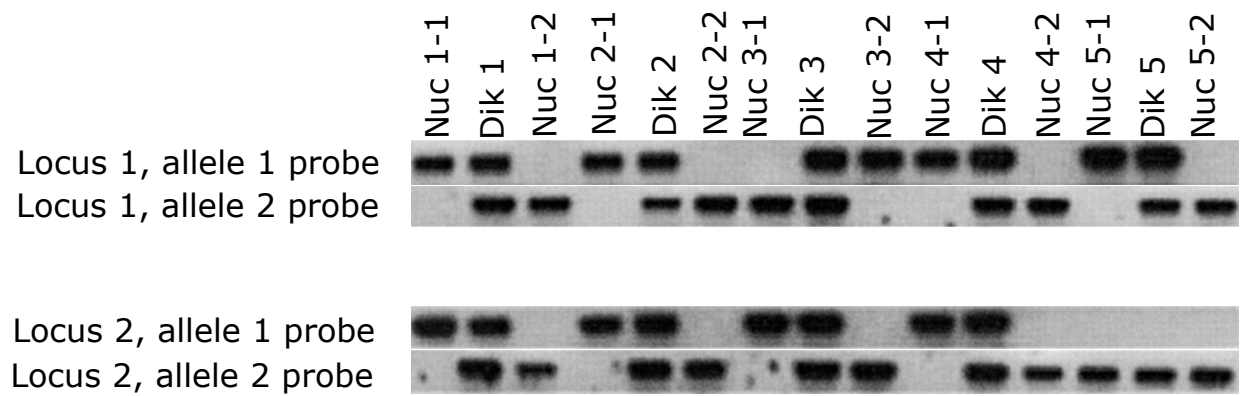


Figure 4 (draft version)

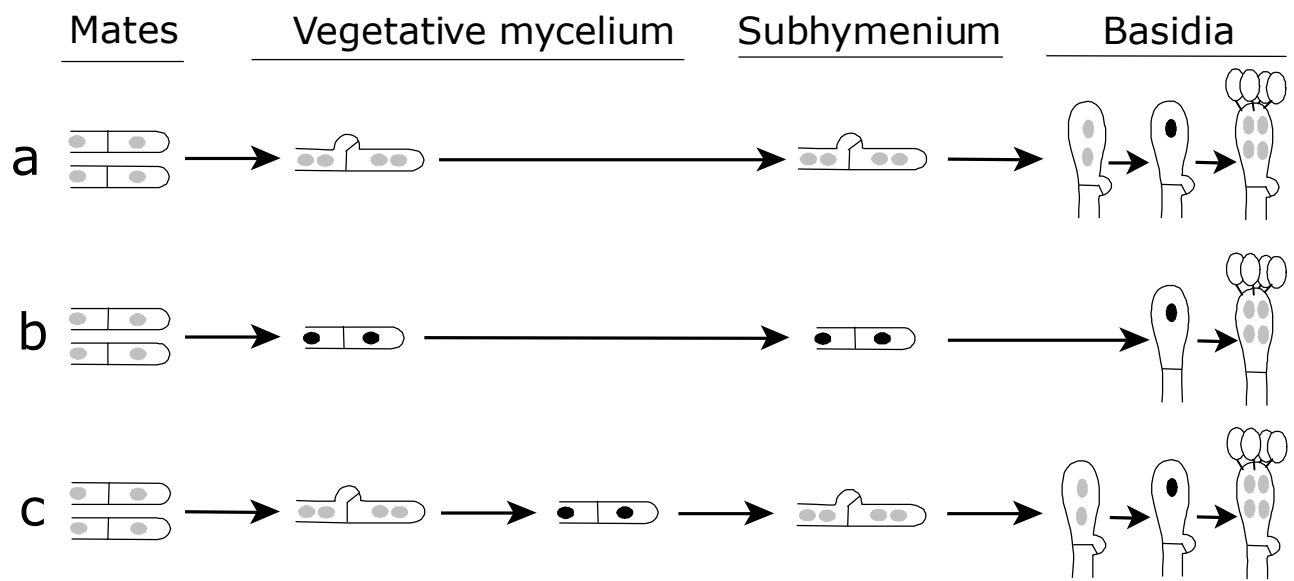


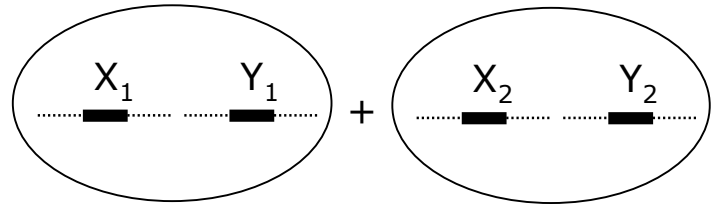
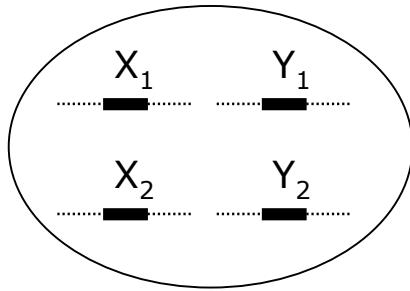
Figure 5 (draft version)



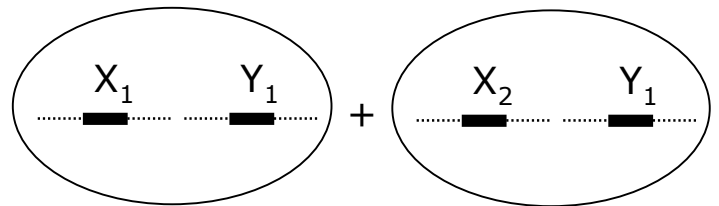
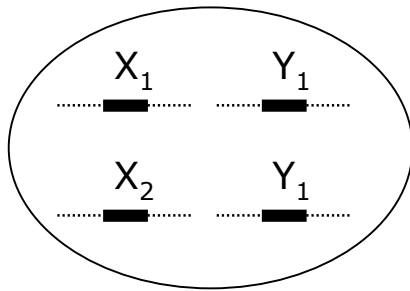
## Diploid

## Dikaryon

No  
exchange



Non-  
reciprocal  
exchange



Reciprocal  
exchange

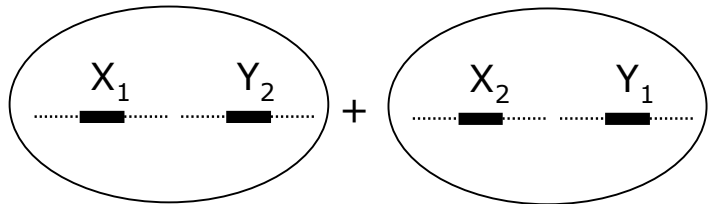
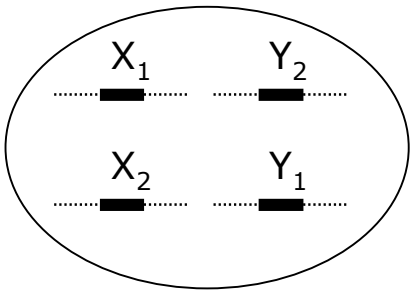


Figure 6 (draft version)