Interspecific hybridization as a driver of fungal evolution and adaptation

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Abstract

Cross-species gene transfer is often associated with bacteria, which have evolved several mechanisms that facilitate horizontal DNA exchange. However, the increased availability of whole-genome sequences has revealed that fungal species also exchange DNA, leading to intertwined lineages, blurred species boundaries or even novel species. In contrast to prokaryotes, fungal DNA exchange originates from interspecific hybridization, where two genomes are merged into a single, often highly unstable, polyploid genome that evolves rapidly into more stable derivatives. The resulting hybrids can display novel combinations of genetic and phenotypic variation that enhance fitness and allow colonization of new niches. Interspecific hybridization led to the emergence of important pathogens of humans and plants (for example, various *Candida* and 'powdery mildew' species, respectively) and industrially important yeasts, such as *Saccharomyces* hybrids that are important in the production of cold-fermented lagers or cold-cellared Belgian ales . In this Review, we discuss the genetic processes and evolutionary implications of fungal interspecific hybridization and highlight some of the best-studied examples. In addition, we explain how hybrids can be used to study molecular mechanisms underlying evolution, adaptation and speciation, and serve as a route towards development of new variants for industrial applications.

Introduction

The traditional view that organisms belonging to different species do not exchange DNA has long been abandoned. In bacteria, this concept was already challenged in 1928, when Frederick Griffith showed experimentally that genetic information (in this case a virulence factor) can be transferred horizontally from one *Streptococcus pneumoniae* strain to another[1]. Later, exchange of genetic information was shown to not be restricted to within-species exchanges. Instead, several independently-evolved asexual mechanisms allow horizontal gene transfer between different species[2]. The prevalence of horizontal gene transfer in bacteria profoundly affects their evolution and adaptation and even casts doubt on the relevance of the concept of a 'species' for some prokaryotic lineages[3].

By contrast, transfer of genetic material between eukaryotic species was often considered a rare event of little evolutionary consequence because the resulting offspring is typically sterile. Nonetheless, botanists have long recognized that some plant species can exchange genetic material and that these events, although rare, are important drivers of plant evolution[4–6]. More recently, the ever-increasing number of sequenced genomes has revealed that many eukaryotic genomes contain loci that originate from other species, suggesting that interspecific DNA exchange is prevalent in all kingdoms of life[7,8], including fungi[9–11] (Fig.1; Table 1). Here, specific horizontal gene transfer events that resemble the exchange of DNA fragments typically observed in bacteria are occasionally reported[12], and some molecular mechanisms have been proposed[13]. However, the majority of cross-species transfer of fungal genetic material originates from a process termed interspecific hybridization, which can either occur through sexual or parasexual mating.

Sexual hybridization is arguably the best-known mechanism of horizontal DNA exchange between individuals. It requires the generation of gametes of various mating types by meiosis, recognition of a compatible mating partner and ultimately cell–cell fusion to yield a zygote that combines (parts of) the DNA from the parents. Although these basic features are shared across the tree of life, fungi have evolved a broad array of specific mechanisms and considerable variation in the timing of karyogamy (nuclear fusion) is observed[14]. In Chytridiomycota and Zygomycota, karyogamy occurs quickly, often directly followed by meiosis[15]. However, in Ascomycota and Basidiomycota, karyogamy is sometimes delayed, resulting in cell lineages that maintain nuclei of both parental mating types. The length of this dikaryotic phase is species-dependent, with some (particularly Basidiomycota) species

maintaining this state until the next sexual cycle begins, whereas other species (such as *Saccharomyces cerevisiae*) induce karyogamy rapidly after mating[16].

In contrast to the sexual cycle, parasexuality is unique to fungi and other single-celled organisms. Parasexual mechanisms enable the transfer of genetic material without the need for meiosis or the development of sexual structures. The process starts with the fusion of vegetative cells or hyphae, leading to multinucleate cells termed heterokaryons. Heterokaryons of incompatible strains can show reduced fitness and genetic instability and consequently often revert back to homokaryons[17]. However, in other cases, heterokaryons can undergo nuclear fusion and mitotic cross-over of chromosomes, often followed by chromosome losses leading to heterogeneous populations of heteroploid hybrids[18].

How can sexual and parasexual mating lead to DNA exchange between species? In most organisms, a range of safeguard mechanisms are in place to prevent hybridization between gametes or vegetative cells of different species. Although such reproductive barriers often emerge haphazardly[19], they can be very effective in maintaining species integrity by impeding the formation or affecting the fertility and viability of a hybrid. However, in fungi, the barriers are often not absolute and their strength largely determines the evolutionary outcome of the hybridization process. Therefore, in this Review, we first provide an overview of the different types of reproductive barriers and their strength. Even if these barriers are overcome, the evolutionary success of hybrids is not guaranteed, as newly formed hybrids often suffer from fitness defects, are inherently unstable and can undergo drastic genomic changes. Then, we next discuss the short-term and long-term implications of interspecific hybridization for the organism's phenome, genome, transcriptome and proteome. We explain how the genomic instability may actually result in a remarkable adaptive potential, allowing hybrids to quickly evolve features that enable the colonization of niches that are inaccessible to both parent species. We conclude by describing the origin and evolutionary trajectory of some important fungal hybrids that formed and thrive in human-associated environments. In particular, a number of recent studies show how hybridization fuelled the emergence of important animal, plant and human pathogens, but also reveal how many benign yeasts that are used in the production of fermented products such as beer, wine and bread have a complex ancestry of hybridization.

[H1] Overcoming species boundaries

In the canonical view of the biological species concept, species are represented as separate populations that are reproductively isolated from each other (reviewed elsewhere[20]).

Reproductive barriers restrict gene flow between species, most notably by preventing the hybridization event itself. However, even when these reproductive barriers are overcome and a hybrid is formed, the evolutionary outcome depends on the presence of barriers between the newly formed hybrid and the parent species. When crossing between the parent species and the hybrid is prevented, the potential for the hybrid to evolve as a distinct population is increased. If these barriers are absent or permissive, newly formed hybrids often engage in repeated backcrossing with one (usually the most abundant) parent species, leading to a dilution of the other parent subgenome, an process termed introgression[21].

In animals, plants and Ascomycota, reproductive barriers are traditionally subdivided into pre-zygotic and post-zygotic barriers. However, this terminology can be misleading in organisms with a long dikaryotic stage (such as Basidiomycota), , so we will use 'pre-mating' and 'post-mating' throughout this Review (Fig.2).

[H2] Pre-mating barriers

For species to hybridize, the first prerequisite is that they occur in the same place at the same time. Geographical, ecological and temporal isolation of parent species are very effective pre-mating barriers (Fig.2). Whereas geographical and ecological isolation are common in fungi, only a few cases of temporal isolation have been described. For example, temporal isolation plays a part in maintaining genetic differentiation amongst *Ampelomyces* spp., which are fungal intracellular mycoparasites that target plant pathogens. Notwithstanding that *Ampelomyces* spp. readily hybridize in a laboratory environment, hybrids are not encountered in nature because of the seasonal occurrence of their hosts[22].

Even if species are not isolated in space or time, other pre-mating barriers can exist, such as favoured selfing or assortative mating. Favoured selfing has been described in *Saccharomyces paradoxus*[23] and *Microbotryum violaceum*[24], and limits the occurrence of interspecific mating. In assortative mating in fungi, gametes can discriminate between conspecific and heterospecific individuals, for example through species-specific pheromones and receptors[25].

In some cases, geographical and ecological barriers to hybridization have been erased by industrialization, globalization, large-scale agriculture and changes in climate. This barrier removal is especially relevant for pathogens, and globalization is hypothesized to be one of the main drivers of their increasing emergence[9,26]. For example, the chytrid fungi *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* are the causative agents of chytridiomycosis, a disease that is causing catastrophic losses in nearly half of all amphibian species[27,28]. Both species originated in Asia[29], but anthropogenic activities fuelled their worldwide spread, creating the opportunity for hybridization between divergent

lineages and the emergence of new, highly virulent genotypes[30]. The increased incidence of interspecific hybridization in previously geographically isolated lineages might be further promoted by 'reinforcement', also known as the 'Wallace effect'[31]. According to the Wallace effect, reproductive isolation is often stronger in sympatric than in allopatric species, suggesting that there is natural selection against hybridization during sympatry[32–35]. The evolutionary benefit of this effect has been ascribed to various factors, such as prevention of spreading viruses[36] or avoiding costly reproduction processes that will otherwise result in inviable offspring[34]. For example, *Neurospora crassa* and *Neurospora intermedia* are often sympatric, but natural hybrids have never been encountered. While mating between these species is possible, *N. crassa* can selectively abort hybrid progeny before zygote formation. In line with the Wallace effect, this occurs at a higher frequency in sympatric populations[34].

[H2] Post-mating barriers

Following mating, the newly formed hybrids may display reduced viability or sterility, which can be attributed to multiple, often concomitant factors. Species that evolved independently can accumulate variation that may not be compatible, so-called 'Bateson–Dobzhansky–Muller incompatibilities' (BDMIs). The deleterious effect of BDMIs mostly originates from negative epistatic interactions that disrupt co-adapted gene complexes[37] and the emergence of BDMIs is affected by the evolutionary history of the parent populations. For example, in *Saccharomyces*, mutations leading to BDMIs arose more rapidly in parent populations experimentally evolved in distinct environments (low-glucose and high-salinity) compared to parent lineages inhabiting similar niches[19,38] . In one case, the incompatibility was pinpointed to an antagonistic epistatic effect between mutations in each parent, more specifically between allelic variants of *PMA1* (encoding a proton-efflux pump) and *MKT1* (encoding a global mRNA regulator)[19]. Whereas the individual mutations are adaptive in the parent lineages, their co-occurrence in hybrids results in a reduced glucose uptake rate. In parasexual reproduction, vegetative incompatibility can arise when conflicting alleles of specific loci termed *het* (heterokaryon) or *vic* (vegetative) are co-expressed in the same hyphal cytoplasm, which can result in the activation of programmed cell death[39].

In addition to incompatibilities linked to one or a handful of loci, sequence divergence between parent genomes can also reduce meiotic crossing-over efficiency, thereby preventing recombination through the anti-recombination machinery. As a result, chromosomes will mis-segregate, leading to generally inviable aneuploid segregants[40]. In *Saccharomyces* hybrids, anti-recombination has been suggested to account for 97% of hybrid sterility[40,41]. However, this form of sterility is sometimes resolved by whole-genome

duplication (WGD) in the hybrid, which provides homologous chromosomes for correct meiotic pairing[42–45]. Similarly, fertility can be regained by loss of heterozygosity (LOH). LOH occurs during long periods of mitotic growth[21] or when meiosis is aborted after it is initiated and cells return to mitotic growth, a process referred to as 'return to growth'[46]. The resulting blocks of homozygosity can facilitate recombination between highly diverged subgenomes, thus promoting correct chromosome segregation. In addition, LOH events at the mating type locus can give rise to mating-proficient diploid hybrids[46]. Nevertheless, even complete sterility does not necessarily limit the evolutionary potential of fungal hybrids, because of their ability to reproduce asexually. This crucial difference from many multicellular eukaryotes, in particular animals, largely explains why fungal hybrids are so common.

[H2] Avoiding hybrid extinction

The evolutionary success of a newly formed hybrid is determined by its fitness as well as whether it is reproductively isolated from the parent species. Reproductive isolation can be caused by various factors, most commonly hybrid ploidy and ecological isolation (for example, occupation of a new niche). Hybrids can display the same ploidy level as the parent species (through fusion of gametes) or the sum of the number of parent chromosome sets (through fusion of cells), generally referred to as allodiploidy (n = 2) or allopolyploidy (n>2). An increase in ploidy compared with the parent species allows immediate reproductive isolation, as the hybrid is often sterile, or because chromosome pairing between hybrid and parents during backcrossing is disrupted. Consequently, the vast majority of currently reported fungal hybrids are allopolyploids.

When hybrid and parent genomes have the same ploidy, balanced pairing of chromosomes during meiosis is possible and successful backcrosses might follow. However, if the sequence divergence of the paired chromosomes is too high to allow cell division, or if other barriers, such as ecological isolation, prevent backcrossing with the parents, the hybrid gains the potential to evolve into a separate species[47,48], a process termed 'homoploid speciation'. For example, in the haploid grass pathogen *Zymoseptoria pseudotritici*, interspecific hybridization between two closely related (3% nucleotide divergence) haploids led to the formation of a (transient) diploid zygote. After meiosis, haploidy was re-established, resulting in persistence of a homoploid hybrid, likely because of host specialization[48].

[H1] Hybrid fitness advantages

For some traits, hybrids sometimes outperform the parent species, a phenomenon termed 'heterosis' or 'hybrid vigour'. Although heterosis is well documented in hybrids, it seems counterintuitive that mixing of genomes from different species will result in superior performance, and for over a century biologists have struggled to formulate a unifying hypothesis of the underlying mechanisms of heterosis[49]. Several models have been proposed. These models are not mutually exclusive and their relative importance likely varies according to phenotype and genotype. The overarching view in the field attributes heterosis to enhancement of growth-promoting pathways[50]. The first, and perhaps most well-known hypothesis, termed 'dominance', was coined in 1908 by Charles Davenport. This hypothesis states that deleterious recessive alleles of one parent can be masked by dominant advantageous alleles of the other parent. Around the same time, the geneticists Edward East and George Shull independently developed the 'overdominance' hypothesis. In this model, heterosis is attributed to increased levels of heterozygosity in the hybrid, referred to as 'heterozygote advantage', which implies that for each gene and each environment, hybrids have a copy of 'the best' parent allele. Throughout the years, several variants of these models have been proposed and validated experimentally, most notably the 'pseudo-overdominance' model. In pseudo-overdominance, complementation occurs for different recessive alleles that are present in close linkage but on opposite members of a pair of homologous chromosomes such that overdominance seems to be operating[50]. Last, heterosis can also be due to newly established favourable interactions in the hybrid between alleles of different loci ('epistasis')[51].

Although these models are widely accepted, they fail to account for all observed cases of heterosis[50,52]. In 2017, a new model was proposed that challenges the view that 'heterosis' and 'genetic incompatibility' are contrasting mechanisms[53]. In this model, incompatibilities in the hybrid perturb regulatory mechanisms that evolved to protect cells against damage or prepare them for future challenges by limiting cell growth. For example, cell cycle checkpoints no longer function correctly and, as a consequence, hybrid cells do not reduce growth in response to mild stressors (for example, moderate ethanol concentrations). Although this model has been experimentally validated in artificial *Saccharomyces* hybrids, it is unclear to what extent such deficiencies in safeguard mechanisms are relevant over long evolutionary timescales and for survival in natural, fluctuating environments.

[H1] Hybrid genomic instability

It is important to note that increased hybrid fitness does not necessarily emerge immediately after hybridization. Instead, the immediate effect of joining two divergent genomes in one nucleus can be dysfunctional. If not fatal, this dysfunction may lead to decreased fitness compared with the parents, whose genomes have each been honed by natural selection[49,54]. At first glance, interspecific hybrids would therefore seem to be likely to be outcompeted by the parent species. However, the genomes of newly formed interspecific hybrids are highly unstable, quickly spawning new variants through various molecular mechanisms that shuffle, amplify, delete or alter the inherited genetic blocks[21]. This immense plasticity can purge deleterious interactions between the two subgenomes and ultimately result in a more stable genotype that bestows increased fitness, a process referred to as 'genome stabilization' (Fig.3).

[H2] Introgression and LOH

A first mechanism by which hybrids can become more fit and stable is backcrossing to one of the parent species. As this process gradually replaces the unstable, sub-optimal hybrid genome with parts of only one parent, it can in a sense be seen as an 'extinction' of the hybrid, as the other parent genome is gradually diluted out. Importantly however, loci of the second parent that provide a fitness benefit are maintained by selection and may eventually reach fixation (adaptive introgression). For example, ~5% of the genome of the wheat pathogen *Zymoseptoria tritici* comprises introgressed regions, including 18 virulence genes[55]. Similarly, large blocks of introgressed DNA from multiple *Neurospora* species have been detected in the sex-determining region (the *mat* locus) of different lineages of the *Neurospora tetrasperma* species complex[56,57].

However, not all introgressed regions are a result of backcrossing. Spontaneous loss of DNA of one of the two parent species can occur during mitotic recombination. For example, a clonal descendant of the ancestral yeast hybrid that founded the *S. cerevisiae* 'Alpechin' lineage, which is associated with olive oil production, retains the ancestral genome structure of the first-generation hybrid, harbouring contiguous *S. cerevisiae* and *S. paradoxus* subgenomes (indicative of the absence of meiosis and backcrossing)[58]. Nevertheless, the genome contains more than one hundred LOH blocks that likely originated from mitotic recombination events and even rescued hybrid fertility[21].

Ultimately, LOH results in a complex mixture of the two parent genomes, with mosaic chromosomes consisting of loci inherited from both parents[10,59,60]. These complex heterozygosity patterns are one of the defining features that is currently used to recognize and study ancestral hybridization events[45,61–63].

The significance of LOH in fungal hybrid evolution and the pace at which it can arise became especially apparent in laboratory evolution experiments[64–67]. When artificial hybrids between *S. cerevisiae* and *Saccharomyces uvarum* were subjected to nutrient limitation, LOH events were observed in about half of populations after only 200 generations[65]. Furthermore, the environment dictated which LOH variants were retained in the population. In cold conditions, the subgenome of the cryotolerant *S. uvarum* was favoured over that of *S. cerevisiae* and vice versa[65,67].

Of note, LOH does not always occur at a large scale. Sometimes, the initial heterozygosity largely persists and seemingly has a role in the hybrid's adaptive potential. For example, the human pathogen *Candida albicans* is highly heterozygous, largely owing to an ancestral interspecific hybridization event[68]. *C. albicans* has on average one heterozygous site every 200–300 bp, although this varies among isolates[69,70]. This heterozygosity seemingly serves as a reservoir for (cryptic) genetic variation, with LOH events occurring at high frequency when cells are subjected to stressors (for example, the therapeutic drug fluconazole), allowing rapid adaptation[70].

[H2] Aneuploidy

Another phenomenon that occurs frequently during genomic stabilization is aneuploidy — the loss or gain of entire chromosomes. Aneuploidies arising during meiosis can lead to sterility and therefore introduce a reproductive barrier (Fig.2). However, aneuploidies might also provide an evolutionary advantage by tweaking gene dosage or purging dominant deleterious alleles, thus allowing swift adaptation to stresses[71–74]. In such cases, aneuploidy is considered a transient state, and the organism often reverts back to euploidy when a more efficient genetic solution (for example, a mutation) is acquired[75]. In addition, aneuploidy can also aid in purging genomic incompatibilities. Consequently, aneuploidy is pervasive in many fungal hybrids. For example, in several strains of the human pathogen *Cryptococcus neoformans × Cryptococcus gattii* (serotype AD), the amplification of chromosome XIII has been linked to increased virulence[76]. In the lager beer yeast hybrid *S. cerevisiae x S. eubayanus*, chromosome copy number varies considerably among strains, ranging from 45 to 79 chromosomes per cell[77]. This karyotype variability is functionally relevant, as variants differing only in chromosomal copy number can show marked differences in industrially-relevant phenotypes, such as flocculation and production of the off-flavour compound diacetyl[77].

[H2] Whole-genome duplication

Another common consequence of interspecific hybridization is WGD, which has been extensively reported in plants[42–45,78]. WGD can restore correct chromosome pairing, thereby enabling meiosis to occur[42]. It has been suggested that interspecific hybridization is the root cause of the well-established WGD in the *S. cerevisiae* lineage[44,45]. This ancient WGD took place ~100My ago and was initially considered to involve autopolyploidization. However, new analyses revealed that the duplication was instead a direct consequence of an ancient interspecific hybridization event, either between two diploid species or between two haploid species that in turn underwent autodiploidization to re-establish fertility[45].

[H2] Mitonuclear compatibility

The mitochondrial genome and its interaction with the nuclear genome can also affect the fate of hybrids. Mitochondrial inheritance during hybridization varies extensively within the fungal kingdom. The majority of Basidiomycota only inherit one of the parent mitotypes during hybridization (uniparental inheritance)[79] and which mitotype is inherited can strongly influence the hybrid's characteristics. For example, the virulence of the hybrid fungus *Heterobasidion annosum*, one of the most important and economically devastating tree pathogens, is a direct consequence of its inherited mitotype[80,81]. In Ascomycota, inheritance is biparental and hybridization results in nuclear–mitochondrial chimeras[82]. However, vegetative segregation leads to the fixation of a single mitotype in the hybrid lineage. Which mitotype is retained can be determined by genetic drift (random changes in population genetic variation due to chance events), but more often there is a preferential inheritance of one mitotype[59]. Losing a mitotype can purge mitonuclear incompatibilities, which are common in fungi[83–86], but positive selection has also been observed. For example, lager yeasts consistently inherited mitochondria of *S. eubayanus*, an observation in line with artificial hybrids evolved in lager beer-like conditions[59,87,88]. The main phenotypic advantage of the *S. eubayanus* mitochondria is cold tolerance, a crucial phenotype for lager beer fermentation[89,90].

[H1] Transcriptome shock in hybrids

Combining divergent genomes in one nucleus can alter the transcription regulatory programme of the parent species, affecting both absolute expression levels and gene regulation. These alterations in expression have been studied extensively in plant and animal hybrids and can include homoeologue expression bias[91–93], subgenome dominance[93–95], inter-species transcriptional rewiring[96–98] and chromatin accessibility modifications[99], collectively referred to as 'transcriptome shock'[100–102] (Fig.4).

In natural hybrids, it is often challenging to disentangle whether transcriptional changes are directly related to hybridization or are due to polyploidization. Furthermore, the rate at which certain changes occur after hybridization is variable, and the immediate transcriptional response before genome stabilization might differ from long-term alterations due to selection or genome restructuring. However, several studies have begun to unravel transcriptome shock in fungi, using both natural and artificial hybrids[52,102–109]. In contrast to plant and animal hybrids, this shock in fungal hybrids seems to be mild, and differences in absolute expression levels between parent species are largely maintained upon hybridization[105,109]. This transcriptional robustness might be associated with limited interaction between the transcriptional networks of the two species, which is likely explained by the generally high divergence between parent species[110]. For example, in artificial *S. cerevisiae* x *S. uvarum* hybrids (20% nucleotide divergence[111]), the vast majority of homoeologous genes (87%) inherited the same expression levels as the corresponding orthologous genes in the parent species. Only 10% of genes showed a smaller expression difference between homoeologues in the hybrid than between orthologues in the parent species (homoeologue expression blending), whereas the remaining 3% showed a greater expression difference between homoeologues than between orthologues (homoeologue expression bias)[105] (Fig.4). However, in some plant hybrids, the number of genes displaying gene expression alterations is much larger, with one third of orthologues in rapeseed[112] and cotton[113] hybrids showing homoeologue expression blending or bias.

In *Saccharomyces* artificial interspecific crosses, the magnitude of orthologue expression differences between parent species is generally larger than homoeologue expression differences in hybrids[105], indicating that transcriptional differences between parent species are partly buffered after hybridization. Similar trends were also observed in a natural allopolyploid hybrid, *Epichloë hybrida* Lp1, which is a grass endophyte that is estimated to have emerged 300,000 years ago[102,114]. However, only 56% of homoeologues in Lp1 inherited the parent expression profiles and over 25% displayed homoeologue expression blending. This discrepancy with the *Saccharomyces* hybrids may be explained by the lower divergence between subgenomes (5% nucleotide divergence) in Lp1, or could be a result of mutations acquired during evolution.

When studying hybrid transcription, it is important to consider that variation in gene expression levels is independent from regulatory variation. Genes that become differently expressed in the hybrid might show conserved regulatory patterns, while conserved gene

expression levels can be caused by different regulatory underpinnings[115]. Regulatory variation between species mainly results from the complex interplay of mutations accumulated in cis-regulatory elements and/or trans-regulatory elements during their divergence[110,116–118]. In fungi, a large fraction of the regulatory variation between parent species remains conserved in the hybrid, suggesting that it originates mostly from cis-acting variation[116]. However, in some cases, regulatory variation is lost in the hybrid, which can be attributed to trans-effects that influence homoeologous genes equally[54,116,119] or cis-elements blending between homoeologues[110] (Fig.4). The latter can occur when genetic divergence between parent genomes is low or because of subgenome homogenization during stabilization. For example, in the hybrid *Trichosporon coremiiforme*, many transcription factor-binding sites are shared between subgenomes, leading to a coordinated regulation of transcription that results in substantial homoeologue expression blending[106]. Furthermore, in *Saccharomyces* artificial interspecific crosses, cis-acting variation shows an additive mode of expression inheritance, whereas trans-acting variation follows a dominant mode that mirrors only one of the parents, mostly associated to limited activity of transcription factors in one of the parents (and therefore also in the corresponding homoeologues)[115].

[H1] Chimeric proteins and protein complexes

Hybridization, and the subsequent genome stabilization, can also affect the properties of proteins and protein complexes. For example, during genome stabilization, LOH and gene conversion can fuel the formation of chimeric genes, potentially leading to proteins with altered properties[120,121]. Laboratory evolution experiments showed that chimeric protein formation is a recurring and potentially adaptive event. For example, during evolution of artificial *S. cerevisiae x S. uvarum* hybrids in ammonium-limited conditions, an adaptive chimeric variant of the high-affinity ammonium permease Mep2 evolved several times independently[122].

Interactions between homoeologous proteins from the parent species (interlogous protein–protein interactions (PPIs)) can lead to chimeric protein complexes, which potentially show altered functionalities (Fig.5). In *Saccharomyces* hybrids, most interlogous PPIs can occur[123,124]. Furthermore, novel PPIs that are not encountered in either of the parent species can emerge, modifying the functionality of the complex. For example, a novel PPI in the Trp2–Trp3 complex had a heterotic effect on tryptophan transport[125]. However, for some complexes, PPIs preferentially occur between proteins from the same parent (intralogous PPIs). These preferential interactions could be caused by sequence divergence between homoeologues or

stoichiometry imbalances of homoeologues[123]. Of note, sometimes even interlogous PPIs can lead to incompatibilities. For example, co-evolution of interacting proteins within the proliferating cell nuclear antigen (PCNA) complex prevent chimerism, leading to hybrid network incompatibilities[126]. However, this incompatibility was observed between proteins from very distant species and its relevance in hybrid speciation is yet to be determined.

[H1] Domesticated and pathogenic hybrids

Given that interspecific hybridization is an evolutionary fast-track to adaptation in the face of sudden environmental changes, it is perhaps not surprising that interspecific hybridization has been pivotal in fungal adaptation to novel niches that were created by humans. These niches range from industrial applications, such as the fermentation processes involved in the production of beer, cheese and wine, to naïve populations affected by introductions of novel pathogens.

[H2] Industrial fermentation

Humans have historically utilized the capacity of fungi to produce a wide variety of fermented products. By establishing standardized fermentation practices, fungi provided humans with a new, continuously available nutrient source. One of the earliest and most important aspects of standardizing fermentation practices is transferring material (including the fungi) from a completed fermentation to start a new batch, a process referred to as 'backslopping'[127]. The continuous cultivation of microbes in such challenging conditions required a rapid and strong adaptation process to the novel niche. In several cases, this adaptation, or 'domestication', was facilitated by interspecific hybridization[10,59,128,129].

Hybrids from a wide range of fungal genera have been isolated from fermentation environments (Table 1; Fig.1). However, interspecific hybridization is especially common in the *Saccharomyces* genus, of which half of the species have been shown to be involved in hybridization events (Fig.1d). Some hybrids even show an ancestry of four separate species (*S. cerevisiae × S. kudriavzevii × S. eubayanus × S. uvarum*)[59]. Most famously, strains used to produce lager-type beers form a distinct allopolyploid hybrid lineage (*S. cerevisiae x S. eubayanus*) named *Saccharomyces pastorianus*, which consists of two sublineages that are named for the region from which they were first isolated: 'Frohberg' and 'Saaz'. As these lineages share some breakpoints in their chimeric chromosomes, it is argued that they originate from a single hybridization event[121,130–132], although this is contested by some researchers[87,133]. Phylogenomic analyses revealed that the *S. cerevisiae* subgenome is most

closely related to beer yeast from continental Europe, and molecular clock analyses dated the hybridization event to the 16th century[10], perfectly coinciding with the origins of lager brewing in Bavaria (Germany). While the exact contribution of each parent species to the hybrid's evolutionary success is yet to be resolved, it is clear that these hybrids acquired complementary parent phenotypes, such as the vigorous fermentation capacity of *S. cerevisiae* and cold tolerance of *S. eubayanus*. Both traits are indispensable during lager production, which was performed in cold cellars during winter[10].

Inspired by the pervasiveness of hybrids in industrial settings, interspecific hybridization is now a well-established strategy to generate superior yeasts for industrial applications[134]. As such, laboratory-bred hybrids for beer, wine, baking, cider, sake, biofuel and commercial enzyme production are now available (Table 2). The added value of these hybrids for industrial processes is diverse. Sometimes, increased fermentation vigour or stress tolerance provides a competitive edge, but most often the advantage relates to the production of non-conventional metabolite profiles (Table 2). In a study in which 31 new lager yeasts were developed, drastic increases in ester production were observed when compared with benchmark strains[88]. Esters are some of the most significant flavour metabolites produced by yeast, imparting fruity and flowery notes to the product[135]. Similarly, hybrids of *S. cerevisiae* with other *Saccharomyces* spp. often produce increased glycerol levels compared with the *S. cerevisiae* parent, an important feature to reduce alcohol content in wines[136]. Most of the studies that develop artificial hybrids mimic hybridization events that also occur in nature but sometimes new species combinations are explored. For example, *S. cerevisiae* x *Saccharomyces mikatae* hybrids have never been isolated from natural environments but laboratory-made hybrids showed properties that are relevant for making wine, including increased ethanol tolerance and a two-fold increase of the rose-like compound 2-phenyl ethyl acetate[137].

Performing hybridization in a laboratory setting has several advantages. First, it allows a wider selection of species combinations, as pre-mating barriers are circumvented. Furthermore, alternative hybridization techniques, such as protoplast fusion, can be used, which even enable intergeneric crossing (Table 2). For example, hybrids between *Scheffersomyces stipitis* and *S. cerevisiae* displayed the robustness of *S. cerevisiae* and xylose utilization of *S. stipitis*, a combination required for second-generation biofuel production[138]. Researchers have further pushed the boundaries of interspecific hybridization by developing a hybrid with the genomic make-up of 6 *Saccharomyces* species[139].

[H2] Pathogenicity

The ability to rapidly respond to environmental changes or invade novel niches is particularly relevant for fungi engaged in symbiotic relationships, such as endophytes, mutualistic symbionts and pathogens[9,140]. Especially for pathogens, swift adaptation is essential in the continuous arms races with the host's immune system. It is therefore not surprising that hybridization is a major driver of these processes, yielding variants with altered virulence or host ranges[9,11]. Such hybridization-driven host expansions pose a severe threat, as they sometimes result in epidemic outbreaks[9]. Here, we discuss three genera that exemplify the key role of hybridization in the emergence of pathogens, one affecting elm trees (*Ophiostoma*), one affecting humans (*Candida*) and one affecting crops (*Blumeria*).

[H3] Dutch elm disease. Dutch elm disease (DED) is generally considered one of the most devastating plant pandemics, as two consecutive pandemics of this fungal infection in the past century led to the death of over a billion elm trees worldwide[141]. The DED pathogen complex consists of three lineages: *Ophiostoma ulmi* (OU) and two subspecies of *Ophiostoma novo-ulmi* (ONU), *Ophiostoma novo-ulmi* subsp. *novo-ulmi* (SSNU) and *Ophiostoma novo-ulmi* subsp. *americana* (SSAM) (Fig.1B). The first pandemic, which was caused by OU, started in the early 20th century and killed 10–40% of elm trees in Europe and North America. In the 1940s, an even more destructive pandemic emerged on both continents, which was caused by the more aggressive SSNU and SSAM lineages and destroyed most remaining mature elm trees. An analysis of 97 DED-causing fungi in 2020 showed how hybridization fuelled the increased virulence of SSNU and SSAM[142]. First, interbreeding between SSNU and SSAM was frequent, as is evident from the abundance of mosaicism in their genomes. Second, SSNU and SSAM showed varying degrees of introgression with OU, with OU genomic content representing 0–8% of their genomes. While functional analyses of these regions are still lacking, they are enriched for genes involved in survival and virulence, such as detoxification and reproduction.

[H3] Human pathogens. Human fungal pathogens are widely scattered across the fungal tree, and most have close non-pathogenic relatives (Fig.1). This indicates that the ability to infect humans can evolve rapidly and arose several times independently. The incidence of fungal infections, and especially those caused by hybrids, is steadily increasing[9]. For example, evidence of allodiploid hybridization has recently been described for *Aspergillus* spp., the filamentous fungi that causes aspergillosis[143]. The hybrid isolates show higher heterogeneity in virulence-related traits compared to related species. In the *Candida* genus, about 30 *Candida* species are able to infect humans, at least 4 of which are hybrids (Fig.1C). The most-studied is *Candida albicans*, a natural commensal of humans that can adopt a pathogenic

behaviour and is responsible for over half of the 73 million annual candidaemia cases[68,144]. While predominantly clonal, evidence of gene flow suggests the existence of (para)sexual hybridization events between diverged populations[145]. Moreover, the species itself originated through an ancient interspecific hybridization event[68]. Similar hybrid origins have been discovered for *Candida inconspicua*[146], *Candida metapsilosis*[61] and *Candida orthopsilosis*[147], the latter consisting of subgroups originating from at least four independent hybridization events between the same parent lineages[148].

[H2] Powdery mildew. Apart from being important human pathogens, fungal infections cause up to a third of all pest-driven crop losses[28]. 'Powdery mildew', an umbrella term for pathogenic fungi that cause similar symptoms after infection, can affect a range of plants and is one of the most widespread plant diseases[149]. The most intensively studied pathogen, *Blumeria graminis*, infects various grasses, including agricultural crops such as barley, rye, and wheat[150]. *B. graminis* is subdivided into several host-specialized sublineages (*'formae speciales'*), and hybridization is an important driver of host expansion. For example, powdery mildew of wheat (*B.g. f. sp. tritici*) was hypothesized to originate from an ancestral interspecific hybridization event[151] that was calculated to have occurred not long after the emergence of wheat bread ~10.000 years ago, suggesting that these pathogens can rapidly exploit new host opportunities triggered by agriculture. This rapid adaptation is also exemplified by the emergence of triticale pathogens. Triticale is an artificial hybrid of wheat (*Triticum* sp.) and rye (*Secale* sp.) that combines the yield and grain quality of wheat with the environmental tolerance of rye, and is resistant to the powdery mildews that infect the parent species. However, in 2001, the aggressive *B. graminis* variety *B. graminis f. sp. triticale* emerged, causing severe triticale losses[152]. Initial studies pointed to host range expansion of *B.g. f. sp. tritici* through mutation of a few genes[153] but thorough genomic investigation revealed instead that *B. graminis f. sp. triticale* arose from at least two independent hybridization events between rye-specific and wheat-specific mildews, followed by recurrent backcrossing to the wheat-specific mildews, demonstrating how pathogen evolution can rapidly mirror evolution on the host side[151].

[H1] Conclusions and outlook

The increasing availability of fungal genome sequencing data, fuelled by projects such as Y1000+[154], the 1002 Yeast Genomes Project[58] and many other genome sequencing initiatives[10,59,142,155], revealed the presence of interspecific hybrids and signatures of past genetic exchange, such as introgressions, in the evolutionary history of many fungal taxa.

Unlike other organisms such as animals, fungal hybridization does not seem to be hindered by a large genetic distance between parent species. For example, *Saccharomyces* hybrids have been identified that resulted from parent species with an average orthologous protein divergence of ~20%, which is roughly equivalent to the distance between humans and chickens[156]. This promiscuous hybridization is at least partly explained by the ability of fungi to propagate asexually after hybridization (allowing infertile hybrids to grow and acquire mutations and adaptations that help overcome possible incompatibilities between the subgenomes), but also by the remarkably similar chromosome karyotype and synteny that are often present between species of the same species complex or genus[37]. However, whereas the number of reported cases of interspecific hybrids and introgressions is growing, they likely represent only the proverbial tip of the iceberg, and several outstanding research questions need to be addressed to ascertain the full relevance of hybridization in fungal evolution and biodiversity.

First, whereas (introgressive) hybridization has been shown to be common across the fungal branch of the tree of life, the frequency of hybridization events at the population level is still unclear. Are interspecific hybrids the result of rare 'jackpot' events or, given the right circumstances, do many different hybrids form but are usually weeded out from the population? Second, most hybrids are detected in industrial or clinical settings, which are also the most intensively sampled niches. Denser sampling of other, more natural environments, particularly those with extreme or fluctuating conditions, would help to establish which environmental factors dictate the evolutionary relevance of interspecific hybridization. Third, extensive gene loss, low divergence among parent species and incomplete lineage sorting can effectively mask hybridization, especially for ancient events. Therefore, efficient, accurate detection of hybridization events in genomes remains challenging. Telomere-to-telomere chromosome assemblies and haplotyping, which are achievable with new long-read sequencing technologies, together with standardized genomic pipelines and new phylogenetic methods, will become invaluable tools to detect and interpret signals of hybrid ancestries throughout the tree of life. Fourth, compared with our understanding of plant or animal ecology, for example, that of fungi and other microbes is still in its infancy. For example, the most common view of the geographical distribution of fungi originates from the Baas Becking hypothesis ("everything is everywhere, but the environment selects"). However, in some cases, hybridization is clearly facilitated by human-associated dispersal of fungal species or populations (for example, fungal pathogens and fermentation-associated species)[10,142]. Therefore, a more systematic investigation of fungal biogeography and how this is affected by globalization and associated phenomena, such as climate change, pollution,

agriculture and human travel, could help to explain past hybridizations and predict future hybridization events.

615 **Tables**

616

617 Table 1. Examples of natural fungal interspecific hybrids.

618

Phylum	Hybrid name	Source	Reference
Chytridiomycota	*Batrachochytrium dendrobatidis*	Animal pathogen	30
Mucoromycota	*Rhizopus* species complex	Plant, animal pathogen and fermentation environment	157
Basidiomycota	*Malassezia furfur*	Human pathogen	158
	Trichosporon ovoides	Plant pathogen	107
	Trichosporon coremiiforme	Plant pathogen	107
	Cryptococcus deneoformans species complex	Human pathogen	159
	Cryptococcus gattii species complex	Human pathogen	159
Ascomycota	*Saccharomyces cerevisiae*	Fermentation environment	45
	S. paradoxus	Natural environment	47
	S. cerevisiae x Saccharomyces eubayanus (S. pastorianus)	Fermentation environment	160
	S. cerevisiae x Saccharomyces uvarum x S. eubayanus (Saccharomyces bayanus)	Fermentation environment	161
	S. cerevisiae x Saccharomyces kudriavzevii	Fermentation environment	10,59
	Saccharomyces uvarum x S. eubayanus	Fermentation environment	10,59
	S. cerevisiae x Saccharomyces paradoxus	Fermentation environment	21
	Dekkera bruxellensis	Fermentation environment	162
	Candida metapsilosis	Human pathogen	61
	Candida orthopsilosis	Human pathogen	147
	Candida albicans	Human commensal and pathogen	68
	Candida inconspicua	Human pathogen	146
	Millerozyma (Pichia) farinosa	Fermentation environment	163
	Zygosaccharomyces parabailii	Fermentation environment	43
	Hanseniaspora opuntiae x Hanseniaspora pseudoguilliermondii	Fermentation environment	164
	Zymoseptoria pseudotritici	Plant pathogen	48
	Coccidioides immitis x Coccidioides posadasii	Human pathogen	165
	Aspergillus latus	Human pathogen	143
	Blumeria graminis f. sp. triticale	Plant pathogen	151
	Botrytis allii	Plant pathogen	166
	Ophiostoma spp.	Plant pathogen	142
	Verticillium longisporum	Plant pathogen	167
	Epichloë hybrida (Lp1)	Plant pathogen	114

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622 Table 2. Examples of artificial hybridization to develop new yeasts for industrial applications

Interspecific hybridization			
Parent 1	**Parent 2**	**Application**	**Ref.**
Saccharomyces cerevisiae Fermentation performance and flavour	*Saccharomyces eubayanus* Cryotolerance and flavour	Beer production	[88]
S. cerevisiae Fermentation performance	*Saccharomyces arboricola, S. eubayanus, and Saccharomyces mikatae* Cryotolerance and flavour	Beer production	[168]
S. cerevisiae Fermentation performance and flavour	*S. eubayanus* Cryotolerance	Cider production	[169]
S. cerevisiae Fermentation performance and H_2S production	*S. cerevisiae x Saccharomyces kudriavzevii* Fermentation performance and flavour	Wine production	[170]
S. cerevisiae Fermentation performance and stress tolerance	*Saccharomyces paradoxus, S. mikatae, S. kudriavzevii, S. arboricola, Saccharomyces uvarum and S. eubayanus* Xylose utilization and toxin tolerance	Biofuel production	[171]
S. cerevisiae Fermentation performance	*S. arboricola* Cryotolerance and aroma	Sake production	[172]
Penicillium expansum High pectinase production	*Penicillium griseoroseum* High pectinase production	Pectinase production	[173]
Aspergillus nidulans High cellulase production	*Aspergillus tubingensis* High cellulase production	Cellulase production	[174]

Intergeneric hybridization			
Parent 1	**Parent 2**	**Application**	**Reference**
S. cerevisiae Fermentation performance and ethanol tolerance	*Scheffersomyces stipitis* Xylose utilization	Biofuel production	[138]
S. cerevisiae Fermentation performance and ethanol tolerance	*Kluyveromyces marxianus* Lactose utilization	Biofuel production	[175]
S. cerevisiae Fermentation performance	*Candida krusei* Flavour	Cider production	[176]
Aspergillus oryzae High chitinase production	*Trichoderma harzianum* High chitinase production	Shellfish waste removal	[177]
Mucor sp. Multi-metal resistance	*Fusarium* sp. Multi-metal resistance	Phytoremediation	[178]

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626 **Figure legends**

627 **Figure 1**. **Occurrence of interspecific hybridization in fungi. a |** Genome-scale fungal tree of
628 life based on 290 genes in 1,644 fungal species and spanning currently known fungal diversity.
629 Tips correspond to species-level ranking and clades including more than two species have
630 been collapsed to genus level. Clades with reported hybridization events are indicated with a
631 dot, the colour of which represents the original source of isolation of the interspecific hybrid
632 (Table 1). **b |** Hybridization events and the direction of introgression (arrows) in the genus
633 *Ophiostoma*, which includes plant pathogens. **c |** Hybridization in the genus *Candida*, which
634 includes human pathogens. **d |** Natural and fermentation-associated hybrids within the
635 *Saccharomyces* genus. Bars indicate the species composition of the hybrids. Adapted with
636 permission from ref.[179]

637

638 **Figure 2**. **Roadmap to overcoming species boundaries.** The successful formation of a hybrid
639 population is prevented (stop signs) by the presence of barriers (grey boxes) that limit hybrid
640 formation (grey arrows). **a |** Pre-mating barriers impede mating between species. These
641 barriers include geographical, ecological and temporal isolation, which keep species physically
642 separated in space and time. A higher preference for intra-tetrad mating (selfing, or self-
643 fertilization) over outcrossing and a higher frequency of mating between conspecific
644 individuals than between heterospecific individuals (assortative mating) reduce the chances
645 of successful hybrid formation[24]. **b |** Post-mating barriers act after hybrid formation.
646 Aneuploidies and genetic incompatibilities between subgenomes can affect fertility, viability
647 and fitness of hybrids. **c |** Introgression or hybrid extinction. When a successful hybrid is
648 formed, a lack of reproductive isolation from the parent species will limit the possibility of
649 diverging as a distinct lineage. **d |** Mechanisms of hybrid persistence. If reproductive isolation
650 is obtained and hybrids are able to outcompete their parent species or occupy a new niche,
651 then a new hybrid lineage can be established.

652

653 **Figure 3. Genome stabilization after hybridization. a |** Schematic view of how an inherently
654 unstable hybrid genome can evolve after the interspecific hybridization event. Hybridization
655 between a haploid parent species and a diploid parent species results in the formation of an
656 allopolyploid (allotriploid) hybrid. Karyotypic changes include whole-genome duplication (for
657 example leading to restored fertility of the hybrid) and the emergence of aneuploids (for
658 example leading to removal of dominant deleterious alleles or genomic incompatibilities).
659 Usually, mitochondria of only one parent are inherited or retained, but as recombination can
660 occur in the early stages of stabilization, chimeric mitochondrial DNA has also been
661 observed[180]. Backcrossing to either of the parent species, usually that which is most abundant

662 in the direct environment of the hybrid, leads to introgression. Recombination can lead to
663 loss-of-heterozygosity (LOH) or translocations (reciprocal or non-reciprocal). Either allelic
664 recombination or ectopic recombination can occur, ultimately leading to a highly chimeric
665 hybrid genome. **b |** Chromosome structure and copy number of *Saccharomyces pastorianus*
666 (CBS 1483). This hybrid between *Saccharomyces cerevisiae* and *Saccharomyces eubayanus,*
667 which likely emerged around 500 years ago[10], is used for commercial lager beer production[130]
668 and displays a highly chimeric hybrid genome. Adapted with permission from ref.[130]
669
670 **Figure 4**. **Transcriptional response to hybridization. a |** Interspecific expression divergence
671 between orthologous alleles in the parent species includes differences in expression levels
672 and transcription regulation, owing to a combination of the effect of cis-acting and trans-
673 acting mutations. After hybridization, the transcriptional response to genome merging can
674 vary across hybrid systems, but in general leads to three non-mutually exclusive outcomes.
675 **b |** Inheritance of parent expression bias. The expression differences between orthologues in
676 the parent species are maintained for the corresponding homoeologous alleles in the hybrid.
677 This phenomenon is mainly due to subgenome-specific cis-acting mutations that preserve
678 interspecific differences. **c |** Homoeologue expression blending. The initial expression
679 differences between orthologues are lost in the hybrid. This loss can be attributed to the
680 presence of strong trans factors that are now shared by homoeologous alleles residing in the
681 same nucleus and/or to cross-talk between cis elements (triangles) due to various
682 mechanisms (for example, low divergence between homoeologous alleles and cis element
683 structural blending during hybrid genome stabilization). 3) **d |** Homoeologue expression bias.
684 The expression differences between orthologues in the parent species are different from
685 those of the corresponding homoeologous alleles in the hybrid. Several factors might
686 contribute to homoeologue expression biases, including loss or inactivation of cis elements in
687 one subgenome (red cross) and/or trans factors affecting the two subgenomes
688 asymmetrically.
689
690 **Figure 5. Assembly of protein complexes in fungal interspecific hybrids**. Interactions
691 between proteins from the same (intralogous) or different (interlogous) parent species can be
692 formed, and novel interactions can emerge. In *Saccharomyces* hybrids, interlogous protein–
693 protein interactions (PPIs) mostly form with equal efficiency to intralogous PPIs, and chimeric
694 complexes are readily assembled. However, in some cases, intralogous PPIs are preferentially
695 formed, leading to parent complex biases. In the most extreme cases, this can lead to reduced
696 fitness or even incompatibilities. Alternatively, novel interactions can provide fitness

advantages in specific environments, thereby contributing to transgressive (extreme) phenotypes observed in hybrids.

700 **Glossary terms**

701

Interspecific hybridization	In this review, defined as hybridization between two or more genetically isolated populations that can usually be generalized as 'species'
Heteroploid	The presence of an abnormal chromosome number in a cell, resulting from either aneuploidy or euploidy
Conspecific	From the same species
Heterospecific	From a different species
Gene flow	Transfer of genetic material from one population to another
Selfing	Mating between gametes from the same diploid
Sympatric	Occurring in the same geographical location
Allopatric	Occurring in a non-overlapping geographical location
Aneuploid	Under- or over-representation of one or more chromosomes in a cell
Euploidy	Chromosomal variation involving the entire set of chromosomes in a cell; for example, polyploidy, the presence of multiple copies of the entire set of chromosomes
Homologous genes	Genes deriving from the same ancestral sequence
Flocculation	A reversible, asexual, calcium-dependent process in which cells adhere to form flocs consisting of thousands of cells
Homoeologue expression bias	Unequal contribution of one homoeologue to the total gene expression
Subgenome dominance	Genome-wide expression skewed towards one subgenome
Orthologous genes	Homologous genes arising from speciation
Homoeologous genes	Corresponding parent orthologues in the hybrid
Cis-regulatory elements	Non-coding regions, such as promoters, transcription factor binding sites and terminators, which are near genes and are thus linked to a single subgenome
Trans-regulatory elements	Elements such as transcription factors, chromatin regulators and signalling molecules, which interact with cis elements but act independently of their own genomic location and are therefore shared by subgenomes residing in the same nucleus
Subgenome homogenization	A process in which subgenomes in a hybrid become more uniform due to genome stabilization, such as by gene conversion
Chimeric genes	Genes consisting of a fusion of the 5′ part of one parent to the 3′ end of the other parent
Ectopic recombination	Recombination between homologous sequences that are not at the same position on homologous chromosomes
Allelic recombination	Recombination between homologous sequences on homologous chromosomes
Synteny	Co-occurrence of loci on the same chromosome among two species, with or without a conserved order

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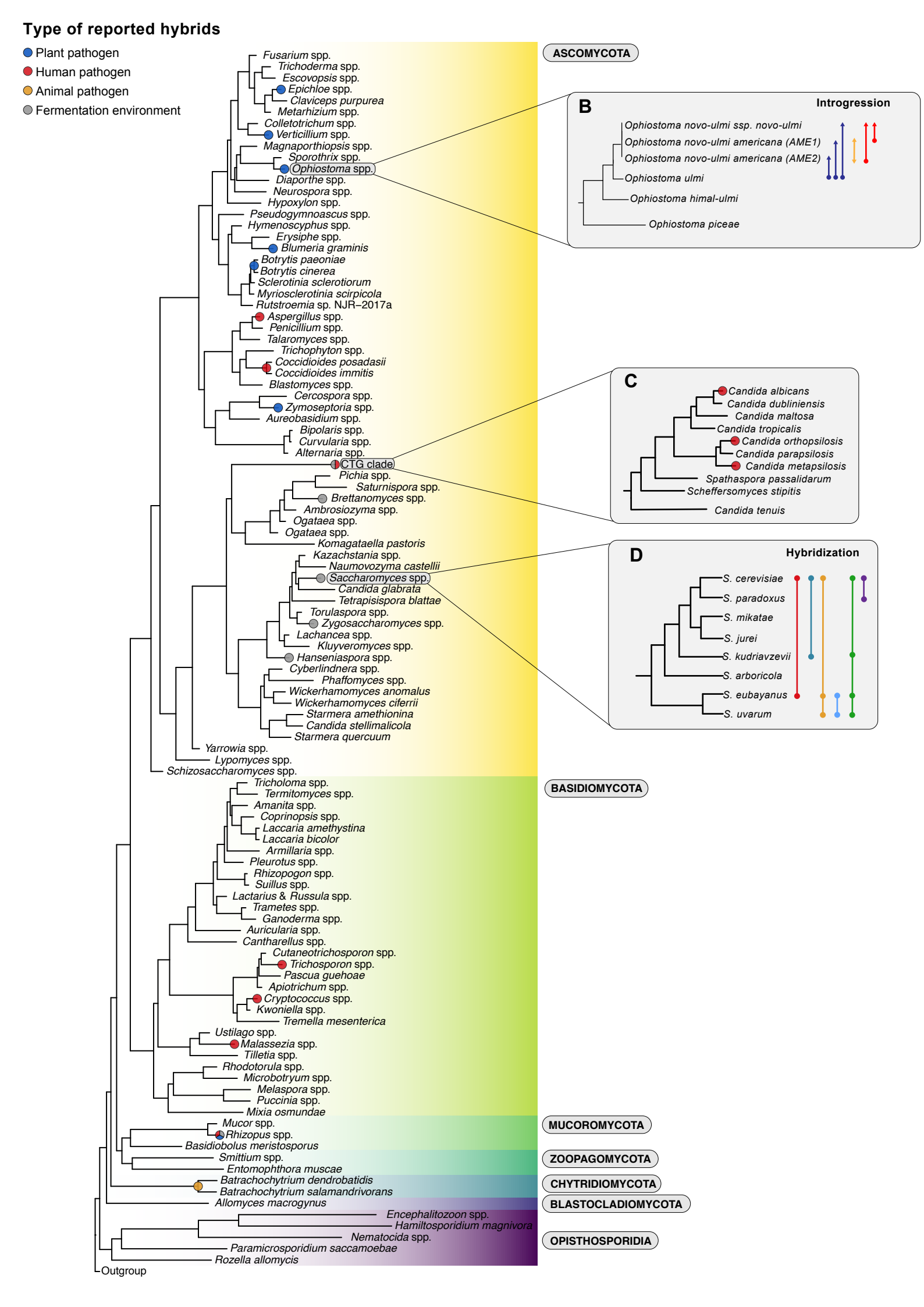
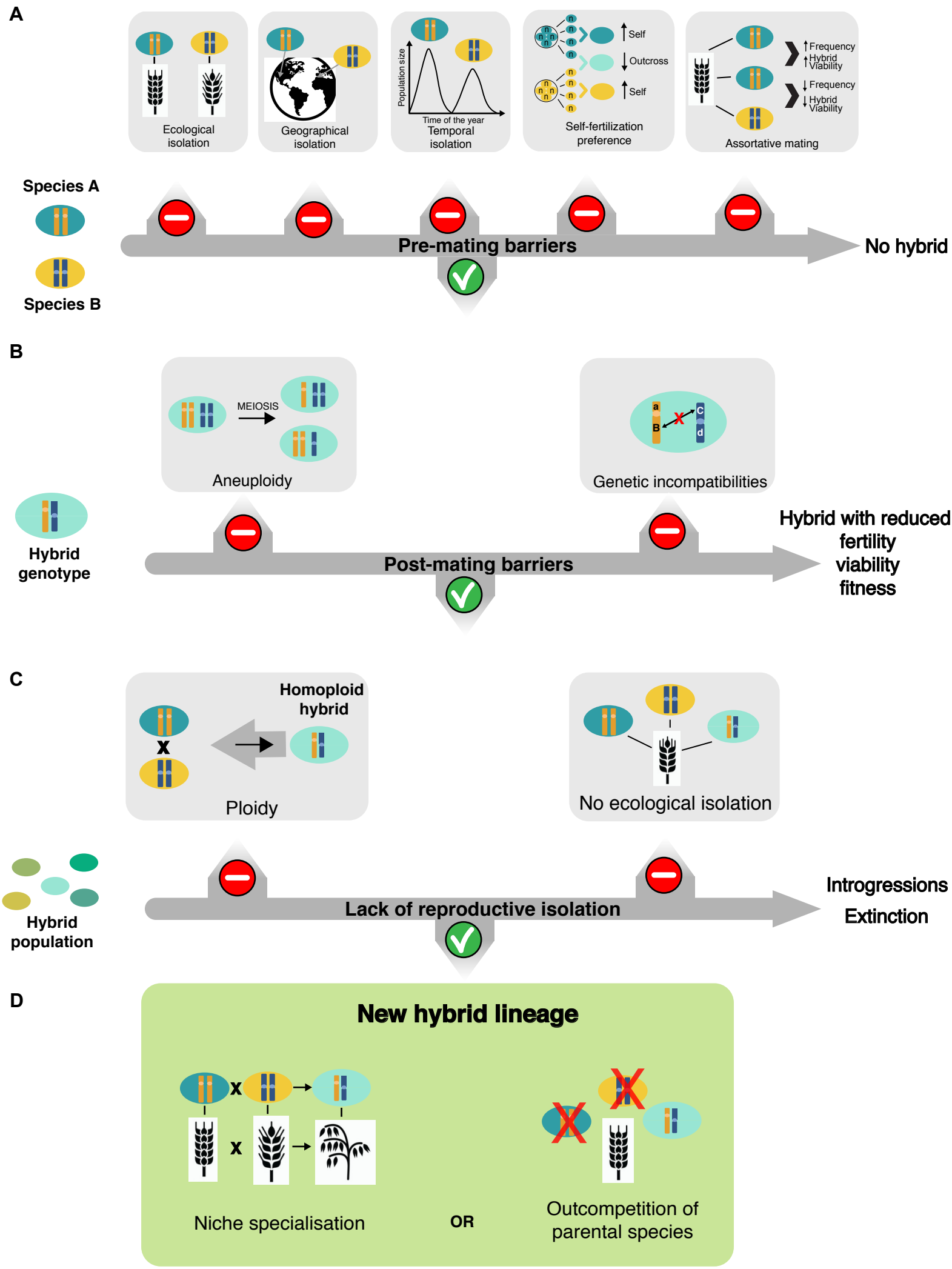
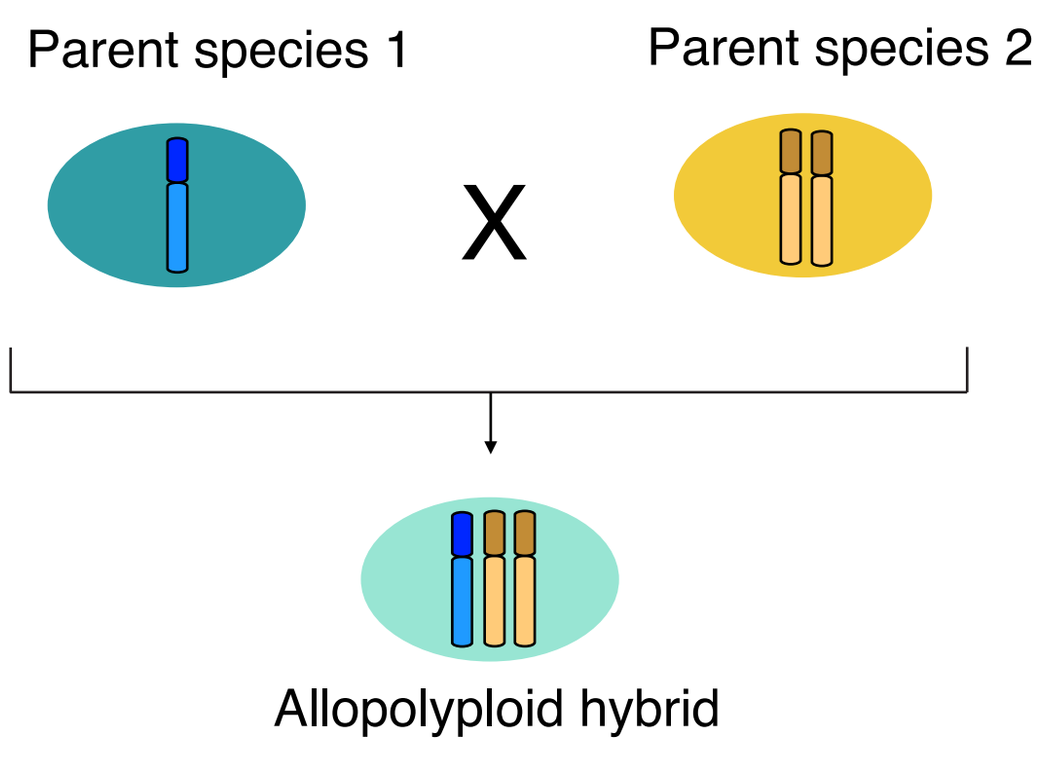

Type of reported hybrids
Plant pathogen
Human pathogen
Animal pathogen
Fermentation environment
ASCOMYCOTA
Fusarium spp.
Trichoderma spp.
Escovopsis spp.
Epichloe spp.
Claviceps purpurea
Metarhizium spp.
Colletotrichum spp.
Verticillium spp.
Magnaporthiopsis spp.
Sporothrix spp.
Ophiostoma spp.
Diaporthe spp.
Neurospora spp.
Hypoxylon spp.
Pseudogymnoascus spp.
Hymenoscyphus spp.
Erysiphe spp.
Blumeria graminis
Botrytis paeoniae
Botrytis cinerea
Sclerotinia sclerotiorum
Myriosclerotinia scirpicola
Rutstroemia sp. NJR–2017a
Aspergillus spp.
Penicillium spp.
Talaromyces spp.
Trichophyton spp.
Coccidioides posadasii
Coccidioides immitis
Blastomyces spp.
Cercospora spp.
Zymoseptoria spp.
Aureobasidium spp.
Bipolaris spp.
Curvularia spp.
Alternaria spp.
CTG clade
Pichia spp.
Saturnispora spp.
Brettanomyces spp.
Ambrosiozyma spp.
Ogataea spp.
Ogataea spp.
Komagataella pastoris
Kazachstania spp.
Naumovozyma castellii
Saccharomyces spp.
Candida glabrata
Tetrapisispora blattae
Torulaspora spp.
Zygosaccharomyces spp.
Lachancea spp.
Kluyveromyces spp.
Hanseniaspora spp.
Cyberlindnera spp.
Phaffomyces spp.
Wickerhamomyces anomalus
Wickerhamomyces ciferrii
Starmera amethionina
Candida stellimalicola
Starmera quercuum
Yarrowia spp.
Lypomyces spp.
Schizosaccharomyces spp.
BASIDIOMYCOTA
Tricholoma spp.
Termitomyces spp.
Amanita spp.
Coprinopsis spp.
Laccaria amethystina
Laccaria bicolor
Armillaria spp.
Pleurotus spp.
Rhizopogon spp.
Suillus spp.
Lactarius & Russula spp.
Trametes spp.
Ganoderma spp.
Auricularia spp.
Cantharellus spp.
Cutaneotrichosporon spp.
Trichosporon spp.
Pascua guehoae
Apiotrichum spp.
Cryptococcus spp.
Kwoniella spp.
Tremella mesenterica
Ustilago spp.
Malassezia spp.
Tilletia spp.
Rhodotorula spp.
Microbotryum spp.
Melaspora spp.
Puccinia spp.
Mixia osmundae
MUCOROMYCOTA
Mucor spp.
Rhizopus spp.
Basidiobolus meristosporus
ZOOPAGOMYCOTA
Smittium spp.
Entomophthora muscae
CHYTRIDIOMYCOTA
Batrachochytrium dendrobatidis
Batrachochytrium salamandrivorans
BLASTOCLADIOMYCOTA
Allomyces macrogynus
OPISTHOSPORIDIA
Encephalitozoon spp.
Hamiltosporidium magnivora
Nematocida spp.
Paramicrosporidium saccamoebae
Rozella allomycis
Outgroup
B
Introgression
Ophiostoma novo-ulmi ssp. novo-ulmi
Ophiostoma novo-ulmi americana (AME1)
Ophiostoma novo-ulmi americana (AME2)
Ophiostoma ulmi
Ophiostoma himal-ulmi
Ophiostoma piceae
C
Candida albicans
Candida dubliniensis
Candida maltosa
Candida tropicalis
Candida orthopsilosis
Candida parapsilosis
Candida metapsilosis
Spathaspora passalidarum
Scheffersomyces stipitis
Candida tenuis
D
Hybridization
S. cerevisiae
S. paradoxus
S. mikatae
S. jurei
S. kudriavzevii
S. arboricola
S. eubayanus
S. uvarum

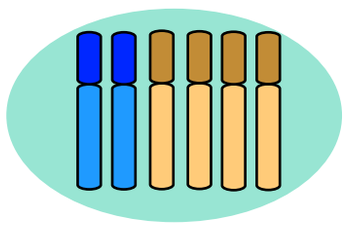

A
Ecological isolation
Geographical isolation
Population size
Time of the year
Temporal isolation
Self
Outcross
Self
Self-fertilization preference
Frequency
Hybrid Viability
Frequency
Hybrid Viability
Assortative mating
Species A
Species B
Pre-mating barriers
No hybrid
B
MEIOSIS
Aneuploidy
Genetic incompatibilities
Hybrid genotype
Post-mating barriers
Hybrid with reduced
fertility
viability
fitness
C
Homoploid hybrid
Ploidy
No ecological isolation
Hybrid population
Lack of reproductive isolation
Introgressions
Extinction
D
New hybrid lineage
Niche specialisation
OR
Outcompetition of parental species

A

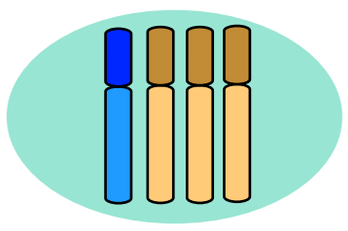


Karyotype

Whole-genome duplication

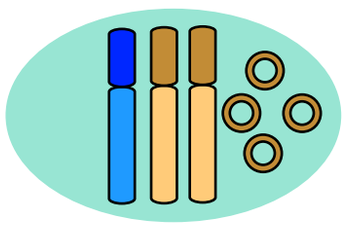


Aneuploidy

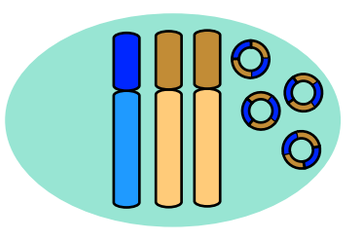


Mitotype

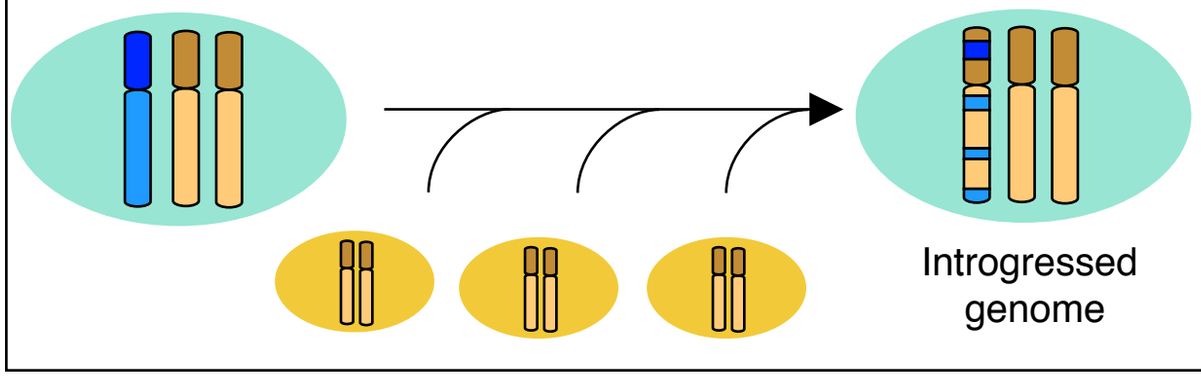
Selective inheritance



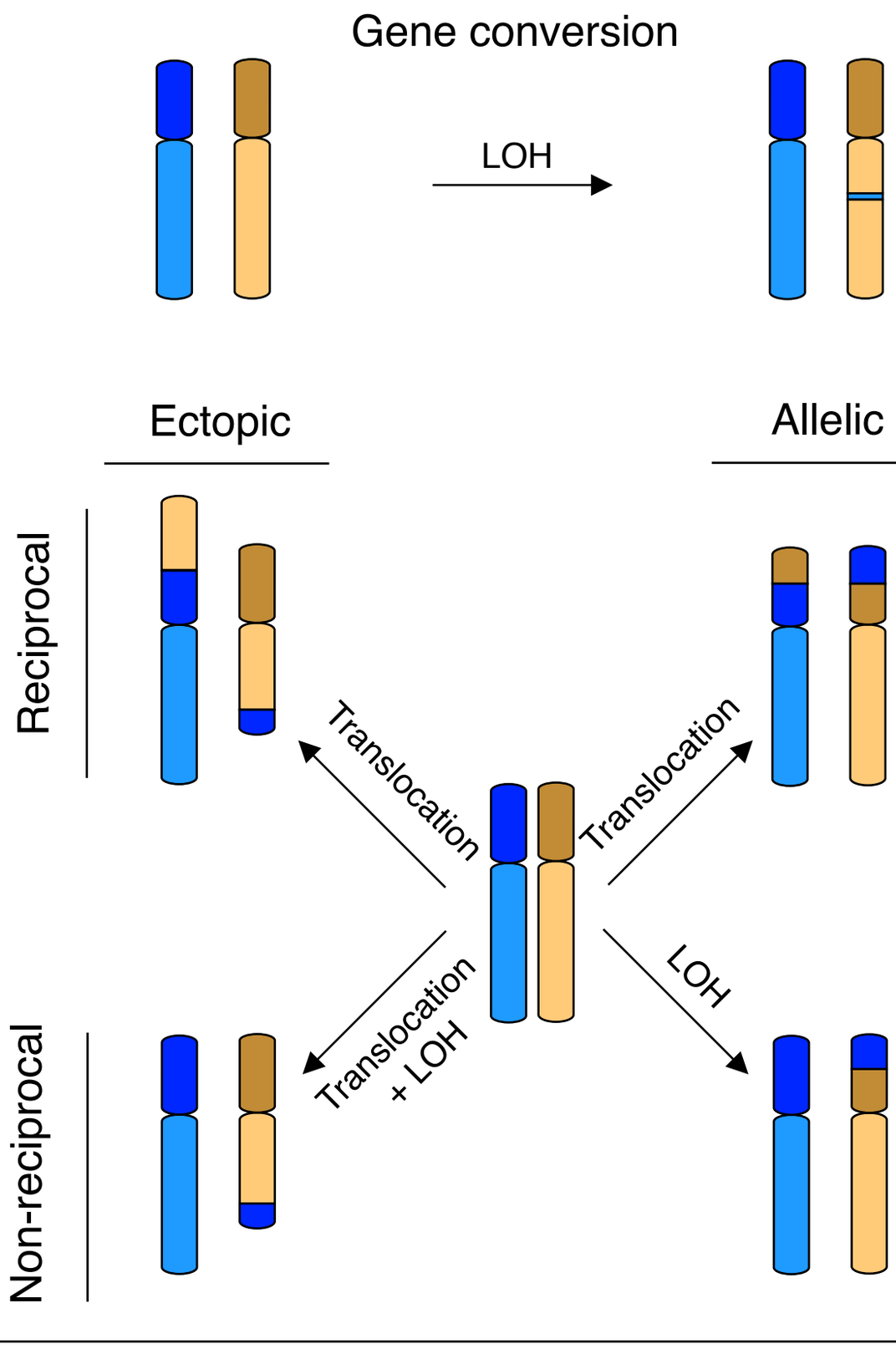
Recombination



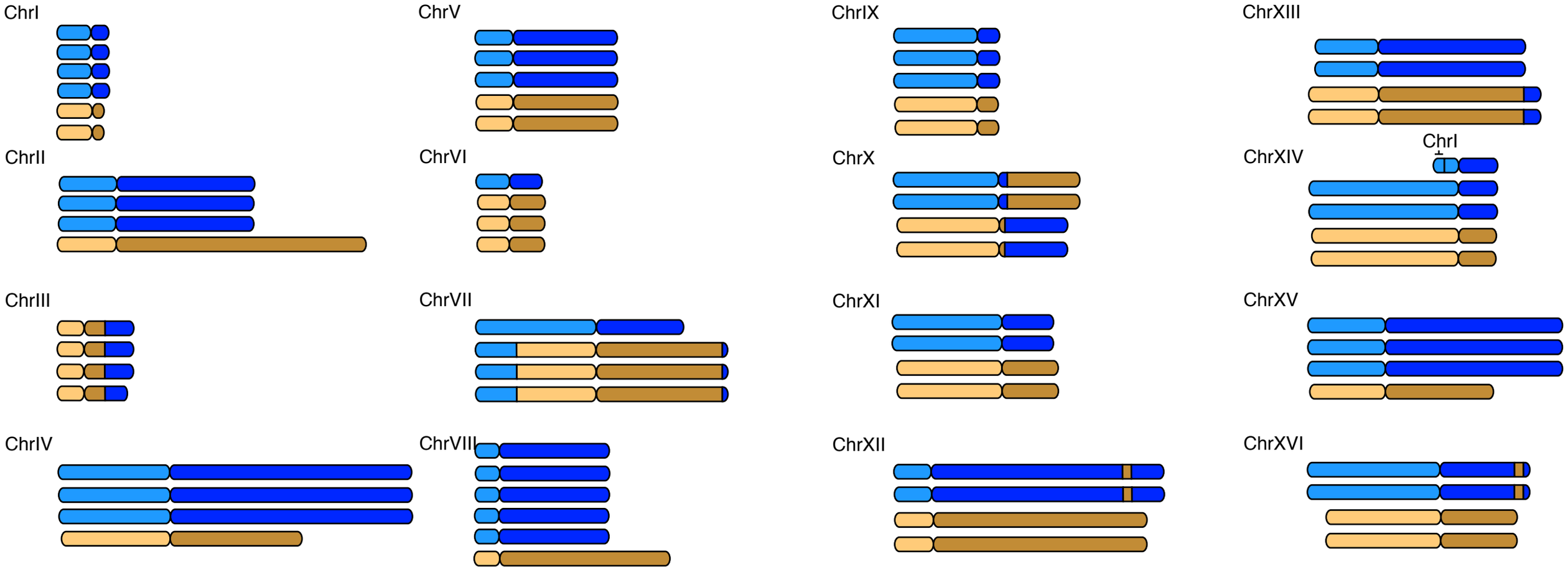
Backcrossing

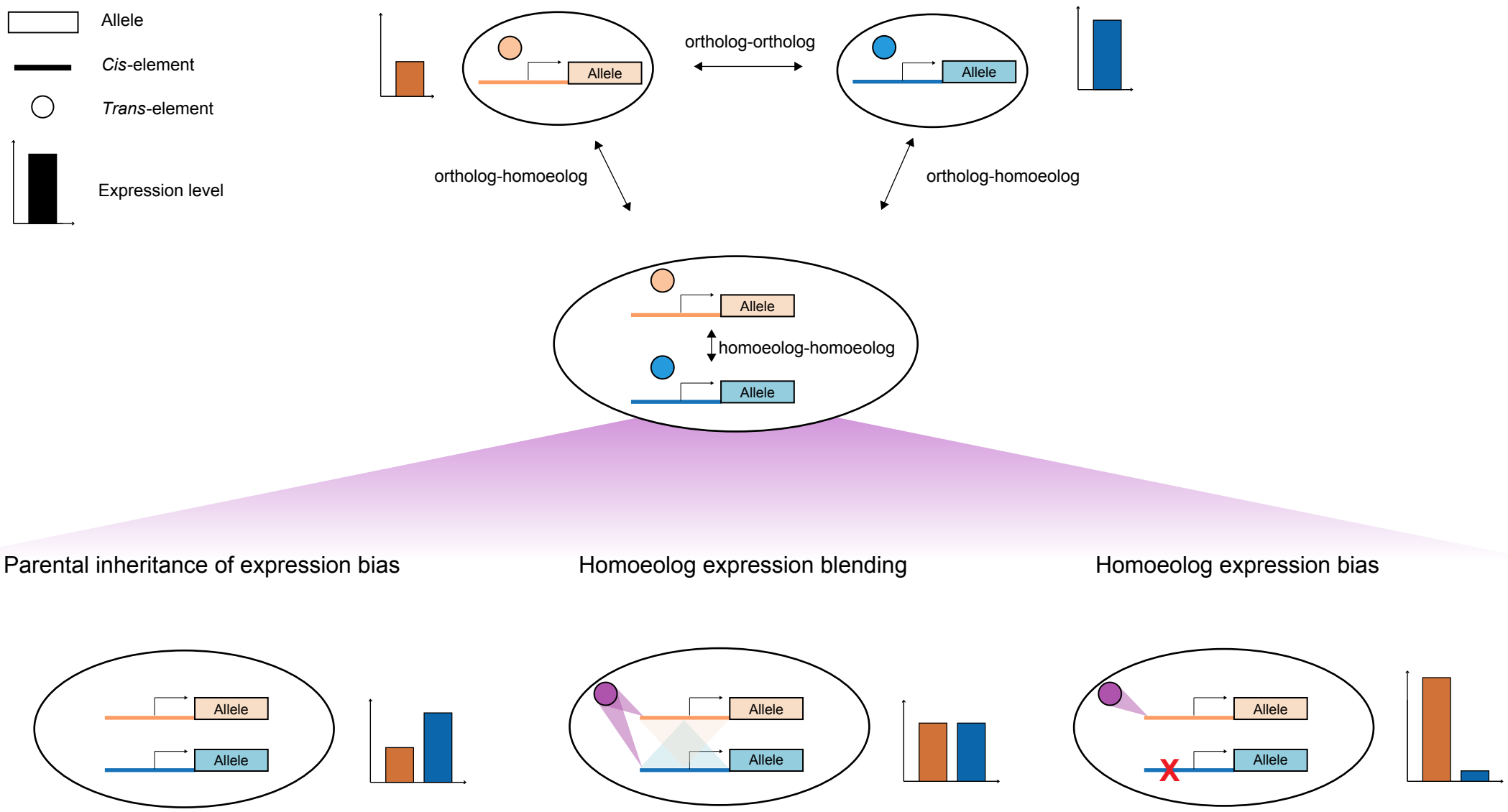


Recombination



B

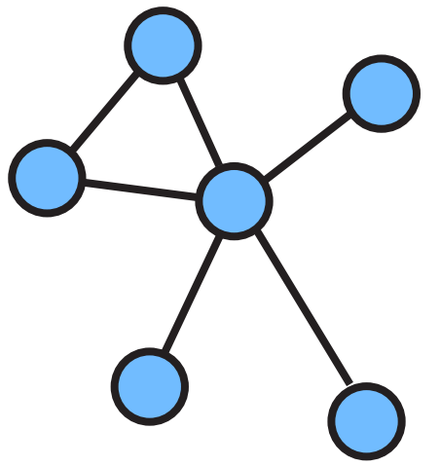



Allele
Cis-element
Trans-element
Expression level
Allele
ortholog-ortholog
Allele
ortholog-homoeolog
ortholog-homoeolog
Allele
homoeolog-homoeolog
Allele
Parental inheritance of expression bias
Homoeolog expression blending
Homoeolog expression bias
Allele
Allele
Allele
Allele
Allele
Allele
X

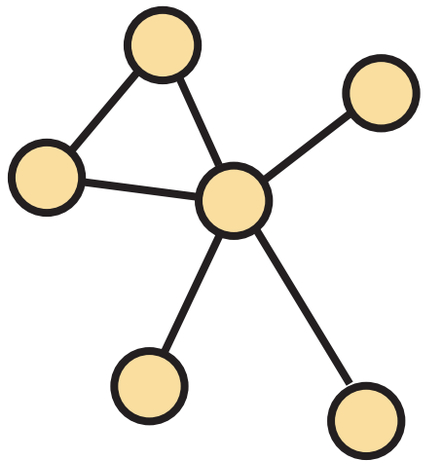
Parents

Intralogous PPI

Interlogous PPI

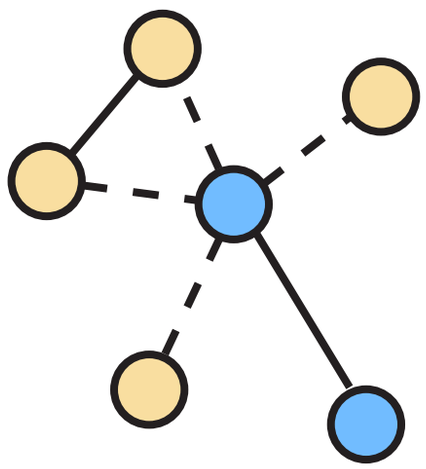


Parent 1

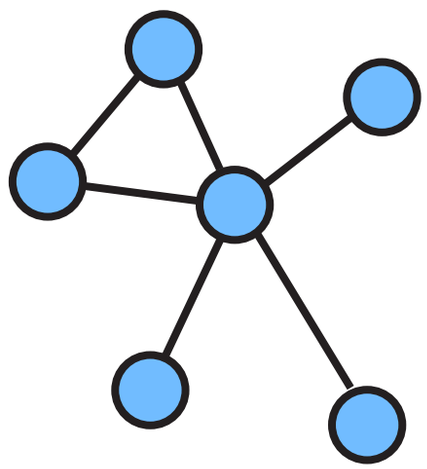


Parent 2

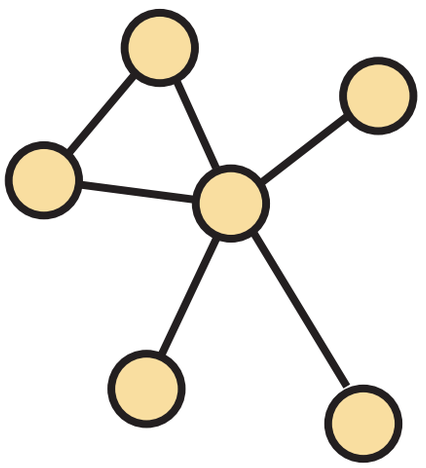
Interspecific hybrids

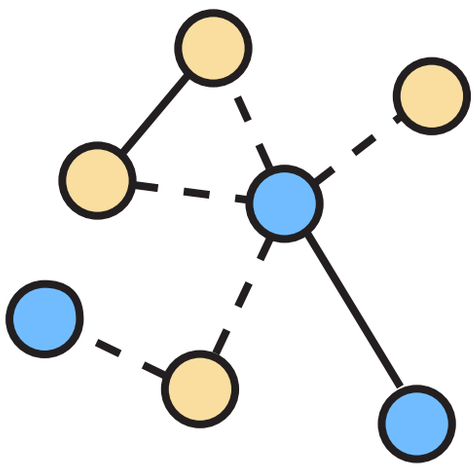


Chimaerism



Parental complex bias

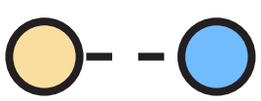




Novel PPIs

Majority of complexes

No measurable fitness effect



PCNA complex

Incompatibilities

Trp2/Trp3 complex

Increased fitness in tryptophan scarcity

Examples in *Saccharomyces*