

Interspecific hybridization impacts host range and pathogenicity of filamentous microbes

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Interspecific hybridization is widely observed within diverse eukaryotic taxa, and is considered an important driver for genome evolution. As hybridization fuels genomic and transcriptional alterations, hybrids are adept to respond to environmental changes or to invade novel niches. This may be particularly relevant for organisms that establish symbiotic relationships with host organisms, such as mutualistic symbionts, endophytes and pathogens. The latter group is especially well-known for engaging in everlasting arms races with their hosts. Illustrated by the increased identification of hybrid pathogens with altered virulence or host ranges when compared with their parental lineages, it appears that hybridization is a strong driver for pathogen evolution, and may thus significantly impact agriculture and natural ecosystems.

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Introduction

Genomes continuously evolve through mechanisms such as mutation, recombination, duplication and uptake of novel DNA upon horizontal gene transfer (HGT). Such plasticity allows organisms to adapt to environmental changes, which is considered particularly important for microbial pathogens that engage in arms races with hosts for continued symbiosis [1]. As a consequence, many pathogens have evolved two-speed genomes with genomic regions implicated in pathogenicity displaying accelerated evolution [2,3]. Interspecific hybridization often concerns two species within the same genus, and is considered an important driver for genome evolution. Hybridizations have been widely observed within various

eukaryotic taxa such as plants, insects, birds, mammals and fungi [4,5], and since the 1990s various interspecific hybrids of filamentous plant pathogens have been described (Table 1). In this review we discuss the impact of interspecific hybridization on the pathogenicity of filamentous microbes.

Hybridization mechanisms

Hybrids may originate from parasexual reproduction, a nonsexual process thought to occur widely in fungi for transferring genetic material without development of sexual structures or meiosis. Parasexual reproduction typically involves hyphal fusion of two strains, followed by nuclear fusion, mitotic recombination and chromosome loss to restore the parental ploidy state [6]. Consequently, interspecific hybrids may originate from nuclear fusion without subsequent immediate chromosome loss [7]. However, interspecific hybridization can also occur through sexual reproduction, when gametes of different species fuse into a viable zygote. For example, hybrids between the sexually compatible causal agents of anther smut disease of different weeds, *Microbotryum lychnididoicae* and *M. silenes-dioicae*, are regularly found in nature [8,9].

Hybridization results in allopolyploidy when the ploidy of hybrids increases with respect to that of the normal ploidy of the parental species (Figure 1). Nevertheless, the hybridization is generally followed by loss of genetic material due to the incompatibility of genes or the discard of genomic ballast, eventually leading to the original ploidy status [10]. The resulting hybrids are therefore considered stable or transient according to the conservation of parental chromosome sets over time (Figure 1). However, during homoploid hybridization, for instance upon hybridization of the haploid gametes of two diploid species, chromosome numbers do not change. For such a hybrid to be viable, the parental chromosomes need to be very similar, and thus the parents closely related, to allow proper mitosis in the offspring [11]. Consequently, homoploid hybrids are often only weakly reproductively isolated from their parents [12], which may contribute to reversion of homoploid hybrids to their original parental genomes and making the hybrid phase transient. Ultimately, this may lead to the transfer of genetic material between hybridized lineages, leading to introgression of DNA of one parent into the other parent's genome.

Table 1

Examples of naturally occurring interspecific hybrid pathogens

	Hybrid species taxon ^a	Division	Parental species 1	Parental species 2	Reported host genera	References
Ascomycota						
<i>Botrytis</i>	<i>B. allii</i>		<i>B. aclada</i>	<i>B. byssoidea</i>	<i>Allium</i>	[46,48]
<i>Ophiostoma</i>			<i>O. novo-ulmi</i>	<i>O. ulmi</i>	<i>Ulmus</i>	[29]
<i>Verticillium</i>	<i>V. longisporum</i>	Lineage A1/D1	A1 ^b	D1 ^b	<i>Beta</i> , <i>Brassica</i> , & <i>Raphanus</i>	[7]
		Lineage A1/D2	A1 ^b	<i>V. dahliae</i>	<i>Armoracia</i>	[7]
		Lineage A1/D3	A1 ^b	<i>V. dahliae</i>	<i>Armoracia</i> & <i>Brassica</i>	[7]
Basidiomycota						
<i>Cryptococcus</i>			<i>C. gattii</i>	<i>C. neoformans</i>	<i>Homo</i>	[46,49]
<i>Melampsora</i>	<i>M. × columbiana</i>		<i>M. medusae</i>	<i>M. occidentalis</i>	<i>Populus</i>	[50,51]
	<i>M. medusae-populina</i>		<i>M. medusae</i>	<i>M. larici-populina</i>	<i>Populus</i>	[52]
<i>Microbotryum</i>			<i>M. lychnidis-dioicae</i>	<i>M. silene-dioicae</i>	<i>Silene</i>	[9]
<i>Heterobasidion</i>			<i>H. annosum</i>	<i>H. irregulare</i>	<i>Pinus</i>	[53]
			<i>H. irregulare</i>	<i>H. occidentale</i>	<i>Juniperus</i> , <i>Pinus</i> , & <i>Larix</i>	[54,55]
Oomycota						
<i>Phytophthora</i>	<i>P. andina</i>		<i>P. infestans</i>	Unknown	<i>Brugmansia</i> & <i>Solanum</i>	[34,56]
	<i>P. ×alni</i>		<i>P. ×multiformis</i>	<i>P. uniformis</i>	<i>Alnus</i>	[31]
	<i>P. ×multiformis</i>		Pm1 ^b	Pm2 ^b	<i>Alnus</i>	[31]
	<i>P. ×pelgrandis</i>		<i>P. cactorum</i>	<i>P. nicotianae</i>	<i>Eriobotrya</i> , <i>Spathiphyllum</i> , <i>Pelargonium</i> & <i>Primula</i>	[57–59]
	<i>P. ×serendipita</i>		<i>P. cactorum</i>	<i>P. hedraiandra</i>	<i>Allium</i> , <i>Dicentra</i> , <i>Idesia</i> , <i>Penstemon</i> , <i>Kalmia</i> & <i>Rhododendron</i>	[44,60]
			<i>P. porri</i>	<i>P. taxon parsley</i>	<i>Allium</i> , <i>Chrysanthemum</i> , <i>Parthenium</i> , & <i>Pastinaca</i>	[10]
<i>Pythium</i>			<i>P. phragmitis</i>	<i>P. phragmiticola</i>	<i>Phragmites</i>	[61,62]

^a Only mentioned if a designated taxon has been provided to the hybrid.

^b Provisional name for hitherto unknown parental species.

Genomic and transcriptomic consequences of hybridization

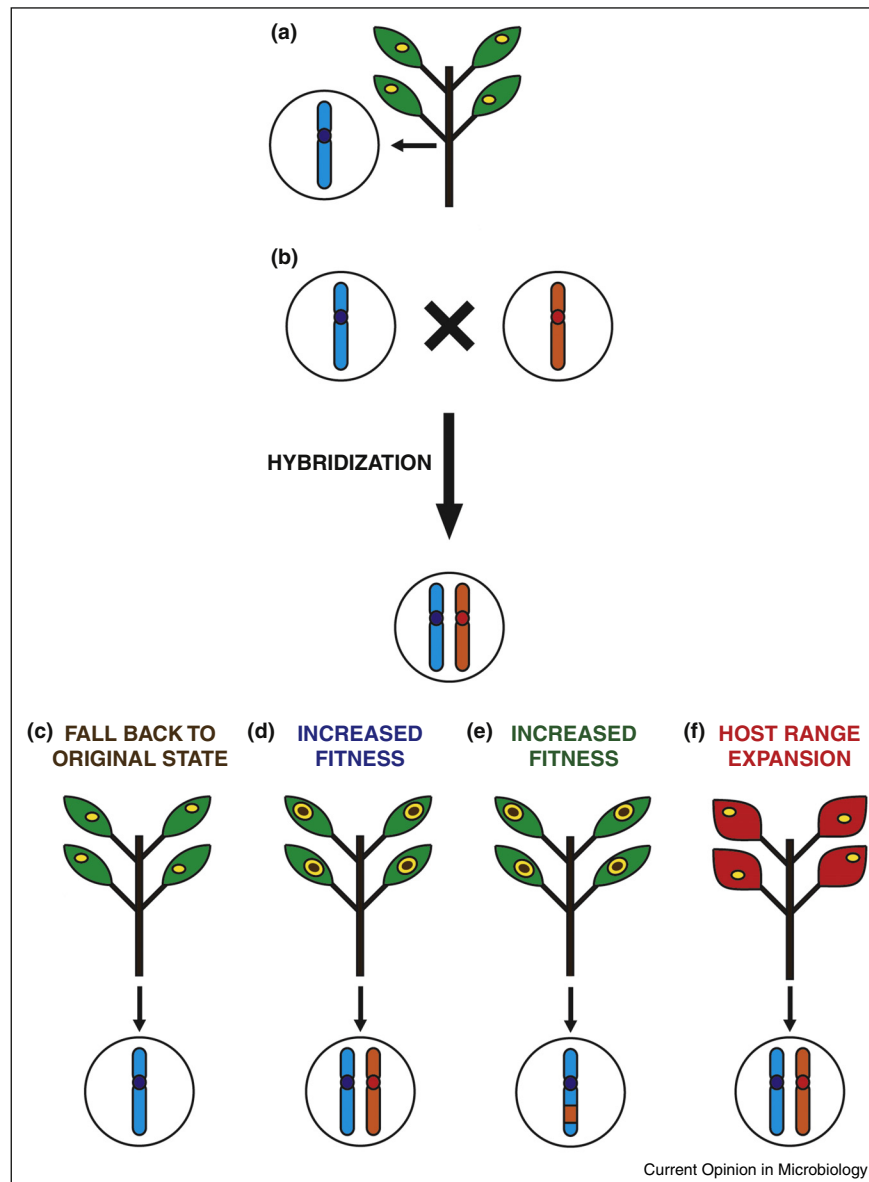
Gene flow from closely related species through hybridization is a saltational, fast mean of genome evolution [13]. Allopolyploidy as a result from hybridization generally leads to chromosomal doubling, thereby inciting major genomic and transcriptomic reorganization also known as genome and transcriptome shock, respectively (Figure 2). In the early stages of allopolyploid evolution, most genes are present in two copies (homeologs), one derived from each parental genome with its own transcriptional regulation. In plant and fungal hybrids, the transcriptional response upon hybridization is often conservative [14,15^{*}]. For instance, in the fungal grass endophyte *Epichloë* polyploid Lp1, an economically important allopolyploid derived from interspecific hybridization between the sexual species *E. typhina* and the asexual relative *Neotyphodium lolii*, over half of the homeologs retained the parental gene expression patterns [15^{*}]. Moreover, if genes were differentially expressed in the parental lineages the difference was often lost in the hybrid, and the vast majority of genes with differential parental expression displayed signs of expression-level dominance, whereby the total homeolog expression level is similar to one of the parents [15^{*}]. While in relatively recently evolved allopolyploids such as *Epichloë* Lp1 gene loss is only observed at low frequency, gene loss is one of the major patterns over longer evolutionary

time frames (Figure 2). Arguably, extensive gene loss and close synteny between duplicated gene regions can mask ancestral processes that played a role in genome evolution. For instance, allopolyploidy was recently revealed to be causative to the whole-genome duplication (WGD) in the baker's yeast *Saccharomyces cerevisiae* that had been initially ascribed to doubling of its ancestor DNA [16^{*}]. Subsequently, *S. cerevisiae* underwent extensive genomic rearrangements and gene loss, eventually resulting in retaining of only ~15% of the duplicated genes [17–19]. In fungi and plants, retained genes often functionally diverge, for example, by increasing differences in gene expression patterns, leading to neo-functionalization when one copy retains the original function while the other copy acquires a novel function, or subfunctionalization when both copies partition the original gene function [20,21].

Hybrid emergence

Although hybridization most frequently occurs between species with close relationship [12], it remains a rare phenomenon as closely related species typically evolved barriers to prevent crossing, especially if they evolved in the same geographical area [22]. However, allopatric species that evolved in isolation or in different geographic regions often display less stringent reproductive barriers. It is therefore not surprising that novel hybrid pathogens have often been associated with microbial introductions in new geographical

Figure 1



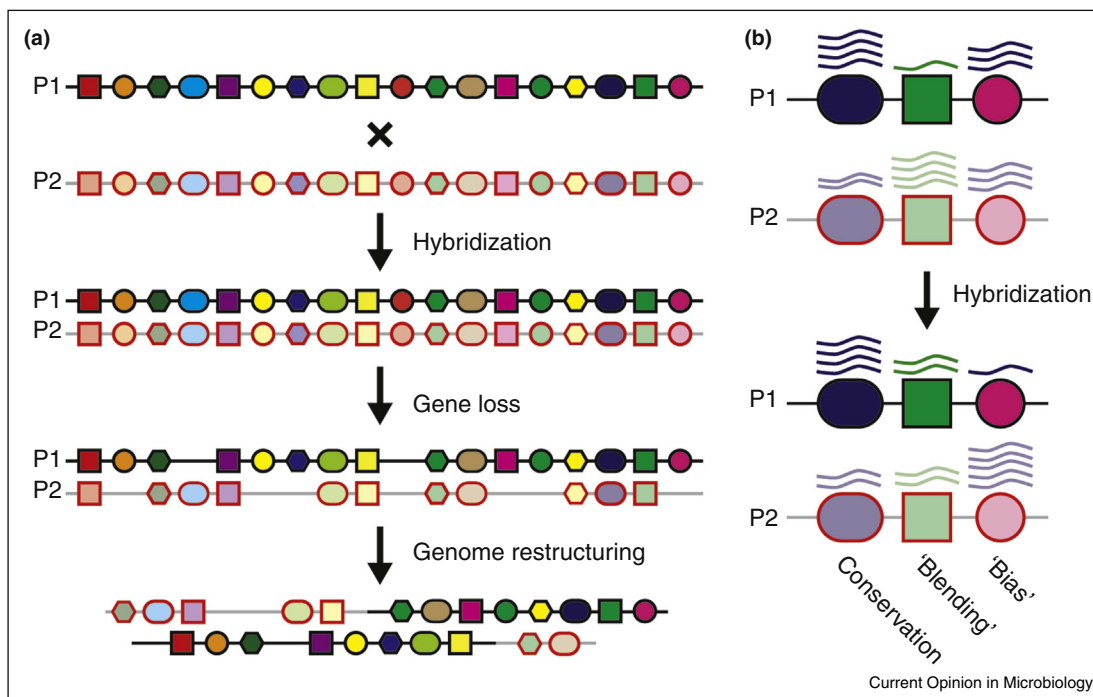
Evolutionary fate of hybrid pathogen genomes. One of the parental lineages (illustrated as a blue chromosome) causes disease on a particular host plant (a). Another, somewhat related, species (illustrated as an orange chromosome) co-occurs in a particular niche, makes physical contact and hybridizes, resulting in a lineage with the cumulative amount of chromosomes (b). Hybridizations with disadvantageous outcomes are evolutionary dead ends. These hybrids are not viable or evolve back to their original genomic state by extensive gene loss (c). Alternatively, plasticity of the hybrid genome enables that hybrids eventually evolve towards three extreme outcomes. Potentially, the combination of the two parental genomes leads to increased fitness on the original host and the hybrid is able to establish as a stable entity (d). Ultimately, this may even lead to displacement of the parental lineage that may consequently even go extinct. Increased aggressiveness may still be accompanied by extensive DNA loss, leading to hybrid pathogen reversion to the original ploidy status while maintaining a proportion of particularly beneficial genetic material (e). In some cases, the hybrid gains the capacity to colonize alternative hosts, leading to host range expansion (f). Ultimately, this may lead to adaptation on a novel host.

areas. For example, hybrids between two allopatric species of the *Heterobasidion* genus that harbours economically important pathogens of Northern Hemisphere conifer forests, the European *H. annosum* and the American *H. irregulare*, emerged in Italy upon the movement of US troops in Europe during World War II [23,24]. Arguably, increased

globalization, trade and traffic promote contacts between geographically separated microbial species, therefore enhancing opportunities for hybridization events [25].

Even in the absence of crossing barriers, mating in many fungi (and thus also hybridization) only occurs under very

Figure 2



Potential genomic and transcriptomic consequences of allopolyploidy. **(a)** Hybridization of two parental genomes (P1 and P2, of which genes (different shapes and colours) are indicated by solid and pastel colours, respectively), initially leads to two copies for each gene (homeologs) in the hybrid. Over longer evolutionary timescales, gene loss in the hybrid is a major evolutionary pattern that, together with genomic rearrangements, ultimately leads to genomic restructuring. **(b)** Transcriptional responses in the hybrid lineage when compared with its parental lineages. Expression patterns in the hybrid are often either directly inherited from the parents ('expression conservation') or, if differentially expressed in the parents, expression differences are lost ('expression blending'). Alternatively, if not differentially expressed in the parents, genes in the hybrid display an expression pattern that differs from the pattern observed in the parental lineages ('expression bias').

particular, often enigmatic conditions. This is illustrated by the recent discovery of the outbreeding behaviour of *S. cerevisiae*, a well-studied model organism of which the reproduction ecology remained largely obscure. Intriguingly, intestines of the social wasp *Polistes dominula* were found to host highly outbred *S. cerevisiae* strains as well as a rare hybrid of *S. cerevisiae* with *S. paradoxus*, the latter of which cannot survive solitarily in wasp intestines and needs to be rescued through interspecific hybridization [26*]. Similarly, the occurrence of mosaic *Albugo candida* genomes with intermixed genetic material of different races, remained puzzling as these races are obligate biotrophic parasites on distinct *Brassicaceous* hosts [27**]. However, infecting sequentially, different races were recently found to be able to colonize the same host, as host-adapted races pave the way for the subsequent colonization by normally avirulent strains. Conceivably, hybrids between *A. candida* strains can arise during such co-colonizations [27**].

As hybridization has an enormous impact on pathogen genome characteristics, illustrated by genome and transcriptome shocks (Figure 2), host species may take a relatively long time to intercept hybrid pathogens,

sometimes leading to the rapid expansion of emerging hybrid pathogens and resulting in epidemic outbreaks. For example, hybridization is thought to have played a pivotal role in the second Dutch elm disease epidemic that started in the early 1970s in Europe [28]. Hybrids between *Ophiostoma novo-ulmi* and *O. ulmi* are thought to have formed a genetic bridge between the two species [29], mediating interspecific gene flow and allowing *O. novo-ulmi* to outcompete *O. ulmi* in Europe eventually [28]. In addition, rapidly expanding hybrids between eastern European and North American *O. novo-ulmi* subspecies *novo-ulmi* and *americana*, respectively, emerged during the geographical overlap of both subspecies during this epidemic [30]. The introduced genetic heterogeneity between subspecies may have facilitated the epidemic proliferation of *O. novo-ulmi* even more.

Hybrids need to be fitter towards a given niche than their parental lineages in order to sustain themselves on the same host (Figure 1). For example, the main causal agent of alder decline, *Phytophthora xalmi*, is the progeny from hybridization between the generally less aggressive parental lineages *P. ×multiformis* and *P. uniformis* [31,32].

However, if the increase in fitness is too significant, hybrids may completely outcompete and replace their parental lineages on a particular host, which could eventually lead to the extinction of particular parental lineages [33]. This may be a plausible explanation for unfound parental lineages underlying numerous hybridization events [7,10,34]. This is the case for the interspecific hybrid causal agent of stem striping on oilseed rape, *Verticillium longisporum* [7,35]. This species originates from at least three separate hybridization events involving three different parental *Verticillium* lineages. One of these lineages has been identified as *V. dahliae*, a species that shows remarkable genomic plasticity [36,37], whereas the two remaining lineages have hitherto not been found. *V. longisporum* is highly adapted to *Brassicaceae* hosts, whereas *V. dahliae* does generally not colonize these plants [35]. Arguably, the unknown *V. longisporum* parents were *Brassicaceae*-specific pathogens that were outcompeted by the *V. longisporum* hybrid.

Probably the best illustrated example of genome evolution and the impact of hybridization on host range stems from the *Zymoseptoria* genus. *Z. pseudotritici* and *Z. tritici* diverged from the last common ancestor with the domestication of wheat in the Middle East, approximately 10 000 years ago [38]. Whereas the wheat-adapted *Z. tritici* spread globally presumably with its host, *Z. pseudotritici* only occurs in the Middle East on various wild grass species. Intriguingly, the genome composition of *Z. pseudotritici* is consistent with a hybridization event that occurred less than 400 sexual generations ago, likely within the last five centuries, due to a sexual cross between two related haploid individuals that both diverged from the *Z. tritici* lineage [39]. Similar to *V. longisporum*, the parental species of *Z. pseudotritici* have never been collected, suggesting that they occur on different hosts as the hybrid or got outcompeted [40]. Thus, hybridization led to the emergence of a unique pathogenic species.

Pathogens on novel hosts through hybridization

As hybridization allows organisms to acquire novel traits to colonize new niches, it may even allow colonization of novel hosts (Figure 1). In the 1960s, a hybrid crop between wheat and rye called triticale was introduced in agriculture. Although initially immune to powdery mildew disease, infections on triticale were reported since the 2000s. It was recently determined that the causal agent, *Blumeria graminis* f.sp. *triticales*, arose upon hybridization of two subspecies of the powdery mildew fungus *B. graminis* that are adapted to wheat and rye, respectively [41^{••}]. Intriguingly, the mosaic genome of *B. graminis* f.sp. *triticales* was determined to have evolved only within the last 50 years, suggesting that it arose on the novel crop.

Introgression of the apple scab resistance gene *Rvi6* from the wild apple *Malus floribunda* into domestic

M. × domestica cultivars permitted the invasion of a virulent population of the apple scab pathogen *Venturia inaequalis* into apple orchards, bringing together environmental and agricultural pathogen lineages that diverged in allopatry several thousand years ago [42]. Presently, the *Rvi6* resistance in orchards is overcome by virulent *V. inaequalis* lineages with heterogeneous genomes, indicating a high amount of gene flow between the two allopatric populations due to mating on the common host [43]. Thus, the introduction of *Rvi6* resistance in apple seventy years ago led to the transfer of virulence from a non-agricultural pathogen population into agriculturally relevant pathogen populations showing that, similar to the previous example, modern agricultural practices may generate novel pathogen lineages.

Although the previous examples concern intraspecific hybrids, similar shifts in host range are observed for interspecific hybrids. For example, a hybrid between *P. cactorum* and *P. hedraiaandra*, called *P. ×serendipita*, infects monocot (*Allium*) and dicot (*Idesia* and *Penstemon*) hosts outside the host range of its parents [44]. Similarly, hybrids between the leek and parsley pathogens *P. porri* and *P. taxon* parsley, respectively, expanded their host range with species from the genera *Pastinaca*, *Chrysanthemum* and *Parthenium* [10]. Hypothetically, observations of hybrid host range shifts may result from their capacity to invade novel niches (hosts), while they failed to compete with their parental lineages on the traditional host.

More extremely, hybridization of two non-pathogenic lineages may even result into the generation of a novel pathogen. This was recently demonstrated for the opportunistic pathogenic yeast *Candida metapsilosis* where a single hybridization event of two non-pathogenic ancestor lineages resulted in a highly heterozygous species. Presently, *C. metapsilosis* reached worldwide distribution as an emerging pathogen of immunocompromised patients [45[•]].

Conclusions

Interspecific hybridization appears a successful evolutionary strategy whereby the combination of two dissimilar genomes promotes diversification in a saltational manner, leading to novel (combinations of) traits, including those relevant to pathogenicity. Particularly successful hybridization events may generate hybrid pathogens that are particularly fit in a given niche, and thus have far-reaching effects on agricultural crops or natural ecosystems. Considering that interspecific hybridization was recently revealed to be causative to the WGD in *S. cerevisiae* that had been initially ascribed to doubling of its ancestor DNA [16[•]], the occurrence of interspecific hybridization may currently be underestimated. Additionally, the number of hybridization events resulting in novel pathogen lineages is expected to increase due to the increased globalization, travel and trade, as well as agricultural practices such as large acreages

of monocultures and hybrid crops, mediating physical contact between geographically separated organisms. Clearly, interspecific hybridization events do not only impact plant pathogenicity, but may affect pathogens of any organism. In addition to the previously mentioned example of *Candida metapsilosis*, the yeast hybrid that is an emerging pathogen of immunocompromised patients, also interspecific hybrids between *Cryptococcus neoformans* and *C. gattii*, both causal agents of pulmonary cryptococcosis and meningitis in various animals, have been reported to occur in humans [46].

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