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A dialogue-like cell communication mechanism is conserved in filamentous ascomycete fungi and mediates interspecies interactions

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In many filamentous fungi, germinating spores cooperate by fusing into supracellular structures, which develop into the mycelial colony. In the model fungus *Neurospora crassa*, this social behavior is mediated by an intriguing mode of communication, in which two fusing cells take turns in signal sending and receiving. Here we show that this dialogue-like cell communication mechanism is highly conserved in distantly related fungal species and mediates interspecies interactions. In mixed populations, cells of *N. crassa* and the phytopathogenic gray mold *Botrytis cinerea* coordinate their behavior over a spatial distance and establish physical contact. Subsequent cell–cell fusion is, however, restricted to germlings of the same species, indicating that species specificity of germling fusion has evolved not on the level of the signal/receptor but at subsequent levels of the fusion process. In *B. cinerea*, fusion and infectious growth are mutually exclusive cellular programs. Remarkably, the presence of *N. crassa* can reprogram this behavior and induce fusion of the gray mold on plant surfaces, potentially weakening its pathogenic potential. In a third fungal species, the nematode-trapping fungus *Arthrobotrys flagrans*, the conserved signaling mechanism mediates vegetative fusion within mycelial colonies but has also been repurposed for the formation of nematode-catching traps. In summary, this study identified the cell dialogue mechanism as a conserved complex trait and revealed that even distantly related fungi possess a common molecular language, which promotes cellular contact formation across species borders.

cell signaling | interspecies interaction | cell fusion | fungi | pathogenicity

Significance

This study reveals that a dialogue-like communication mechanism, which mediates cell–cell fusion in filamentous fungi, is a conserved complex trait. It allows the communication and behavioral coordination of cells of distantly related species and mediates their mutual attraction and subsequent physical contact, although interspecies fusion does not occur. Through the activation of this signaling machinery, one species can reprogram the developmental program of the other fungus. These data promote our understanding of microbial communication, illustrate the mechanism of repurposing of existing building blocks in cellular evolution, revive the hypothesis of vegetative fusion as an avenue of horizontal gene transfer in fungi, and establish the idea of developmental reprogramming as a tool for controlling fungi.

In natural ecosystems, all organisms typically maintain numerous interactions within and beyond species boundaries. These relations can be of competing or beneficial nature with often fluent transitions depending on the specific environmental conditions (1, 2). Even potentially advantageous interactions hold the risk of detrimental consequences and must therefore be controlled by staunch friend–foe recognition mechanisms. The evolution and molecular basis of these control mechanisms remain some of the most fascinating and challenging questions in the life sciences.

In microbial populations, single-cell organisms often take on social behavior characterized by coordinated growth and development. Communication between interacting microorganisms typically involves the secretion of chemical compounds, such as signaling molecules or inhibitors (3, 4). An extreme example of cellular cooperation is the fusion of germinating fungal spores into supracellular networks, which further develop into the mycelial colony. In this process, genetically identical cells direct their growth toward each other and fuse, thereby rendering their individuality. Numerous consecutive fusion events result in the formation of larger-scale functional units, which conquer their territories in a coordinated manner (5). Major benefits of this cooperative behavior include pooling of the resources provided by the individual spores and the avoidance of direct competition between disconnected but genetically identical colonies (6).

On the downside, however, cooperation via cell–cell fusion poses the risk of the transmission of infectious elements, such as viruses, between individuals. If genetic differences between the fusion partners exist, even the takeover of all cellular resources by one genotype is possible (7). In order to avoid such detrimental consequences, fungi have evolved multilayered allorecognition systems, which ensure that only genetically compatible and oftentimes identical individuals merge into supracellular units. Cell–cell fusion is a multistep process that includes the induction of fusion competence, recognition and communication of potential partner cells, directed growth, the establishment of cell–cell contact, cell wall reconstruction, and finally plasma membrane fusion. Checkpoints of the allorecognition systems exist at several of these

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different distinct steps (8). The existence of such a variety of fine-tuned control mechanisms suggests that fungi aim to prevent nonself-fusion at all costs, emphasizing the importance of self- versus nonself-recognition. The molecular basis of the described control mechanisms has been mostly investigated in the model fungus *Neurospora crassa*, which has advanced as one of the major research models for studying eukaryotic cell–cell fusion (9).

Fusing spore germlings of this fungus employ an intriguing cell–cell communication mechanism, in which the two fusion partners coordinately alternate between two physiological stages, probably representing signal sending and receiving (10). The current working model suggests that the cells take turns in signal emission and perception, resembling a cellular dialogue. On the molecular level, signal translation and integration are mediated by an intrinsic network of conserved but also fungal-specific factors, including two mitogen-activated protein (MAP) kinase pathways, NADPH oxidase complexes, and the conserved striatin-interacting phosphatase and kinase (STRIPAK) signaling complex (11, 12). The physiological switches of the two communicating cells are characterized by the alternating membrane recruitment of the MAK-2 MAP kinase module and the SO (soft) protein (10). While MAP kinase cascades are highly conserved in eukaryotic organisms, where they typically translate extracellular signals into appropriate cellular responses, the SO protein is only conserved in filamentous ascomycete fungi, and its molecular function in cell–cell communication remains insufficiently understood (13, 14). During the fusion related interaction of two spore germlings, the proteins accumulate and disappear at the membrane of the growing cell tips with a phase of 6 to 12 min until cell–cell contact has been established and cell fusion is initiated. The alternating membrane recruitment of MAK-2 and SO occurs in exact antiphase in the two cells, illustrating the coordination of the cellular behavior over a spatial distance (10). The same molecular mechanism also mediates the fusion of hyphal branches within mature mycelial colonies, where hyphal fusion is increasing the interconnectivity of the mycelium (15). A conserved role of MAK-2 and SO homologous proteins has been reported for several filamentous fungi with different lifestyles, including the saprotrophic species *Sordaria macrospora*, the endophyte *Epichloe festucae*, and the plant pathogen *Fusarium oxysporum*. The potential conservation of the intriguing cell dialogue mechanism has remained, however, an open question for many years.

In this study we provide evidence that the cell dialogue mechanism first observed in *N. crassa* is highly conserved in two distantly related fungi with different lifestyles: the plant-pathogenic gray mold *Botrytis cinerea* and the nematode-trapping species *Arthrobotrys flagrans*. Despite the strict fungal self-/nonself-concepts, the cell dialogue mechanism even mediates common interactions between germlings of *N. crassa* and *B. cinerea*, indicating that fungi possess a common molecular language. However, interspecies cell–cell contacts fail to result in fusion, suggesting that despite conserved precontact signaling, molecular mechanisms evolved that prevent interspecies mergers. Cocultivation with *N. crassa* induces cell fusion in *B. cinerea* under conditions that usually suppress this cellular behavior in the gray mold, suggesting interspecies developmental reprogramming. Finally, we show that *A. flagrans* employs the cell dialogue mechanism for the formation of nematode-catching traps, illustrating how the evolution of new capacities relies on the repurposing of existing molecular programs.

Results

The BcPro40 Protein and the MAP Kinase BMP1 Are Essential for Germling Fusion in *B. cinerea*. Earlier studies indicated that spore germlings of *B. cinerea* readily undergo mutual interactions and fusion, similar to *N. crassa* and many other filamentous ascomycete species (16). Typically, this fusion occurs through specialized cellular protrusions, so-called conidial anastomosis tubes (CATs), which are thinner and shorter than germ tubes. To test if the molecular machinery mediating this cellular interaction is conserved between *B. cinerea* and *N. crassa*, we analyzed if the proteins homologous to MAK-2 and SO of the red bread mold are also essential for germling fusion in *B. cinerea*. In contrast to the well-described MAK-2 homologous protein BMP1 (Bcin02g08170), the SO homologous BcPro40 (Bcin01g06080) has only been briefly described (17). We therefore conducted a more detailed analysis of the role of BcPro40 during germling fusion in *B. cinerea*. A sequence alignment of SO and BcPro40 revealed that both proteins share about 59% sequence identity with low conservation in the N-terminal and high conservation in the C-terminal half of the polypeptides. The typical domain structure found in SO homologs of several fungi is also conserved in *B. cinerea* (*SI Appendix*, Fig. S1). BcPro40 comprises 1,204 amino acid residues and is encoded by a gene (*bcpro40*) containing a 4,567-bp open reading frame with two predicted introns. Sequencing of cDNA and transcript database analysis confirmed the position and size of the introns and gave no indication of alternative transcripts. To test the role of BcPro40 in vegetative spore germling fusion, a Δ*bcpro40* knockout mutant was created by a gene replacement strategy (*SI Appendix*, Fig. S1 *C* and *D*). In order to ensure that potentially observed deficiencies of this isolate were caused by the loss of *bcpro40*, the gene was reintroduced into the mutant strain to create a complemented isolate. To further facilitate cell biological studies, the *bcpro40* sequence was fused to the green fluorescent protein (GFP) encoding DNA sequence. In a parallel approach, the open reading frame fused to *gfp* was expressed under control of the constitutive *oliC* promoter, which is routinely used in cell biological studies in *B. cinerea* (18). Both constructs were separately integrated at the *bcniiA* gene locus of the mutant strain. The gene replacement mutant Δ*bmp1* of *B. cinerea*, which lacks the homolog of the *N. crassa* MAP kinase MAK-2, was available from an earlier study (19). The same complementation strategy involving expression of *gfp* fusion constructs from the native or the constitutive promoter at the *bcniiA* locus was applied to the *bmp1* mutant. General phenotypic characterization revealed reduced growth of the Δ*bmp1* mutant but normal growth rates for Δ*bcpro40* (*SI Appendix*, Fig. S2*A*). The formation of vegetative spores was significantly affected in both isolates (*SI Appendix*, Fig. S2*B*). All complemented isolates grew and sporulated in a way comparable to the wild-type reference strain B05.10, indicating that the developmental deficiencies of the mutants are caused by loss of the respective gene and that the different GFP fusion constructs are fully functional (*SI Appendix*, Fig. S2 *A* and *B*). To test germling fusion, conidia of the two gene knockout mutants, two respective complemented isolates, and the wild-type reference strain were plated on synthetic minimal medium. In a preexperiment, we determined a time point of 15 h after inoculation as the optimal time point for the analysis of cell–cell interactions (*SI Appendix*, Fig. S2*C*). Spore germination was comparable in all tested strains, indicating that BcPro40 and BMP1 are dispensable for germ tube formation (Fig. 1*A*). However, while in the wild-type and the two

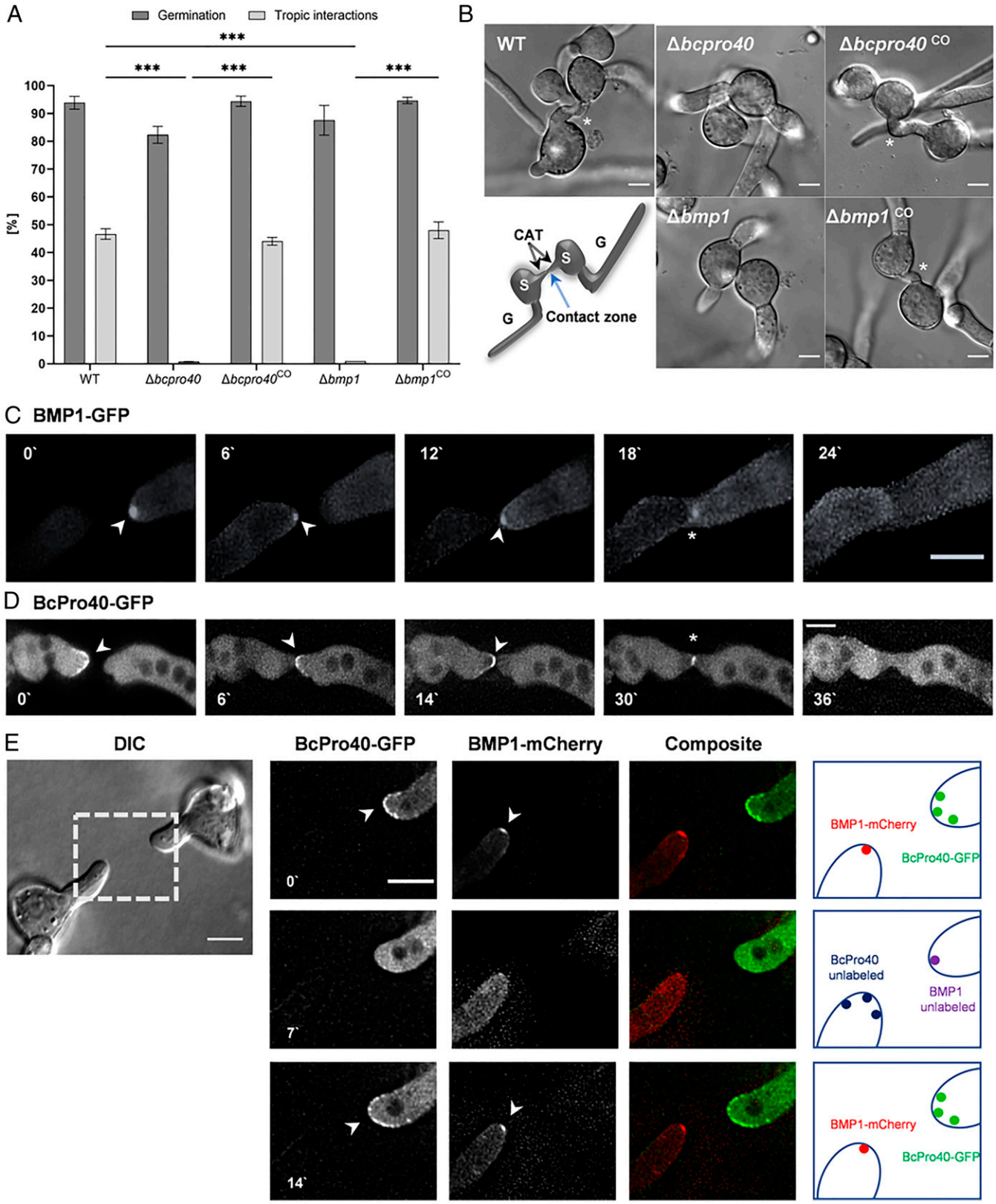

Fig. 1. Germling fusion in *B. cinerea*. (*A*) Quantification of tropic interactions and germination. Wild-type (WT), Δ*bcpro40*, Δ*bcpro40*^{CO} (complemented Δ*bcpro40* mutant), Δ*bmp1*, and Δ*bmp1*^{CO} (complemented Δ*bmp1* mutant). Error bars indicate SD calculated from three independent experiments (each n~100). ***$P < 0.001$. (*B*) Representative images of germlings of the different strains (asterisks indicate fusion bridges). S, spore; G, germ tube. (*C*) Time lapse of BMP1-GFP localization during germling interaction (arrowhead, protein accumulation; asterisk, fusion site). Comparable observations were made for multiple cell pairs ($n > 30$). (*D*) Time lapse of BcPro40-GFP localization during germling interaction (arrowhead, protein accumulation; asterisk, fusion site). Comparable observations were made for multiple cell pairs ($n > 10$). (*E*) Time lapse images of two fusing cells expressing BMP1-mCherry (left cell) and BcPro40-GFP (right cell). Note that only one fluorophore is visualized per cell; i.e., when the signal is absent from the tip, the untagged oscillatory protein (BMP1/BcPro40) is recruited (see schematic to the right). Similar observations were made several times ($n > 3$). (All scale bars: 5 μm.).

complemented strains, fusion-related cell–cell interactions were readily observed with comparable frequencies in all three strains, these interactions were fully absent in the Δ*bcpro40* and Δ*bmp1* mutant strains (Fig. 1 *A* and *B*). These data indicate that BcPro40 and BMP1 are essential for vegetative cell–cell fusion in *B. cinerea*, similar to the role of their homologs in *N. crassa* and other filamentous fungi.

The Cell Dialogue Mechanism Is Conserved in *B. cinerea*. In *N. crassa*, the interaction of two germlings undergoing mutual attraction and subsequent fusion is characterized by the highly coordinated, alternating recruitment of SO and the BMP1 homologous MAP kinase MAK-2 to the plasma membrane of the growing tips (10). To test the subcellular dynamics of the two proteins during germling interactions of *B. cinerea*, the strains expressing GFP-labeled BcPro40 or BMP1 were investigated. All tested GFP fusion constructs resulted in a detectable fluorescent signal. Relative quantification of the fluorescent signal revealed higher signal intensities when the *gfp* constructs were expressed from the constitutive than from the native promoter (ca. 5 times higher for BMP1 and ca. 10 times higher for BcPro40). In noninteracting cells, both proteins appeared evenly distributed in the cytoplasm, while the MAP kinase also accumulated in nuclei (*SI Appendix*, Fig. S3*A*). Importantly, BMP1-GFP also strongly accumulated in a punctuate structure at one of the two growing tips in interacting germlings (Fig. 1*C*). Time lapse microscopy revealed that this membrane-associated accumulation was highly dynamic and oscillated in a phase of ∼11 to 15 min. Interestingly, the same oscillatory dynamic was observed in the partner cell, however, in exact antiphase, such that the highest signal intensity in the first cell tip correlated with the lowest intensity in the second cell (Fig. 1*C*; *SI Appendix*, Fig. S3*B*; and Movie S1). Once the two cells established physical contact, the protein accumulated at the contact zone. After cell–cell contact, the BMP1-GFP signal took on a ring-shaped appearance, suggesting the formation of a fusion pore, before it disappeared after fusion of the two cells (*SI Appendix*, Fig. S3 *C* and *D* and Movie S2). Expression of the BMP1-GFP fusion construct from the native and constitutive promoters resulted in comparable observations, excluding overexpression artifacts (*SI Appendix*, Fig. S3*E*).

The subcellular localization of the BcPro40-GFP fusion protein showed comparable dynamics during germling interactions. However, the protein accumulated in a crescent shape at the growing cell tip rather than in a punctuate structure (Fig. 1*D*). Similar to BMP1-GFP, the membrane recruitment of BcPro40-GFP also oscillated in antiphase of ∼11 to 15 min in the two partner cells before the protein accumulated at the cell contact zone after physical contact of the germlings (Fig. 1*D* and *SI Appendix*, Fig. S4 *A* and *B*). Since expression of the BcPro40-GFP construct from the constitutive P*oliC* promotor did also not influence the protein dynamics as compared to the fusion construct under control of the promoter from the *bcpro40* gene (Fig. 1*D*; *SI Appendix*, Fig. S4*C*; and Movie S3), overexpression strains were used for all further localization experiments.

In *N. crassa*, SO and the MAP kinase MAK-2 are recruited to the plasma membrane in a highly coordinated alternating fashion. To test if in *B. cinerea* the two proteins also alternate at the cell tip or, alternatively, colocalize, a strain expressing BMP1 fused to the red fluorescent protein mCherry protein was constructed. The localization pattern of mCherry-labeled BMP1 was identical to that of the GFP-tagged MAP kinase (Fig. 1*E*). Spores of the BMP1-mCherry expressing isolate were mixed with even numbers of conidia expressing BcPro40-GFP. After 12 h of incubation, germling pairs consisting of a red and green fluorescent cell were analyzed by fluorescent microscopy. In both cells the tip recruitment of BcPro40-GFP and BMP1-mCherry oscillated at the two opposing cell tips in parallel phases, indicating that also in *B. cinerea* the proteins alternate in their membrane localization in the individual cells (Fig. 1*E*).

Taken together, these data indicate that during their interaction, the two *B. cinerea* cells switch between two physiological stages in a highly coordinated manner. This behavior is mostly identical to the cellular dynamics of germling fusion in *N. crassa*, suggesting that the dialogue-like cell–cell communication mechanism is highly conserved between these distantly related fungi.

***N. crassa* and *B. cinerea* Undergo Interspecies Interactions Mediated by the Cell Dialogue Signaling Mechanism.** The MAP kinases MAK-2 and BMP1 are most likely involved in translating the postulated cell communication signal into the appropriate cellular response. So far, the nature of this signal and its putative, cognate receptor remain unknown. Since the cell dialogue mechanism appears to be conserved between *N. crassa* and *B. cinerea*, we reasoned that this mechanistic conservation allows testing of whether the postulated signal is species-specific or also conserved among the filamentous ascomycete species. We hypothesized that a conserved signal and receptor would promote interspecies interactions through the cell dialogue mechanism, while species-specific factors would allow efficient self-/nonself-recognition of germinating spores. To test this hypothesis, *N. crassa* and *B. cinerea* spores were mixed in equal amounts and analyzed for the occurrence of interspecies interactions. In order to unambiguously attribute the germinating spores to one of the two species, a wild-type *N. crassa* strain was mixed with a cytoplasmic GFP-expressing isolate of *B. cinerea*. Since spores of *N. crassa* germinate significantly faster than those of *B. cinerea*, the latter one was given a head start, ensuring that the peak of cell–cell interactions within the mixed cell population occurred in the same time window for both species. The interaction within and between the two species, indicated by nonrandom cell–cell contacts, was quantified by light and fluorescent microscopy. In addition to the expected intraspecies interactions, a significant proportion of interspecies interactions between *N. crassa* and *B. cinerea* was also detected in these mixed cell populations (Fig. 2 *A* and *B*). While intraspecies contacts represented the majority of all interactions (ca. 49% for *N. crassa* and 33% for *B. cinerea*), interspecies communications occurred with a frequency of about 16% (Fig. 2*B*). This observation suggests that these two distantly related fungal species are able to locate and mutually attract each other, suggesting a sufficient level of conservation of the proposed involved signal/receptor pair. In all observed cases of interspecies interactions, contact between the germlings of *N. crassa* and *B. cinerea* resulted in growth arrest (Fig. 2*A*), suggesting that the so far unknown signals, which terminate the cellular program of directed growth, also function across the species border. To test if the interspecies interaction also involves the cell dialogue mechanism, spores of *N. crassa* expressing either MAK-2-GFP or SO-GFP were confronted with *B. cinerea* spore germlings. Strong and robust oscillatory recruitment of the MAP kinase to the plasma membrane of the *N. crassa* cell growing toward *B. cinerea* was observed, indicating that the coordinated switch in the cellular behavior is also the basis of interspecies interactions (*SI Appendix*, Fig. S5*A*). The detected SO signal was, however, weaker than in intraspecies interactions, and the obtained data were less conclusive,

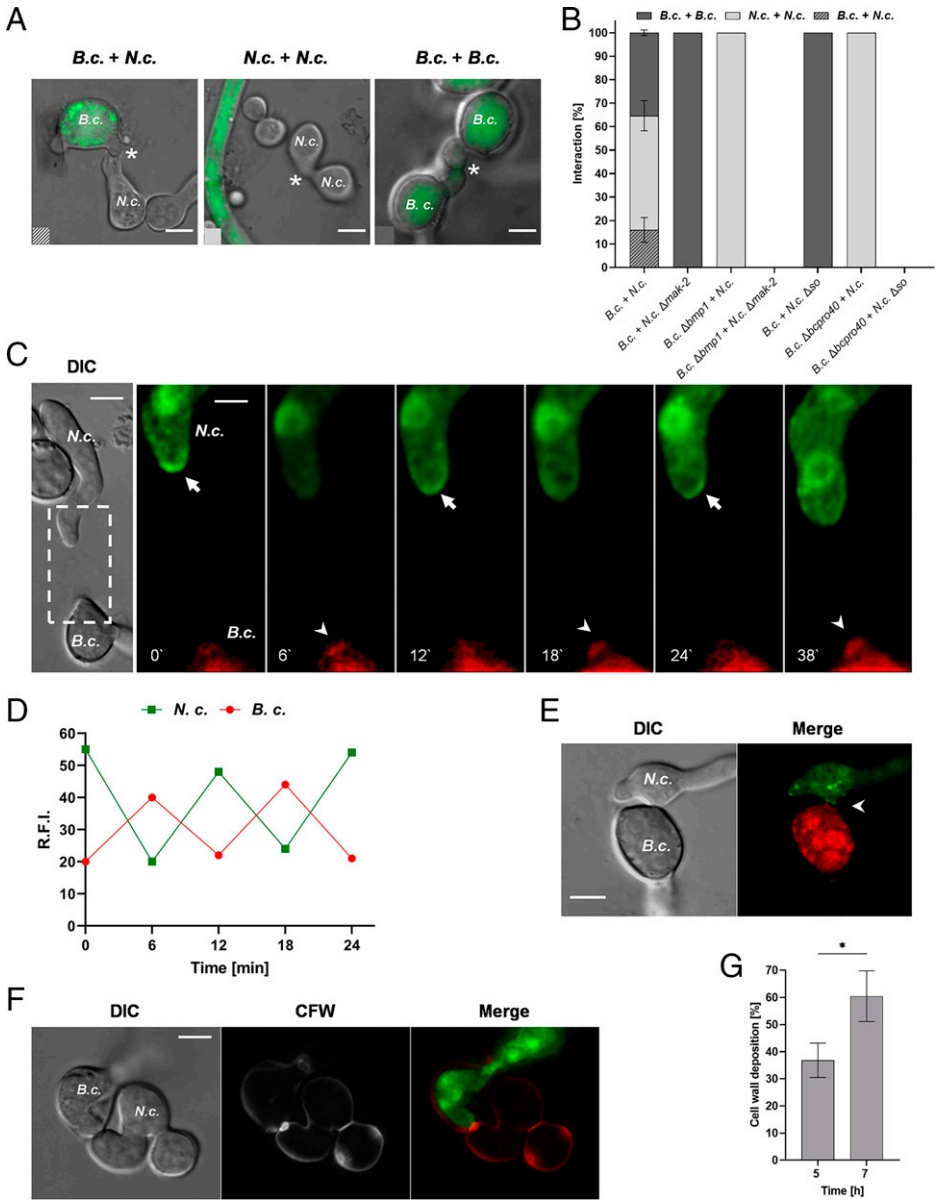

Fig. 2. *B. cinerea* and *N. crassa* undergo interspecies interactions. (*A*) Representative images showing the different kinds of cell–cell interactions in mixed assays of *N. crassa* wild-type (*N.c.*) and *B. cinerea* expressing cytoplasmic GFP (*B.c.*). Asterisks indicate contact sites. The square in the bottom left corner indicates the color code used in *B*. (*B*) Quantification of the three kinds of interactions in mixes of different strains, calculated as percentage of overall detected interactions. Strains are indicated at the *x* axis. Error bars represent the SD calculated from three independent experiments (n = ~50 interactions each). (*C*) Dynamics of BMP1-mCherry (*B.c.*) and MAK-2-GFP (*N.c.*) in an interspecies interaction. Arrows indicate accumulation of MAK-2, and arrowheads indicate accumulation of BMP1. Comparable observations were made multiple times (n = 5). (*D*) Relative fluorescence intensity (RFI) measured at the cell tips shown in *C*. (*E*) Cell–cell contact between cells of the same strains shown in *D*. Note that no accumulation of SO-GFP/BMP1-mCherry signal is detected at the contact area. (*F*) CFW staining of an interspecies cell pair (*N.c.* wild-type and *B.c.* expressing BMP1-GFP). Note that cell wall material is accumulated at the contact site (arrowhead). (*G*) Quantification of cell wall accumulation at the contact site calculated as percentage of interspecies cell pairs exhibiting an increased CFW signal at the contact zone. Error bars indicate SD from three independent experiments (n = ~40 each). (All scale bars: 5 μm.)

suggesting some differences between the two types of cellular interactions (*SI Appendix*, Fig. S5*B*). Similarly, robust oscillating membrane recruitment of BMP1 during interspecies interactions was exhibited by the *B. cinerea* germling (*SI Appendix*, Fig. S5*C* and Movie S4).

To test if the cells undergoing these interspecies interactions also coordinate their behavior in a way similar to the intraspecies communication, *N. crassa* spore germlings expressing MAK-2-GFP were coincubated with *B. cinerea* germlings expressing BMP1-mCherry. In interspecies pairings, coordinated alternating recruitment of the MAP kinases to the plasma membrane of the growing cell tips was observed, indicating that vegetative cells of these different fungal species are capable of coordinating their cellular behavior over the spatial distance (Fig. 2 *C* and *D*). In contrast to intraspecies fusion pairs, however, no clear accumulation of the MAP kinases at the fusion point was observed (Fig. 2*E*).

We therefore asked whether these interactions would also result in subsequent cell–cell fusion. To answer this question, populations of *N. crassa* spores expressing cytoplasmic GFP were mixed with nonlabeled wild-type *B. cinerea* germlings and observed over extended periods of time. Successful cell–cell fusion would be indicated by the transfer of the fluorescent signal from one cell to the other. Cell fusions between *N. crassa* and *B. cinerea* were never detected (*SI Appendix*, Fig. S5*D*), suggesting that the cell fusion program is either not activated or

actively suppressed in these interspecies pairings. Interestingly, staining with calcofluor-white (CFW) revealed increased cell wall deposition at the cell–cell contact zone in the *N. crassa* cell, in about 40% of interspecies cell pairs. Over time, this number increased, suggesting an increase in cell wall deposition (Fig. 2*F*). A comparable accumulation of cell wall material is never observed in the intraspecies pairings (*SI Appendix*, Fig. S5 *E* and *F*), suggesting that when in physical contact with *B. cinerea*, *N. crassa* actively enforces the separation of the nonidentical cells of these two species.

Based on the combined interspecies interaction data, we hypothesize that a conserved signal/receptor pair functions in these distantly related fungi and that downstream checkpoints exist, which efficiently prevent interspecies cell–cell merger.

BcPro40 Is Dispensable for Pathogenicity of *B. cinerea* on Common Bean. A plethora of data from different fungi indicate that several of the main factors mediating vegetative cell–cell fusion are also essential for pathogenic growth and development. Examples include the MAP kinases homologous to MAK-2 in several pathogenic fungi, including BMP1 in *B. cinerea* (20–22). To test a potential role of BcPro40 for pathogenic growth of *B. cinerea*, leaves of the common bean *Phaseolus vulgaris* were inoculated with spore suspensions of the wild-type reference strain, the Δ*bcpro40* mutant, and the complemented isolate. Virulence of the strains was determined by measuring the diameter of the forming necrotic area. No significant differences were observed between the three strains, indicating that in contrast to BMP1, BcPro40 is dispensable for pathogenic growth and development of the gray mold on this host plant (*SI Appendix*, Fig. S6 *A* and *B*).

We had previously shown that *B. cinerea* is undergoing either cell–cell fusion or pathogenic growth depending on the properties of the plant cell surface it is growing on (16). While on hydrophobic plant surfaces it is behaving as a pathogen and is forming infectious cellular structures, it is undergoing cell–cell fusion on hydrophilic surfaces without infecting the plant cells. Based on these observations we had hypothesized that infectious growth and cell–cell fusion are two alternative, mutually exclusive developmental programs and that, as a consequence, on hydrophobic surfaces, no cell–cell fusion signals are produced by this fungus (16). After observing that *B. cinerea* responds to signals secreted by a *N. crassa* cells, we reasoned that confronting the two fungi on a hydrophobic plant surface might override the pathogenicity program of *B. cinerea* and induce interspecies and maybe even intraspecies cell–cell fusion in the gray mold on hydrophobic layers. To test this hypothesis, we first compared the fusion frequency of *N. crassa* wild-type cells on a hydrophilic or hydrophobic plant surface. For this, minimal agar medium was covered with a single-cell epidermal layer obtained from onion bulbs (*Allium cepa*). These plant cell layers possess a hydrophilic and a hydrophobic side, so that positioning them in one or the other direction provides a hydrophilic or hydrophobic growth surface. Germinating conidia of *N. crassa* showed on both different surface qualities exactly the same behavior and readily underwent mutual attraction and fusion (*SI Appendix*, Fig. S6*C*). As previously reported, *B. cinerea* germlings underwent fusion exclusively on the hydrophilic side and only formed infection structures on the hydrophobic layer (Fig. 3*B* and *SI Appendix*, Fig. S6*D*). On the hydrophilic side, the interaction was mediated by the previously described cell dialogue mechanism (*SI Appendix*, Fig. S6*E*). Remarkably, when spore germlings of both species were cocultivated on the hydrophobic plant cell layer, the formation of CATs and intraspecies and interspecies interactions of *B. cinerea* were frequently observed, indicating that the presence of *N. crassa* induced the cellular fusion program in the gray mold (Fig. 3 *A* and *B*). To exclude the possibility that potential nutrient starvation caused by competition with *N. crassa* is inducing this effect, we tested the behavior of *B. cinerea* germlings growing by themselves on hydrophobic onion epidermal layers placed on nutrient-free starvation medium. No fusion-related cellular interactions were observed, suggesting that the induction of these reactions in the mixed assays is not an effect produced by nutrient competition between the two species (*SI Appendix*, Fig. S7*A*). We also tested the inducing ability of the Δ*so* and Δ*mak-2* mutants of *N. crassa*. While Δ*so* induced fusion in *B. cinerea* comparable to the wild-type, Δ*mak-2* mostly failed to reprogram the gray mold (Fig. 3 *A* and *B*).

We reasoned that if the presence of *N. crassa* might (partially) reprogram *B. cinerea* from infectious growth to cell–cell fusion, the red bread mold would also reduce the virulence of the gray mold on its host plants. To test this hypothesis, spores of the two fungi were mixed and applied to detached bean leaves and leaves of intact plants of the model species *Arabidopsis thaliana*. In the presence of *N. crassa*, plant infection judged by the formation of necrotic areas was significantly reduced on both host species (Fig. 3 *C* and *D* and *SI Appendix*, Fig. S8 *A*–*C*). In the sole presence of *N. crassa*, no symptoms of plant cell death were observed. Similarly, inoculation with *N. crassa* did not induce the expression of a pathogen-responsive reporter gene in *A. thaliana*, while an infection by *B. cinerea* significantly increased expression levels (*SI Appendix*, Fig. S8*D*). The reporter is based on regulatory sequences of *DJ1E*, a gene induced in the presence of various necrotrophic and biotrophic fungal, oomycete, and bacterial species (23). Together, these observations suggest that the reduced pathogenicity of *B. cinerea* in the mixed assays was not caused by increased defense reactions of the host tissue (*SI Appendix*, Fig. S8*C*). Light microscopy revealed that similar to the results for the onion epidermal layer, fusion of *B. cinerea* was absent on the bean leaves when the fungus was inoculated alone (0 ± 0%) but was induced in the presence of *N. crassa* (9 ± 2.6%) (Fig. 3*E*). In contrast to the reprogramming observed on onion epidermis, however, the Δ*mak-2* mutant of the red bread mold also suppressed pathogenicity (Fig. 3 *C* and *D*). Together, these data suggest that reprogramming by *N. crassa* might reduce the virulence of *B. cinerea*.

The Cell Dialogue Mechanism Has Been Repurposed for Trap Formation in the Nematode Trapping Fungus *A. flagrans*. Hyphal fusion is employed throughout the fungal kingdom for different developmental purposes. In nematode trapping fungi, trap formation includes fusion of a hyphal branch with a short peg formed by the leading hypha, resulting in the formation of a ring-like hyphal structure. Our earlier study indicated that the SO-homologous SofT is essential for this cell fusion process in *A. flagrans* (24). Based on our observation that the cell dialogue signaling mechanism is highly conserved in filamentous fungi, we therefore hypothesized that *A. flagrans* also employs this process for vegetative hyphal fusion and might have repurposed it for trap formation. Spore germling fusion was not apparent under laboratory growth conditions, but hyphal fusion was readily observed in the wild-type reference strain. In gene knockout mutants for *sofT* and the *mak-2*–homologous *makB* gene, hyphal fusion was fully absent, indicating that the respective proteins are also essential for vegetative fusion in this fungus (*SI Appendix*, Fig. S9 *A* and *B*). Subcellular localization

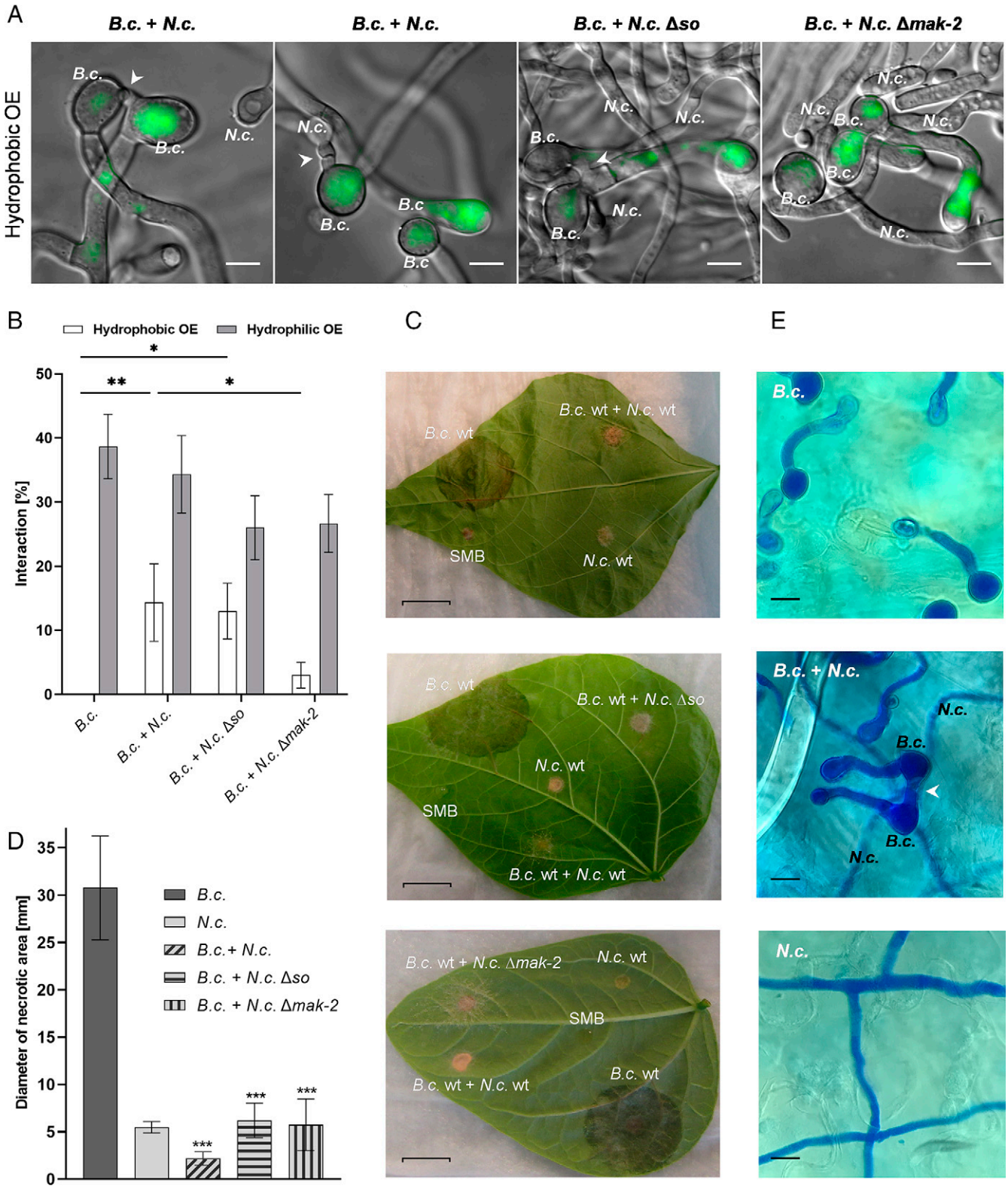

Fig. 3. *N. crassa* induces cell fusion in *B. cinerea* during pathogenic growth. (*A*) Interspecies mix of *B. cinerea* expressing cytoplasmic GFP (*B.c.*) and different *N. crassa* strains (*N.c.*) incubated on the hydrophobic side of an onion epidermis (OE). Arrowheads indicate cell–cell interactions. For images of cells growing on the hydrophilic OE, see *SI Appendix*, Fig. S7*B*. Scale bars: 5 μm. (*B*) Quantification of cell fusion of *B. cinerea* on the hydrophobic or hydrophilic OE in different strain mixes. Error bars indicate SD calculated from three independent experiments (each $n = \sim 100$). $^{**}P < 0.01$; $^{*}P < 0.05$. (*C*) Infection assay showing French bean leaves inoculated with individual and mixed *B. cinerea* wild-type (*B.c.*) and *N. crassa* (*N.c.*) wild-type and mutant strains. Controls and test combinations were inoculated on the same leaf. SMB, buffer control. Scale bars: 1 cm. (*D*) Quantification of the diameter of the necrotic area produced by the different inocula on bean leaves. Error bars indicate the SD calculated from three different experiments ($n = 5$ leaves in each experiment). $^{***}P < 0.001$. (*E*) Microscopic analysis of fungal growth on bean leaves inoculated with individual and mixed *B. cinerea* wild-type (*B.c.*) and *N. crassa* (*N.c.*) wild-type strains 16 h after inoculation. Fungal cells were stained by lactophenol cotton blue. Arrowhead indicates cell–cell fusion Scale bars: 5 μm.

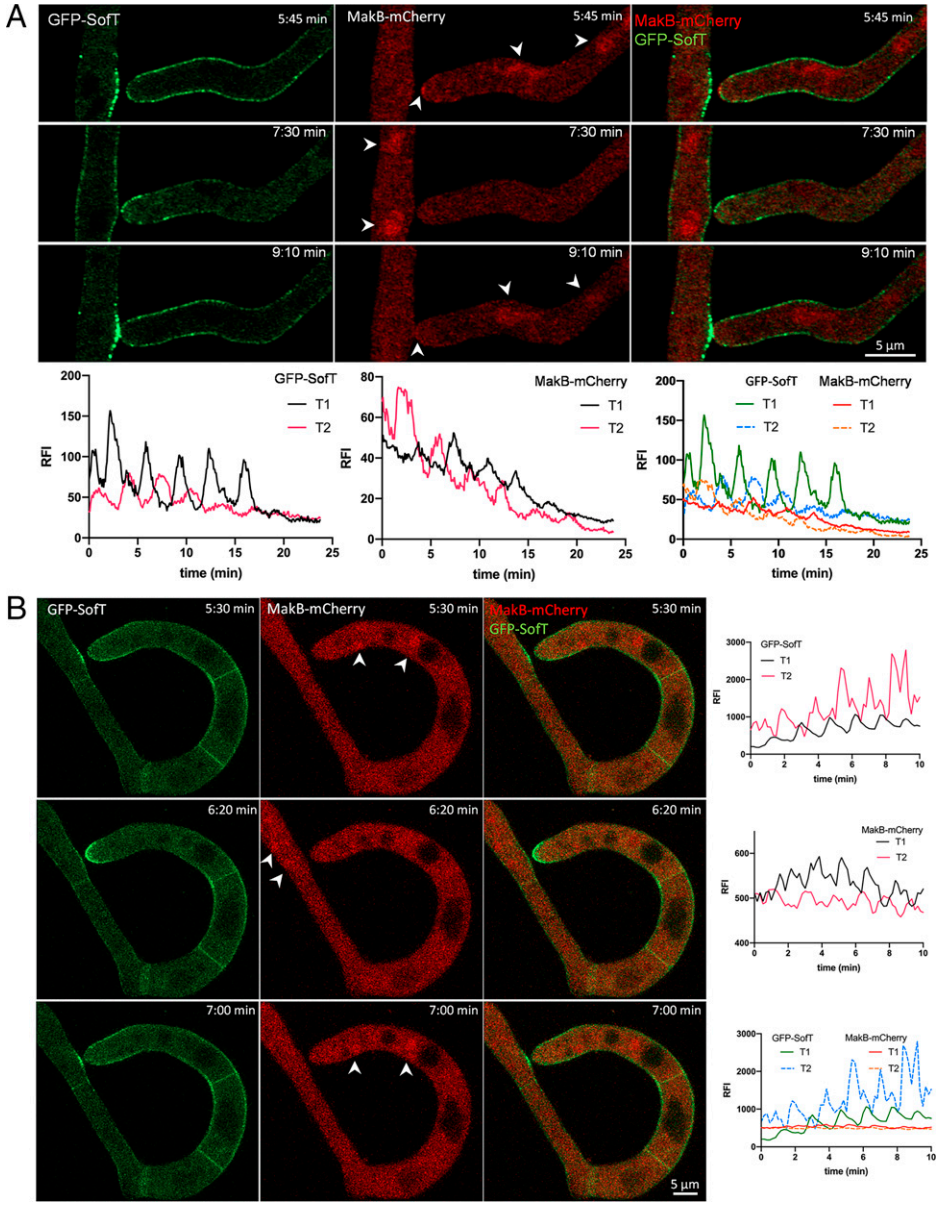

Fig. 4. The conserved cell dialogue mechanism mediates hyphal fusion and trap formation in *A. flagrans*. (*A*) (*Top*) Time course of GFP-SofT (depicted in green) and MakB-mCherry (depicted in red) localization during a hyphal fusion event. (*Bottom*) Quantification of RFI (*y* axis) in the interacting zone over time (*x* axis, in minutes). T1, left hypha; T2, right hypha. Arrows indicate the localization of MakB-mCherry in nuclei or at the tip of the interacting cells. (*B*) Time course of GFP-SofT and MakB-mCherry localization during trap morphogenesis. *x* axis and *y* axis in the graph showing RFI are identical to those in *A*. Arrows indicate the localization of MakB-mCherry in nuclei of the interacting cells.

of SofT revealed robust oscillating membrane recruitment of the protein to the plasma membrane of fusing cell tips, in an oscillation phase comparable to the ones observed in *N. crassa* and *B. cinerea* (Fig. 4*A*). Recruitment of the MAP kinase to the plasma membrane was more difficult to observe because of low expression levels. Interestingly, however, MakB clearly accumulated in the nuclei in a rhythmic, oscillating manner in exact antiphase to SofT membrane recruitment, a dynamic localization pattern observed neither in *N. crassa* nor in *B. cinerea* (Fig. 4*A*). Together, these data reveal that also in *A. flagrans,* the fusing cells coordinately alternate between two physiological stages, indicating that the cell dialogue mechanism is conserved. To test if this process also mediates trap formation, formation of these structures was analyzed in the Δ*makB* mutant. Similar to the Δ*sofT* mutant, fusion during trap formation was deficient, and the rings would not close. Despite this morphological deficiency, the traps were still sticky and allowed trapping of nematodes (*SI Appendix*, Fig. S9 *C* and *D*). Subcellular localization of SofT revealed robust oscillatory recruitment of the protein to the membrane of the fusion tips, with a shorter phase than observed in hyphal fusion. Similar to hyphal fusion, MakB was more difficult to detect at the membrane during the process but again accumulated in the nuclei in antiphase to SofT membrane recruitment (Fig. 4*B*). Taken together, these data indicate that the growing cell tips in trap formation also undergo coordinated, rapid switching in their physiological stage, the hallmark for the cell dialogue communication process. Trap formation differs from germling and hyphal fusion in the sense that the two fusing tips originate from the same leading hyphae with a distance of typically four compartments. In most ascomycete fungi, including *A. flagrans*, the septal pores of actively growing hyphae are open, thereby allowing rapid cytoplasmic flux. We hypothesized that during trap formation, however, the septal pores must be closed, in order for

the physiological switches of the closely neighboring cell tips to occur. Light microscopy revealed that indeed, the septal pores of the three septa within a trap are closed by Woronin bodies, the organelles typically employed by ascomycete fungi for pore occlusion (*SI Appendix*, Fig. S10 *A* and *B*). To test the functional role of this organelle, we created a Δ*hexA* mutant in *A. flagrans*, lacking the gene for the main Woronin body protein. Consistent with our hypothesis, trap formation in this mutant showed a deficiency comparable to the Δ*sofT* and the Δ*makB* mutant, such that cell fusion was absent and the rings would not close (*SI Appendix*, Fig. S10 *C–F*). While MakB oscillating translocation into the nuclei was still observed, SofT recruitment to the plasma membrane was absent in this mutant (*SI Appendix*, Fig. S10 *G* and *H*). Taken together, these data indicate that the cell dialogue mechanism has been repurposed by *A. flagrans*, in order to form specified three-dimensional cellular structures.

Discussion

A Conserved Cell–Cell Communication Mechanism. Our data indicate that homologs of the *N. crassa* SO protein and the MAK-2 MAP kinase have conserved functions during cell–cell communication and fusion in the distantly related fungal species *A. flagrans* and *B. cinerea*. Studies in *N. crassa* and other filamentous ascomycete fungi revealed that both proteins are part of an intrinsic signaling network consisting of two MAP kinase modules, two NADPH oxidase complexes, and the conserved STRIPAK complex (11). In addition to cell–cell communication and fusion, this signaling network governs a plethora of fungal developmental decisions, including pathogenicity, fruiting body development, and directed growth in response to environmental cues. This network therefore appears to function as a central and conserved signaling hub controlling fungal biology (11, 25). Strikingly, the highly coordinated subcellular dynamics of the SO and MAK-2 homologs during vegetative cell fusion are almost identical in the three species investigated in this study. Mathematical modeling suggested that the coordinated behavior of the fusion partners in *N. crassa* requires intertwined positive and negative feedback loops and a pulse-like signaling release (26). These specific dynamics would allow communication via a single chemoattractant/receptor system, while avoiding self-stimulation. Based on our observations, we hypothesize that the signaling network and even its specific, excitable dynamics are highly conserved in filamentous ascomycete fungi, probably because these dynamics are so specific that their evolution would quickly result in a loss of their main features, namely, the absence of self-stimulation despite signaling through a single signal/receptor system. The cell dialogue mechanism therefore appears to represent a complex trait with conservation throughout ascomycete evolution.

The Cell Dialogue Mechanism Mediates Interspecies Interaction. The high conservation of the cell dialogue mechanism is strikingly illustrated by the cross-species interactions of *N. crassa* and *B. cinerea* spore germlings. During this interspecies interaction, the homologous MAP kinases MAK-2 and BMP1 are recruited to the plasma membrane in a highly coordinated alternating manner. This finding indicates that the two fungi are able to coordinate their cellular behavior over a spatial distance, despite an evolutionary distance between these two species that is comparable to that between human and salmon. The so far unknown cell communication signal and its cognate receptor therefore appear to be also conserved and not species-specific. This notion stands in stark contrast to the recent description of various communication and compatibility groups for germling fusion in *N. crassa*, which suggested extremely high specificity for these cellular interactions (8). As a potential explanation of this conundrum, we propose that specificity could not be reached by the evolution of the signal/receptor system, because of the aforementioned high need for conservation of this complex trait. Instead, downstream allorecognition mechanisms have evolved that efficiently prevent interspecies cell–cell merger. Similar observations were made in the animal kingdom, where interspecies chemoattraction between egg and sperm of different mammals occurs. Here too the involved cell–cell communication signals and receptors are conserved, and cell fusion is usually blocked after cell–cell contact (27). Within the species *N. crassa*, allorecognition checkpoints exist at different stages of the cell–cell fusion process, including communication, cell wall remodeling, and cytoplasmic mixing (8). In the case of the *N. crassa*/*B. cinerea* interaction, this allorecognition functions at the stage of cell–cell contact. Interestingly, growth arrest is still induced after cell–cell contact in interspecies pairs, indicating that cell contact recognition is still functional in these pairings. Loss of this recognition would result in continuous adhering growth of the two cells, as described for germling pairs of the Δ*erg-2* mutant of *N. crassa* (28). The subsequent cell wall remodeling step, however, fails to be initiated, and instead, cell wall reinforcement at the cell contact zone frequently occurs in the *N. crassa* partner cell. Morphologically, this fusion block is strongly reminiscent of the intraspecies block mediated by the *cwr-1* and *cwr-2* genetic loci in *N. crassa* (29). Interestingly, however, these factors are not conserved in *B. cinerea*, suggesting that a different signaling system is in place in the interspecies pairings. Another system for the prevention of nonself-fusion of mature fungal colonies is the so-called vegetative incompatibility. Comparative analysis of the genetic basis of this allorecognition system in different fungi revealed that even in closely related species the mediating factors are often only poorly conserved (30). Taken together, these findings suggest that in contrast to the very highly conserved cell–cell communication machinery, allorecognition is a very quickly evolving process, which has independently been acquired by numerous fungal species.

The unexpected finding of interspecies interactions is of very high relevance for various aspects of fungal biology. Assuming that a block in interspecies fusion is genetically encoded, its failure will occur with the same frequency as loss of function mutations of the respective genetic loci. In addition, vegetative incompatibility can be inactive at certain developmental stages, for example, during sexual fusion of genetically different mating partners (31). Interspecies allorecognition fusion blocks might therefore not be triggered under every environmental condition, including physical or chemical stress or starvation. Failure or inactivation of this block would result in interspecies fusion, which could represent a conceivable avenue for horizontal gene transfer. Although horizontal gene transfer significantly contributes to the evolution of fungi, its cellular and mechanistic basis remains unknown (32). Throughout the literature, vegetative cell–cell fusion has been repeatedly discussed as a potential mechanism for horizontal gene transfer; however, experimental proof is still lacking (33–35). Potential evidence of intraspecies cell merger was found within the genus *Colletotrichum*, a clade, however, with ambiguous species boundaries (36, 37). Our findings that even distantly related fungi are able to establish physical contact via the cell dialogue mechanism now revive and very strongly promote the old hypothesis of vegetative fusion as an avenue of interspecies genetic exchange. In addition, cell dialogue-mediated interspecies interaction could have

also contributed to the evolution of mycoparasitic species, which grow toward their prey fungi in order to establish physical contact and digest their cellular content. Consistent with this notion, strains of the mycoparasitic genus *Trichoderma* that lack factors homologous to proteins of the cell dialogue signaling network are also affected in their parasitic capacity (38). In summary, we hypothesize that the highly conserved cell dialogue mechanism has evolved early in the ascomycete clade and has been repurposed for several new capacities, thereby contributing to the characteristic adaptability of fungi.

Another striking example supporting this hypothesis is the repurposing of the cell dialogue mechanism for trap formation in *A. flagrans*. Similar to germling and hyphal fusion, this type of cell merger involves two growing cell tips. In this case, however, both tips emerge from the same hyphae in very close distance, typically just four to five hyphal compartments apart. The mathematical model of the cell dialogue process requires specific spatial and temporal dynamics of cytoplasmic proteins in order to create the proposed positive and negative feedback loops (26). We therefore hypothesized that cytoplasmic continuity between the cellular compartments involved in trap formation is impossible. Consistent with this idea, we found that during trap formation, the septal pores become occluded by the Woronin body, which originally functions in the plugging of septal pores after hyphal injury (39). The *hexA* mutant lacking this structure strikingly exhibits the same phenotype as mutants lacking *sofT* and *makB*. We hypothesize that this defect is caused by the inability to maintain the coordinated, alternating switches and their underlying positive and negative feedback loops in a continuous cytoplasm. Consistent with this idea, SofT is not recruited to the plasma membrane in the *hexA* mutant. The translocation of MakB into the nucleus, however, still occurs in this strain, indicating that parts of the signaling dynamics are still functional. Overall, the robust alternating localization of the MAP kinase to the nuclei, which has not been observed in *N. crassa* and *B. cinerea*, indicates that although the cell dialogue mechanism seems to be highly conserved, the exact subcellular dynamics of individual molecular factors are adjusted to the specifics of the species. Studies in another nematode trapping fungus, *Arthrobotrys oligospora*, revealed that also in this species the *makB* and *hexA* homologs are required for trap formation (40, 41). We therefore hypothesize that here too the cell dialogue mechanism governs this developmental process. Taken together, trap formation impressively illustrates how during evolution, existing building blocks are repurposed and newly combined in order to gain new capacities.

The Presence of *N. crassa* Might Reprogram *B. cinerea*. In the phytopathogenic gray mold *B. cinerea*, germling fusion and infectious growth appear to be alternative, mutually exclusive developmental programs (16). Pathogenic behavior is induced on hydrophobic surfaces, while fusion occurs in a hydrophilic environment, reflecting the specific adaptation as a leaf pathogen. In contrast, interactions of *N. crassa* germlings are not influenced by the growth surface, consistent with its saprophytic lifestyle. Interestingly, however, the presence of *N. crassa* induces the fusion program and subsequent intraspecies and interspecies interactions of *B. cinerea* on hydrophobic surfaces. This reprogramming likely occurs on the level of fusion competency indicated by the formation of CATs in the presence of *N. crassa*. This effect is not caused by competition of nutrients, since even on starvation medium, *B. cinerea* does not form CATs or fuse on hydrophobic surfaces. In addition, the *N. crassa mak-2* mutant fails to induce this behavior on onion epidermal layers, in contrast to the *so* mutant or the wild-type. The fact that the *so* and *mak-2* mutants have similar growth characteristics supports the idea of a specific effect. It is a very tempting hypothesis that the fusion signals produced by *N. crassa* might reprogram the development of *B. cinerea* and suppress its pathogenicity. Consistent with this notion, virulence of *B. cinerea* is reduced on host plants in the presence of *N. crassa*, and germling fusion is induced. Here, however, no difference between the *so* and *mak-2* mutants was observed, suggesting that the interaction of the two fungi differs to some extent on living versus dead plant material. So far, the exact roles of the different signaling proteins in signal emission and perception remain unclear. It is, however, obvious that the pathways do not function in a strict linear manner but are wired into positive and negative feedback loops, which amplify a potential continuous, basic signaling of germinating spores into the observed robust alternating communication once fusion pairs are established (26). It is therefore not unlikely that the respective mutants still undergo basic signaling on plant surfaces resulting in the observed behavior.

Based on our observation of induced fusion in *B. cinerea* by the presence of *N. crassa*, we hypothesize that fungal developmental decisions can be reprogrammed by the appropriate chemical signals. Proof of this idea will require the identification and purification of the so far unknown cell–cell communication signal. If the purified compound would indeed reprogram *B. cinerea* and thereby decrease virulence, developmental reprogramming might hold rich potential for new fungal control strategies.

Materials and Methods

Strains and Growth Conditions. Fungal strains used in this study are listed in *SI Appendix*, Table S1. *N. crassa* was routinely grown on Vogel's minimal medium (42), with supplements added when required. *B. cinerea* was grown on complete medium or Vogels minimal medium, as previously described (16). *A. flagrans* was cultured at 28 °C on Potato Dextrose Agar (PDA) as previously described (24).

Cell–cell fusion assays on minimal agar medium or onion epidermal layers were conducted as described earlier (15, 16). For details, see *SI Appendix, Materials and Methods*.

Transformation of Fungal Strains. Fungal transformation was conducted as described earlier (16, 24, 43). For details of the strain construction, see *SI Appendix, Materials and Methods*.

Live-Cell Imaging. Cells were observed on a Zeiss Observer 2.1 microscope using Nomarski optics with a Plan-Neofluar 100×/1.30 oil immersion objective (420493-9900) with an LED (CoolLED pE4000) as a light source for fluorescence microscopy. Images were obtained with a PCO Edge 5.5 Gold (16 bit) camera controlled by a modified version of 4-D microscopy software programmed by Ralf Schnabel and Christian Hennig (44). Simple image analyses were performed with Fiji (ImageJ). Images were obtained as stacks and deconvolved using Huygens deconvolution software (Scientific Volume Imaging, Netherlands). Alternatively, cells were observed on a Zeiss LSM900 microscope with Plan Apochromat 63×/1.4 Oil objective and Airyscan 2 detector in superresolution mode. Images were acquired as z stacks and converted into maximum intensity projections. Analysis of relative fluorescent intensity was performed by the ZEISS microscope software ZEN Blue. For conventional fluorescence microscopy, a Zeiss AxioImager Z.1 microscope with Plan-Apochromat 63×/1.4 Oil Differential Interference Contrast (DIC), EC Plan-Neofluar 40×/0.75, Enhanced Contrast (EC) Plan-Neofluar 20×/0.50, or EC Plan-Neofluar 10×/0.30 objective and an AxioCamMR were used. Images were taken using the ZEN Blue edition.

Pathogenicity Assays. Virulence of *B. cinerea* was tested on the host plants *P. vulgaris* and *A. thaliana*. For details, see *SI Appendix, Materials and Methods*. Trap morphogenesis in *A. flagrans* was induced by coincubation of around 100

to 500 *C. elegans* individuals with fungal spores. The fungal cell wall was stained using CFW (fluorescent brightener 28, Sigma-Aldrich) as described earlier (24).

Statistical Analysis. Statistical data analysis was performed with GraphPad Prism (version 8.3.1) using unpaired, two-tailed Student's *t* tests, to evaluate statistically significant differences in fungal growth, cell–cell fusion, and pathogenicity.

Data Availability. All study data are included in the article and/or supporting information.

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1. A. Casadevall, L. A. Pirofski, Host-pathogen interactions: Redefining the basic concepts of virulence and pathogenicity. *Infect. Immun.* **67**, 3703–3713 (1999).
2. J. M. Lang, M. E. Benbow, Species interactions and competition. *Nature Educ. Knowledge* **4**, 8 (2013).
3. V. Troselj, P. Cao, D. Wall, Cell-cell recognition and social networking in bacteria. *Environ. Microbiol.* **20**, 923–933 (2018).
4. A. Sharma, P. Singh, B. K. Sarmah, S. P. Nandi, Quorum sensing: Its role in microbial social networking. *Res. Microbiol.* **171**, 159–164 (2020).
5. M. Gabriela Roca, N. D. Read, A. E. Wheals, Conidial anastomosis tubes in filamentous fungi. *FEMS Microbiol. Lett.* **249**, 191–198 (2005).
6. F. Richard, N. L. Glass, A. Pringle, Cooperation among germinating spores facilitates the growth of the fungus, *Neurospora crassa*. *Biol. Lett.* **8**, 419–422 (2012).
7. N. L. Glass, I. Kaneko, Fatal attraction: Nonself recognition and heterokaryon incompatibility in filamentous fungi. *Eukaryot. Cell* **2**, 1–8 (2003).
8. A. P. Gonçalves, N. L. Glass, Fungal social barriers: To fuse, or not to fuse, that is the question. *Commun. Integr. Biol.* **13**, 39–42 (2020).
9. P. S. Aguilar *et al.*, Genetic basis of cell-cell fusion mechanisms. *Trends Genet.* **29**, 427–437 (2013).
10. A. Fleissner, A. C. Leeder, M. G. Roca, N. D. Read, N. L. Glass, Oscillatory recruitment of signaling proteins to cell tips promotes coordinated behavior during cell fusion. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 19387–19392 (2009).
11. M. S. Fischer, N. L. Glass, Communicate and fuse: How filamentous fungi establish and maintain an interconnected mycelial network. *Front. Microbiol.* **10**, 619 (2019).
12. U. Kück, D. Radchenko, I. Teichert, STRIPAK, a highly conserved signaling complex, controls multiple eukaryotic cellular and developmental processes and is linked with human diseases. *Biol. Chem.* 10.1515/hsz-2019-0173 (2019).
13. A. Fleissner *et al.*, The so locus is required for vegetative cell fusion and postfertilization events in *Neurospora crassa*. *Eukaryot. Cell* **4**, 920–930 (2005).
14. I. Teichert *et al.*, PRO40 is a scaffold protein of the cell wall integrity pathway, linking the MAP kinase module to the upstream activator protein kinase C. *PLoS Genet.* **10**, e1004582 (2014).
15. A. Serrano *et al.*, Spatio-temporal MAPK dynamics mediate cell behavior coordination during fungal somatic cell fusion. *J. Cell Sci.* **131**, jcs213462 (2018).
16. M. G. Roca, M. Weichert, U. Siegmund, P. Tudzynski, A. Fleissner, Germling fusion via conidial anastomosis tubes in the grey mould Botrytis cinerea requires NADPH oxidase activity. *Fungal Biol.* **116**, 379–387 (2012).
17. Y. Yin *et al.*, The MAPK kinase BcMkk1 suppresses oxalic acid biosynthesis via impeding phosphorylation of BcRim15 by BcSch9 in *Botrytis cinerea*. *PLoS Pathog.* **14**, e1007285 (2018).
18. J. Schumacher, Tools for *Botrytis cinerea*: New expression vectors make the gray mold fungus more accessible to cell biology approaches. *Fungal Genet. Biol.* **49**, 483–497 (2012).
19. G. Doehlemann, P. Berndt, M. Hahn, Different signalling pathways involving a Galpha protein, cAMP and a MAP kinase control germination of *Botrytis cinerea* conidia. *Mol. Microbiol.* **59**, 821–835 (2006).
20. J. R. Xu, Map kinases in fungal pathogens. *Fungal Genet. Biol.* **31**, 137–152 (2000).
21. M. Leroch, N. Mueller, I. Hinsenkamp, M. Hahn, The signalling mucin Msb2 regulates surface sensing and host penetration via BMP1 MAP kinase signalling in *Botrytis cinerea*. *Mol. Plant Pathol.* **16**, 787–798 (2015).
22. L. Zheng, M. Campbell, J. Murphy, S. Lam, J. R. Xu, The BMP1 gene is essential for pathogenicity in the gray mold fungus *Botrytis cinerea*. *Mol. Plant Microbe Interact.* **13**, 724–732 (2000).
23. M. Lehmeyer *et al.*, Functional dissection of a strong and specific microbe-associated molecular pattern-responsive synthetic promoter. *Plant Biotechnol. J.* **14**, 61–71 (2016).
24. L. Youssar *et al.*, Intercellular communication is required for trap formation in the nematode-trapping fungus *Duddingtonia flagrans*. *PLoS Genet.* **15**, e1008029 (2019).
25. A. Fleißner, S. Herzog, Signal exchange and integration during self-fusion in filamentous fungi. *Semin. Cell Dev. Biol.* **57**, 76–83 (2016).
26. A. B. Goryachev, A. Lichius, G. D. Wright, N. D. Read, Excitable behavior can explain the "ping-pong" mode of communication between cells using the same chemoattractant. *BioEssays* **34**, 259–266 (2012).
27. F. Sun *et al.*, Lack of species-specificity in mammalian sperm chemotaxis. *Dev. Biol.* **255**, 423–427 (2003).
28. M. Weichert *et al.*, Accumulation of specific sterol precursors targets a MAP kinase cascade mediating cell-cell recognition and fusion. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 11877–11882 (2016).
29. A. P. Gonçalves *et al.*, Allorecognition upon fungal cell-cell contact determines social cooperation and impacts the acquisition of multicellularity. *Curr. Biol.* **29**, 3006–3017.e3 (2019).
30. M. Paoletti, Vegetative incompatibility in fungi: From recognition to cell death, whatever does the trick. *Fungal Biol. Rev.* **30**, 152–162 (2016).
31. N. L. Glass, G. A. Kuldau, Mating type and vegetative incompatibility in filamentous ascomycetes. *Annu. Rev. Phytopathol.* **30**, 201–224 (1992).
32. S. Herzog, H. Brinkmann, M. Vences, A. Fleißner, Evidence of repeated horizontal transfer of sterol C-5 desaturase encoding genes among dikarya fungi. *Mol. Phylogenet. Evol.* **150**, 106850 (2020).
33. T. L. Friesen *et al.*, Emergence of a new disease as a result of interspecific virulence gene transfer. *Nat. Genet.* **38**, 953–956 (2006).
34. I. R. Sanders, Rapid disease emergence through horizontal gene transfer between eukaryotes. *Trends Ecol. Evol.* **21**, 656–658 (2006).
35. F. H. Ishikawa *et al.*, Heterokaryon incompatibility is suppressed following conidial anastomosis tube fusion in a fungal plant pathogen. *PLoS One* **7**, e31175 (2012).
36. L. L. da Silva, H. L. A. Moreno, H. L. N. Correia, M. F. Santana, M. V. de Queiroz, Colletotrichum: Species complexes, lifestyle, and peculiarities of some sources of genetic variability. *Appl. Microbiol. Biotechnol.* **104**, 1891–1904 (2020).
37. M. G. Roca *et al.*, Conidial anastomosis fusion between *Colletotrichum* species. *Mycol. Res.* **108**, 1320–1326 (2004).
38. S. Gruber, S. Zeilinger, The transcription factor Ste12 mediates the regulatory role of the Tmk1 MAP kinase in mycoparasitism and vegetative hyphal fusion in the filamentous fungus *Trichoderma atroviride*. *PLoS One* **9**, e111636 (2014).
39. G. Jedd, N. H. Chua, A new self-assembled peroxisomal vesicle required for efficient resealing of the plasma membrane. *Nat. Cell Biol.* **2**, 226–231 (2000).
40. S. A. Chen, H. C. Lin, F. C. Schroeder, Y. P. Hsueh, Prey sensing and response in a nematode-trapping fungus is governed by the MAPK pheromone response pathway. *Genetics* **217**, iyaa008 (2021).
41. L. Liang *et al.*, The Woronin body in the nematophagous fungus *Arthrobotrys oligospora* is essential for trap formation and efficient pathogenesis. *Fungal Biol.* **121**, 11–20 (2017).
42. J. H. Vogel, A convenient growth medium for Neurospora (medium N). *Microbial Genetics Bulletin* **13**, 42–43 (1956).
43. A. Serrano, A. Fleißner, "The art of networking: Vegetative hyphal fusion in filamentous ascomycete fungi" in *The Mycota*, J. Wendlend, Ed. (Springer, Cham, 2015), vol. **1**, pp. 133–153.
44. R. Schnabel, H. Hutter, D. Moerman, H. Schnabel, Assessing normal embryogenesis in *Caenorhabditis elegans* using a 4D microscope: Variability of development and regional specification. *Dev. Biol.* **184**, 234–265 (1997).