Supplementary Materials

Investigating the smuts: common cues, signaling pathways, and the role of MAT in dimorphic switching and pathogenesis

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Figure S1 Alignment of bE (HD1) protein sequences. The bE protein sequence from *Ustilago maydis* (UMAG_00577) was used as a reference sequence to determine orthologs in all 16 Ustilaginomycotina genomes. Then, all orthologous sequences were used for the alignment using the MUSCLE algorithm performed in MEGA-X. Note that there are two duplicated bE genes in *Meira miltonrushii*. Species abbreviations are as follows: Acain, *Acaromyces ingoldii*; Cergu, *Ceraceosorus guamensis*; Exova, *Exobasidium vaccinii*; Jamro, *Jaminaea rosea*; Malgl, *Malassezia globosa*; Malsy, *Malassezia sympodialis*; Meimi, *Meira miltonrushii*; Psean, *Pseudozyma antarctica*; Psehu, *Pseudozyma hubeiensis*; Psegl, *Pseudomicrostroma glucosiphilum*; Spore, *Sporisorium reilianum*; Tescy, *Testicularia cyperi*; Tilan, *Tilletiaria anomala*; Tilwa, *Tilletiopsis washingtonensis*; Ustma, *Ustilago maydis*; Viopa, *Violaceomyces palustris*. 
Figure S2 Alignment of bW (HD2) protein sequences. The bW protein sequence from Ustilago maydis (UMAG_00578) was used as a reference sequence to determine orthologs in all 16 Ustilaginomycotina genomes. Then, all orthologous sequences were used for the alignment using the MUSCLE algorithm performed in MEGA-X. Species abbreviations are as follows: Acain, Acaromyces ingoldii; Cergu, Ceraceosorus guamensis; Exova, Exobasidium vaccinii; Jamro, Jaminaea rosea; Malgl, Malassezia globosa; Malsy, Malassezia sympodialis; Meimi, Meira miltonrushii; Psea, Pseudozyma antarctica; Psehu, Pseudozyma hubeiensis; Pseil, Pseudomicrostroma glucosiphilum; Spore, Sporisorium reilianum; Tescy, Testicularia cyperi; Tilan, Tilletiaria anomala; Tilwa, Tilletiopsis washingtonensis; Ustma, Ustilago maydis; Viopa, Violaceomyces palustris.
Figure S3 Gene phylogeny of pheromone receptors Pra1. The pheromone receptor protein sequence from *Ustilago maydis* (UMAG\_02383) was used as a reference sequence to determine orthologs in all 16 Ustilaginomycotina genomes. All orthologous sequences, plus the ones from each MAT a allele in *Ustilago maydis* and *Sporisorium reilianum*, were retrieved for the analyses. The protein sequences were aligned using the MUSCLE algorithm, and then used for phylogenetic reconstruction through the Neighbor-joining method with JTT as a substitution model and a gamma distribution as a rate heterogeneity among sites. The 1000 replicates of bootstrapping were used as an indicator for node supports. Allele types of pheromone receptors are classified based on monophyletic relationships with reference sequences from *U. maydis* and *S. reilianum*: UMAG\_02383 for the a1 allele in *U. maydis*, CAI59749 for the a1 allele in *S. reilianum*, P31303 for the a2 allele in *U. maydis*, Spore\_3761 for the a2 allele in *S. reilianum*, and CAI59763 for the a3 allele in *S. reilianum*. Species abbreviations are as follows: Acain, *Acaromyces ingoldii*; Cersp, *Ceraceosorus guamensis*; Exova, *Exobasidium vacciniii*; Jamsp, *Jaminaea rosea*; Malgl, *Malassezia globosa*; Malsy, *Malassezia sympodialis*; Meimi, *Meira miltonrushi*; Psean, *Pseudozyma antarctica*; Psehu, *Pseudozyma hubeiensis*; Rhodsp, *Pseudomicrostroma glucosiphilum*; Spore, *Sporisorium reilianum*; Tescy, *Testicularia cyperti*; Tilan, *Tilletia anomala*; Tilwa, *Tilletiopsis washingtonensis*; Ustma, *Ustilago maydis*; Viopa, *Violaceomyces palustris*. Bar: 0.2 substitution/site.
Table S1  A list of known fungal dimorphism genes from *Ustilago maydis* literature

| Categories                  | Gene name | Function                                                                 | References |
|-----------------------------|-----------|---------------------------------------------------------------------------|------------|
| Receptors                   | *Pra1*    | Pheromone receptor                                                        | [1]        |
|                             | *Msb2*    | Transmembrane mucin, multicopy suppressor of a budding defect             | [2]        |
|                             | *Sho1*    | Osmosensor transmembrane protein                                          | [2]        |
|                             | *Ump2*    | Ammonium transporter                                                      | [3]        |
| cAMP/PKA pathway            | *Gpa3*    | G protein alpha subunit                                                   | [4]        |
|                             | *Bpp1*    | G protein beta subunit                                                    | [5]        |
|                             | *Uac1*    | Adenylate cyclase                                                         | [6]        |
|                             | *Ubc1*    | Regulatory subunit of cAMP-dependent protein kinase A                     | [6,7]      |
|                             | *Adr1*    | Catalytic subunit of cAMP-dependent protein kinase A                      | [8]        |
|                             | *Uka1*    | cAMP-dependent protein kinase A                                           | [8]        |
|                             | *Umple1/2*| Phosphodiesterases                                                        | [9]        |
|                             | *Ucn1*    | Antagonist phosphatase of PKA                                             | [10]       |
| MAPK pathway                | *Ubc2*    | Pheromone-responsive SH3 domain protein                                   | [11,12]    |
|                             | *Kpp2/Ubc3*| MAP kinase                                                               | [13,14]    |
|                             | *Kpp4/Ubc4*| MAP kinase kinase                                                         | [15,16]    |
|                             | *Kpp6*    | MAP kinase                                                               | [17]       |
|                             | *Fuz7/Ubc5*| MAP kinase kinase                                                         | [16,18]    |
|                             | *Rok1*    | Dual specificity protein phosphatase                                       | [19]       |
|                             | *Crk1*    | MAP kinase                                                               | [20]       |
| GTPase-mediated signaling   | *Ras1, Ras2*| *Ras* family GTPase                                                       | [21,22]    |
|                             | *Sql2*    | Cdc25-like guanyl nucleotide exchange factor                              | [22]       |
|                             | *Rho1*    | *Rho* family GTPase                                                       | [23]       |
|                             | *Pdc1*    | 14-3-3 homolog                                                           | [24]       |
|                             | *Cln4*    | PAK family kinase                                                         | [25]       |
|                             | *Rac1*    | *Rho* family GTPase                                                       | [26]       |
| Transcriptional regulator   | *Biz1*    | *b* locus-dependent Zn finger transcription factor                        | [27]       |
|                             | *Hap2*    | CCAAT-box binding protein                                                 | [28]       |
|                             | *Rop1*    | High-mobility-group (HMG) domain transcription factor                     | [29]       |
|                             | *Prf1*    | Pheromone response factor functioning as a transcription factor           | [30,31]    |
|                             | *Rhf1*    | *b* locus-dependent Zn finger transcription factor                        | [32]       |
|                             | *Cib1*    | *b* locus-dependent Zn finger transcription factor                        | [33]       |
|                             | *Gcn5*    | Histone acetyltransferase                                                 | [34]       |
|                             | *Hos2*    | Histone deacetylase                                                       | [35]       |
|                             | *Tup1*    | General transcriptional repressor                                         | [36]       |
|                             | *Pac2*    | WOPR family transcriptional repressor                                     | [36]       |
|                             | *Ros1*    | WOPR family transcriptional regulator                                     | [37]       |
|                             | *Nit2*    | GATA transcription factor responsive to low nitrogen                     | [38]       |
| Other downstream molecular players | Med1 | Transcription factor | References |
|-----------------------------------|------|----------------------|------------|
| Hgl1                              | Putative regulatory protein | [39] |
| Rak1                              | Seven-WD40 repeat motif protein | [40] |
| Mgc5                              | Class V myosin | [41] |
| Kin1, Kin3                        | Kinesin-1 and 3 required for hyphal growth | [42] |
| Rrm4, Khd4                        | RNA-binding protein for filamentous growth | [43] |
| Clb2                              | B-type cyclin 2 | [44] |
| Chs5                              | Chitin synthase V | [45] |
| Chs7                              | Chitin synthase | [46] |
| Mcs1                              | Myosin chitin synthase 1 | [47] |
| Clp1                              | Function in nuclei distribution during cell division in dikaryon | [48] |
| Yup1                              | t-SNARE protein for vesicular cycling | [49] |
| Sep3                              | Septin 3 as an effector of cAMP/PKA pathway | [50] |
| Tea1, Tea4                        | Cell end markers | [51] |

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