Genetic and Molecular Evidence for a Tetrapolar Mating System in the Edible Mushroom, *Grifola frondosa*

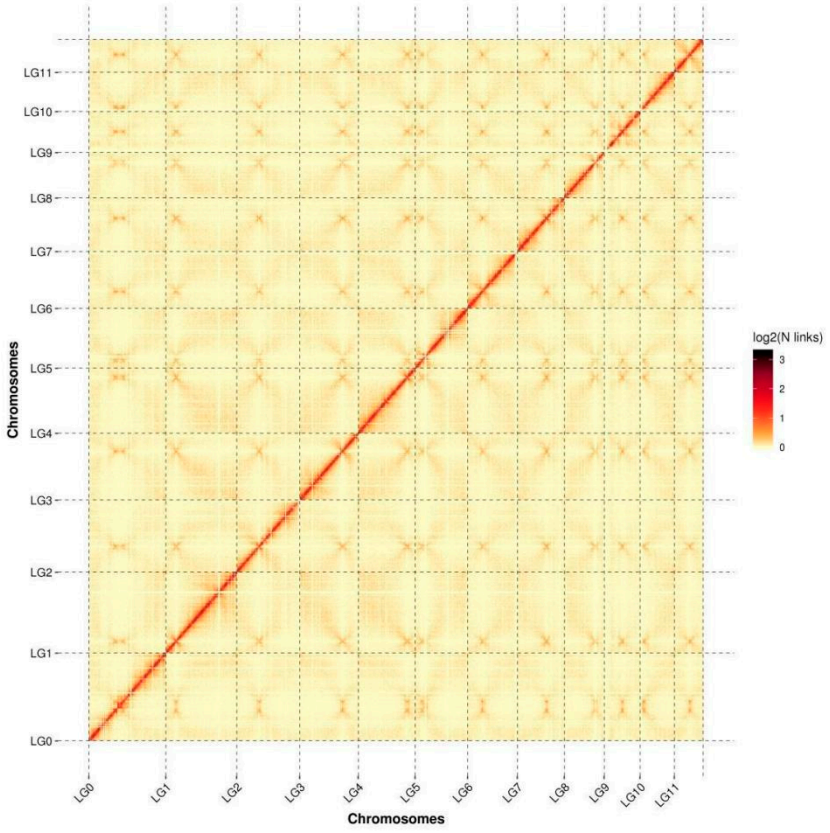
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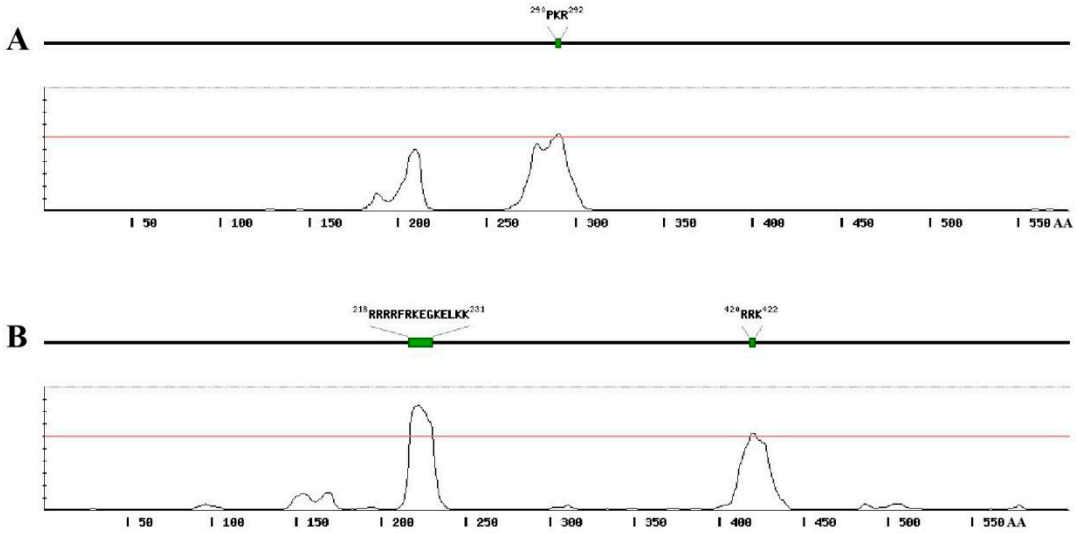
Supplementary Material



Supplementary Figure S1. Collecting basidiospores of *Grifola frondosa* dikaryotic strains LMXY (**A**) and LMCZ (**B**).



Supplementary Figure S2. Intensity signal heat map of the Hi-C chromosome.
Note: 'Lachesis Group' (LG) stands for the chromosome.



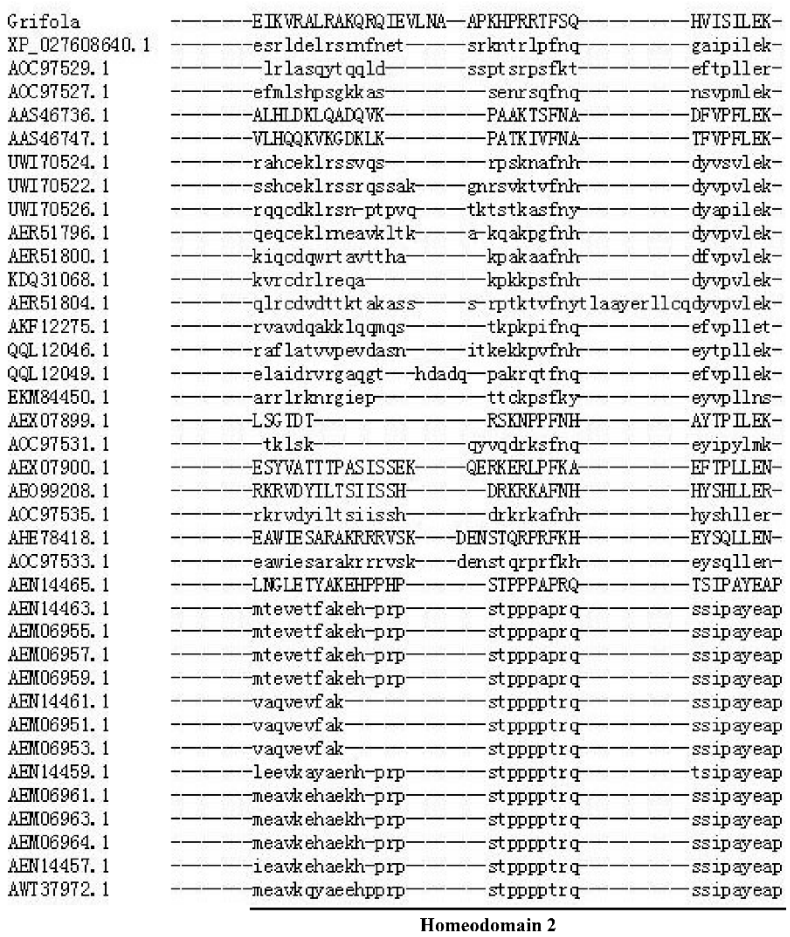
Supplementary Figure S3. Nuclear localization signal analysis of HD1 (**A**) and HD2 (**B**).

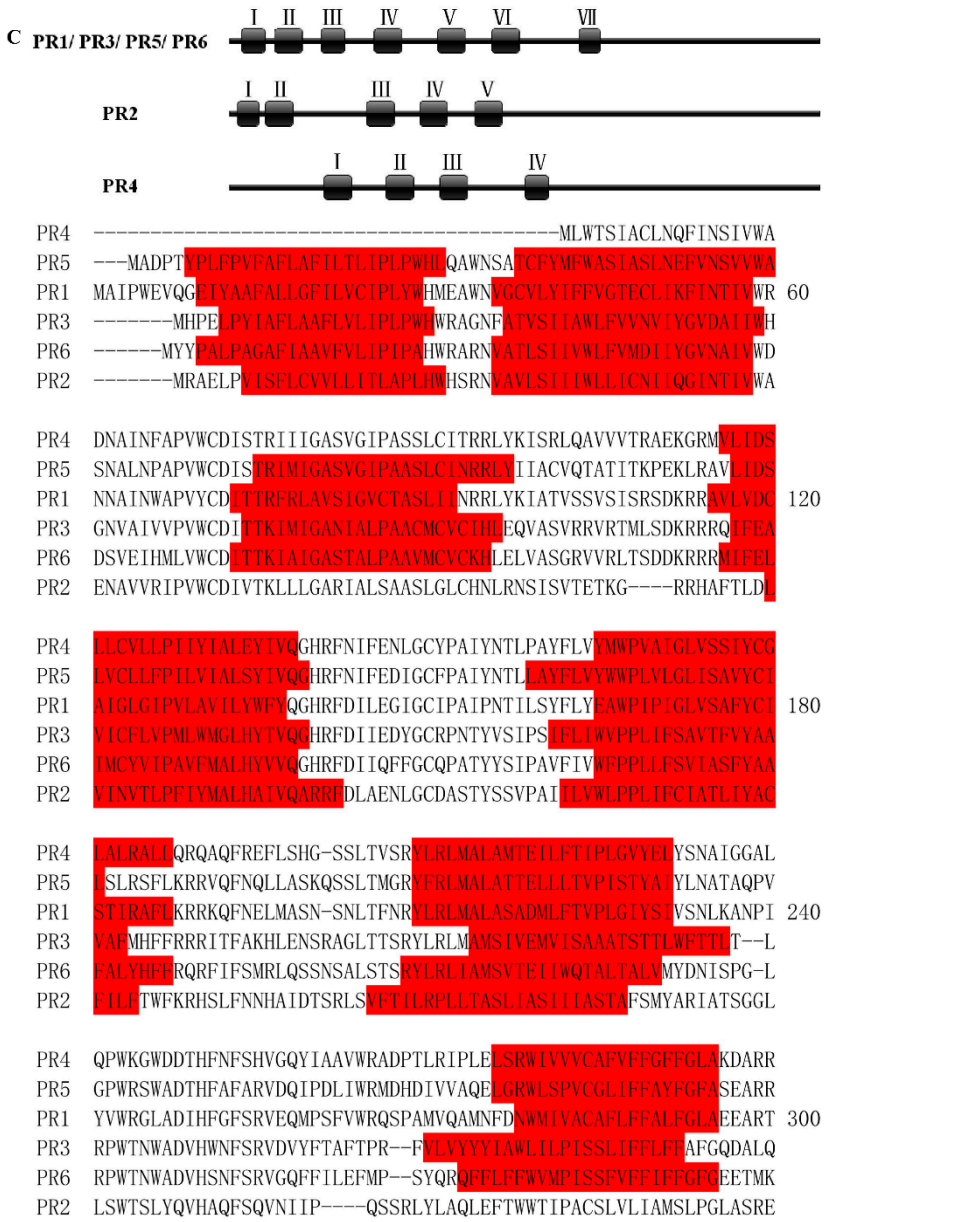
Note: Horizontal red line depicts the chosen posterior threshold of 0.6.

A

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Grifola              -------askltlad-sspyphsspgss-------------------ksnvs
XP_027608639.1       -------prqaytlqgtsdfaatheydhntisssslds--slpyrdies--dvqssve
AEO99207.1           -tvaryiqpaydwllhnlhnpypssavresisrst-----gcsrkdidnwfidvrkrig
AOC97528.1           ---ppytkvtydwlvsnlhnpyptkkvkqaistqc-----qcpekdidawfvdvrrrig
AHE78417.1           ---psyiplacdwllrnlhnpypsssqkdtianas-----gtprqnidswfvdarrrig
AAS46746.1           ---ppyippayswllrnihnpypskghklsisret-----ttslasidtwflnirrrig
AAS46737.1           ---psyiapaykwllrnlhapypskkqkslisrqt-----stsvhhidtwflnvrrrig
EMBOSS_003           ---ppyiapaykwllknirnpypsketkklisrgt-----gtslqnidvwflnarrrig
AER51795.1           ---ppyiapaykwllknihnpypsketkelisrgt-----gtslqnidawflnarrrig
EMBOSS_004           ---tpyiapafkwllknihnpypsrqtkelisrgt-----gtsiqnvdawflnarrrig
AER51803.1           ---spyiapaykwllknicnpypsketkelisrgt-----gtslqnidawflnarrrig
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QQL12047.1           ---ppyiepayrwllqnihnpypakatreeicrqh-----sylrkavdtwfiearkrmg
AOC97526.1           ---ssyttaasswlldhlanpypstsvrqklasta-----gvprkdvdrwftdargkig
EMBOSS_001           ---slyvesayrwlvdnlhnpypskqlrvhlakks-----gsavkdidswftdvrrrig
AEX07898.1           ---sayikpayewllanlhnpypsivirnhlakes-----kcsrqvidswftdirkrig
XP_006455003.1       ---alyikqayswlmqnlsrnpyppkevreaiarka-----gsdpkhverwfgdvrkrmg
XP_007326024.1       ---apyikqayswlvqnlsrnpyppkevreaiarka-----gsdpkhverwfgdarkrmg
XP_009540980.1       ---lipteavyswllanlhypypsasmkkelaasc-----gttveyiaslfndtrerig
XP_007326022.1       ---rrdvslaaewlsknffrnpypsstvrdrishqs-----rwnrkdvdawfteatrrig
AOC97536.1           ---payisvaytwllqnlhnpypsmrvkdsiakst-----gtnrkdieawfvdarkrig
AEN14464.1           -nlpsyippsytwllsnlhnpypstsirdsiasst-----ntprrlidawfvdvrrrig
EMBOSS_002           gnlpsyippsytwllsnlhnpypsttirdtiastt-----ntprrlidawfvdvrrrig
AEN14456.1           gnlpsyippsytwllsnlhnpypsttirdtiastt-----ntprrlidawfvdvrrrig
AEM06950.1           gnlpsyippsytwllsnlhnpypsttirdtiastt-----ntprrlidawfvdvrrrig
AEM06960.1           gnlpsyippsytwllsnlhnpypsttirdtiastt-----ntprrlidawfvdvrrrig
AWT37973.1           gnlpsyippsytwllsnlhnpypsttirdtiastt-----ntprrlidawfvdvrrrig
AEN14458.1           -nlpsyippsytwllsnlhnpypsttirdtiastt-----ntprrlidawfvdvrrrig
QKI37336.1           ---fhfvqpllqfvldnicnpypcaeqkrnllaqcaaagwtsvtqrkledwmrnmargkmg
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                                                    .
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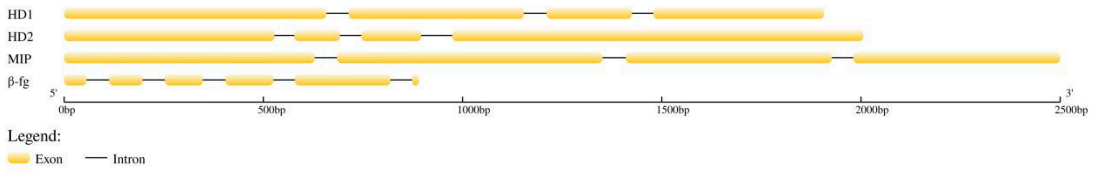
B


Grifola
XP_027608640.1
AOC97529.1
AOC97527.1
AAS46736.1
AAS46747.1
UWI70524.1
UWI70522.1
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AEM06951.1
AEM06953.1
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Homeodomain 2
Homeodomain 2

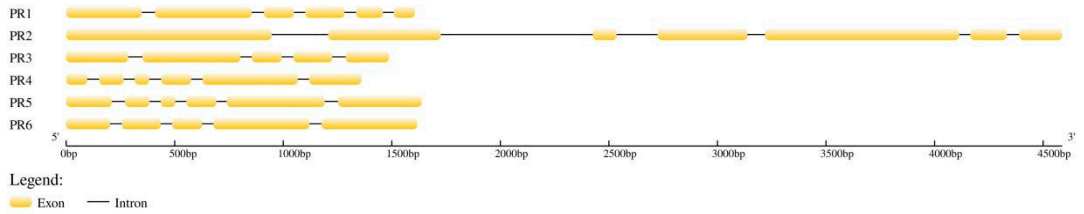


Supplementary Figure S4. Amino acid sequence alignments of HD1 (**A**), HD2 (**B**) and PR (**C**).

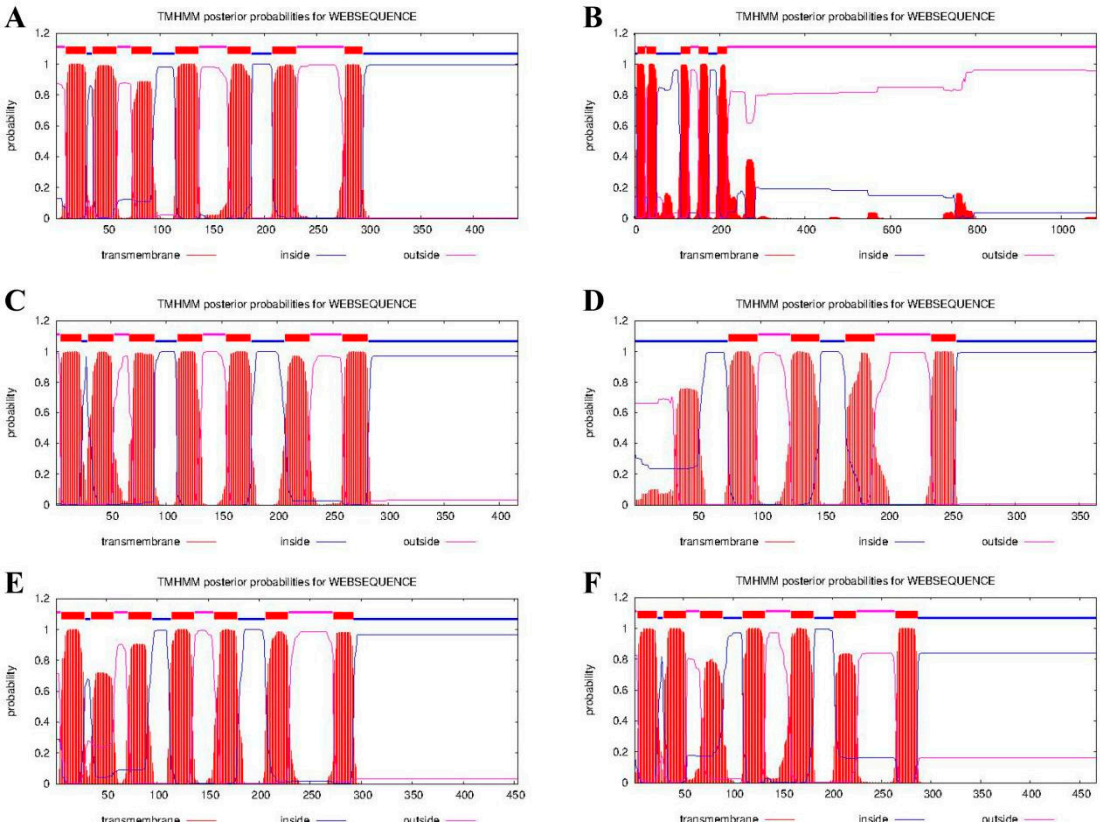
Note: A and B show parts of the alignment of HD1 and HD2, respectively; black boxes and red highlightings in C indicate approximate organization of transmembrane helix structures.



Supplementary Figure S5. Structures of genes at Mate A loci.



Supplementary Figure S6. Structures of the six pheromone receptor genes.



Supplementary Figure S7. Transmembrane structure of the six pheromone receptors.A: PR1; B: PR2; C: PR3; D: PR4; E: PR5; F: PR6.

>PP1

MSDSTSAISTNSTKCSNTYVHCLLATVLCRSPAQMNASFAPVLQLITEPATYSSMSIKIR

>PP2

MIPRIFILCVLFSSTARFVKCGTVVDGMVELMTCDSPSEPLAEQCVSACQPVRDSQVCGQKAACTCAAAP
PAAVWVCMQCQFHTAQDAREPGSAFIVTLRMNAYSRLCGEPLVDETITSALGVSASQERNTTVKMRELP
GICFYGLPEVTIVDRPAASSGRFGLATYGLFFSVFVMAVVMYEL

>PP3

MDDIFTPITIIEEEDHEGPSIPVDEDHPFNFPAYCVIAACSSDWRVPDTRTFGWYDGFLILERDSDWSSWP

>PP4

MDEFSQLINEFSELVDGVSQLVALPRVVTRIGGDDGVPVNEDSISRAGIYCIIGVRSNLPFPSLSNLSLI

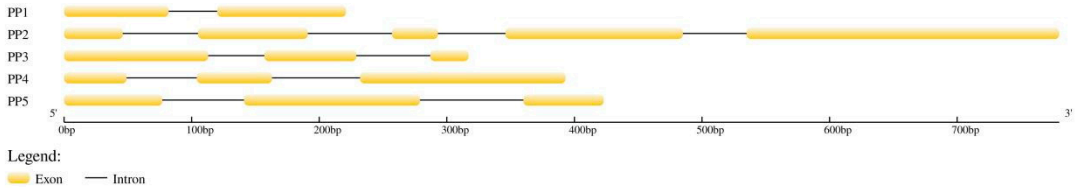
MQQFVCGISYRVLLYLPYH

>PP5

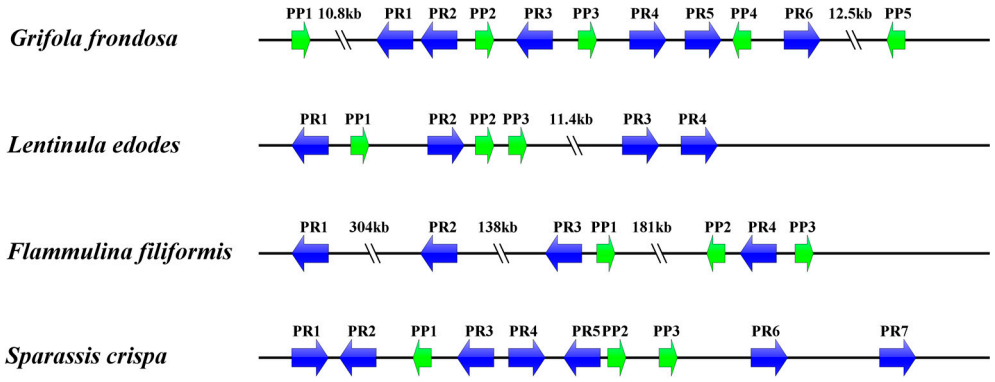
MVGTPYWMAPEVVKQKEYGAKALYLIATNGTPTLKKPEALSRELKGFLSVCLCVDVKSRATADELLEHE
FLKKACALSGLAPLLRFKNKQAS

Supplementary Figure S8. The amino acid sequences of pheromone precursor proteins.

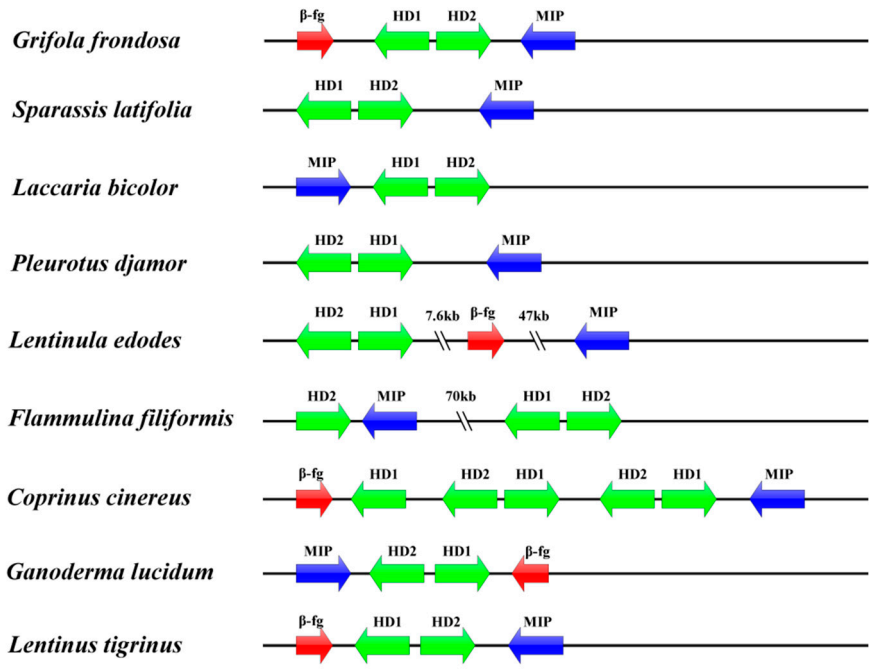
Note: The conserved sequences are highlighted in yellow.



Supplementary Figure S9. Gene structures of five pheromone precursor genes.



Supplementary Figure S10. Synteny around the pheromone receptors and pheromone precursors of some edible fungi.



Supplementary Figure S11. Synteny around the HD locus of some edible fungi.