Convergent evolution of a fused sexual cycle promotes the haploid lifestyle

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Sexual reproduction is restricted to eukaryotic species and involves the fusion of haploid gametes to form a diploid cell that subsequently undergoes meiosis to generate recombinant haploid forms. This process has been extensively studied in the unicellular yeast Saccharomyces cerevisiae, which exhibits separate regulatory control over mating and meiosis. Here we address the mechanism of sexual reproduction in the related hemiascomycete species Candida lusitaniae. We demonstrate that, in contrast to S. cerevisiae, C. lusitaniae exhibits a highly integrated sexual program in which the programs regulating mating and meiosis have fused. Profiling of the C. lusitaniae sexual cycle revealed that gene expression patterns during mating and meiosis were overlapping, indicative of co-regulation. This was particularly evident for genes involved in pheromone MAPK signalling, which were highly induced throughout the sexual cycle of C. lusitaniae.

Furthermore, genetic analysis showed that the orthologue of IME2, a ‘diploid-specific’ factor in S. cerevisiae, C. lusitaniae and STE12, the master regulator of S. cerevisiae mating3,4, were each required for progression through both mating and meiosis in C. lusitaniae. Together, our results establish that sexual reproduction has undergone significant rewiring between S. cerevisiae and C. lusitaniae, and that a concerted sexual cycle operates in C. lusitaniae that is more reminiscent of the distantly related ascomycete, Schizosaccharomyces pombe. We discuss these results in light of the evolution of sexual reproduction in yeast, and propose that regulatory coupling of mating and meiosis has evolved multiple times as an adaptation to promote the haploid lifestyle.

Sexual reproduction is divided into two programs: gamete fusion, in which haploid cells merge to form a diploid cell, and gametogenesis, in which a specialized cell division, meiosis, generates recombinant haploid gametes. Sexual reproduction in S. cerevisiae involves mating between cells of opposite mating types, generating diploid products that subsequently undergo meiosis upon nutritional limitation12–14. It is unclear, however, how representative this sexual cycle is of that in other yeast species. Here we examine regulation of sexual reproduction in the related hemiascomycete Candida lusitaniae, a member of the Candida clade of human pathogens9. C. lusitaniae was recently shown to have a complete sexual cycle despite lacking orthologues of key meiosis genes such as IME1, encoding the master transcriptional regulator of meiosis in S. cerevisiae7–9. In addition, whereas S. cerevisiae is predominantly diploid, C. lusitaniae cells preferentially exist in the haploid form and therefore exhibit a transient diploid state9,10.

Transcriptional profiling of C. lusitaniae cells progressing through mating and meiotic programs was performed and compared to those of S. cerevisiae. In total, 406 genes were induced more than fourfold during C. lusitaniae mating, including highly conserved MAPK genes that regulate pheromone signalling in diverse fungal species12–14 (Fig. 1a and Extended Data Fig. 1). Interestingly, we also observed elevated expression of several genes that are orthologues of ‘meiosis-specific’ genes in S. cerevisiae, including SPO11, REC8 and IME2 (refs 1, 14–16) (Fig. 1a). We similarly performed expression profiling on C. lusitaniae diploid a/z cells induced to enter meiosis. Cells became enlarged by 8 h, exhibited a morphological change by 12 h, and by 18–36 h dyd (two-cell) spores were formed, as is typical of this species (Extended Data Fig. 2a–c). Profiling revealed that a total of 618 genes were induced during C. lusitaniae meiosis, compared to 480 genes during S. cerevisiae meiosis12. In S. cerevisiae, meiotic gene expression includes early, middle and late stages of expression12. We were similarly able to distinguish three temporal classes of meiotic gene expression in C. lusitaniae (Fig. 1b, d and Extended Data Fig. 2d). In total, we observed increased expression (threefold) of 255 early genes, 307 middle genes and 56 late genes during C. lusitaniae meiosis (Fig. 1e). Thus, despite lacking an orthologue of IME1, many downstream components of meiosis are regulated in a similar stage-specific manner in C. lusitaniae as in S. cerevisiae.

The most striking aspect of C. lusitaniae meiosis was the expression of many genes whose orthologues are expressed specifically during mating in S. cerevisiae. In particular, multiple components of the pheromone MAPK signalling cascade were highly induced, including the terminal transcription factor STE12 (Fig. 1c, f). In addition to MAPK genes, pheromone, pheromone receptor and pheromone processing genes were also induced in C. lusitaniae meiosis (Extended Data Fig. 1). We therefore surmised that MAPK signalling might have a role in regulating C. lusitaniae meiosis, in addition to its conserved function in directing cell–cell communication and conjugation.

Given that program-specific expression of many S. cerevisiae mating and meiosis genes does not occur in C. lusitaniae orthologues, genetic experiments were performed to determine whether this transcriptional rewiring has functional consequences for the sexual cycle. First, the role of C. lusitaniae ime2 in mating and meiosis was analysed. S. cerevisiae Ime2 (inducer of meiosis 2) is a conserved serine/threonine kinase that acts in tandem with the cyclin-dependent kinase Cdk1 to promote meiosis12,13,14,15. As predicted on the basis of IME2 function in S. cerevisiae, loss of IME2 (CLUG_00015) in C. lusitaniae blocked meiosis; wild-type diploid cells formed haploid progeny with ~40% efficiency after 3 days, whereas haploids were formed by <1% of ime2A/ime2A cells (Fig. 2a). In addition, whereas wild-type diploids generated dyad spores, ime2A/ime2A mutants failed to sporulate (Fig. 2b). These results establish a conserved role for IME2 in regulating meiosis in hemiascomycete yeast, even in species for which IME1 is absent. Profiling of C. lusitaniae ime2A/ime2A mutants revealed that most early meiosis genes were still induced in this background, whereas induction of many middle and late meiosis genes was lost (Extended Data Fig. 3). We also note that induction of NDT80 (CLUG_05634) and genes encoding cell-cycle regulators was compromised in the ime2A/ime2A mutant (Extended Data Fig. 3b). NDT80 is responsible for the induction of middle meiosis genes in S. cerevisiae, where its expression is dependent on Ime2 (ref. 20). The diminished expression of NDT80, together with the loss of expression of cell-cycle genes, is probably responsible for the inability of C. lusitaniae ime2A/ime2A cells to proceed through meiosis.

We next addressed the role of IME2 in C. lusitaniae mating. In contrast to S. cerevisiae, in which IME2 has no role in mating, C. lusitaniae

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IME2 was induced during mating (Fig. 2c) and was required for the efficient formation of mating products. Thus, co-incubation of C. lusitaniae a and α cells resulted in approximately 0.5% of cells forming a/α diploids, whereas bilateral crosses between ime2Δ a and ime2Δ α cells resulted in a mating frequency of less than 0.03% (a 16-fold decrease, Fig. 2d). Unilateral crosses between wild type and ime2Δ mutants showed mating frequencies similar to those between wild-type partners (Fig. 2d). Complementation by reintegration of the IME2 gene into the mutant background restored mating competency in the bilateral cross (Extended Data Fig. 4). These results establish a novel role for IME2 in C. lusitaniae mating, and indicate that the ime2Δ defect is limited to bilateral crosses. The latter observation is consistent with a late role for Ime2 during mating following the fusion of a and α partner cells. Microscopic analysis of ime2Δ crosses showed the formation of zygote structures, further supporting a late role for Ime2 during mating (Fig. 2e), and additional studies will be required to address the precise role of Ime2 in this process.

Next, we investigated the role of pheromone MAPK signalling and the transcription factor Ste12 in the C. lusitaniae sexual cycle. First, we tested whether C. lusitaniae cells undergoing meiosis actively secrete pheromone. A pheromone reporter assay was developed in which the transcription factor Ste12 in the related species C. lusitaniae was induced during mating following the fusion of a and α partner cells. Induction of MAPK pathway genes (blue text) during meiosis is shown in the figure. Co-incubation of the yellow fluorescent protein (YFP)-labelled reporter strain with C. lusitaniae cells undergoing meiosis showed induction of a morphological response in the reporter cells (Fig. 3b), demonstrating that C. lusitaniae cells are actively secreting α pheromone during meiosis.

STE12 is the master regulator of mating and pheromone signalling in S. cerevisiae and many other hemiascomycetes, including Candida species11,12. To test the role of STE12 in the sexual cycle of C. lusitaniae we constructed haploid and diploid ste12Δ mutants. Loss of STE12 (CLUG_02576) in haploid cells led to a complete block in zygote formation and mating between a and α cells (Fig. 3, c), as expected based on previous studies13. Surprisingly, however, loss of STE12 in C. lusitaniae a/α diploids also led to a block in meiosis and sporulation. Thus, formation of meiotic progeny was reduced approximately 100-fold in ste12Δ/ste12Δ mutants (Fig. 3e) and spore formation was abolished (Fig. 3f and Extended Data Fig. 2c). Reintegation of the STE12 gene into the ste12Δ/ste12Δ background restored the ability to undergo meiosis (Extended Data Fig. 5). Expression profiling revealed that the meiotic transcriptional
profile was essentially absent from ste12Δ/ste12Δ mutants (Fig. 3g). Our results therefore establish that STE12 has a critical role in regulating both mating and meiosis in C. lusitaniae, in contrast to S. cerevisiae where STE12 specifically regulates mating only.

Taken together, our studies demonstrate that a fundamental rewiring of the sexual cycle has occurred between the two hemiascomycetes, S. cerevisiae and C. lusitaniae. Mating and meiosis are distinct programs in S. cerevisiae, yet these programs are integrated in C. lusitaniae (Fig. 4a and Extended Data Fig. 7). This is evident both from transcriptional profiles that reveal overlap of gene expression patterns in mating and meiosis, as well as genetic analysis of key regulators of the sexual cycle. In particular, whereas STE12 and IME2 are necessary for S. cerevisiae mating and meiosis, respectively, the C. lusitaniae orthologues are required for efficient progress through both stages of the sexual cycle.

The regulation of sexual reproduction in C. lusitaniae has notable parallels to that in the distantly related ascomycete S. pombe, even though these lineages diverged from one another more than 330 million years ago13. In S. pombe, mating and meiosis are also tightly coupled and both are dependent on pheromone MAPK signalling and the downstream transcriptional regulator, Ste11 (refs 24, 25). In C. lusitaniae, we show that the pheromone MAPK-associated transcription factor STE12 is similarly essential for both mating and meiosis. Thus, in both S. pombe and C. lusitaniae, the programs regulating mating and meiosis are fused (Fig. 4a).

To address if Ste12 also regulates meiosis in other hemiascomycete species, we deleted the STE12 orthologue from related sexual species including S. cerevisiae, Kluyveromyces lactis, Pichia pastoris and Yarrowia lipolytica (Extended Data Fig. 6). In each species loss of STE12 failed to block meiosis, indicating that the meiotic function of STE12 evolved relatively recently in the C. lusitaniae lineage (Fig. 4b). In support of this hypothesis we note that C. lusitaniae, but not the other hemiascomycete species, lost the transcriptional regulator α2 during evolution8,9. The α2 gene acts to prevent expression of haploid-specific genes (including MAPK genes) in diploid α/α cells in diverse yeast species26,27. Thus, as cells, n = 3. e. Loss of STE12 inhibited the formation of meiotic haploid progeny (4-day time course, D1–D4). *P < 0.05, unequal variance t-test, n = 3. f. Absence of meiotic spores (white asterisks) in C. lusitaniae cells lacking STE12, n = 5. g. Gene expression changes when C. lusitaniae haploid, diploid or ste12Δ/ste12Δ strains are incubated on PDA medium. Deletion of STE12 abolished most meiosis-specific gene expression changes. Where appropriate, scale bars, 5 μm; data represented as mean ± s.e.m. All n values indicate number of biological replicates.

![Figure 3](https://example.com/figure3.png)

**Figure 3** | STE12 is essential for both mating and meiosis in C. lusitaniae. a. Schematic of assay to detect pheromone secretion from C. lusitaniae cells. C. lusitaniae α/α cells were co-incubated with YFP-labelled C. albicans α cells expressing the C. lusitaniae α pheromone receptor. b. C. albicans cells generate polarized mating projections (arrows) in response to C. lusitaniae pheromone, demonstrating that C. lusitaniae cells actively secrete pheromone during meiosis. c, d. Deletion of STE12 abolishes mating in C. lusitaniae (4-day time course, D1–D4). *P < 0.05, Kruskal–Wallis, n = 3. d. Mating occurs between wild-type C. lusitaniae cells (arrow) but not between ste12Δ mutants.
previously proposed8,9, the loss of a2 could have preceded the rewiring of meiotic control in C. lusitaniae. In particular, we propose that it facilitated MAPK signalling and enabled Ste12 to assume control of meiosis in this species.

Why have such distinct modes of sexual regulation evolved in diverse unicellular yeasts? The most parsimonious explanation is that these differences reflect a species’ preference for one ploidy state over another. Both C. lusitaniae and S. pombe, despite being highly divergent, have haploid lifestyles that contrast sharply with the predominantly diploid lifestyle of S. cerevisiae. Co-regulation of the transcriptional programs controlling mating and meiosis therefore ensures that cells, once mated, immediately enter meiosis and return to the haploid state. This could represent an adaptive advantage for haploid cells in both C. lusitaniae and S. pombe. Alternatively, the selective pressure to keep these two sexual programs separate may have been lost during evolution, leading to the amalgamation of these programs in the two species. Although the relative advantages of haploidy and diploidy continue to be debated28,29, it is clear that distinct transcriptional circuits can evolve to promote either haploid or diploid lifestyles. That this mode of regulation evolved independently in S. pombe and C. lusitaniae further suggests that examples of fused sexual cycles will be found throughout the fungal tree of life.

METHODS SUMMARY

Gene expression profiling. For mating and meiosis, C. lusitaniae cells were grown on YPD medium (non-inducing) or PDA medium (inductive for mating/meiosis). RNA was isolated from cells using the Ribopure-Yeast Kit (Life Technologies) and treated with Turbo DNase I (Ambion). Complementary DNA was synthesized using medium selective for a or o, or r, and analysed phenotypes. R.K.S., C.M.S. and R.J.B. were involved in study design and performed transcriptional profiling experiments. S.E.T. and R.J.B. constructed strains and analysed phenotypes. R.K.S. and C.M.S. constructed strains, analysed phenotypes and wrote the manuscript. This work was supported by National Science Foundation Grant MCB1021120 (to R.J.B.), by National Institutes of Health R01 Grant AI081704 (to R.J.B.), and by an Investigator in the Pathogenesis of Infectious Disease Award from the Burroughs Wellcome Fund (to R.J.B.).

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METHODS

Media and reagents. Standard laboratory media were prepared as previously described11. Potato dextrose agar (PDA) was prepared as a 10% dilution of standard PDA medium (Becton Dickinson) that was solidified using 1.45% Bacto Agar (Becton Dickinson)48. Nourseothricin-resistant transformants were selected on YPD (yeast peptone dextrose) medium containing 200 mg ml-1 nourseothricin, as previously described49. Kanamycin-resistant transformants were selected on YPD medium supplemented with 50 μg ml-1 kanamycin. Liquid yeast peptone sorbitol (YPS) was prepared similar to YPD; however, 2% sorbitol was used in the place of dextrose. Haploid progeny from sporulation assays were selected on YPD medium supplemented with 10 μg ml-1 cycloheximide. SC-Maltose consisted of synthetic complete medium supplemented with 2% maltose. Malt medium (2% malt extract, 3% glucose) was used to induce mating and meiosis in K. lactis. Pichia pastoris mating/sporulation medium consisted of sodium acetate medium (0.5% sodium acetate, 1% potassium acetate, 1% glucose, 2% agar)50. Yarrowia lipolytica sporulation was achieved using V8 sporulation medium (40% V8 juice, 0.8% yeast extract, 1.6% agar, pH 6.5).

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METHODS

Strain construction. All strains constructed for this study are listed in Supplementary Table 1, and oligonucleotides used are listed in Supplementary Table 2. Strain construction. All strains constructed for this study are listed in Supplementary Table 1, and oligonucleotides used are listed in Supplementary Table 2. Strain construction.

To delete the STE12 gene, RSY411 was transformed with pRB278. pRB278 was linearized using PshAI and transformed into RSY437. Integration at the endogenous IME2 locus was checked by PCR using primers RS224/BL70. Diploid ime2Δ/ime2Δ strains RS427, RS428 and RS429 were generated by mating haploid RSY406 and RSY568 strains. Strains were verified by flow cytometry, PCR verification of MLT a/h configuration and prototrophy.

Diploid STE12 mutant strains were constructed in RSY432. The 5′ flank of STE12 was PCR amplified using oligonucleotides RS323/RS324. The 5′ flank and 3′ flanks of the gene were amplified by PCR using oligonucleotides RS66/67 and RS68/69. PCR using primers RS399/RS400 was used to verify the absence of STE12.

The C. lusitaniae STE12 gene was reintegrated into a ste12Δ/ste12Δ strain (RSY664). The STE12 ORF, together with -1 kb of the promoter, was PCR amplified using oligonucleotides RS401/RS402. The PCR product was digested with KpnI and AapI and ligated together. Transferring the resulting construct was linearized by using BmgBI and transformed into RSY564 to generate complemented strains RSY601, RSY602, and RSY603. Accurate integration of the construct was determined by PCR using oligonucleotides RS403/RS435.

Construction of K. lactis strains. To obtain diploid K. lactis strains, CAY571 and CAY572 were mated on malt medium at 30°C and selected on medium lacking tryptophan and uracil to generate RSY467, RSY468, RSY469, RSY470 and RSY478. K. lactis mutants were generated using an electroporation protocol previously described51. To make a gene deletion cassette, STE12 5′ and 3′ homologous flanks were generated by PCR of genomic DNA using oligonucleotides BL1581/BL1568 and BL1569/BL1582 respectively. Nourseothricin and kanamycin resistance genes were amplified by PCR using plasmids pKANmx4 and pNATmx4 (ref. 37) with primers BL1313 and BL1414. Resistance genes were fused to target gene-flanking regions by fusion PCR using oligonucleotides BL1581/BL1582. This created two linear DNA constructs for transformation, STE12-NAT and STE12-KAN. STE12 double mutants were generated by two sequential rounds of transformation. RSY478 was first transformed with the STE12-KAN cassette to obtain RSY551 and RSY555. Accurate integration was verified by PCR across 5′ and 3′ junctions using oligonucleotides BL1500/BL1329 and BL1501/BL1330. RSY531 and RSY535 were then transformed with the STE12-NAT1 construct to obtain STE12Δ/ste12Δ Δste12Δ strains RSY571/RSY572 and RSY574, respectively. Loss of the STE12 ORF was verified by PCR (BL1498/BL1499). Haploid K. lactis STE12 mutants were generated by transforming CAY571 with the STE12-NAT1 cassette to generate RSY567 and RSY568. CAY572 was transformed with STE12-KAN to generate RSY550 and with STE12-NAT to generate RSY570.

Construction of P. pastoris and Y. lipolytica strains. To delete the STE12 gene in Pichia pastoris, 5′ and 3′ flanks of the gene were amplified by PCR using oligonucleotides 2357/2358 and 2359/2360. HYG or NAT selection markers were PCR amplified using oligonucleotides BL1309/BL1310 from plasmids pNATMX4 and pNATMX4. The PCR product was digested with SacII and KpnI and cloned into pINA156 to generate STE12-NAT1. STE12 double mutants were transformed into P. pastoris strains JC304 and JC306 using an established protocol52.

To delete the STE12 gene in Yarrowia lipolytica, the STE12 ORF and flanking regions were first amplified by PCR using oligonucleotides BL2618/BL2619 and cloned into pCR-Blunt II-TOPO (Invitrogen) to generate pBR339. The LEU2 gene was also PCR amplified from Y. lipolytica using oligonucleotides BL2620/BL2621 and cloned into pCR-BluntII-TOPO. LEU2 was then excised from this vector using BamHI/KpnI and used to replace the STE12 ORF in pBR340. A cassette was generated by amplifying HindIII/EcoRV and used to transform Y. lipolytica to delete the STE12 gene. The URA3 gene was also amplified from vector pINA156 (gift from C. Lisset-Flores Mauriz) using primers BL2622/BL2643 and cloned into pSC-B-amp/kan (Agilent). URA3 was excised from this vector and used to replace STE12 in vector pBR341. Subsequently, a cassette was released from this vector.
using HindIII/EcoRV and transformed into Y. lipolytica to delete STE12. Transformations in Y. lipolytica were achieved using the lithium acetate method of Barth and Gaillardin40.

**Construction of S. cerevisiae strains.** Diploid S. cerevisiae strains were generated by mating CAY4584 and CAY4585 on YPD medium for 24 h, then selecting for diploid products on SCD medium lacking tryptophan and adenine. The resulting mating product, CAY4595, was verified as being diploid by its ability to sporulate and PCR of the MAT locus using primers 2461/2562 (MATα1) and 2565/2566 (MATα1). Subsequent transformations were performed as described41. To delete the STE12 gene, oligonucleotides BL2468/BL2469 were used to PCR amplify selection markers from pNATMX4 and pKANMX4 and contained sequences that were complementary to the 5’ and 3’ regions of the STE12 ORF. Transformation using a NATMX4-STE12 PCR construct generated strain CAY4770, which was PCR checked using primers BL329/BL2470 and BL330/BL2471. CAY4770 was transformed with a KANMX4-STE12 PCR construct to generate CAY4808, a ste12Δ/ste12Δ deletion mutant. Loss of the ORF was confirmed by PCR with primers BL2472/BL2473.

**Microarray design.** C. lusitaniae genomic sequences were downloaded from the Broad Institute (http://www.broadinstitute.org) to design a custom 8 × 15 K microarray using Agilent eArray. Each array had a minimum of two independent 60-mer oligonucleotides designed against the 5,941 open reading frames encoded by the C. lusitaniae genome, resulting in a total of 11,882 unique oligonucleotides on each microarray.

**Cell preparation for microarray analysis.** To analyse meiosis, diploid C. lusitaniae strains were grown on PDA (meiosis-inducing) or YPD (non-inducing) medium. Cells from wild-type strain RSY432 were removed after 0.5, 2, 4, 8, 12, 18, 24 and 30 h on solid PDA medium and flash frozen in liquid nitrogen. For ime2Δ/ime2Δ and ste12Δ/ste12Δ profiling, cells from strain RSY428 and CAY4240 were incubated on solid PDA medium for 0, 12, 18, and 24 h and flash frozen. RSY432 cell aliquots were collected after 2 h incubation on YPD and used as a reference for all meiosis-profiling experiments. For mating profiles, 6.6 × 10⁶ RSY284 (α) and RSY411 (γ) cells were co-incubated on PDA medium for 0, 4 and 12 h and frozen. For comparison, 6.6 × 10⁶ RSY410 (α) and RSY406 (γ) cells were co-incubated on PDA medium for 4 h to profile ime2Δ mating expression patterns. RSY284 α cells incubated on YPD for 4 h were used as a reference sample for mating profiles.

**Microarray analysis.** RNA was isolated from cells using the Ribopure yeast RNA extraction kit (Ambion). RNA was treated with turbo DNase (Ambion) and evaluated for quality and purity using an Agilent Bioanalyzer. Approximately 10 μg of total RNA was used for each cDNA synthesis reaction. Random oligonucleotides (pdN₉) as well as dT₉ oligos were annealed to total RNA. cDNA was synthesized using Superscript III reverse transcriptase (Invitrogen) in reaction mixes containing 0.25 mM diithiothreitol (DTT) and 0.5 mM total deoxyribonucleotide phosphates added in a ratio of 3.2 aminolinyldUTP/dNTPs. cDNA reactions were stopped by incubating on YPD for 4 h were used as a reference sample for mating profiles.

**Statistical analysis of mating and meiosis data.** Analysis of C. lusitaniae meiosis data was performed on log-transformed data. Data were normally distributed (Anderson–Darling test) and homoscedastic (P > 0.05, Levene’s test for homogeneity of variance), and one way ANOVA was performed. In ime2Δ mating and ste12Δ mating experiments, the data either was not normally distributed or displayed heteroscedasticity, and a Kruskal–Wallis test was performed to compare across wild-type, unilateral, and bilateral crosses. In ste12Δ/ste12Δ mutants using an unequal variance t-test. **Phenome detection assay.** A wild-type C. lusitaniae diploid strain and C. albicans CAY3705 strain were cultured in SCD medium at 24 °C overnight. CAY3705 contains a chimaeric STE2 phenotype receptor; the majority of the receptor is derived from an upstream STE2 gene and the C-terminal region is derived from C. albicans STE2. Previous studies have shown that C. albicans cells expressing the chimaeric receptor can respond to synthetic C. lusitaniae α-pheromone41. CAY3705 is also expressing a fluorescent nuclear reporter (a histone HTB–yellow fluorescent protein fusion protein). Cells were washed in water and 4 × 10⁷ C. lusitaniae cells
mixed with $0.4 \times 10^7$ CAY3705 cells and plated onto PDA plates at 24°C. Cells were removed from PDA plates after 14–18 h incubation and imaged using a Zeiss Observer Z1 microscope.

**Microscopy.** Differential interference contrast (DIC) and fluorescent images of cells were captured using a Zeiss inverted microscope (Axio Observer. Z1) fitted with an AxioCam HR. Images were processed with AxioVision Rel. 4.8 (Zeiss). For analysis of *C. lusitaniae* cell morphology during meiosis, diploid *a/α* cells were incubated on PDA medium at 24°C and samples taken at the indicated time points in Extended Data Fig. 2c and examined microscopically. At least 300 cells per strain were examined for a change in morphology (defined as polarized growth of the cell) or for spore formation.

**Defining *C. lusitaniae* gene expression changes during meiosis.** Fold induction of *C. lusitaniae* meiotic genes demonstrating expression patterns characteristic of early, middle and late meiosis were determined by evaluating microarray data. Genes were characterized as being induced during meiosis if they exhibited more than threefold induction in haploid *a* and *α* cells incubated on PDA medium for 30 min, 2 h or 24 h. This analysis generated a set of 618 genes. The resulting list of genes was then compared to genes induced during *ime2/Dme*/*ime2* meiosis data to determine the number of meiosis-specific genes dependent on Ime2 (Extended Data Fig. 3e). This same set of 618 genes was used to compare to gene induction and to determine the number of meiosis-specific genes dependent on Ime2 (Extended Data Fig. 3e). For spore formation.

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**Analysing MAPK gene expression during *C. lusitaniae* meiosis.** Average fold gene induction was calculated by averaging expression changes of *S. cerevisiae* MAPK genes (STE20, STE11, STE7, FUS3, STE12, STE2, STE3, GPA1, STE4, STE18, MFALPHA and KSS1) or their *C. lusitaniae* orthologues during meiosis. *S. cerevisiae* gene expression data was from ref. 30 and was enhanced threefold to be comparable to expression changes in *C. lusitaniae* genes.

**RT–PCR.** WT strains RSY411 and RSY284 were mated on PDA at 24°C. Aliquots of mating mixes were snap frozen after 0 min, 30 min, 2 h, 4 h and 24 h of co-incubation. pdN9 was annealed to 2 μg of RNA extracted from the mating mixes. First strand cDNA was synthesis was performed with GoScript reverse transcriptase (Promega). 75 ng of the resulting cDNA was PCR amplified for *IME2* and *ACT1* expression using oligonucleotides RSY183/RSY184 and RS333/RS334.

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Extended Data Figure 1 | Schematic showing induction of pheromone-processing genes (a) and pheromone MAPK genes (b) during both mating and meiosis in *C. lusitaniae*. Expression changes highlight genes induced during co-incubation of a and α cells on PDA medium (mating), as well as during growth of diploid a/α cells on PDA medium (induces meiosis and sporulation). In contrast, these genes are mating-specific in the related yeast *S. cerevisiae*. Scale indicates fold change for gene expression.
Extended Data Figure 2 | Temporal analysis of meiosis in C. lusitaniae.

**a.** C. lusitaniae a/α cells divide as stable diploid cells on YPD medium, n = 3.

**b.** On PDA medium, a/α cells are induced to enter meiosis and dyad spores (asterisks) begin to appear at 18 h, n = 3.

**c.** Time course of meiosis in wild type, ste12Δ-ste12Δ and STE12-complemented C. lusitaniae strains. A morphology change (polarized growth) is evident in wild-type diploid strains grown on PDA medium starting at 12 h, whereas spore formation is apparent at 18–36 h. In the ste12Δ-ste12Δ mutant, both sporulation and morphology change are absent.

**d.** Global transcriptional profile of meiosis in C. lusitaniae showing induced genes (yellow) and repressed genes (blue). C. lusitaniae diploid a/α cells (RSY432) were grown on PDA (sporulating) medium and expression changes compared to those on YPD (non-sporulating) medium. Genes changing more than four fold in expression are shown. All n values represent number of biological replicates. Scale indicates fold expression changes.
Extended Data Figure 3 | Transcriptional profiling of C. lusitaniae wild type and ime2Δ mutants during mating and meiosis. a, Full profile of wild-type and ime2Δ/ime2Δ strains during meiosis indicates that many transcriptional changes occur in ime2Δ/ime2Δ mutants as in wild-type strains. b, Analysis of cell cycle regulating genes during C. lusitaniae meiosis. Several cell cycle genes are induced in wild-type cells undergoing meiosis but not in ime2Δ/ime2Δ mutants (for example, APC4, CDC3, CDC10 and CDC14). c, Comparative expression of early (IME2, IME4, SPO11), middle (REC102, CDC3, SPS4, SPS1, NDT80, SWM1) and late (DIT1, DIT2) meiosis genes. Expression changes scaled as in Extended Data Fig. 1. d, The mating profiles of wild-type and ime2Δ strains were very similar. e, Comparison of meiosis genes induced in wild type and ime2Δ/ime2Δ mutants.
Extended Data Figure 4 | Reintegration of IME2 restores mating efficiency. IME2 was reintegrated at the endogenous locus in an ime2Δ mutant (RSY437). Mating frequency was quantified by monitoring the formation of prototrophic products from auxotrophic parents. WT cross, RSY411 × RSY284, ime2Δ mutant cross, RSY406 × RSY437, IME2 complemented cross, RSY406 × CAY5022. Differences between the WT cross and the ime2Δ mutant cross, and between the IME2 complemented cross and the ime2Δ mutant cross were both significant. n = 9 (3 biological replicates in triplicate), P < 0.001, Kruskal–Wallis test. Data are representative of mean ± s.e.m.
Extended Data Figure 5  |  Schematic of genetically marked *C. lusitaniae* strains and control of meiosis by STE12. a, *C. lusitaniae* mating experiments were performed between RSY284 (a strain) and RSY411 (α strain) and mating products selected based on auxotrophic makers. b, The a/α diploid strain RSY432 was induced to undergo meiosis on PDA medium and meiotic progeny identified by their red colour on YPD medium (ade^2^ colonies) or by growth on medium containing cycloheximide (CHX-resistant colonies). c, Diploid a/α strains of *C. lusitaniae* were incubated on PDA medium for 3 days and analysed for the frequency of formation of meiotic progeny. Two independent ste12/D/ste12/D mutants were constructed in the a/α background and tested for meiotic progeny using both the CHXR and ADE2 markers. Mutants lacking STE12 were unable to undergo meiosis to produce haploid progeny, while reintegration of STE12 into the mutant background restored meiosis. Differences between both wild-type strains and ste12/D/ste12/D mutants, and between ste12/D/ste12/D mutants and STE12-complemented strains were significant (n = 3 biological replicates, P < 0.05, student’s t-test, two tailed). Data representative of mean ± s.e.m.
Extended Data Figure 6 | Analysis of STE12 function in mating and meiosis in diverse hemiascomycete species. The STE12 gene was deleted from haploid and diploid strains of *K. lactis*, *P. pastoris* and *S. cerevisiae*. The resultant strains were tested both for mating competency and the formation of meiotic progeny. Whereas deletion of STE12 blocked mating in haploid strains of all three species, loss of STE12 from diploid α/α strains did not have a significant effect on the formation of meiotic progeny. Thus, *C. lusitaniae* is unique among the hemiascomycete species tested in that STE12 is essential for meiosis only in this species. *K. lactis* mating and meiosis, *n* = 2, data combined for 3 independent mutants. *P. pastoris* mating, *n* = 3. *P. pastoris* meiosis *n* = 4. *S. cerevisiae* meiosis *n* = 3. All data presented as mean ± s.e.m. All *n* values represent number of biological replicates.
Extended Data Figure 7 | Rewiring of the genetic programs that control sexual reproduction in hemiascomycetes. In many hemiascomycete species, including the model yeast *S. cerevisiae*, mating and meiosis are controlled by two distinct transcriptional programs with *STE12* as the master regulator of mating and *IME2* as a key regulator of meiosis. However, in the opportunistic pathogen *C. lusitaniae*, *STE12* has retained its role in regulating mating, but has also acquired control over meiosis. Similarly, *C. lusitaniae IME2* has a conserved role in regulating mating, but also has a role in promoting mating. The programs controlling these two processes have therefore fused in *C. lusitaniae*, perhaps to facilitate a transient diploid state and more efficient return to the predominant haploid state.