A

Species	Sequence of mature pheromone	Copies in genome
Saccharomyces cerevisiae	YIIKGVFWDPAC(S-farnesyl)	1
	YIIKGLFWDPAC(S-farnesyl)	1
Vanderwaltozyma polyspora	YIVKGWFWDPEC(S-farnesyl)	3
Kluyveromyces lactis	WIIPGFVWVPQC(S-farnesyl)	1
Komagatella pastoris	SNQVYYGGNRYLALYGC(S-farnesyl)	1
	SHQVYHNGNKYLALYGC(S-farnesyl)	1
Candida albicans	AVRSVSTGNCCSTC(S-farnesyl)	1
Schizosaccharomyces pombe	YTPKVPYMC(S-farnesyl)	3

B

CAAX motif identified from	Position A_1	Position A_2	Position X
Known Ascomycota pheromones	A I L M S T V	A I L M S T V	A C L M Q S V
Mutational analysis in *S. cerevisiae*	A D G I L S T V	A G H I L M N Q S T V	A C G I L M Q S N V
Final algorithmic filter	A D G I L S T V	A G H I L M N Q S T V	A C G I L M Q S N V

Figure S1. Yeast peptide pheromones share common maturation motifs. Related to Figure 1. (**A**) Lipidated peptide pheromones (*Sc***a**-factor like) of yeast species (Saccharomycotina) verified by biochemistry or genetics. Yeasts have different numbers of genes in their genomes encoding identical or near-identical mature pheromone sequences. Note that *S. cerevisiae* and *K. pastoris* each have two pheromone genes, differing from each other by a single amino acid change. (**B**) The farnesylation [CAAX] motif is defined by cysteine that is S-farnesylated, followed by two aliphatic residues (AA) and a final variable residue (X). (Top row) Known yeast pheromones provide a collection of possible AAX amino acid residues to identify unannotated pheromone CAAX motifs in the genome. (Middle row) To ensure that the filter is not biased by the few known pheromones we expanded the dictionaries used in our filter to include alternate residues that maintain farnesylation in *S. cerevisiae*[22-24]. Our final filter thus searches for all possible [CAAX-stop] motifs with A_1, A_2, X being any of the residues in the bottom row.

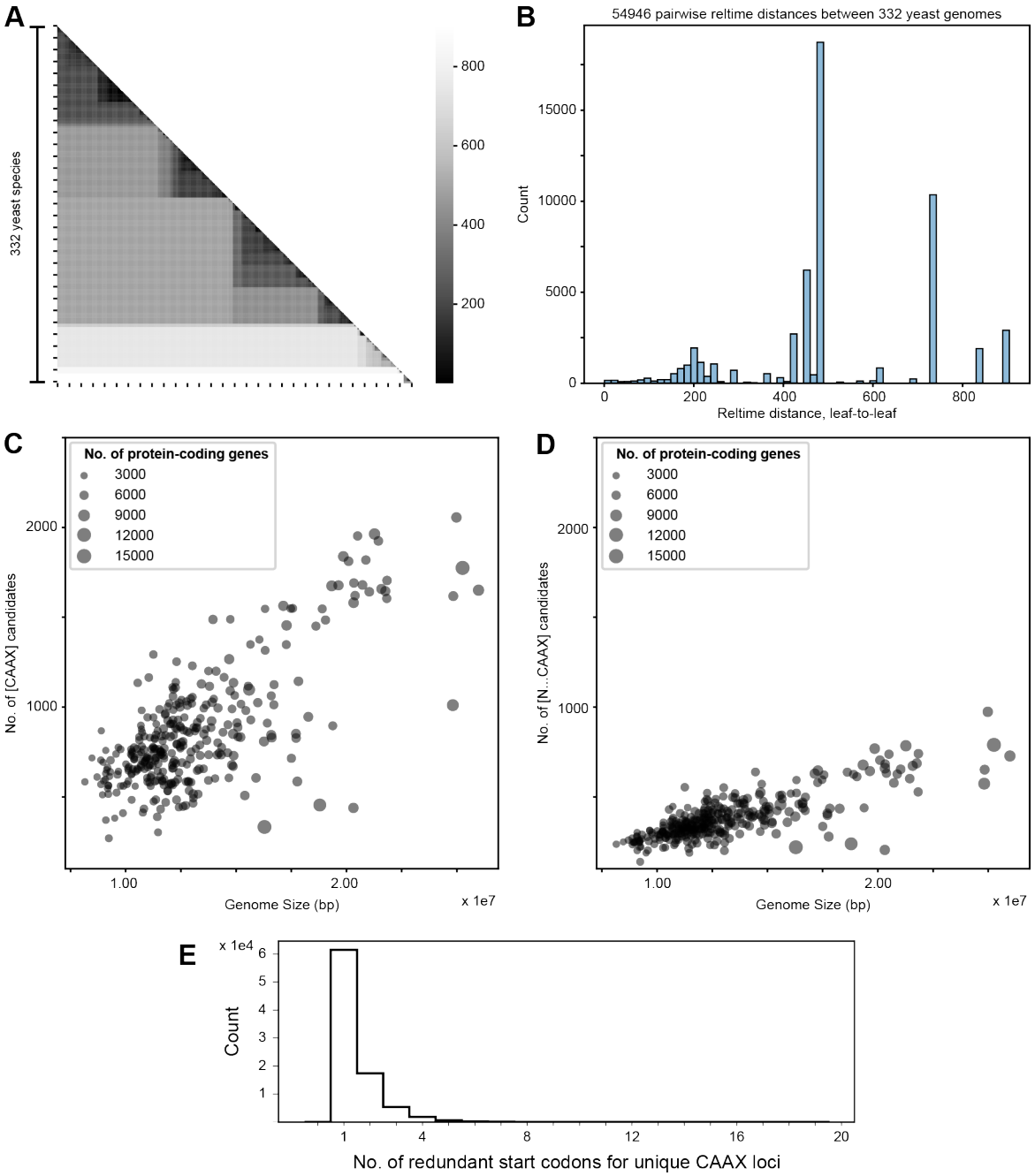
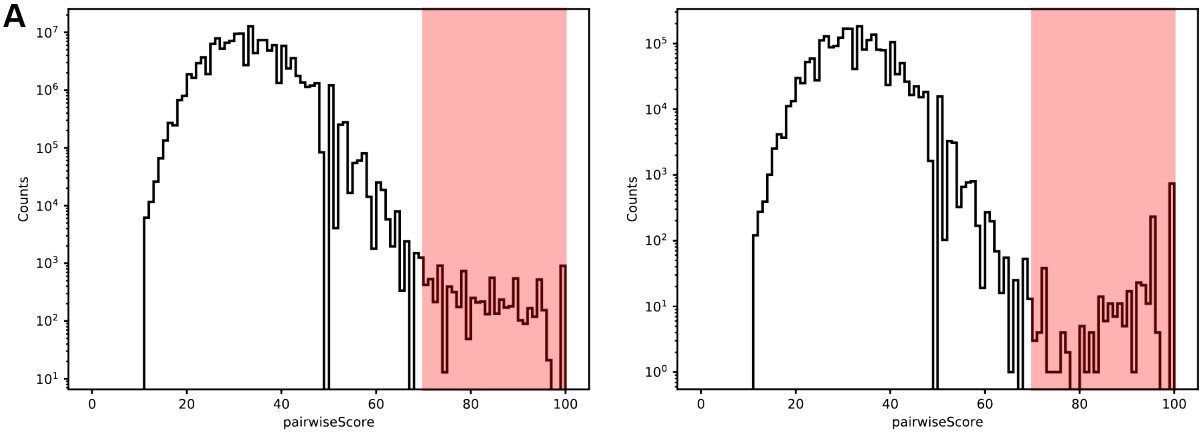


Figure S2. Successive filters reduce the number of candidate pheromone loci. Related to Figure 2. (**A**) Matrix heatmap showing the pairwise distances amongst 332 sequenced yeast genomes evaluated using a time-calibrated species divergence tree[27] produced by the RelTime algorithm[27, 59]. The scale bar indicates pairwise distance between extant species in millions of years. (**B**) Histogram of phylogenetic distances between pairs of species from the heatmap confirms a hierarchical connection between clades, with yeasts within the same clade more related to each other than to species from different clades. (**C, D**) Scatter plots of the number of [CAAX] candidates (**C**) and [N…CAAX] candidates (**D**) plotted against the genome size, where each circle represents a genome, and the diameter of the circles represents the number of annotated protein-coding genes in the genome (overlapping circles produce darker grays).

Adding a second filter (the requirement of an upstream proteolytic site in frame with a C-terminal prenylation motif [CAAX] to generate the paired motifs [N…CAAX]) reduces the number of candidate pheromone loci. (**E**) The number of start codons in frame and within 300 bp upstream of a unique CAAX sequence that satisfies the [N…CAAX] paired motifs.

A



B

Phylogroup	Clade name	No. species with identified pheromone	No. species with no identified pheromone	Species with no identified pheromone
phylogroup 1	Debaryomycetaceae / Metschnikowiaceae	70	21	*yHMPu5000034988_candida_fructus_160519, lodderomyces_elongisporus, yHMPu5000035297_priceomyces_castillae_160928, scheffersomyces_stipitis, candida_sojae, yHMPu5000035335_candida_blattae_160928, yHMPu5000034632_candida_athensensis_160519, clavispora_lusitaniae, wickerhamia_fluorescens, hyphopichia_burtonii, yHMPu5000034986_candida_oregonensis_160519 yHMPu5000035662_meyerozyma_caribbica_160613, yHMPu5000035296_priceomyces_carsonii_160928 yHMPu5000034973_danielozyma_ontarioensis_160519, millerozyma_acaciae, meyerozyma_guilliermondii, yHMPu5000041855_candida_ascalaphidarum_160928, yHMPu5000034606_priceomyces_medius_160519, candida_tenuis priceomyces_haplophilus, yHMPu5000041713_debaryomyces_maramus_160928*
phylogroup 10	Pichiaceae	41	11	*yHMPu5000034614_saturnispora_silvae_160519, ogataea_methanolica, yHMPu5000034625_pichia_kudriavzevii_160519, yHMPu5000034886_ogataea_trehalophila_160519, yHMPu5000034622_pichia_occidentalis_160519, yHMPu5000034904_ogataea_nonfermentans_160519, pichia_membranifaciens, yHMPu5000034903_ogataea_naganishii_160519, yHMPu5000034611_saturnispora_mendoncae_160519, candida_succiphila, candida_arabinofermentans*
phylogroup 13	Saccharomycodaceae	8	0	
phylogroup 14	Saccharomycetaceae	71	0	
phylogroup 17	Phaffomycetaceae	30	0	
phylogroup 22	*Yarrowia*	5	0	

C

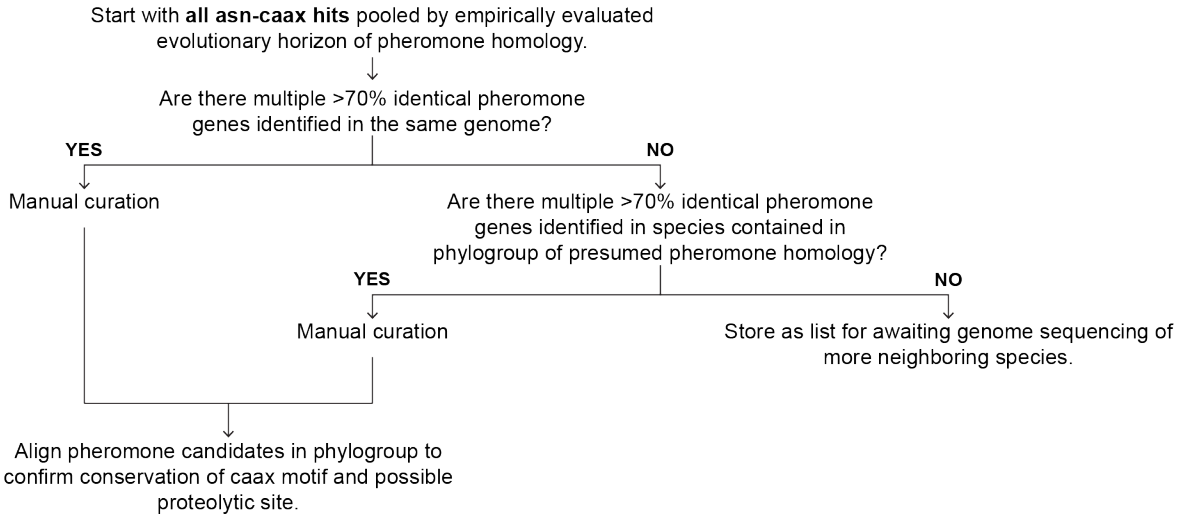


Figure S3. Manual curation from candidates that have homologous copies within a phylogroup identifies good pheromone candidates in most species. Related to Figure 3. (**A**) Histograms of pairwise sequence identity between all [N…CAAX] candidates (calculated from potential proteolysis site to stop) identified from genomes within a phylogroup where pheromones are expected to be conserved. Because these species should have homologous pheromones, their pheromones are likely found among candidate pairs that are more than 85% identical (red shaded region). Sample histograms from phylogroup 14 (Saccharomycetaceae) and phylogroup 22 (Yarrowia) are shown. (**B**) Tabulated list of large phylogroups (with more than 5 species) where a pheromone candidate has been identified by criteria-based curation. Species with no pheromone identified by curation are listed. (**C**) Decision tree describing criterion-based procedure to identify candidate pheromones from all [N…CAAX] candidates.

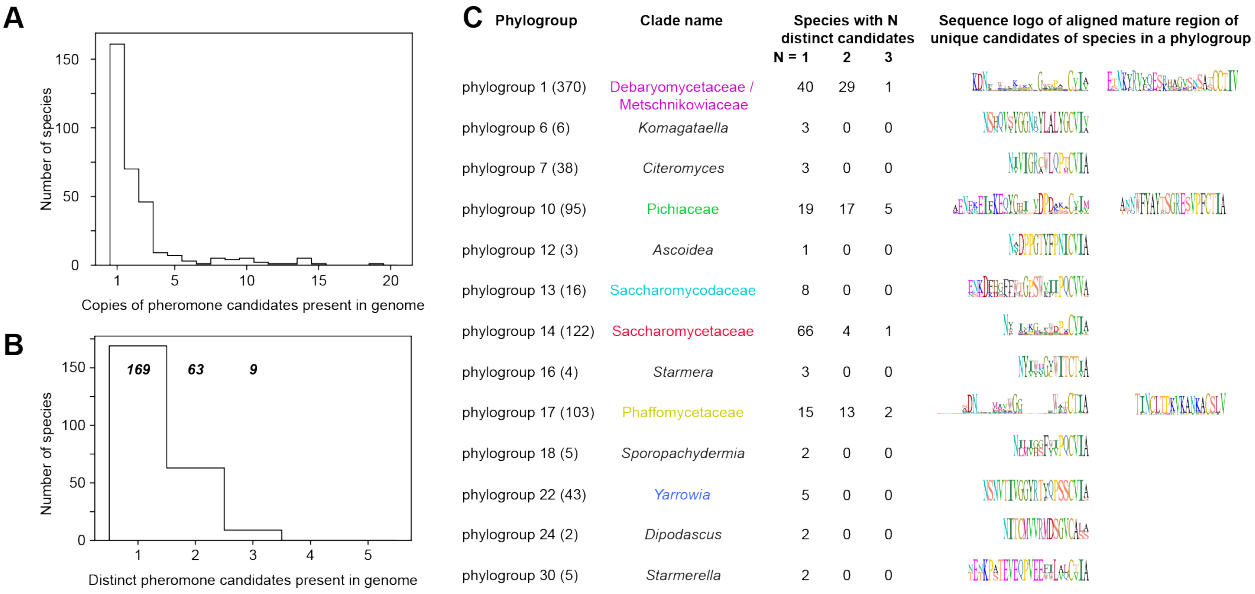


Figure S4. Curation identifies a unique pheromone candidate in most genomes. Related to Figure 4. (**A**) Histogram of number of per-genome distinct candidates identified across 241 yeast genomes. Of the 812 candidates that pass curation, 322 are unique with their in-genome copies accounting for the remaining candidates. (**B**) Candidates are distributed across phylogroups with more than one species with a unique candidate identified in most species. Species have at most 3 unique candidates that must be experimentally tested to identify the true pheromone. Sequence logo of the aligned candidate regions of the best candidates in each phylogroup separated into non-similar groups. The sequence includes the predicted proteolytic site (N), mature pheromone region and farnesylation motifs.

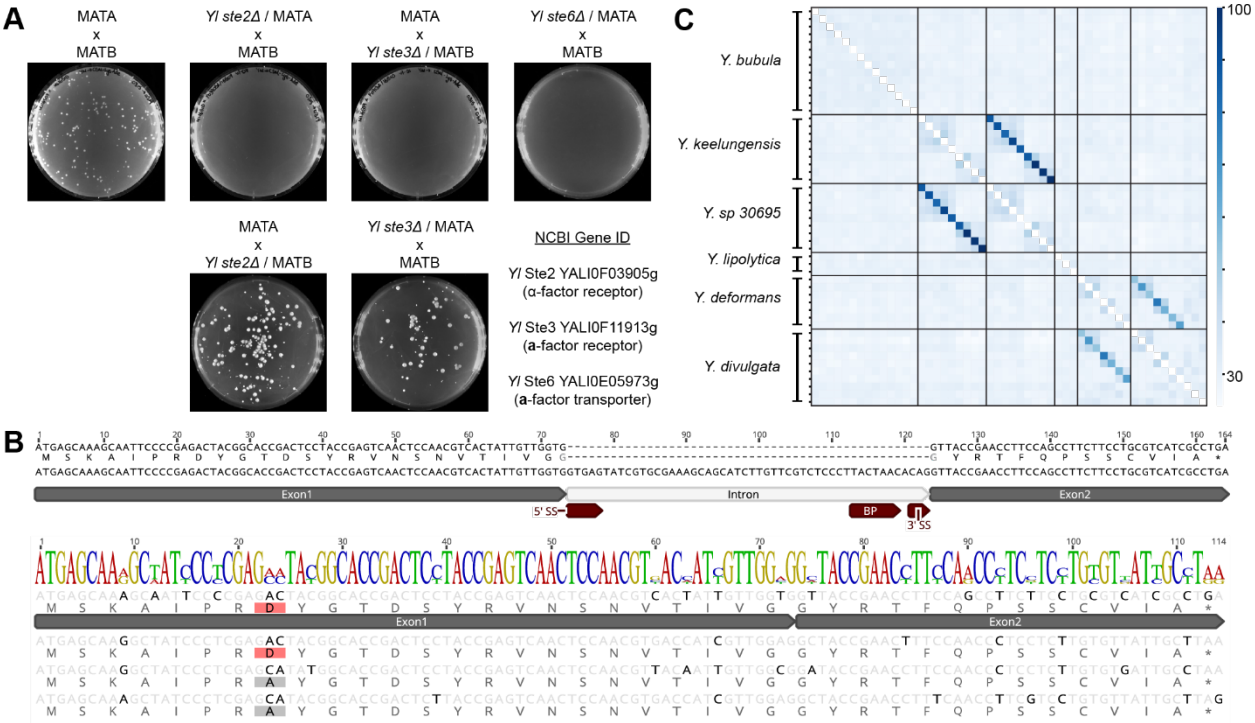


Figure S5. *Y. lipolytica* mating-type MATA is functionally homologous to *S. cerevisiae* MATa and MATB is functionally homologous to MATα. Related to Figure 5. (**A**) Plating of *Y. lipolytica* mating mixtures from semi-quantitative mating experiments on medium on which only diploids can grow. Deletions of homologs of MAT**a** specific mating genes (α-factor receptor, Ste2 / YALI0F03905g and the **a**-factor exporter, Ste6 / YALI0E05973g) prevent detectable mating in the *Y. lipolytica* MATA haploid background. Correspondingly, deletion of a homolog of a MATα specific mating gene (**a**-factor receptor, Ste3 / YALI0F11913g) prevents detectable mating in the *Y. lipolytica* MATB haploid. (**B**) *Y. lipolytica* genomic locus *YlMFA4* encodes the candidate pheromone and contains a predicted intron. (Top) Genomic locus in *Y. lipolytica* that contains a predicted intron identified by motifs (5' splice site (5' ss), branch point (BP), and 3' splice site (3' ss)) conserved across *Y. lipolytica* introns[26]. The corresponding cDNA and its translation (single-letter amino acid code) is aligned to the locus. (Bottom) The cDNA from intron-containing pheromone is aligned to the three intron-less genomic loci identified from the *Y. lipolytica* genome, showing a number of synonymous variants (darker letters) with only a single non-synonymous variant (gray and red boxes) in the N-terminal region (consistent with the pattern of variation across all Yarrowia; Figure 6A). (**C**) Aligning the 1 kbp upstream and downstream flanking DNA sequences of pheromones across the Yarrowia lineage (Figure 5A) is a measure of synteny of pheromone genes across Yarrowia genomes. Each row and column correspond to pheromone candidates in Yarrowia species with between 3 to 14 copies grouped by genome in the same order as in Figure 5A. Pairwise % identity from the alignment of the flanking regions of each locus is displayed as a heat-map marking group of pheromones contained in each species. The low off-diagonal values within candidates of a species are consistent with no candidate within a species being double-counted. The high off-diagonal values all represent syntenic candidates in the two most-closely related pairs of species (*Y. divulgata* and *Y. deformans*; *Y. keelungensis* and *Y. sp. 30695*).

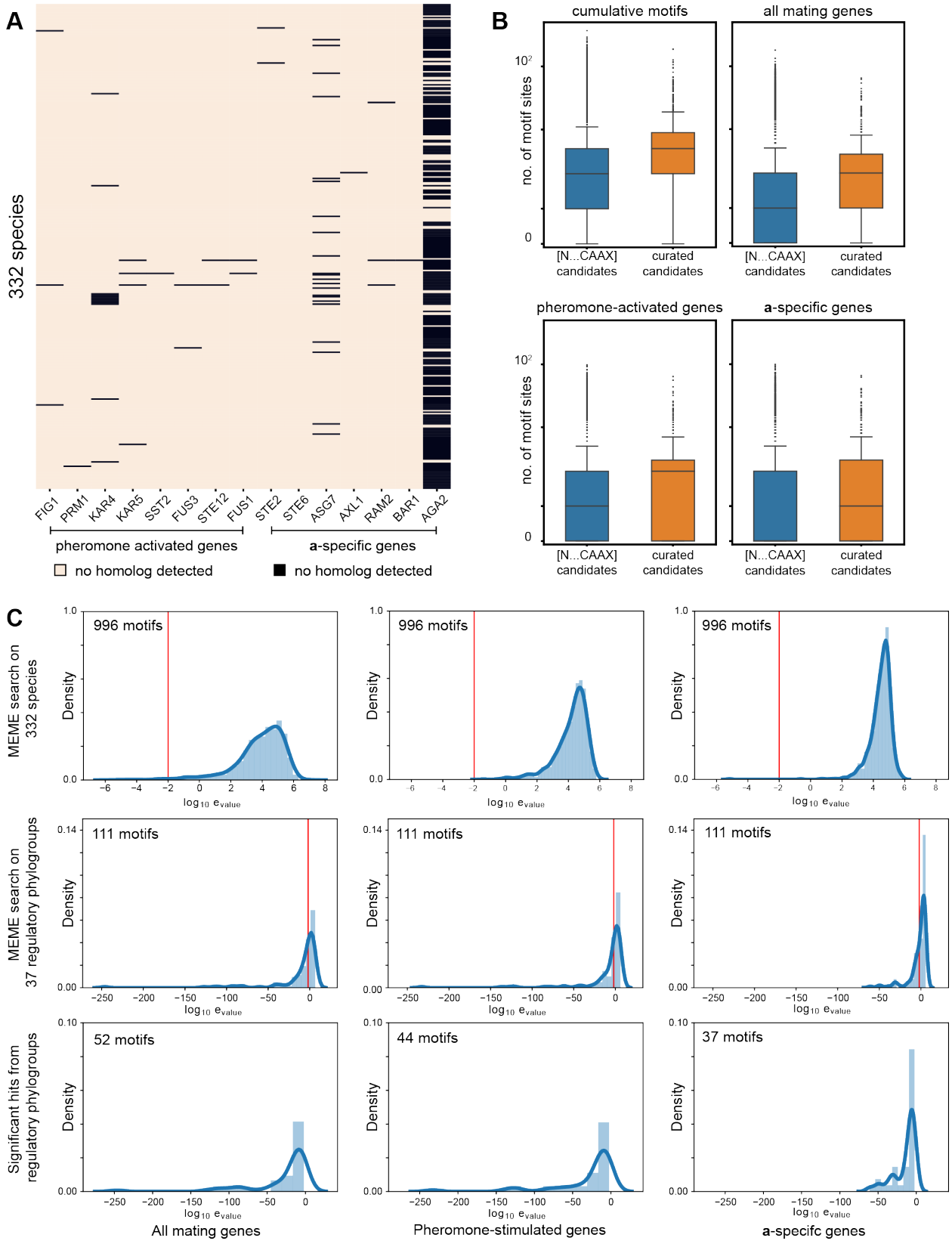

A
332 species
FIG1
PRM1
KAR4
KAR5
SST2
FUS3
STE12
FUS1
STE2
STE6
ASG7
AXL1
RAM2
BAR1
AGA2
pheromone activated genes
a-specific genes
no homolog detected
no homolog detected
B
cumulative motifs
all mating genes
pheromone-activated genes
a-specific genes
no. of motif sites
[N...CAAX] candidates
curated candidates
C
MEME search on 332 species
MEME search on 37 regulatory phylogroups
Significant hits from regulatory phylogroups
996 motifs
111 motifs
52 motifs
44 motifs
37 motifs
Density
log10 evalue
All mating genes
Pheromone-stimulated genes
a-specifc genes

Figure S6. Best pheromone candidates are weakly associated with motifs that regulate other mating genes. Related to Figure 3, Figure 4, Table 1 and Table S1. (**A**) We considered 15 genes essential to mating in *S. cerevisiae* that are either induced by pheromone stimulation or specifically induced in **a**-like haploid cells (see labels at bottom). The figure represents the sequences of 332 yeast genomes (rows) and the detection of homologs of each of these 15 specific mating genes (columns), with a black bar showing the failure to detect a homolog of the mating gene in a particular genome. (**B**) We built phylogenetic groups assuming a maximum evolutionary distance at which mating genes share conserved regulatory motifs, operationally defined by the divergence time of *S. cerevisiae* and *Vanderwaltozyma polyspora*, whose pheromone genes share conserved regulatory DNA sequences[45] (Figure S7). Although true candidates are not clearly identified by the presence of mating regulatory motifs in their promoters, there is a noticeable enrichment of motifs identified using all mating genes, pheromone-activated genes, **a**-specific genes, or the sum of all identified motifs of every class (cumulative) upstream of manually curated candidates (orange) compared to the remaining candidates that have a homologous copy in genomes of the conserved-pheromone phylogroup (blue). Each box plot represents the distribution of motif counts upstream of every candidate, with the midline representing the median, the box and whiskers representing quartiles, and the dots representing outliers. The enrichment of MEME identified motifs upstream of curated candidates are found to be significant using a two-sample Kolmogorov-Smirnov test. (**C**) Histograms of e-values of MEME motifs identified in promoters of all mating genes (left), pheromone-activated genes (middle) or **a**-specific genes (right). (Top row) Motifs identified when analysis is done in individual species. This analysis does not identify many significant MEME motifs (e-value < 0.01, red line highlighting significance threshold). (Middle row) When all mating gene promoters are pooled across species in conserved-mating-regulation phylogroups (290 species across 37 phylogroups), several significant motifs are identified (e-value < 0.01, red line highlighting significance threshold). (Bottom row) Histograms of e-values of significant MEME motifs identified from analyzing all 290 genomes from 37 mating regulation conserved phylogroups collectively for all mating genes (52 motifs), pheromone-stimulated genes (44 motifs) and a-specific genes (37 motifs) in 266 genomes.

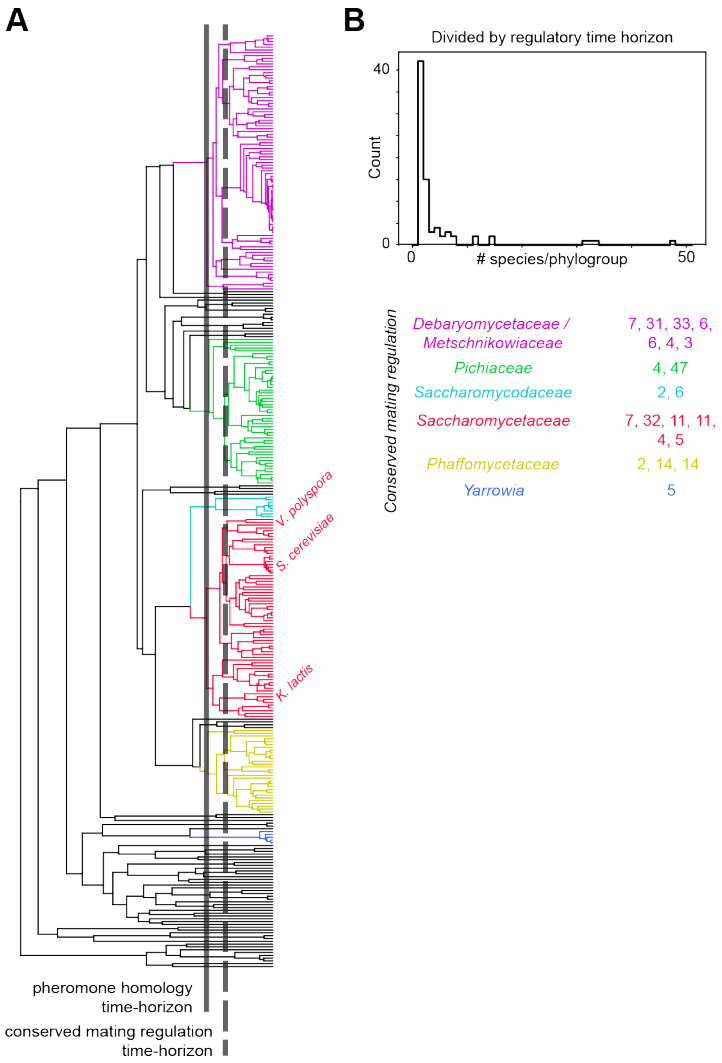


Figure S7. Phylogenetically related species can be grouped based on conservation of regulatory elements of mating genes. Related to Figure 3, Table 1 and Table S1. (**A**) Phylogenetic tree describing the evolutionary relationship of 332 yeasts with two horizons indicated. The dashed grey line indicates the assumed maximum evolutionary distance at which species are expected to share mating regulation, operationally defined by the divergence time of *S. cerevisiae* and *Vanderwaltozyma polyspora*, whose pheromone genes share conserved regulatory DNA sequences[45]. (**B**) Phylogroups expected to share mating regulation are smaller than those of conserved pheromones, with the larger clades divided between multiple phylogroups as indicated; 290 genomes are distributed across 37 mating regulation phylogroups of at least 2 species; there are also 42 singleton species.

Species name	Number of predicted pheromone genes	Mature pheromone sequence
Yarrowia lipolytica CLIB122	4	SNVTIVGGYRTFQPSSC
Yarrowia deformans JCM1694 BCIW[NCBI]	7	SNVTIVGGYRTYQPSSC
Yarrowia divulgata YADI0[NCBI]	10	SNVTIVGGYRTYQPSSC
Yarrowia keelungensis JCM14894 BCJD[NCBI]	9	SNVTIVGGYRTYQPSSC
Yarrowia sp. JCM30695 BCLX[NCBI]	9	SNVTIVGGYRTYQPSSC
Yarrowia bubula YABU0[NCBI]	14	SNVTIVGGYRTYQPSSC

Table S3. Conserved pheromone candidates in Yarrowia clade. Related to Figure 5 and Table S4.

Species name	Chromosome[coordinates]*	Pheromone candidate sequence
Yarrowia lipolytica CLIB122	YALI0F[2139263-2139374]	MSKAIPRAYGTDSYRVNSNVTIVGGYRTFQPSSCVIA*
	YALI0F[2134586-2134697]	MSKAIPRAYGTDSYRVNSNVTIVGGYRTFQPSSCVIA*
	YALI0E[1012402-1012291]	MSKAIPRDYGTDSYRVNSNVTIVGGYRTFQPSSCVIA*
	YALI0F[1323505-1323342]	MSKAIPRDYGTDSYRVNSNVTIVGGYRTFQPSSCVIA*
Yarrowia_deformans_JCM_1694_BCIW_NCBI	BCIW01000013.1[569522-569414]	MSVVKREYGNESYRVNSNVTIVGGYRTYQPSSCIIA*
	BCIW01000004.1[359951-360221]	MIMETTETPALRASIRYQKPPLQTHINISNSLLQFPIKQP TPTTTITTTTTTTIMSVVKREYGNQSYRVNSNVTIVGGYR TYQPSSCVIA*
	BCIW01000017.1[66377-66647]	MTYNFIGSSYLEHYQVASNTSPSKYKYLPSTILELQQTTK TTTTPITTTTTTTTMSVVKREYGNDSYRVNSNVTIVGGYR TYQPSSCVIA*
	BCIW01000004.1[144986-144878]	MSVVKREYGNDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCIW01000004.1[511956-512064]	MSVVKREYGNESYRVNSNVTIVGGYRTYQPSSCVIA*
	BCIW01000010.1[912729-912837]	MSVVKREYGNESYRVNSNVTIVGGYRTYQPSSCVIA*
	BCIW01000008.1[69294-69186]	MSVVKREYGNDSYRVNSNVTIVGGYRTYQPSSCVIA*
Yarrowia_divulgata_YADI0_NCBI	flattened_line_47[116579-116471]	MSVVKREYGNESYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_167[3926-4034]	MSVVKREYGNESYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_147[29621-29513]	MSVVKREYGNESYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_346[7340-7448]	MSVVKREYGNESYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_141[50636-50744]	MSVVKREYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_19[220678-220786]	MSVVKREYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_79[12173-12281]	MSVVKREYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_368[2146-2038]	MSVVKREYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_159[57250-57358]	MSVVKREYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_195[32625-32733]	MSVVKREYGNQSYRVNSNVTIVGGYRTYQPSSCVIS*
Yarrowia_keelungensis_JCM_14894_BCJD_NCBI	BCJD01000001.1[540603-540381]	MDSRQYNNFYSPPQQYKYHLNPPLFLHQTSTTTTTTTMGS AIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCJD01000001.1[242794-242683]	MGSAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCJD01000006.1[423585-423474]	MGSAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCJD01000001.1[402036-401925]	MGSAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCJD01000001.1[264198-264309]	MGSAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCJD01000003.1[907356-907467]	MGEAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCJD01000004.1[1838215-1838326]	MGEAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCJD01000002.1[338601-338490]	MGSAIPRRYGTASYRVNSNVTIVGGYRTYQPSSCVIS*
	BCJD01000001.1[1692828-1692717]	MGSAIPRRYGTASYRVNSNVTIVGGYRTYQPSSCVIA*
Yarrowia_sp._JCM_30695_BCLX_NCBI	BCLX01000001.1[2795617-2795728]	MGSAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCLX01000001.1[3085794-3085905]	MGSAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCLX01000007.1[1018344-1018455]	MGSAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCLX01000001.1[2934643-2934754]	MGSAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCLX01000001.1[3073012-3072901]	MGSAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCLX01000005.1[1822017-1821906]	MGEAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCLX01000002.1[3456539-3456650]	MGEAIPRAYGTDSYRVNSNVTIVGGYRTYQPSSCVIA*
	BCLX01000001.1[1641875-1641986]	MGSAIPRRYGTASYRVNSNVTIVGGYRTYQPSSCVIA*
	BCLX01000002.1[771285-771174]	MGSAIPRRYGTASYRVNSNVTIVGGYRTYQPSSCVIS*
Yarrowia_bubula_YABU0_NCBI	flattened_line_177[13360-13477]	MSGSKAIPRDYGNESYRVNSNVTIVGGYRTYQPSSCVIA*

	flattened_line_4[53746-53920]	MSTPVFIFHQTTTTTTTTTMSGSKAIPRDYGNESYRVNSN VTIVGGYRTYQPSSCVIA*
	flattened_line_73[126719-126836]	MSGSKAIPRDYGNESYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_135[11955-12072]	MSGSKAIPRDYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_57[58408-58171]	MYPSLKCSFSAKSILHCFILQYINPSSPPQITTKHHTLSL MSGSKAIPRDYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_247[9255-9372]	MSGSKAIPRDYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_4[407758-407641]	MSGSKAIPRDYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_169[18491-18608]	MSGSKAIPRDYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_29[80548-80431]	MAGSKAIPRDYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_0[542840-542723]	MSGSKAIPRNYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_95[139800-139917]	MSGSKAIPRNYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_227[37480-37597]	MSGSKAIPRNYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_129[79430-79547]	MSGSKAIPRNYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*
	flattened_line_55[78869-78986]	MSGSRAIPRNYGNQSYRVNSNVTIVGGYRTYQPSSCVIA*

Table S4. Pheromone candidates identified in *Yarrowia* strains in this study. Related to Figure 5 and Table S3.

*Contig/chromosome identifier and genome coordinates are provided for reference.

***Y. lipolytica* strain label**	**Genotype***	**Source**
ML16507	*MATA; ura3-302; leu2-270; lys1-11; Ku70Δ*	Gift from Joshua Truehart (DSM ltd.)
ML16510	*MATB; ura2-6861; leu2-3; ade1; Ku70Δ*	Gift from Joshua Truehart (DSM ltd.)
yaliSS001	ML16507; *Ylste2Δ:LEU2Yl*	
yaliSS005	ML16507; *Ylste6Δ:LEU2Yl*	This study
yaliSS007	ML16507; *Ylmfa2Δ:URA3Yl*	This study
yaliSS009	ML16507; *Ylmfa1Δ:URA3Yl*	This study
yaliSS011	ML16507; *Ylmfa3Δ:URA3Yl*	This study
yaliSS017, yaliSS018	ML16507; *Ylmfa1Δ:LEU2Yl*, *Ylmfa2Δ:URA3Yl*	This study
yaliSS019, yaliSS020	ML16507; *Ylmfa2Δ:URA3Yl*, *Ylmfa3Δ:LEU2Yl*	This study
yaliSS021, yaliSS022	ML16507; *Ylmfa1Δ:LEU2Yl*, *Ylmfa3Δ:URA3Yl*	This study
yaliSS023, yaliSS024	ML16507; *Ylmfa2Δ:LEU2Yl*, *Ylmfa3Δ:URA3Yl*	This study
yaliSS027, yaliSS028	ML16507; *Ylmfa1Δ*	This study
yaliSS029, yaliSS030	ML16507; *Ylmfa1Δ*, *Ylmfa3Δ:LEU2Yl*	This study
yaliSS031, yaliSS032	ML16507; *Ylmfa1Δ*, *Ylmfa2Δ:URA3Yl*, *Ylmfa3Δ:LEU2Yl*	This study
yaliSS035, yaliSS036	ML16507; *Ylmfa1Δ, Ylmfa4Δ*	This study
yaliSS037	ML16507; *Ylmfa1Δ, Ylmfa2Δ:LEU2Yl, Ylmfa4Δ*	This study
yaliSS039, yaliSS040	ML16507; *Ylmfa1Δ, Ylmfa3Δ:LEU2Yl, Ylmfa4Δ*	This study
yaliSS041	ML16507; *Ylmfa1Δ, Ylmfa2Δ:LEU2Yl, Ylmfa3Δ:URA3Yl, Ylmfa4Δ*	This study
yaliSS045, yaliSS046	ML16507; *Ylmfa4Δ*	This study
yaliSS047	ML16507; *Ylmfa1Δ, Ylmfa2Δ:LEU2Yl, Ylmfa3Δ, Ylmfa4Δ*	This study

Label	**Chromosomal position****
YlMFA1	Yali0F_6‖[forward][2134586-2134685-2134697]
YlMFA2	Yali0F_6‖[forward][2139263-2139362-2139374]
YlMFA3	Yali0E_5‖[reverse][1012402-1012303-1012291]
YlMFA4	Yali0F_6‖[reverse][1323505-1323356-1323344]

Table S5 *Y. lipolytica* strains used in this study. Related to STAR Methods.

*In *Y. lipolytica*, MATA and MATB mating-types are equivalent to MAT**a** and MATα of *S. cerevisiae* respectively.

**Candidates located by contigID‖[strand]["Start-Cys-Stop" position in contig].

Primer	Sequence	Purpose
oSS10_121	TGCTCTGCATCATGGGTCGT	PCR amplification of *YlMFA1* locus – forward primer.
oSS10_124	AAGGACGGTAGCGTCAAGCA	PCR amplification of *YlMFA1* locus – reverse primer.
oSS10_129	GCCATTGCTCCTCCCACAAC	PCR amplification of *YlMFA2* locus – forward primer.
oSS10_132	CCATCTACGCCGAGGGAGTC	PCR amplification of *YlMFA2* locus – reverse primer.
oSS10_137	TTACGGCGGCTTTGAGACGA	PCR amplification of *YlMFA3* locus – forward primer.
oSS10_141	TGACCGAGATTGGAGACGCC	PCR amplification of *YlMFA3* locus – reverse primer.
oSS10_213	AGCACACATTCGCTTGTCTCAAAT	PCR amplification of *YlMFA4* locus – forward primer.
oSS10_216	CCCGTTGGTTTCGGTATCAAGAAG	PCR amplification of *YlMFA4* locus – reverse primer.

Table S6 Primers used for verifying genome deletions. Related to STAR Methods.

NOTE: Primers were used to check for genome deletions of pheromone candidates.