Microbiology

Asymmetric mating behavior of isogamous budding yeast

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Anisogamy, the size difference between small male and large female gametes, is known to enable selection for sexual dimorphism and behavioral differences between sexes. Nevertheless, even isogamous species exhibit molecular asymmetries between mating types, which are known to ensure their self-incompatibility. Here, we show that different properties of the pheromones secreted by the MATα and MATα mating types of budding yeast lead to asymmetry in their behavioral responses during mating in mixed haploid populations, which resemble behavioral asymmetries between gametes in anisogamous organisms. MATα behaves as a random searcher that is stimulated in proportion to the fraction of MATα partner cells within the population, whereas MATα behaves as a short-range directional distance sensor. Mathematical modeling suggests that the observed asymmetric responses can enhance efficiency of mating and might thus provide a selective advantage. Our results demonstrate that the emergence of asymmetric mating behavior did not require anisogamy-based sexual selection.

Introduction

Anisogamy is commonly considered to be the ultimate determinant of the behavioral differences observed between the male and female organisms. Most existing evolutionary models suggest that size difference (anisogamy) between gametes—with abundant small male gametes and few large female gametes—gives opportunity for sexual selection and, consequently, asymmetric sexual behavior (Fig. 1A) (1–3). Hence, in isogamous species, including those that have self-incompatible mating types, the behavior of different gametes is assumed to be identical (4, 5). Even the well-investigated example of brown alga Ectocarpus, where similarly sized gametes exhibit clear behavioral asymmetry, might be explained by mild but significant bias in both size and relative abundance of the two different gametes (mild anisogamy) and by the ancestor of Ectocarpus being anisogamous (6, 7). Nevertheless, different responses to mating pheromones could potentially lead to asymmetries between gametes even in isogamous organisms, such as those observed in the green alga Closterium (8) or in the diatom Seminavis robusta (9, 10).

Fungi include both anisogamous and isogamous species (11), being thus attractive models to study the emergence of asymmetric mating behavior. Similar to other isogamous unicellular fungi, yeast Saccharomyces cerevisiae can exist as diploids and haploids, both capable of reproducing asexually. A sexual cycle happens when diploids produce a tetrad of haploid spores, which mate to produce new diploids either directly inside the spore sac (ascus) or as individual haploids after germination (12, 13). Although much less frequent in Saccharomyces than intratetrad mating (14, 15), the latter scenario is important to enable outcrossing, preventing inbreeding depression (16), and facilitating environmental adaptation (17), and it can occur, for example, upon ascus degradation within and dispersal by insect vector (13, 18–20).

Besides their MAT locus, MATα or MATα, haploid cells of both mating types are genetically (and also morphologically) identical.

Self-incompatibility between the two mating types is ensured at the molecular level, with distinct pheromone receptors, pheromone processing/secretion pathways, and partner-specific aggregation proteins being expressed by MATα and MATα cells (Fig. 1B). Two features of this molecular asymmetry might have potential consequences for mating behavior. First, α-factor is considerably more hydrophobic than α-factor due to its prenylation (21). Second, MATα cells secrete specific Bar1 peptidase that cleaves α-factor, which reshapes the sensory input function for these cells (22–25). These asymmetric features appear to be conserved in ascomycetes, including model yeast species Candida albicans and Schizosaccharomyces pombe.

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Fig. 1. Asymmetric pheromone signaling in S. cerevisiae and emergence of asymmetric sexual behavior. (A) Possible routes of emergence of asymmetry in mating behavior, starting from mating between two self-incompatible but morphologically identical (isogamous) gametes/ mating types. Anisogamy is commonly assumed to have evolved due to competition between gametes and selection for high encounter frequency under gamete limitation. Subsequently, different abundances and sizes of gametes lead to sexual selection of additional dimorphisms and to asymmetric sexual behavior. Alternatively, asymmetric mating behavior might have emerged already in isogamous organisms due to molecular asymmetries between self-incompatible gametes. (B) Asymmetric mating determinants of the mating types in Saccharomyces cerevisiae. Molecular components expressed by MATα or MATα cells are shown in red and blue, respectively, and include mating pheromones (α-factor and α-factor), their receptors (Ste2 and Ste3), mating type-specific agglutinins (Aga1-Aga2 and Sag1), and diffusible protease Bar1 secreted by MATα cells. Hydrophobic chain indicates prenylation of α-factor.
(26, 27). Although these molecular asymmetries are well known, the α- and α-factor response pathways are essentially identical downstream of their respective receptors (28). It is thus assumed that the responses of the two mating types of *S. cerevisiae* to potential mates are behaviorally symmetric (29), at least for intratetrad mating within the ascus where gametes are located in immediate proximity and present at a fixed ratio of 2 MATα and 2 MATα haploid cells.

Here, we investigated whether molecular asymmetries between MATα and MATα cells might lead to mating type–specific behavioral patterns during mating in mixed haploid populations, where the distance between cells and the composition of the mating population are variable. To ensure efficient mating under these conditions that are relevant for outcrossing, haploid gametes might need to respond to both the mate availability and its location. We observed that responses of MATα and MATα cells are indeed highly asymmetric, apparently due to the different diffusivity of the α- and α-factors as well as secretion of Bar1. Furthermore, our computer simulations suggested that this behavioral asymmetry maximizes the efficiency of mating over a wide range of conditions, indicating that it might have been under evolutionary selection already before the establishment of anisogamy.

### RESULTS

**MATα and MATα cells respond differently to population parameters and proximity of the mate**

We first compared the signaling responses of MATα and MATα cells to varying density and fraction of mating partners within a well-stirred and nonaggregating coculture by quantifying the expression of the pheromone-responsive P_FUS1-GFP reporter gene using flow cytometry (see Materials and Methods). As reported previously (25), MATα cells responded primarily to the fraction rather than to density of MATα cells within the population (Fig. 2, data file S1, and see Materials and Methods for statistical analysis). This “sex-ratio” sensing depends on Bar1-mediated attenuation of MATα response as a function of its own abundance (25). Contrary to that—but consistent with apparent absence of a diffusible α-factor–specific protease—MATα response depended on MATα density but not on the ratio between the mating types (Fig. 2 and see Materials and Methods for statistical analysis). When mating success is primarily determined by random collisions (e.g., in stirred environment), sex-ratio sensing enables MATα cells to estimate the probability of mating success, and in these environments, it constitutes a more efficient strategy to regulate investment of resources into mating than density sensing (25). We therefore hypothesized that the response of MATα cells might provide an advantage under different conditions, such as mating on the surface, where locating and reaching out to the mate is critical.

To test this idea, we used fluorescence microscopy to measure pheromone responses in mixed cultures of MATα and MATα cells cocoincubated on glass surface (see Materials and Methods). When responses for individual cells were plotted as a function of cell distance \(d_{\text{MM}}\) to the closest cell of opposite mating type, we observed a clear distance dependence for MATα responses over a prolonged period of coincubation (Fig. 3, A and B; Fig. S1, A and B; and data file S2). In contrast, MATα responses were essentially distance independent (see statistical analyses in fig. S1B). We thus conclude that, in an unstirred (static) environment with random distribution of mates, poorly diffusible α-factor signals the distance to the mate, whereas highly diffusible α-factor rather serves as a global indicator of relative mate availability.

To better understand the functional implications of this asymmetric sensing strategy, we investigated whether morphological responses during mating in a static environment might also be mating type specific. Since one of the known phenotypes induced by low levels of pheromone signaling in *S. cerevisiae* is bipolar budding resulting in elongated cell clusters (30), we determined shapes of cell clusters formed in a mixed culture of MATα and MATα cells. This was done by fitting ellipses to individual cell clusters and analyzing the ratios between the major (longer) and minor (shorter) axes (Fig. 3C). Over the time course of coincubation, a substantial fraction of MATα cells indeed developed elongated cell clusters, while MATα cells showed almost exclusively axial budding and consequently produced mostly roundish cell clusters (Fig. 3D, figs. S1C with statistical analysis and S2, and data file S3). Therefore, highly diffusible and globally available α-factor seems to stimulate MATα cells isotropically to initiate bipolar budding and consequently cell-cluster elongation, whereas α-factor concentration, apart from areas in immediate vicinity to emitter cells, does not reach the threshold level that is required to initiate this pattern in MATα cells. Notably, bipolar budding could nevertheless be clearly observed in MATα cells exposed to synthetic α-factor (fig. S3), confirming that MATα cells can exhibit this behavior given sufficient stimulation. Thus, the observed asymmetry in budding behavior apparently stems from differences in spatial distribution of the pheromones rather than from some intrinsic mating type–specific differences in capability to perform bipolar budding.

Chemotropic growth is another phenotype induced by pheromones at concentrations higher than those necessary for stimulation of bipolar budding, and this behavior was well studied for MATα cells exposed to artificial gradients of α-factor (31). When occurrences of...
Steven Anders et al., 2021; 7: eabf8404 | 11 June 2021

Details of image analysis and fig. S1 for additional data and statistical analyses.

Frequencies of cell-cluster elongation over time for each mating type. (Fig. 3E) Chemotropic cells with (black arrows) or without (yellow arrow) direct contacts to mates toward which the elongated projection was directed. Scale bars, 10 µm.

Asymmetric behavior is predicted to maximize efficiency of mating in unstimulated environment over a wide range of conditions.

We hypothesized that the observed mating type-specific behavioral responses could reflect different strategies of mate search pursued by MATa and MATα cells, where randomly oriented elongation of MATα cell clusters enables a random search behavior that increases encounter rates (30), and MATα cell response allows their directional polarization and chemotropic growth toward a close mate. Therefore, we asked whether asymmetric behavioral responses could perform comparatively better than symmetric ones. For this, we built a mathematical model that compares different strategies of mating response: symmetric populations composed of either distance-dependent (local) or distance-independent (global) responders or a mixed asymmetric population of local and global responders (see section S1). In our simulations, local responders (modeling MATα behavior) divide accurately in the direction of their closest partner only if it is found within short sensing distance (Da), and they produce roundish colonies via axial budding otherwise. In contrast, global responders (modeling MATa behavior) produce farther-reaching filamentous colonies via bipolar budding when stimulated but choose the direction of their expansion at random (Fig. 4A). A control scenario of random encounters, where cells bud axially and in random directions, was also simulated. Successful mating, which was assumed to happen each time two partner cells touched or overlapped with each other, was monitored during four rounds (=generations) of simulated haploid cell division.

We first focused on the effect of these different search strategies on the probability for a pair of mating partners or their descendants to mate as a function of the initial distance between them. For simplicity, here we assumed that global responders are always stimulated and thus show bipolar budding. We observed that symmetric local search strategy is most successful at short intercellular distances and at early time points, whereas symmetric global search strategy is superior at larger distances and at later time points (Fig. 4B). Notably, mixed asymmetric response combined advantages of both symmetric strategies, being always nearly as efficient as the most successful strategy. The success rate for the mixed strategy was also less sensitive to the value of the sensing distance Da than for the symmetric local response (Fig. 4B, top and bottom panels). This might represent an advantage in environments where steepness of the a-factor gradient could vary due to differences in diffusion and stirring.

We then considered mating in a population of randomly distributed cells, while accounting for the concomitant asymmetry in the pathway induction for the two mating types. For global responders, bipolar budding was induced with a probability proportional to the global relative abundance of mates (ratio sensing). For local responders, a perfect aiming toward the partner cell was induced when the distance to their closest partner was ≤Da (distance sensing). When simulations were performed for populations with different initial compositions, the symmetric local and asymmetric strategies both achieved the highest overall mating efficiency over the entire range of density values and throughout the course of the simulation (Fig. 4C and fig. S4A). The symmetric global responders performed poorly, although still better than nonresponders. If increased pheromone production by induced cells (32) was included...
**Fig. 4. Asymmetric mating behavior is predicted to confer advantage on surfaces.** (A) Rules for modeling mating behavior of global ratio (G-cells, MAT\(\alpha\)) and local distance (L-cells, MAT\(\alpha\)) sensors. For axial or bipolar budding, daughter cells were produced at minimal or maximal distance from the preceding daughter without overlaps (left), resulting respectively in roundish or elongated microcolonies (middle). Color intensity indicates cell generation (number of preceding cell divisions). Key features (right) are: (i) stimulated G-cells exhibit bipolar budding; (ii) L-cells bud axially if distance to the closest partner \(d_{\text{min}}\) > sensing distance \(D_1\) (left branch), otherwise the daughter is directed toward the closest partner (right branch); (iii) overlapping opposite type cells “mate” (magenta outline in right branch). (B) Dependence of mating success on the initial distance of two partners with L-L (Local-Local, blue), L-G (Local-Global, orange), and G-G (Global-Global, red) strategies for \(D_1 = 1.5\) (top) and \(D_1 = 2.5\) (bottom) and different generations. Control simulations with nonresponding L-cells are shown in black dashed lines. Note that G-G and control strategies remain unaffected by \(D_1\). (C) Mating success of the different strategies [see (B)] in mixed populations with random spatial distribution and varying mating ratio. For local-global mating, cell type 1 refers to L-cells. Simulation results are shown for three different total initial cell densities after three generations, with constant \(D_1 = 2\) (top) and induction-dependent \(D_1\) between 2 and 3 (bottom). See fig. S4 for the complete set of simulations and section S1 for model description.

in our model as an induction-dependent increase in the critical distance \(D_1\), then the mating success was highest for the asymmetric strategy, particularly at lower population densities (Fig. 4C, bottom and fig. S4B). This occurs because induction of local emitter cells (MAT\(\alpha\) in our case) by globally available \(\alpha\)-factor results in increased \(\alpha\)-factor production, thus extending local gradients around MAT\(\alpha\) cells when mating partners are available.

**Discussion**

The emergence of differences in mating behavior between gametes was one on the key events in the evolution of higher eukaryotes. Current models (1–5, 33) assume that behavioral asymmetry between gametes of different sexes emerged as a consequence of anisogamy, which itself evolved as a consequence of gamete competition (34) and/or gamete limitation (35). Although gamete competition is likely to be the stronger selective force in the evolution of anisogamy (33), selection for high gamete encounter rates under conditions of gamete limitation might also be important (36, 37).

By investigating gamete behavior in the yeast *S. cerevisiae*, here, we demonstrated that, even in a morphologically isogamous organism, behavioral differences between the two mating types can emerge from molecular asymmetries in pheromone signaling. During mating on a surface, MAT\(\alpha\) and MAT\(\alpha\) cells of *S. cerevisiae* apparently pursue very different strategies of finding mating partners. While MAT\(\alpha\) cannot efficiently sense distance and thus direction toward a potential mate because of high diffusivity of \(\alpha\)-factor, it undergoes a transition from axial to bipolar budding dependent on the fraction of mating partners in the population to produce elongated cell clusters. Although these clusters are nondirectional, they enable random search for a mating partner. In contrast, MAT\(\alpha\) only responds when in close proximity to a mating partner but can polarize directionally toward it over short distances. Although this asymmetry in searching behavior resembles differences between motile male and sessile female gametes that are typical for oogamy, the most advanced form of anisogamy, it relies solely on the differences between diffusivity and processing of the two mating pheromones.

Mathematical modeling further suggested that the asymmetric mating behavior of *S. cerevisiae* gametes ensures high frequencies of mating encounters over a wide range of population densities. In our simulations, behavioral asymmetry was particularly beneficial for increasing encounter rates at low gamete densities, such as those during outcrossing in yeast. Although the frequency of outcrossing in *Saccharomyces* is much lower than that of the high-density/short-distance mating, either within tetrads or upon mating type switching in a mother cell after producing a daughter (haplo-selling) (13–15), outcrossing may be favored under certain conditions (13, 18–20). Moreover, since at high densities/short distances it is similarly efficient as the best (MAT\(\alpha\)-type) symmetric strategy, the asymmetric behavior may not be counter selected during intratetrad mating or haplo-selling, even considering potential benefits of this self-fertilization (12, 38). The observed behavioral asymmetry is also likely to be common among ascomycetes including those with naturally haploid life styles, which all exhibit similar molecular asymmetry of the mating pathway (27, 39, 40).

We thus conclude that basic asymmetric mating behavior might emerge independently of anisogamy and therefore classical sexual selection and thus be common among isogamous unicellular organisms (8, 9). Although our hypothesis that mating asymmetry in ascomycetes provides evolutionary selective advantage by increasing the frequency of mating encounters remains to be tested in the context of population genetics models, it is conceptually similar to the selection for anisogamy proposed by the gamete limitation model (35). Asymmetric signaling was further proposed to contribute to maintaining binary mating types against reversals to unisexuality or mating type proliferation in isogamous organisms (41), and signaling-dependent behavioral asymmetries could further reinforce this stability. The basidiomycetes—sister clade of ascomycetes—lack the...
asymmetry in pheromone hydrophobicity and display both facultative unisexuality and multiple mating types (11, 42). Eukaryotic mating is ancient and extremely diverse (43), with examples of reversed evolutionary transitions, including self-incompatibility to unisexuality and anisogamy to isogamy (7, 11). Particularly in eukaryotic microorganisms, no mating strategy seems absolutely advantageous over another, and evolutionary trajectories might rather correlate with particular lifestyles (5). While it remains to be explored whether and how asymmetric mating behavior might have contributed to the emergence of anisogamy, our work suggests that, at least in some lineages of isogamous microorganisms, this asymmetry predated anisogamy rather than being a consequence of it.

MATERIALS AND METHODS

Strains and growth conditions

*S. cerevisiae* strains used in this study are derivatives of SEY6210a (*MATα leu2-3,112 ura3-52 his3Δ200 trp1Δ901 lys2-801 suc2Δ9*) or SEY6210 (*MATα, otherwise identical to SEY6210a*) (44) and are listed in Table S1. Fluorescent protein reporters were genomically integrated. Generally, the low-fluorescence synthetic defined (LoFlo-SD) medium for growing yeast in liquid was composed of LoFlo–yeast nitrogen base (Formedium) with complete supplement mix (Formedium) and 2% glucose. Routinely, cells from glycerol stocks or agar plates were inoculated in LoFlo-SD and incubated overnight at 30°C on an orbital shaker at 200 rpm for 12 to 16 hours. Overnight cultures were diluted 1:20 to 1:100 and grown as before to reach the exponential growth phase with a doubling time of ~100 min.

Coincubation in shaking culture

In each coculture under shaking conditions, only one of both mating types contained a GFP reporter gene controlled by the pheromone-responsive FUS1 promoter (P\_FUS1-GFP), i.e., *MATα* and *MATα* responses were measured in different cocultures. For analysis, mating types in a coculture were distinguished by being GFP fluorescence positive or negative (see fig. S5). To ensure homogeneous mixing of *MATα* and *MATα* cells and prevent cell aggregation, the *MATα* cells used in these experiments were deleted for the gene encoding the α-agglutinin subunit Aga2. Separate cultures of *MATα* (γAA198 or γAA277) and *MATα* cells (SEY6210 or γAA156) were grown in 100-ml flasks at 30°C as described above, washed once with LoFlo-SD, and resuspended in fresh LoFlo-SD medium. The OD\_600 (optical density at 600 nm) was determined, and the suspensions were mixed (γAA198 with SEY6210 for measuring *MATα* responses and γAA277 with γAA156 for measuring *MATα* responses) and adjusted to indicated densities and sex ratios in a final volume of 1 ml. Cell mixtures were incubated in 24-well plates (Costar) at 30°C with orbital shaking at 200 rpm. The reporter gene expression was analyzed by flow cytometry immediately after sampling.

Coincubation on glass surface

For coincubation on glass surfaces, both mating types contained a P\_FUS1-mNeongreen reporter gene and additionally expressed mCherry (*MATα*) or mTurquoise (*MATα*), both of which were controlled by constitutive TDH3 promoter. Separate cultures of *MATα* and *MATα* cells were grown in 1 ml of LoFlo-SD in 24-well plates at 25°C. Cell suspensions were diluted in LoFlo-SD to OD\_600 = 0.0075 and briefly sonicated. Cell suspensions were mixed in 1:1 ratio by transferring equal volumes to a 96-well glass-bottom plate (Greiner Bio-One) coated with type-IV concanavalin A (Sigma-Aldrich). Typically, 100 μl of each *MATα* and *MATα* suspensions were added in each well, briefly mixed by pipetting up and down, and briefly centrifuged to force cell decantation. Between three and four replicate wells were used per biological sample with eight fields of view per well acquired during microscopy. Image acquisition by fluorescence microscopy was started approximately 5 min after mixing mating types.

Stimulation with synthetic α-factor

Cell suspensions were transferred to a 96-well glass-bottom plate (Matrical Bioscience), and image acquisition was started after allowing cells to settle down gravitationally for approximately 5 min. For time-lapse experiments using stimulation with synthetic pheromones, wells of the glass-bottom plate were coated with type-IV concanavalin A (Sigma-Aldrich) before the transfer of cell suspensions. Synthetic α-factor dilutions (BioCat Heidelberg) were prepared as 11× stocks in methanol. Image acquisition by fluorescence microscopy was started immediately after pheromone addition and repeated periodically at defined time intervals over the course of several hours.

Flow cytometry

Flow cytometry measurements were performed on an LSRFortessa flow cytometer (BD Biosciences). Cell suspensions were injected from a 96-well plate (Greiner Bio-One) with a high-throughput (HT) sampler. GFP fluorescence was measured with a laser of 488-nm wavelength. Using the BD FACS DIVA software (BD Biosciences), cells were gated in an forward scatter area/side scatter area (FSC-A/SSC-A) plot to exclude debris. GFP-positive cells (either *MATα* or *MATα*, depending on the experiment) were gated manually, and the mean fluorescence of this population was used for further analysis (see fig. S5 and data are available in data file S1). Fluorescence distributions in the respective GFP-positive populations were unimodal (fig. S5).

Fluorescence microscopy

Fluorescence microscopy was performed on a wide-field fluorescence microscope (Nikon Ti-E) equipped with a solid-state white-light light-emitting diode source (Sola SE-II), a motorized stage, a 40× dry objective (Nikon Plan Apo 40× Lambda, numerical aperture 0.95), a scientific complementary metal-oxide semiconductor camera (Andor Zyla), and an incubator with heater controller (Digital Pixel). The following excitation (Ex) and emission (Em) filters were used for acquiring fluorescence protein signals: mCherry (Ex, 575/25; Em, 647/57 nm), mNeongreen (Ex, 513/17; Em, 542/27 nm); GFP (Ex, 470/40; Em, 525/50 nm), and mTurquoise (Ex, 438/24; Em, 480/40 nm). Images were taken periodically. For coincubation experiments, microscopy was performed at 25°C.

Image and data analysis

For analysis of distance-dependent reporter gene expression (Fig. 3B), single-cell segmentation was done on bright-field images using CellProfiler version 2 (45), and the defined masks were used to measure single-cell intensities in the corresponding fluorescence images. Raw data extracted by CellProfiler were further processed using the statistical software R version 3 (46). Identified objects without pathway reporter mNeongreen fluorescence (i.e., below basal activity) were removed from further analysis by manually applying a threshold; typically, those were objects erroneously classified as...
cells by the cell segmentation procedure. Next, mNeonGreen traces averaged over single cells within individual fields of view were plotted, and clearly outlying fields of view were removed manually from further analysis. Subsequently, mCherry fluorescence (label for MATa cells) was plotted against mTurquoise fluorescence (MATα), and three gates within this plot were defined manually to assign cell types MATa, MATα, and diploid MATa/MATα to individual cells. Cells outside any of the defined gates were not considered for further analysis. For each defined MATa and MATα cell, center-to-center distance \( d_{\text{min}} \) to the closest cell of opposite mating type was determined; cells being closer to the image border than \( d_{\text{min}} \) · 0.9 were excluded from further analyses. Further analysis was restricted to data with \( d_{\text{min}} \) < 45 μm and time < 300 min. For each experiment, single-cell mNeonGreen fluorescence values were normalized to its median within the experiment, and those normalized values were used for plotting and statistical analyses. Plots were generated with the ggplot2 package for R (47). Single-cell data are available in data file S2.

For analysis of cell-cluster elongation (Fig. 3D), mCherry and mTurquoise fluorescence images were analyzed using Fiji 1.5 (48, 49). After background subtraction and Gaussian blurring, fluorescence images were converted into binary images by Otsu thresholding algorithm. On these binary images, the “Analyze Particles” command was used to fit ellipses to the cell clusters. The data of the fitted ellipses were exported and further analyzed in R. The single-cluster data used for plotting and statistical analysis are available in data file S3.

For analysis of chemotropism (Fig. 3F), cells with elongated (“chemotropic”) shapes were identified visually on bright-field images and classified in two categories, depending on whether they had been in direct contact with the targeted cell before development of the elongated projection. The analysis was performed on bright-field images to avoid human bias toward a mating type. After categorization, mating types were assigned to individual cells by overlaying bright-field images with fluorescence images, and the proportions of mating types within each category were calculated. Data are available in data file S4.

Statistical analysis
For evaluation of data acquired for stirring cocultures (Fig. 2), pathway responses were fitted by means of MATLAB function “fitglm” to two different generalized linear models, with one model describing pathway responses as being dependent on (absolute) partner densities (model 1) and the other assuming dependence on partner fraction and total cell density (model 2; for detailed description, see section S2). The quality of the models was evaluated by comparing the sum of the squared errors (SSEs) and the skewness of the distribution of the raw residuals (\( m_3 \)). While SSE measures the total distance between the model and the data, the absolute value of the skewness of the residuals indicates the level of systematic bias in this distance. All fit results are listed in table S2. While for MATa cell responses, SSE’s were similar for both models, the residuals were strongly skewed only for model 1 (fig. S6A and table S2). Thus, according to the \( m_3 \) metric, model 2 better describes MATa cell responses. By contrast, according to both SSE and \( m_3 \) metrics, MATα responses are consistent only with model 1 (table S2 and fig. S6B).

SUPPLEMENTARY MATERIALS
Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/24/eabf8404/DC1

View/request a protocol for this paper from Bio-protocol.

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