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Courtship in *Saccharomyces cerevisiae*: an Early Cell-Cell Interaction during Mating

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During conjugation in *Saccharomyces cerevisiae*, two cells of opposite mating type (*MAT*a and *MAT*α) fuse to form a diploid zygote. Conjugation requires that each cell locate an appropriate mating partner. To investigate how yeast cells select a mating partner, we developed a competition mating assay in which wild-type *MAT*α cells have a choice of two *MAT*a cell mating partners. We first demonstrated that sterile *MAT*α*1* cells (expressing no a- or α-specific gene products) do not compete with fertile *MAT*a cells in the assay; hence, wild-type *MAT*a and *MAT*α cells can efficiently locate an appropriate mating partner. Second, we showed that a *MAT*a strain need not be fertile to compete with a fertile *MAT*a strain in the assay. This result defines an early step in conjugation, which we term courtship. We showed that the ability to agglutinate is not necessary in *MAT*a cells for courtship but that production of a-pheromone and response to α-pheromone are necessary. Thus, *MAT*a cells must not only transmit but must also receive and then respond to information for effective courtship; hence, there is a "conversation" between the courting cells. We showed that the only α-pheromone-induced response necessary in *MAT*a cells for courtship is production of a-pheromone. In all cases tested, a strain producing a higher level of a-pheromone was more proficient in courtship than one producing a lower level. We propose that during courtship, a *MAT*α cell selects the adjacent *MAT*a cell producing the highest level of a-pheromone.

In *Saccharomyces cerevisiae*, two haploid cells of opposite mating type, **a** and α, fuse to form a diploid zygote during the process of conjugation (mating) (reviewed in references 13 and 41). When **a** and α cells come in contact, they detect each other's presence by responding to the peptide hormone (**a**- or α-pheromone) that is produced by the opposite cell type. The responses to pheromone include transcriptional activation of the gene products necessary for mating, induction of cell-type-specific agglutinability, and arrest of cell division in the G_1 phase of the cell cycle (13). It is essential that both **a** and α cells induce responses in cells of opposite mating type for conjugation to occur, since either **a** or α cells that do not produce pheromone are completely sterile (30, 35).

Conjugation involves localized cellular activity. Once agglutinated and arrested in G_1, at least one cell of a mating pair produces a projection toward the other cell, the cell walls fuse in this region, and a hole is created by breakdown of the juxtaposed walls (6, 42). Breakdown of the cell wall between fusing cells must be precisely localized since the cells remain under high hydrostatic pressure and would lyse if their walls were indiscriminately digested. The cell membranes and then the nuclei fuse through the pore joining the cells (6).

During conjugation, the two cells of a mating pair communicate via the peptide hormones, **a**- and α-pheromone to induce gene expression. It seems likely that some form of intercellular communication might be necessary to establish the location of the morphogenetic events of conjugation. That is, in addition to activating mating functions in the other cell, does the inducing cell also indicate that it is the intended mating partner? The purpose of this paper is to report an assay (the competition mating assay) designed to investigate whether or not such communication exists and, if so, what the nature of the substances are which mediate this communication.

There is evidence that a mechanism does exist which allows cells to efficiently locate an appropriate mating partner. When pairs of **a** and α cells are placed together by micromanipulation, 45 to 90% of the pairs form zygotes (17, 37, 38). If either cell of a mating pair was unable to determine the position of the other and either cell restricted the events of cell fusion to a random or a predetermined position on its surface, then the probability of forming a diploid would be much less than 50%. It appears therefore that at least one cell of a mating pair is able to indicate that it is the intended mating partner and then by some mechanism to induce the events of cell fusion at the site where the cells are apposed.

The competition mating assay was designed to further test this conclusion and, if supported, to determine the mechanism by which cells locate an appropriate mating partner. We showed that under the conditions of the competition mating assay (which is a mass mating experiment), cells locate an appropriate mating partner as efficiently as when they are placed together in isolation by micromanipulation. Moreover, we showed than an **a** cell does not have to be fertile to be recognized as a potential mating partner in the competition mating assay; therefore, this assay defines an early step in the mating process which we term courtship. Finally, we showed that **a**- pheromone is the major signaling molecule with which an **a** cell courts an α cell.

MATERIALS AND METHODS

Strains and plasmids. The strains used in this study are shown in Table 1. Those of the 381G background are isogenic or congenic with the strain 381G *MAT***a** *cry1 ade2-1° his4-580^a lys2° trp1^a tyr1° SUP4-3*(Ts) (23). Challenger **a** and target **a** cells, defined in Results, are designated throughout the paper as $\mathbf{a}_c$ and $\mathbf{a}_t$ cells, respectively.

The YCp*MAT*α plasmid was constructed by F. Cross and

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TABLE 1. Yeast strains used in this study

Strain	Genotype	Source or reference(s)
381G	α *cry1 ade2 his4 lys2 trp1 tyr1 SUP4*[a,b]	23
3268-2-3	**a** *cry1 ade6 his4 lys2 trp1 sst2-1 SUP4*[b]	Hartwell Lab Collection
3271-19-3	**a** *ade6 his4 lys2 trp1 SUP4*[b]	Hartwell Lab Collection
3666-5	**a** *cry1 ade2 his4 lys2 trp1 tyr1 cyh2 ste2-5*[(ts)] *bar1-1 SUP4*[b]	26
4202-2-4	**a** *cry1 ade6 his4 lys2 trp1 bar1-1 SUP4*[b]	Hartwell Lab Collection
4226-7-2	**a** *cry1 ade2 his4 lys2 trp1 tyr1 bar1-1 ste4-3*[(ts)] *SUP4*[b]	Hartwell Lab Collection
4270-28	**a** *cry1 ade2 his4 lys2 trp1 tyr1 bar1-1 cyh2 ste5-3*[(ts)] *SUP4*[b]	26
5577-2-2	**a** *cry1 lys2 arg4 SUP4*[b]	This work
7608-1-3	**a** *cry1 his4 tyr1 SUP4*[b]	This work
7608-2-4	α *cry1 trp1 tyr1 SUP4*[b]	This work
7608-7-3	α *cry1 his4 tyr1 SUP4*[b]	This work
7608-10-2	**a** *cry1 ade2 ade6 trp1 tyr1 SUP4*[b]	This work
7608-12-3	α *cry1 ade6 his4 tyr1 cyh2 SUP4*[b]	This work
7609-1-1	**a** *cry1 his4 lys2 trp1 tyr1 ura2 SUP4*[b]	This work
7609-9-1	**a** *cry1 ade2 lys2 trp1 tyr1 cyh2 SUP4*[b]	This work
7609-5-3	**a** *cry1 ade2 his4 lys2 ura2 cyh2 SUP4*[b]	This work
7609-6-4	α *cry1 lys2 tyr1 ura2 SUP4*[b]	This work
7609-10-3	α *cry1 tyr1 can*[r] *SUP4*[b]	This work
7609-9-4	**a** *cry1 lys2 ura2 cyh2 SUP4*[b]	This work
7609-8-1	α *cry1 his4 lys2 ura2 cyh2 can*[r] *SUP4*[b]	This work
7609-5-2	α *cry1 ade2 lys2 trp1 tyr1 cyh2 SUP4*[b]	This work
7611-1	α *cry1 his4 lys2 tyr1 ura2 leu2-3,112 cyh2 SUP4*[b]	This work
7611-3	α *cry1 his4 lys2 trp1 tyr1 ura2 leu2-3,112 cyh2 SUP4*[b]	This work
7611-6	**a** *cry1 his4 lys2 trp1 tyr1 ura3-52 leu2-3,112 cyh2 SUP4*[b]	This work
7612-3-1	α *cry1 his4 lys2 tyr1 ura2 sst2-1 SUP4*[b]	This work
7623-4-4	**a** *cry1 ade2 his4 lys2 trp1 cyh2 ste2-5*[ts] *SUP4*[b]	This work
7623-16-4	**a** *cry1 his4 lys2 trp1 leu2-3,112 cyh2 ste2-5*[ts] *bar1-1 SUP4*[b]	This work
7623-16-3	**a** *cry1 ade2 his4 lys2 trp1 ura3 leu2-3,112 cyh2 SUP4*[b]	This work
DJ213-6-3	**a** *cry1 ade2 his4 lys2 trp1 tyr1 ura3-52 leu2-3,112 ste2-10::LEU2 SUP4*[b]	28
7413-3-3	**a** *cry1 ade2 his4 lys2 trp1 tyr1 ura3-52 leu2-3,112 SUP4*[b]	28
351b[101]-4C	**a** *cry1 leu2-3,112 ura3-52 trp1 lys2 cyh2 can1 bar1-1 scg1::LEU2 SUP4/YCp50DAF1-1*[b]	Fred Cross
SY972	**a** *matα1*(*Xho*I linker 189) *leu2 ura3 trp1 his4-519 can1*	49, 54
SY762	(HR125-5d) **a** *leu2-3,112 ura3-52 trp1 his3 his4 gal2*	59
YY609	**a** *leu2-3,112 ura3-52 trp1 his3 his4 gal2 ste6::lacZ*	Susan Michaelis
SM1229	**a** *his4 trp1 leu2 ura3 can1 mfa1::LEU2 mfa2::URA3*	35
SM1188	**a** *his4 trp1 leu2 ura3 can1 ste14::TRP1*	Susan Michaelis
SM1058	**a** *his4 trp1 leu2 ura3 can1*	49
H1171	**a** cry[r] *lys2 tyr1 ura3 leu2 his3/4? trp1 ste16-1*	43, 59
W303-1A	**a** *ade2-1 trp1-1 leu2-3,112 ura3-1 his3-11,15 can1*	Janet Kurjan
LA192	(**a**-specific agglutination-defective mutant derived from W303-1A)	Janet Kurjan

[a] Alleles of 381G strain markers are given in Materials and Methods.
[b] 381G strain background.

contains the *Eco*RI-*Hin*dIII fragment of *MAT*α inserted between the *Eco*RI and *Hin*dIII sites of YCp50. The YEp *MFA2* plasmid contains the 1.75-kilobase *Hin*dIII fragment containing *MFA2* (35) inserted into the *Hin*dIII site of YEp352 (24).

Media and transformation. Liquid cultures were grown in synthetic complete medium containing adenine, uracil, amino acids, and 0.1% Casamino Acids (Difco Laboratories, Detroit, Mich.) (48) unless the strain contained a plasmid, in which case the cultures were grown in synthetic medium lacking uracil. Selective plates contained synthetic medium with 2% agar (Difco) but lacked certain amino acids, adenine, or uracil. The Noble agar plates used for competition mating assays contained synthetic complete medium with 2% Noble agar (Sigma Chemical Co., St. Louis, Mo.).

The lithium acetate method was used to introduce plasmids into yeast cells (25). Plasmid DNA (1 to 5 μg) was used with 40 μg of sonicated calf thymus DNA as the carrier.

Competition mating assay. α, $\mathbf{a}_t$, and $\mathbf{a}_c$ cells grown to the mid-logarithmic phase (5×10^6 to 1.5×10^7 cells per ml) were mixed together and filtered onto 25-mm filters (0.45-μm pore size; Millipore Corp., Bedford, Mass.), and the filters were placed on Noble agar plates. The total number of cells on each filter was 3×10^7, and the fraction of α cells was 1 to 2%. After 3.5 h (23°C) or 2.5 h (34°C), the number of diploids formed between α and $\mathbf{a}_t$ cells was determined by selecting prototrophs on appropriate plates. The numbers of α, $\mathbf{a}_t$, and $\mathbf{a}_c$ cells were determined at the same time by plating on selective plates. For each filter, D (the fraction of α cells that had formed diploids with $\mathbf{a}_t$) and r (the ratio of $\mathbf{a}_t$ cells to total **a** cells) were computed. E (the mating efficiency of α with $\mathbf{a}_t$ cells) was determined by calculating $D(r)$ for $r = 1$. The function $D(r)$ versus r was generated for a given $\mathbf{a}_c$ strain by repeating the mating experiment for r values between 0.01 and 1. To quantify the ability of a given $\mathbf{a}_c$ strain to compete, we computed the competition index (CI). CI was calculated for a given $\mathbf{a}_c$ strain by using the formula $CI = (E \times r)/D(r)$ for $0.001 < r \leq 0.02$. Hence, CI is unity for an $\mathbf{a}_c$ strain which is a good competitor, and $CI < 1$ for a noncompetitive $\mathbf{a}_c$ strain.

Establishing conditions of competition mating assay. For the competition mating assay, the time at which the majority of diploids had been formed but had not divided was determined. A total of 3×10^7 **a** and 3×10^5 α cells grown to the mid-logarithmic phase were mixed together and placed on each of a series of nitrocellulose filters. The filters were

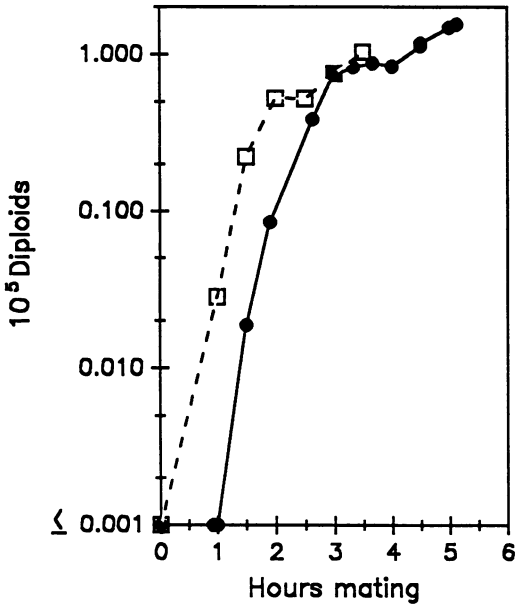


FIG. 1. Time course of mating. A total of 3×10^5 α cells and 3×10^7 **a** cells were mixed together and allowed to mate at 23 or 34°C for the amount of time indicated as described in the text. The number of diploids formed is plotted against time of mating at 23°C (●) and 34°C (□). At 23°C, strains 381G α and 5577-2-2 **a** were used, and at 34°C, strains 7609-5-2 α and 5577-2-2 **a** were used.

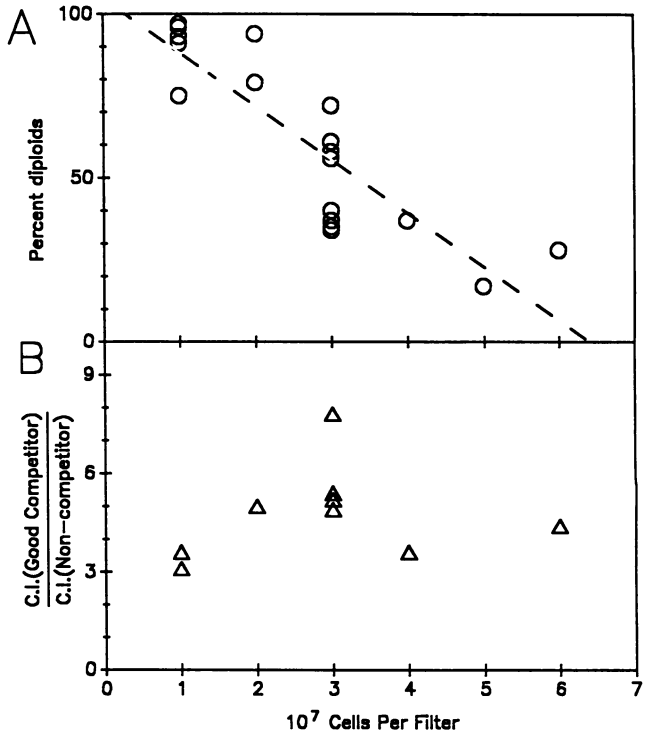


FIG. 2. Optimum number of cells per filter. (A) Efficiency of mating as a function of number of cells on the filter. **a** and α cells in a ratio of 100:1 were allowed to mate for 3.5 h at 23°C, and the percentage of α cells that had formed diploids was plotted against the total initial number of cells placed on the filter as described in the text. The dashed line is the linear line of regression generated by Sigmaplot. (B) Ability to distinguish good competition from non-competition as a function of cell number. The competition mating assay was performed with different total numbers of cells, with 7608-13-2 α cells making up 1% of the total cells. Competition matings were performed with both a good competitor (7413-3-3) and a noncompetitor (DJ213-6-3 *ste2-10*) $\mathbf{a}_c$ strain at each cell density. The $\mathbf{a}_t$ strain was 7609-5-3. The CI for 7413-3-3 (good competitor) and that for DJ213-6-3 (noncompetitor) were computed as described in the text, and the ratio was plotted against the initial total cell number.

incubated on nutrient agar plates at either 23 or 34°C for a given time, and the number of diploids was determined (Fig. 1). These and other experiments indicated that the rapid rate of increase in the number of diploids due to cell fusion decreases by 3.5 h at 23°C and by 2.5 h at 34°C. A previous study showed that at 23°C, the majority of zygotes had not divided from their first bud at 3.5 h (22).

The goal of the competition mating experiments was to distinguish an $\mathbf{a}_c$ strain which is a good competitor from one which is a noncompetitor and hence to maximize the ratio of the CI value of a good competitor to that of a noncompetitor. Theoretically, the more neighbors a given cell has, the larger this ratio of CI values will be. Based on the size of yeast cells (7 by 4.5 mm on average), the area of the filter and the equations worked out by Rogers and Bussey (45), it was determined that there would be only about one layer of cells on the filter for a total cell number of 6×10^6, the number of cells commonly used in quantitative mating experiments (16). Thus, none of the cells would have the maximum number of 12 adjacent cells. Experiments were performed to determine the effects of using larger numbers of cells on the filter. Both efficiency of mating and the ratio CI (good competitor)/CI (noncompetitor) were monitored (Fig. 2). The decrease in mating efficiency with increase in cell number may result from poor diffusion of nutrients to cells in the top layers (Fig. 2A). There did not appear to be a great difference in the ratio CI (good competitor)/CI (noncompetitor) for the range of cell densities tested (Fig. 2B). Since 3×10^7 cells per filter is the maximum number that can be used while maintaining a reasonable mating efficiency, this number of cells was used in the competition mating assay. For 3×10^7 cells per filter, the formula of Rogers and Bussey (45) predicts that there are four to eight layers of cells.

Theoretically, the number of α cells in the mating assay should be very small compared with the total number of cells to ensure that each α cell is completely surrounded by **a** cells. To determine the effect of using different numbers of α cells, we performed the competition mating assay with α strain 7608-13-2, $\mathbf{a}_t$ strain 7609-5-3, and either noncompetitor $\mathbf{a}_c$ strain DJ213-6-3 or good competitor $\mathbf{a}_c$ strain 7413-3-3. The total number of cells was 3×10^7, and $\mathbf{a}_t$ cells made up 2% of the total cells. The ratio of the CI of $\mathbf{a}_c$ strain 7413-3-3 to the CI of $\mathbf{a}_c$ strain DJ213-6-3 was 5.5 when the percentage of α cells was 0.3, 5.4 when α cells made up 1% of the total cells, and 6.3 when α cells composed 2% of the total cells. Hence, there was no significant difference in the ability to distinguish the CI of a good competitor from that of a noncompetitor when the proportion of α cells was in the range 0.3 to 2%. Thus, the α cells make up 1 to 2% of the total cell number in the competition mating assay.

α-Pheromone induction and Northern (RNA) analysis. Cultures were grown overnight at 34°C to the mid-logarithmic phase (5×10^6 to 1×10^7 cells per ml) and diluted to 5×10^6 cells per ml. The cultures were split into two aliquots of 50 ml each, and α-pheromone (Sigma) was added to one aliquot at a final concentration of 4×10^{-8} M. After 20 min of incubation at 34°C, the cultures were placed on ice and then prepared for RNA extraction.

Total RNA was isolated as described previously (18). For each sample, 10 μg of total RNA was denatured by glyoxal and dimethyl sulfoxide and electrophoresed through a 1.5% agarose gel (31). Transfer to Nytran and hybridization was performed as described previously (10, 31). Filters were washed four times in 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–1% sodium dodecyl sulfate and four times in TE (31). Washed filters were autoradiographed with preflashed Kodak XAR-2 film and a Cronex Lightning-Plus intensifying screen at −70°C. In some cases no intensifying screen was used.

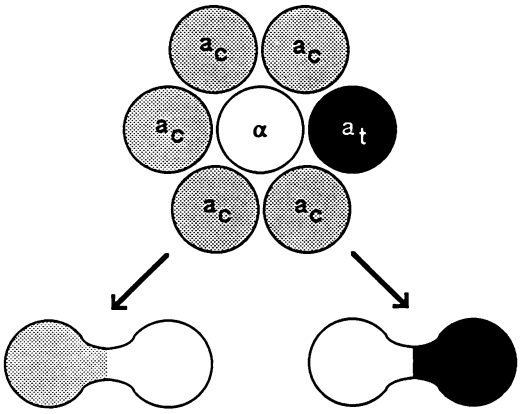
The probes were prepared by hexanucleotide-primed labeling of linear DNA fragments as described previously (19, 20). A 200-μCi sample of [^{32}P]ATP (Dupont, NEN Research Products, Boston, Mass.) was added to the labeling reaction, which was allowed to incubate at room temperature for at least 4 h or overnight.

Agglutination and α-pheromone assays. Agglutination assays were performed as described previously (28). To quantify a-pheromone production, we grew **a** cells to saturation in either synthetic medium lacking uracil (approximately 2 × 10^8 cells per ml) or in YM-1 medium (22) (approximately 5 × 10^8 cells per ml). Cells were removed by centrifugation, and the supernatant was heated at 65°C for 15 min to kill any remaining cells. A 2-ml sample of supernatant was concentrated by ultrafiltration through an Amicon YM-30 membrane as described previously (35). A total of 2 × 10^6 7612-3-1 α *sst2* cells were inoculated into 3 ml of synthetic complete medium containing 0.7% low-melting-temperature agarose (FMC Bioproducts) which was then poured onto synthetic complete plates and allowed to harden. Serial 1/2 dilutions of the concentrated **a**-pheromone supernatants were spotted onto these plates and incubated at 23°C for 2 or 3 days. Growth inhibition of the cells in the lawn occurred if a sufficient amount of **a**-pheromone was present in a spot. The highest dilution at which there was growth inhibition indicated the relative amount of **a**-pheromone present in the original culture.

RESULTS

Competition mating assay. The competition mating assay was designed to investigate whether or not yeast cells can efficiently locate an appropriate mating partner and, if so, to determine which gene products are necessary for this process. The assay is a mass mating experiment in which α cells are challenged to mate with two different types of **a** cells, $\mathbf{a}_t$ and $\mathbf{a}_c$. The number of diploids formed between α cells and the $\mathbf{a}_t$ cells in the presence of the $\mathbf{a}_c$ cells is determined. The number of diploids formed depends on the ability of the $\mathbf{a}_c$ cells to compete with the $\mathbf{a}_t$ cells in attracting the α cells for mating. If $\mathbf{a}_c$ is a good competitor, we expect fewer diploids between α and $\mathbf{a}_t$ than if $\mathbf{a}_c$ is unable to compete. If wild-type yeast cells can efficiently locate an appropriate partner during mating, then a sterile *matα1* strain (which expresses no **a**- or α-specific gene products [41, 50]) should be unable to compete. However, if the α cells cannot distinguish between the wild-type $\mathbf{a}_t$ cells and the sterile $\mathbf{a}_c$ cells, then the sterile *matα1* cells should be good competitors.

Competition mating assay: theoretical model. To predict the outcomes of a competition mating experiment for a good competitor and a noncompetitor, we developed theoretical expectations for the experiment based on a simplified model of the experimental situation (Fig. 3). We assumed that each α cell is surrounded only by **a** cells in three dimensions. The number of **a** cells adjacent to a given α cell is denoted n, and in the situation in which ellipsoid cells are packed together perfectly, n is 12 (45). The **a** and α cells are allowed to form diploids for a given time. The ratio of $\mathbf{a}_t$ cells to total **a** cells is denoted r, and for a given value of r, the number of diploids formed between α and $\mathbf{a}_t$ cells is determined. The fraction of α cells that form diploids with $\mathbf{a}_t$, expressed as a percentage, is denoted $D(r)$. If each α cell selects either an $\mathbf{a}_t$ or an $\mathbf{a}_c$ cell as a mating partner with equal frequency, then $\mathbf{a}_c$ is a good competitor and $D(r) = E \times r$, where E is the mating efficiency of α cells with $\mathbf{a}_t$ cells expressed as a percentage. E is a constant determined from $D(r)$ when $r = 1$, that is, when no $\mathbf{a}_c$ cells are present. On the other hand, if $\mathbf{a}_c$ is a noncompetitor, each α cell which has at least one adjacent $\mathbf{a}_t$ cell will select this adjacent $\mathbf{a}_t$ cell as a mating partner with efficiency E (the remaining α cells do not mate with an $\mathbf{a}_t$ cell) and $D(r) = E \times (1 - e^{-nr})$. It is assumed that the probability of there being at least one $\mathbf{a}_t$ cell adjacent to a given α cell is Poisson distributed with mean nr, and no provision is made for cell division during the mating period. The theoretical curves $D(r)$ for a good competitor and a noncompetitor are shown in Fig. 4. To quantify the ability to compete for a given $\mathbf{a}_c$ strain, we computed the CI. CI is defined as CI = $(E \times r)/D(r)$, $0.001 < r \leq 0.02$. (It can be



	Frequency of α x a_c diploid	Frequency of α x a_t diploid
Non-competitor	0	1
Fertile Good Competitor	(n-1)/n	1/n
Sterile Good Competitor	0	1/n

FIG. 3. Theoretical model of competition mating assay. In the model, it is assumed that each α cell is surrounded by **a** cells. Shown here is a situation in which only one of the adjacent **a** cells is an $\mathbf{a}_t$ cell (denoted by a black circle in the diagram). The remaining adjacent **a** cells are the $\mathbf{a}_c$ cells (shaded grey). The α cell is shown in white in the center. n denotes the number of **a** cells adjacent to a given α cell. If the α cell is attracted only to the wild-type $\mathbf{a}_t$ cell and not to the $\mathbf{a}_c$ cells, then the $\mathbf{a}_c$ strain is a noncompetitor and a diploid is formed between each α cell that has at least one adjacent $\mathbf{a}_t$ cell. If $\mathbf{a}_t$ and $\mathbf{a}_c$ cells have equivalent abilities to attract the α cell, then the $\mathbf{a}_c$ strain is a good competitor and the number of diploids formed between the α and the $\mathbf{a}_t$ cells is proportional to the fraction of total **a** cells that are $\mathbf{a}_t$ cells. Hence, for a good competitor, the frequency of diploids formed between α and $\mathbf{a}_t$ cells is n times less than that for a noncompetitor. An α cell forms a diploid with an $\mathbf{a}_c$ cell only when the good competitor is fertile.

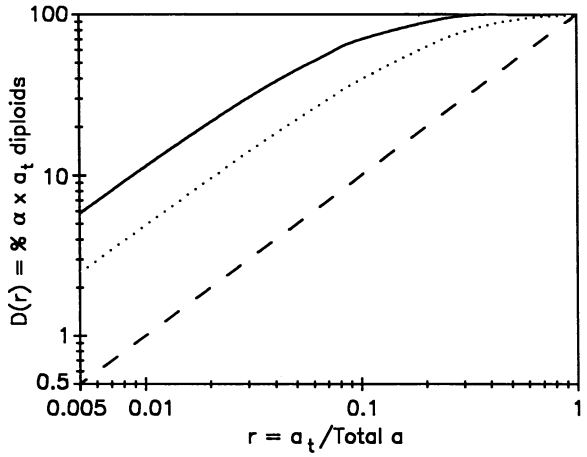


FIG. 4. Theoretical curves $D(r)$ for good competitor and noncompetitor $\mathbf{a}_c$ strains. The function $D(r)$ for a good competitor is $D(r) = E \times r$, and that for a noncompetitor is $D(r) = E \times (1 - e^{-rn})$. Two noncompetitor curves as shown with different values for n. The theoretical maximum value of n is 12. Note that the shapes of the two noncompetitor curves are similar and that only their displacement from the good competitor curves varies. ——, Noncompetitor, $n = 12$; ·····, noncompetitor, $n = 5$; —, good competitor.

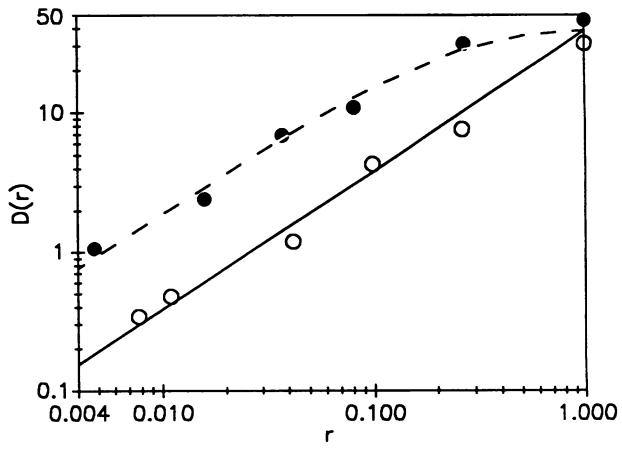


FIG. 5. Competition mating assay was performed with $\mathbf{a}_c$ strain SY972 *matα1* (●) or wild-type strain 7609-9-1 **a** (○) as described in the text. In this and further experiments, the values CI = 0.25 ± 0.06 for SY972 *matα1* and CI = 0.98 ± 0.19 for wild-type **a** strains (7609-1-1 or 7609-9-1) were obtained (mean and standard error [SE] of six determinations for each $\mathbf{a}_c$). ——, Theoretical noncompetitor curve, $E = 38.5\%$, $n = 5$; ——, theoretical good competitor curve, $E = 38.5\%$.

shown that in this range of r values, CI varies by less than 5% and can therefore be considered constant.) Hence, the CI is the expected value of $D(r)$ for a good competitor divided by the observed value of $D(r)$ for the particular $\mathbf{a}_c$ strain being tested. For all values of r and n, the model predicts that CI = 1 for a good competitor $\mathbf{a}_c$ strain (for example, if $\mathbf{a}_c$ is a wild-type **a** strain). For $r = 0.02$ and $n = 12$, the Poisson distribution predicts that approximately 90% of α cells adjacent to at least one $\mathbf{a}_t$ cell have only one neighboring $\mathbf{a}_t$ cell (as shown in Fig. 3), that approximately 10% have exactly two adjacent $\mathbf{a}_t$ cells, and hence that CI = 0.093 for a noncompetitive $\mathbf{a}_c$ strain. Although the theoretical maximum for n is 12, the model holds for any value of n greater than 1. For example, Fig. 4 shows the theoretically predicted curve for $n = 5$. The model predicts that for a noncompetitor, CI = 0.21 when $n = 5$.

Yeast cells can efficiently locate an appropriate mating partner. To determine whether these theoretical expectations hold in an actual mating experiment, we performed the competition mating assay using either a wild-type **a** or a *matα1* strain as $\mathbf{a}_c$. The wild-type $\mathbf{a}_c$ strain used was isogenic to the $\mathbf{a}_t$ strain, differing only by auxotrophic markers, so the two should be equivalent in ability to mate with the α strain. This $\mathbf{a}_c$ strain should therefore provide the experimental curve $D(r)$ for a good competitor, when an α cell mates with equal likelihood with an $\mathbf{a}_t$ or $\mathbf{a}_c$ cell. A *matα1* mutant strain was chosen as a standard noncompetitor since it expresses no **a**- nor α-specific genes (41, 50).

The results of this experiment are shown in Fig. 5. When $\mathbf{a}_c$ is wild type, the percentage of diploids formed between α and $\mathbf{a}_t$ cells [$D(r)$] is directly proportional to r. Thus, the experimental curve $D(r)$ for a wild-type $\mathbf{a}_c$ strain corresponded to that expected for a good competitor. The curve $D(r)$ obtained when $\mathbf{a}_c$ was the *matα1* strain was similar to that predicted for a theoretical noncompetitor with $n = 4$ to 5. Hence, the results obtained from competition mating experiments conformed reasonably well to those predicted by the theoretical model.

The value for n obtained in an actual experiment when $\mathbf{a}_c$ was the *matα1* strain was less than the theoretical maximum value of 12. A value of 12 for n predicts a CI of 0.09 for a noncompetitor, whereas we observed a slightly larger value (CI = 0.25 ± 0.06) corresponding to a value of $n = 4$ to 5 for the *matα1* strain. We considered two possible explanations for this deviation from the expected CI. First, it is possible that the *matα1* strain competes weakly and is therefore not an adequate standard for a noncompetitor. Although it does not express **a**- or α-specific gene products, a *matα1* strain does express the haploid-specific (mating type homozygous-specific) gene products, which could potentially play a role in competition. If so, then an **a**/α diploid strain, which does not express the haploid-specific genes, should have a lower CI than the *matα1* strain. To test this hypothesis, an **a** *ste14* strain and an **a** *ste2-10* strain (which are also noncompetitors like *matα1*; see Tables 5 and 6) as well as a wild-type **a** strain were transformed with a *MATα CEN* plasmid (YCp*MATα*). The transformed **a**(YCp*MATα*) strains all had nearly the same CIs as *matα1* (see Tables 5 and 6) (Fig. 1), indicating that haploid-specific genes have no effect on competition at the level of sensitivity of the assay. Therefore, we consider it unlikely that the *matα1* strain is a weak competitor. We favor an alternative explanation for the discrepancy between the expected and observed values for n, which is that the theoretical model is an oversimplification of the experimental situation. First, in a competition mating experiment, cell number does not remain constant but instead approximately doubles over the course of the mating period. Thus, many α cells will have at least one adjacent α-cell neighbor rather than having all **a**-cell neighbors, and neighbors will change position during the experiment. Second, for an α cell to have 12 **a**-cell neighbors, it must be in an interior layer of cells on the filter. Since the number of layers is between four and eight, 25 to 50% of the cells are in the top and bottom layers with, theoretically, only nine neighbors. Third, it is assumed in the theoretical model that the cells are packed uniformly. This assumption is probably not valid, since in an asynchronous population, the cells are not uniform in shape. We do not know how small distances between cells affect competition, and hence all neighbors may not be equally effective. These factors would result in a lower value for n than the

TABLE 2. Good competition is separate from fertility

Temp of mating (°C)	a_c	Mating efficiency[a]	CI ± SE[b]
34	Wild type **a**[c]	1.0	1.07 ± 0.45 (14)
34	SY972 *matα1*	0.00012	0.21 ± 0.064 (9)
34	*ste2-5 bar1* **a**[d]	0.000089	0.92 ± 0.27 (14)
34	*ste2-5 BAR1* **a**[e]	0.000071	0.39 ± 0.13 (5)
23	Wild type **a**[f]	1.0	1.01 ± 0.22 (4)
23	*scg1* **a**(YCp*DAF*1-1)	0.00039	0.96 ± 0.16 (3)

[a] Relative mating efficiency of the a_c strain with the wild-type α strain in the assay; each value is an average of three to nine determinations.
[b] Competition mating experiments were performed as described in Materials and Methods with wild-type strain 7609-5-3 or 7608-10-2 as the a_t strain and wild-type strain 7608-13-2, 7611-3, 7609-10-3, or 7609-6-4 as α. CI was calculated as described in the text and is expressed as the mean and SE of the number of trials indicated in brackets.
[c] Strain 7609-9-1 or 7623-16-3.
[d] Strain 3666-5 or 7623-16.4.
[e] Strain 7623-4-4.
[f] Strain 7413-3-3.

TABLE 3. Genes not required for good competition

a_c	CI ± SE[a]
4202-2-4 *bar1* **a**	1.58 ± 0.27 (6)
3271-19-3 *BAR1* **a**[b]	0.60 ± 0.11 (5)
3268-2-3 *sst2* **a**	0.64 ± 0.11 (6)
7609-5-3 *SST2* **a**[b]	0.69 ± 0.17 (6)
La192 **a**[c]	1.83 ± 0.31 (3)
W303-1A **a**[b]	2.54 ± 0.29 (3)

[a] See footnote *b* of Table 2. α strains used were 7608-7-3 or 7609-6-4 (lines 1 and 2), 7608-7-3 (lines 3 and 4), and 7608-13-2 (lines 5 and 6). a_t strains used were 7609-9-4 or 7608-1-3 (lines 1 and 2), 7609-9-4 (lines 3 and 4), and 7608-1-3 (lines 5 and 6).
[b] Each strain noted is isogenic to the mutant strain listed directly above it.
[c] La192 was isolated by J. Kurjan and P. Lipke in a search for strains defective in **a**-specific agglutinability.

theoretical maximum of 12, and therefore a higher CI for a noncompetitor than expected.

Since a *matα1* strain is a noncompetitor in the assay, we conclude that wild-type a_t and α cells can efficiently locate an appropriate mating partner during conjugation. This conclusion agrees with the results of the micromanipulation mating experiments in which a high mating efficiency was observed for isolated pairs of cells (see Introduction).

Good competition is separate from fertility. To use the competition mating assay to study the mechanism by which yeast cells select a mating partner, it is necessary to establish that this assay is not simply monitoring the ability of the a_c cells to mate. That the property of good competition is separate from the property of fertility is demonstrated by the fact that there are sterile strains which compete as well as a wild-type **a** strain in the competition mating assay: *ste2-5 bar1* and *scg1*(YCp*DAF*1-1). These strains were identified as sterile good competitors in the course of testing a large number of mating-defective **a** strains for their ability to compete (see below). *ste2-5* is a mutation in the α-pheromone receptor which confers a temperature-sensitive mating defect on **a** cells (26, 27). The competition mating assay was performed at 34°C with a wild-type **a**, *ste2-5 bar1* **a**, or *matα1* strain as the a_c strain (Table 2). As before, the *matα1* strain did not compete. However, the *ste2-5 bar1* strain was a good competitor, even though its mating efficiency was 10^{-4} that of the wild-type a_c strain. The *bar1* mutation was necessary to achieve good competition of *ste2-5 bar1* strains, since a *ste2-5 BAR1* strain was a poor competitor (Table 2).

The *scg1* mutation causes constitutive activation of the pheromone response pathway (14, 36) and therefore is lethal in a haploid. An *scg1*(YCp*DAF*1-1) strain is viable since the dominant *DAF1-1* mutation prevents haploid cells from arresting cell division in G_1 in response to activation of the mating pathway (12). This strain has a low mating efficiency and produces a high constitutive level of *FUS1* transcript (F. Cross, personal communication). We hypothesized that this strain might constitutively express a gene product(s) necessary for good competition and therefore be able to compete despite being sterile. This mutant strain did in fact compete as well as a wild-type **a** strain, yet mated with an efficiency 10^{-3} that of a wild-type strain. Finally, the *ste2-10*(YEp *MFA*2) strain, which mated at a frequency of $<10^{-5}$ relative to wild type, also competed as well as a wild-type **a** strain (see Table 7). The motivation for testing this strain is discussed below. All three mutant strains were sterile in that they did not form diploid progeny; furthermore, all three strains failed to form zygotes with wild-type α cells (data not shown). Thus, mating is blocked before cell fusion. These mutants which are able to compete but are unable to mate must be participating with the α cells in an early, pre-cell fusion step of conjugation that prevents the α cells from mating with the wild-type a_t cells. This early step of conjugation, defined by the competition mating assay, we term courtship.

Courtship ability of mating-defective a strains. A series of sterile or mating-defective **a** strains were tested in the competition mating assay for their courtship ability. In each experiment, the mutant **a** strain and its isogenic wild-type parent were tested as a_c strains (Table 3; see Tables 5 and 6).

Gene products whose absence makes **a** cells supersensitive to α-pheromone (*BAR1* and *SST2*) were not required for good competition. An *sst2-1* **a** strain competed as well as a wild-type **a** strain (Table 3). *sst2* cells mate at reduced efficiency (5% of wild-type level) and are defective in recovery from treatment with α-pheromone (8, 9, 15). *bar1* cells had a CI that was significantly greater than unity, indicating that they were slightly better competitors than wild-type **a** cells. In agreement with this result, a *ste2-5 bar1* strain was a better competitor than a *ste2-5 BAR1* strain (Table 2). *bar1* cells are unable to proteolytically degrade α-pheromone (11) but mated at the same efficiency as wild-type cells in the competition mating assay. Since a response to α-pheromone is necessary for good competition (see below) and *bar1* cells are more sensitive to α-pheromone (8), they may produce the response needed more quickly or to a greater degree than wild-type **a** cells.

The La192 **a** strain is defective in agglutination (Table 4) and represents the major complementation group of **a**-specific agglutination-defective mutants (A. Roy and J. Kurjan, personal communication). The agglutination index of La192 after induction with 10^{-7} M α-pheromone was 0.038, while the parent strain W303-1A had an agglutination index of 0.79 under similar conditions. Both these strains were good competitors (Table 3). Thus, courtship does not require agglutination of **a** and α cells. This result is interesting since the agglutinins are the only known mating-specific cell surface molecules (13) and would be in a good position to participate in cell-cell recognition processes.

Theoretically, the CI of a wild-type strain should be 1. Many of the wild-type a_c strains tested, however, had a CI that was reproducibly greater or less than 1 (e.g., Table 3, lines 2, 4, and 6). The results presented in Table 7 indicate

TABLE 4. Agglutinability of wild-type and mutant strains

Strain	α-Factor concn (M)	Agglutination index[a]
W303-1A **a**	0	0.0
	10^{-9}	0.28
	10^{-8}	0.55
	10^{-7}	0.79
La192 **a**	0	0.0
	10^{-9}	0.037
	10^{-8}	−0.076
	10^{-7}	0.038

[a] Agglutination assay and calculation of agglutination index are described in Mateirals and Methods.

that an $\mathbf{a}_c$ strain producing more **a**-pheromone than the $\mathbf{a}_t$ strain had a CI greater than 1. Perhaps the wild-type strains with CIs greater than 1 produce more **a**-pheromone than the $\mathbf{a}_t$ strain, while the strains which have CIs of less than 1 produce less **a**-pheromone. In fact, the wild-type strain SM1058 (which has a CI of 1.66 ± 0.61 [Table 5]) constitutively produces more **a**-pheromone in a halo assay than the wild-type strains 3271-19-3 and 7609-9-1 (which have CIs of 0.6 ± 0.11 and 0.88 ± 0.13, respectively [Table 3]) (data not shown).

Production of a-pheromone and response to α-pheromone are necessary in a cells for courtship. Four strains that fail to produce **a**-pheromone [*ste6*, *ste14*, *ram*(*ste16-1*), and *mfa1 mfa2*] were tested as $\mathbf{a}_c$ strains. All were noncompetitors (Table 5). *MFA1* and *MFA2* are the structural genes for **a**-pheromone, and the *mfa1 mfa2* strain contains deletions of both of these genes (4, 35). *RAM*(*STE16*) is necessary for the addition of palmitic acid to RAS proteins and may also be necessary for fatty acid modification of **a**-pheromone (1, 43). The *ram*(*ste16-1*) strain is temperature sensitive for growth and defective in **a**-pheromone production at all temperatures (59). The *STE6* gene product may be involved in secretion of **a**-pheromone (29a, 34), and the *STE14* gene product is thought to be involved in **a**-pheromone processing (7, 59). All these strains responded normally to α-pheromone but were sterile. These results imply that production of biologically active **a**-pheromone is necessary for courtship.

STE2 encodes a component of the α-pheromone receptor in **a** cells (3, 5, 26, 27, 32, 40). *ste2-10* is a deletion of this gene and is defective in all responses to α-pheromone tested

TABLE 5. **a**-Pheromone production is required for good competition

$\mathbf{a}_c$	CI ± SE[a]
YY609 *ste6***a**	0.22 ± 0.087 (10)
SY762 *STE6* **a**[b]	2.23 ± 0.71 (7)
SM1229 *mfa1 mfa2* **a**	0.24 ± 0.81 (14)
SM1188 *ste14* **a**	0.17 ± 0.069 (6)
H1171 *ram*(*ste16-1*) **a**	0.28 ± 0.028 (3)
SM1058 wild-type **a**[b]	1.66 ± 0.61 (14)
SM1188 *ste14* **a**(YCp*MAT*α)	0.21 ± 0.11 (6)
SM1188 *ste14* **a**(YCp50)	0.18 ± 0.062 (6)

[a] See footnote *b* of Table 2. The α strains used were 7608-13-2 or 7609-9-4 (lines 1 and 2), 7608-13-2 or 7609-5-2 (lines 3 to 6), and 7609-8-1 or 7611-1 (lines 7 and 8). The $\mathbf{a}_t$ strains used were 7609-5-3, 7609-9-4, or 5577-2-2 (lines 1 and 2), 5577-2-2, 7611-6, or 7609-9-4 (lines 3 to 6), and 7608-10-2 (lines 7 and 8).

[b] SY762 is a wild-type **a** strain isogenic to YY609, and SM1058 is a wild-type **a** strain isogenic to the three strains listed above it.

TABLE 6. Response to α-pheromone is necessary for good competition

$\mathbf{a}_c$	CI ± SE[a]
DJ213-6-3 *ste2-10* **a**	0.17 ± 0.038 (12)
7413-3-3 or 7609-9-1 *STE2* **a**	0.83 ± 0.26 (12)
4226-7-2 *ste4-3* **a** (23°C)	1.14 ± 0.020 (3)
4270-28 *ste5-3* **a** (23°C)	2.15 ± 0.25 (3)
7609-9-1 *STE4 STE5* **a** (23°C)	1.31 ± 0.26 (3)
4226-7-2 *ste4-3* **a** (34°C)	0.10 ± 0.015 (3)
4270-28 *ste5-3* **a** (34°C)	0.25 ± 0.066 (3)
7609-9-1 *STE4 STE5* **a** (34°C)	0.88 ± 0.13 (3)
7413-3-3 **a**(YCp50)	1.51 (2)
7413-3-3 **a**(YCp*MAT*α)	0.27 (2)
DJ213-6-3 *ste2-10* **a**(YCp50)	0.35 (2)
DJ213-6-3 *ste2-10* **a**(YCp*MAT*α)	0.26 (2)

[a] See footnote *b* of Table 2. The α strains used were 7608-13-2, 7609-8-1, or 7608-2-4 (lines 1 and 2) and 7608-13-2 (lines 3 to 12). The $\mathbf{a}_t$ strains used were 7609-5-3 or 7608-1-3 (lines 1 and 2) and 7609-5-3 (lines 3 to 12).

(28). The *ste2-10* strain was a noncompetitor in the assay (Table 6). *STE4* and *STE5* are necessary for response to α-pheromone (23). *STE4* may be the β subunit of the G protein mediating signal transduction (57), and *STE5* acts downstream of *STE4* in the signal transduction pathway (2). Both *STE4* and *STE5* were necessary in **a** cells for courtship (Table 6). These results imply that a response to α-pheromone is necessary in **a** cells for courtship. It is interesting that in contrast to *ste2-10*, the temperature-sensitive *ste2-5 bar1* **a** strain described above was a good competitor. This difference is not due to the *bar1* mutation, since in the competition mating assay, *ste2-10* and *ste2-10 bar1* $\mathbf{a}_c$ strains have identical CIs and are therefore equally poor competitors (data not shown). Hence, this result indicates that the *ste2-5* receptor is performing a function in providing the response necessary in **a** cells for courtship, even though it is incapable of providing enough function to permit mating.

ste2-10 and all the **a**-pheromoneless strains had similar CIs, suggesting that these strains are equally defective in courtship. However, these mutations are in different strain backgrounds. Since the wild-type parent strains have different CIs, it is clear that strain background differences influence the CI, so the CIs of mutants in different backgrounds cannot be directly compared. However, if we assume that all **a**/α strains are equivalent noncompetitors, then we can compare the CI of a mutant strain with that of an isogenic **a**/α strain. The assumption that all **a**/α strains are equally poor competitors is reasonable, since they express no haploid-, **a**-, or α-specific genes (41). *ste14* and *ste2-10* were transformed with a YCp*MAT*α plasmid to produce **a**/α strains isogenic to each mutant strain. The results for *ste14* (Table 5) and for *ste2-10* (Table 6) show that the CI for the isogenic **a**/α strain was similar to that for the untransformed mutant **a** strain in both cases. Therefore, both the **a**-pheromoneless strains and the receptorless *ste2-10* strain are indeed equally defective in courtship at the level of sensitivity of this assay.

The fact that production of **a**-pheromone as well as response to α-pheromone are both necessary in **a** cells for courtship implies that **a** cells must not only signal the α cell (with **a**-pheromone) but must also receive and then respond to the α-cell signal (α-pheromone) for effective courtship. Hence, one might say that there is a conversation between

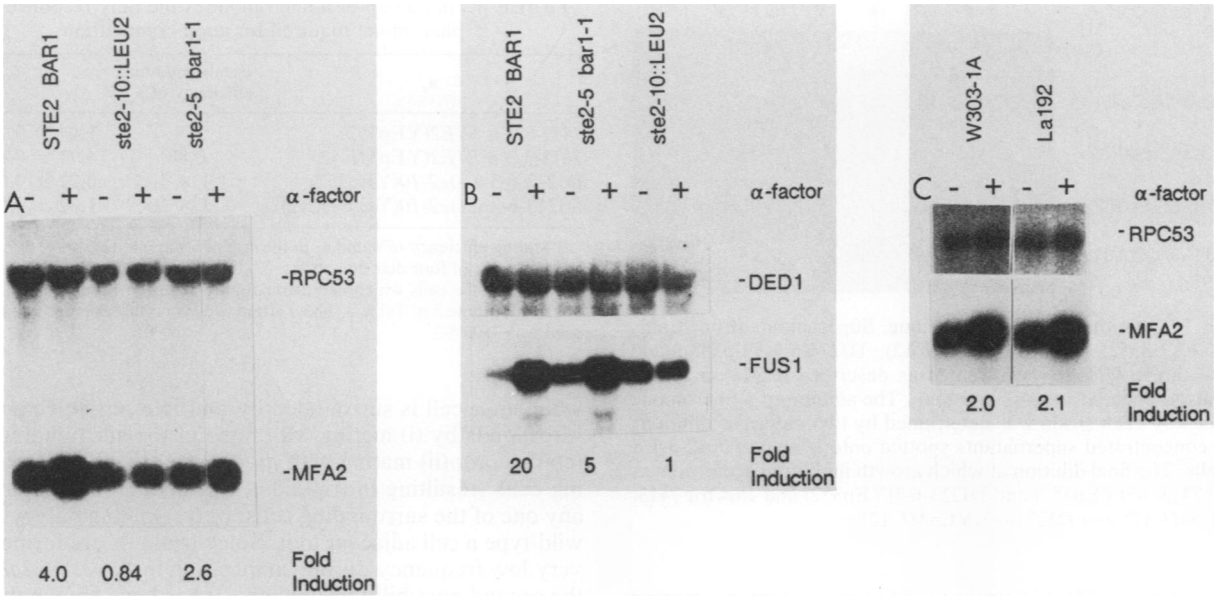


FIG. 6. Induction of FUS1 and MFA2 mRNA in **a** cells treated with α-pheromone. (A) Strains 7413-3-3 **a** (wild type), DJ213-6-3 **a** (*ste2-10*), and 3666-5 **a** (*ste2-5 bar1*) were treated with α-pheromone (+α-factor) or not treated (−α-factor) for 20 min at 34°C, and total RNA was isolated as described in Materials and Methods. ^{32}P-labeled linear DNA fragments containing either *MFA2* (4, 35) or *RPC53* (encoding a subunit of RNA polymerase III [44]) were used as probes (see Materials and Methods). *RPC53* was used as a control to quantify the amount of RNA loaded per lane. The positions of the *MFA2* and *RPC53* transcripts are indicated. The fold induction of *MFA2* mRNA in response to α-pheromone is shown for each strain. RNA was quantitated with a Quick Scan Jr. (Helena Laboratories). (B) RNA samples are identical to those described in panel A. The probes used were ^{32}P-labeled linear DNA fragments containing *FUS1* (33, 54) or *DED1* (52); the filter was first probed with *FUS1* and then stripped and reprobed with *DED1*, which was used to quantify the amount of RNA per lane. The constitutive level of *FUS1* transcript for strains *ste2-5 bar1* and *ste2-10*::*LEU2* was approximately fivefold higher than that of the wild-type strain. In other experiments, the constitutive level was similar to that of wild type; the reason for this variability is not known but may be due to low levels of pheromone present in the cultures before treatment with α-pheromone owing to low-frequency heterothallic mating-type switching. (C) Strains W303-1A and La192 were treated with α-pheromone or not treated, and RNA was isolated as described in Materials and Methods. The *MFA2* and *RPC53* probes are described in the legend to panel A, and the positions of the transcripts are indicated. The fold induction of *MFA2* mRNA in response to α-pheromone is shown.

a and α cells during courtship. These results raise the question of what response must be made by the **a** cells during courtship.

High-level expression of a-pheromone is necessary for good competition. What response to α-pheromone is necessary for good competition? It is known that in **a** cells, production of **a**-pheromone is increased in response to α-pheromone (51). Since production of **a**-pheromone is necessary for good competition, we hypothesized that the response to α-pheromone necessary for good competition is induction of **a**-pheromone. A prediction of this hypothesis is that the *ste2-5 bar1* strain (which is a good competitor) can be induced to increase expression of its **a**-pheromone, whereas the receptor deletion *ste2-10* strain would be unable to do so. There is evidence that transcription of the **a**-pheromone genes is induced by α-pheromone since they contain a consensus sequence that has been shown to mediate pheromone induction (4, 29, 55). Also, it has been shown that in a strain carrying an *MFA2-lacZ* fusion gene, β-galactosidase is induced three- to fivefold (K. Kubo, S. Michaelis, and I. Herskowitz, personal communication). As a first step in testing the hypothesis given above, we performed Northern analysis on the *ste2-5 bar1*, *ste2-10*, and wild-type **a** strains with and without treatment with α-pheromone. Both *FUS1* and *MFA2* transcripts were induced in the wild-type and *ste2-5 bar1* **a** strains but not in the *ste2-10* strain (Fig. 6A and B). In addition, the agglutination-defective La192 strain which is also a good competitor showed normal induction of *MFA2* (Fig. 6C). These results are consistent with the hypothesis.

A second prediction of the hypothesis is that the receptor deletion strain (*ste2-10*) which is a noncompetitor would become a good competitor if it was engineered to produce a high level of **a**-pheromone. To test this prediction, we transformed a *ste2-10* strain with a 2μm plasmid carrying the *MFA2* gene (*YEpMFA2*). This strain overproduced **a**-pheromone constitutively at a level four- to eightfold above the constitutive level of a wild-type **a** strain, determined by halo assay and by the dilution endpoints of supernatants (Fig. 7). This level of **a**-pheromone production is similar to the reported level of **a**-pheromone produced by α-pheromone-treated **a** cells (51). The *ste2-10*(YEp*MFA2*) strain was as good a competitor as the isogenic wild-type **a** strain (Table 7). Since the receptor deletion strain *ste2-10*(YEp*MFA2*) is incapable of responding to α-pheromone, we conclude that induction of expression of **a**-pheromone is the only response to α-pheromone necessary in wild-type **a** cells for good competition when $\mathbf{a}_t$ is wild type.

Since good competition is manifest by a decrease in mating efficiency between the wild-type α and $\mathbf{a}_t$ cells in the competition mating assay, it is possible that the *ste2-10*(YEp*MFA2*) cells inhibit the mating of the α and $\mathbf{a}_t$ cells by some means other than interacting directly with the α cells. The *ste2-10*(YEp*MFA2*) strain (apparently a good competitor) differs from the *ste2-10* strain (a noncompetitor) only in that it overproduces **a**-pheromone. Hence, the only way that

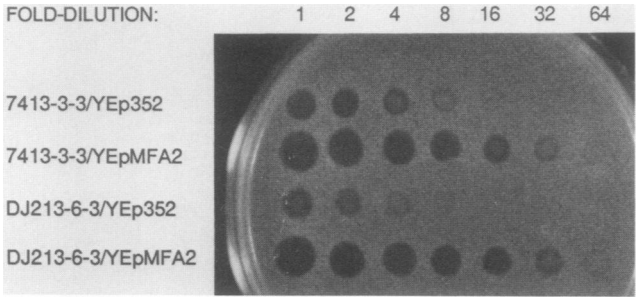


FIG. 7. Quantitation of **a**-pheromone. Supernatants from strains 7413-3-3(YEp352), 7413-3-3(YEp*MFA2*), DJ213-6-3(YEp352), and DJ213-6-3(YEp*MFA2*) were treated as described in Materials and Methods to concentrate **a**-pheromone. The amount of **a**-pheromone produced by each strain was determined by twofold serial dilutions of the concentrated supernatants spotted onto a lawn of 7612-3-1 α *sst2* cells. The final dilution at which growth inhibition occurred was 1/8 for 7413-3-3(YEp352) and DJ213-6-3(YEp352) and 1/64 for 7413-3-3(YEp*MFA2*) and DJ213-6-3(YEp*MFA2*).

the *ste2-10*(YEp*MFA2*) strain could indirectly inhibit mating is if excess **a**-pheromone inhibits mating. This explanation is not true, however, since the mating efficiency between α and the wild-type $\mathbf{a}_c$ strain carrying YEp*MFA2* (which also overproduces **a**-pheromone [Fig. 7]) is the same as that between α and the same strain carrying YEp352 (Tables 7 and 8).

Of all the $\mathbf{a}_c$ strains that we tested in the competition mating assay, those which produced less **a**-pheromone than the $\mathbf{a}_t$ strain were poor competitors, whereas those $\mathbf{a}_c$ strains which produced the same level of **a**-pheromone as the $\mathbf{a}_t$ strain were good competitors. We propose that the ability of an $\mathbf{a}_c$ strain to compete is determined solely by the level of **a**-pheromone that it produces relative to the $\mathbf{a}_t$ strain. If this hypothesis is correct, we predict that an $\mathbf{a}_c$ strain producing more **a**-pheromone than the $\mathbf{a}_t$ strain will be a super-competitor with a high CI. The 7413-3-3 **a** *STE2*(YEp*MFA2*) strain probably produces a higher α-pheromone-induced level of **a**-pheromone than a wild-type **a** strain. We have not shown this directly, but we have shown that in response to α-pheromone this strain produces a much higher level of *MFA2* transcript than a wild-type **a** strain (data not shown). Hence, we postulate that the 7413-3-3 **a** *STE2*(YEp*MFA2*) strain is an **a**-pheromone-hyperinducing strain. Consistent with the hypothesis given above, the CI obtained when the challenger $\mathbf{a}_c$ strain was the hyperinducing strain was considerably greater than unity, indicating that it is a better competitor than a wild-type $\mathbf{a}_c$ strain (Table 7, line 2). A further prediction of the hypothesis is that a wild-type strain would be a poor competitor when $\mathbf{a}_t$ is the hyperinducing strain. This second prediction was also borne out (Table 8). When $\mathbf{a}_t$ was the **a**-pheromone-hyperinducing strain [7413-3-3 **a** *STE2*(YEp*MFA2*)], a wild-type $\mathbf{a}_c$ strain (expressing the normal level of **a**-pheromone) was a poor competitor, whereas this same wild-type $\mathbf{a}_c$ strain was a good competitor when $\mathbf{a}_t$ produced the normal level of **a**-pheromone [i.e., when $\mathbf{a}_t$ was 7413-3-3 **a** *STE2*(YEp352)].

TABLE 7. Induction of **a**-pheromone is the only response to α-pheromone required for good competition

$\mathbf{a}_c$	Relative mating efficiency of $\mathbf{a}_c$[a]	CI ± SE[b]
7413-3-3 **a** *STE2*(YEp352)	1	1.44 ± 0.15 (5)
7413-3-3 **a** *STE2*(YEp*MFA2*)	0.88	4.71 ± 0.89 (5)
DJ213-6-3 **a** *ste2-10*(YEp352)	$<1.1 \times 10^{-5}$	0.21 ± 0.034 (6)
DJ213-6-3 **a** *ste2-10*(YEp*MFA2*)	$<1.1 \times 10^{-5}$	1.32 ± 0.060 (5)

[a] Mating efficiency of α and $\mathbf{a}_c$ in the competition mating assay. Each value is an average of four determinations. Mating efficiency was calculated as the percentage of α cells which had formed diploids. 1 represents 32%.

[b] See footnote *b* of Table 2. The α strain used was 7609-6-4, and the $\mathbf{a}_t$ strain used was 7609-5-3.

DISCUSSION

Yeast cells efficiently locate a partner during mating. In a mating mixture, when α cells are surrounded by two different types of **a** cells, wild-type **a** cells and sterile **a** cells, there are formally four possible outcomes. It could be that when an α cell is surrounded by multiple possible partners, it responds by (i) mating with none of the surrounding cells (confusion); (ii) mating with more than one of the surrounding cells (resulting in triploids); (iii) mating at random with any one of the surrounding cells; or (iv) mating only with the wild-type **a** cell adjacent to it. Since triploids are formed at a very low frequency during conjugation in *S. cerevisiae* (45), the second possibility is unlikely. It has been shown that the fourth possibility is the actual outcome when the sterile strain is *ste2*, *ste4*, *ste5*, or *mfa1 mfa2* (23, 35). Our results confirmed this conclusion (Tables 2 and 7; data not shown). This result can be explained by one of two models. In the first model, α cells cannot distinguish between wild-type and sterile **a** cells as mating partners, but those α cells that initiate mating with a sterile **a** cell are unable to complete the mating process. In the second model, α cells are able to distinguish between the wild-type and sterile **a** cells and only initiate mating with a wild-type **a** cell. We used the competition mating assay to distinguish these models. In this assay, α cells were challenged to mate with a mixture of $\mathbf{a}_t$ cells (usually wild type) and $\mathbf{a}_c$ cells which were sterile or mating defective. The number of diploids between α and $\mathbf{a}_t$ cells was monitored, thereby assessing the ability of the $\mathbf{a}_c$ cells to compete with the wild-type $\mathbf{a}_t$ cells for recognition by the α cells. When $\mathbf{a}_c$ was the sterile *matα1* strain, mating between the α and $\mathbf{a}_t$ cells was much greater than when $\mathbf{a}_c$ was wild type. This result implies that an α cell is able to select a wild-type $\mathbf{a}_t$ cell as a mating partner (or that an $\mathbf{a}_t$ cell is able to select an α cell). The *matα1* cells were not recognized as mating partners. Thus, in a mass mating experiment, as in the micromanipulation mating experiments described above (see Introduction), wild-type cells can efficiently locate an appropriate mating partner.

In both the competition mating experiments and the micromanipulation mating experiments, yeast cells of opposite mating type were placed together on a solid support. Since yeast cells are nonmotile, we assume that they were unable to move during these mating experiments. The fact that yeast cells can efficiently form diploids under these conditions implies that there is not a predetermined site for cell fusion on the surfaces of the cells. This situation is in contrast to that for *Chlamydomonas* species, in which cell fusion during mating occurs at a particular site between the bases of the flagella (39). Since *Chlamydomonas* cells are motile, having a predetermined site does not preclude a high mating efficiency, as it would in yeasts.

Courtship. Most sterile strains that we tested as $\mathbf{a}_c$ strains in the competition mating assay had the same phenotype as *matα1*; they did not prevent the wild-type α and $\mathbf{a}_t$ cells from efficiently forming diploids. However, a small class of sterile mutants had a phenotype identical to that of a wild-type **a**

TABLE 8. Wild-type **a** is a noncompetitor when $\mathbf{a}_t$ overexpresses **a**-pheromone

$\mathbf{a}_t$ strain	$\mathbf{a}_c$ strain	CI ± SE[a]	E^b
7413-3-3 **a**(YEp*MFA2*)	7413-3-3 **a** *STE2*	0.195 ± 0.021 (3)	46.2
7413-3-3 **a**(YEp352)	7413-3-3 **a** *STE2*	0.580 ± 0.026 (3)	48.3
7413-3-3 **a**(YEp*MFA2*)	DJ213-6-3 **a** *ste2-10*	0.089 ± 0.02 (3)	51.4
7413-3-3 **a**(YEp352)	DJ213-6-3 **a** *ste2-10*	0.115 ± 0.017 (3)	47.9

[a] See footnote *b* of Table 2. The α strain used was 7636-6-1.
[b] Mating efficiency of the α with the $\mathbf{a}_t$ strain in the competition mating assay (expressed as a percentage of α cells) when $r = 1$ (see Materials and Methods).

strain when used as the $\mathbf{a}_c$ strain in the competition mating assay. When the $\mathbf{a}_c$ strain was one of these sterile strains, the mating efficiency between the α and $\mathbf{a}_t$ strains was reduced to the same level as when $\mathbf{a}_c$ was a wild-type strain. We propose that these sterile cells which are good competitors are participating in an early step of conjugation that precludes mating of the α cell with the $\mathbf{a}_t$ cell but that a subsequent step of the mating process is blocked. Hence, the existence of sterile good competitors defines a step of conjugation which we term courtship. This conclusion implies that there is a direct interaction between an α cell and a sterile competitive $\mathbf{a}_c$ cell during courtship. However, it could be that these sterile competitive $\mathbf{a}_c$ strains cause a decrease in the efficiency of mating between the α and $\mathbf{a}_t$ cells through the production of an inhibitor of mating. The results in Table 7 argue against this interpretation. The *ste2-10*(YEp*MFA2*) strain was a good competitor, whereas the *ste2-10*(YEp352) strain was a noncompetitor. Yet these two strains differ only in the amounts of **a**-pheromone that they produce. That overproduction of **a**-pheromone does not inhibit mating was shown by the fact that a wild-type strain carrying YEp352 had the same mating efficiency as the same strain carrying YEp*MFA2* (Tables 7 and 8). We conclude that the decrease in mating efficiency between the α and $\mathbf{a}_t$ cells caused by the $\mathbf{a}_c$ strain *ste2-10*(YEp*MFA2*) is caused by an interaction between α cells and these $\mathbf{a}_c$ cells. A second comparison also supports this conclusion. A *ste2-5 bar1* strain was a good competitor, whereas a *ste2-5 BAR1* strain was a poor competitor (although not as poor as *ste2-10* or *matα1*). This difference was not the result of the *bar1* mutation in the $\mathbf{a}_c$ strain acting through the production of an inhibitor to reduce the mating efficiency of the α with the $\mathbf{a}_t$ strain, since *ste2-10 bar1* and *ste2-10 BAR1* strains both have the same CI (data not shown).

It would be interesting to study the nature of the courtship interaction. We have microscopically examined mating mixtures containing a wild-type α strain and a sterile competitive **a** strain [*ste2-10*(YEp*MFA2*)] and were unable to detect any association between the **a** and α cells (data not shown). Thus, courtship does not appear to be due to a stable association of the α cells and these courtship-proficient **a** cells. It would be interesting to observe a single α cell surrounded by **a** cells and to monitor the position of intracellular structures (such as the spindle pole body) or a cell-associated molecule (such as chitin, acid phosphatase, the **a** agglutinin, or FUS1 protein) that is known to be localized during the mating response (21, 46, 54, 56). Perhaps courtship involves reorganization of certain intracellular components.

The α-pheromone-induced level of a-pheromone is necessary in a cells for courtship. To determine the genetic components necessary in **a** cells for courtship, we tested a series of mating-defective **a** strains in the competition mating assay. Production of **a**-pheromone is necessary for courtship since the strains *ste6*, *ste14*, *ram*, and *mfa1 mfa2* which are defective in production of **a**-pheromone (7, 35, 59) were defective in courtship. A response to α-pheromone is necessary for courtship since a strain with a deletion of the α-pheromone receptor gene (*ste2-10*) and strains *ste4* and *ste5* which are defective in transduction of the pheromone-generated signal (2, 23, 57) were defective in courtship. We demonstrated that the only response to α-pheromone necessary for courtship is induction of **a**-pheromone by the fact that a *ste2-10* strain carrying the plasmid YEp*MFA2* which is receptor defective but constitutively produces a high level of **a**-pheromone (similar to the α-pheromone-induced level of **a**-pheromone) is proficient in courtship.

The identification of three sterile strains that were proficient in courtship [*ste2-5 bar1*, *ste2-10*(YEp*MFA2*), and *scg1*(YCp*DAF*1-1)] established that courtship is a step in the process of mating separable from conjugation itself. The first two (and probably the third also) produced a high induced or constitutive level of **a**-pheromone. The *ste2-5 bar1* strain displayed near-normal transcriptional induction of **a**-pheromone in response to α-pheromone, and the *ste2-10*(YEp *MFA2*) strain constitutively expressed a high level of **a**-pheromone. The *scg1*(YCp*DAF*1-1) strain expresses the *FUS1* transcript constitutively at a high level (F. Cross, personal communication), and since induction of all pheromone-inducible genes probably occurs by the same pathway (29, 55), this strain probably produces **a**-pheromone constitutively at a high level.

Is production of an induced level of **a**-pheromone sufficient for courtship? Our results showed that the only response to α-pheromone necessary for courtship is production of the α-pheromone-induced level of **a**-pheromone. However, there might be other constitutively expressed gene products that are necessary for courtship. Whether production of **a**-pheromone by an **a** cell is the only **a**-specific (haploid-specific) gene product necessary for courtship could be determined by expressing **a**-pheromone in a *matα1* (**a**/α) strain. However, since *MFA1*, *MFA2*, *STE6*, and perhaps *STE14* are **a**-specific genes (35, 58), this experiment would require expressing all these genes under non-cell-type-regulated promoters in a *matα1* (**a**/α) strain.

Mechanism of courtship. We performed three types of competition mating experiments in which the two **a** strains produced (1) no **a**-pheromone versus wild-type induced level (e.g., when $\mathbf{a}_c$ was *mfa1 mfa2* and $\mathbf{a}_t$ was wild type); (2) wild-type uninduced level versus wild-type induced level (e.g., when $\mathbf{a}_c$ was *ste2-10* and $\mathbf{a}_t$ was wild type); and (3) wild-type induced level versus overexpressed induced level [e.g., when $\mathbf{a}_c$ was wild type and $\mathbf{a}_t$ was the **a**-pheromone-hyperinducing strain 7413-3-3(YEp*MFA2*)]. In each case, the **a** cell producing the higher amount of **a**-pheromone was the preferred mating partner.

It is likely that the α cell is playing an active role in choosing a mating partner during courtship, since the recep-

tor deletion *ste2-10*(YEp*MFA2*) strain is able to court but is completely unable to respond to the presence of the α cell (by all criteria that currently exist). If so, the data summarized above show that an α cell selects the **a** cell producing the highest level of **a**-pheromone as a mating partner. The results of experiment 2 above show that α cells can distinguish between **a** cells which produce the constitutive and the α-pheromone-induced levels of **a**-pheromone. It has been reported that this difference is approximately 10-fold (51). Furthermore, α cells distinguish these two types of **a** cells as well as they distinguish between a wild-type **a** cell and one producing no **a**-pheromone at all (at the level of sensitivity of the assay). Experiment 3 rules out the possibility that any cell producing **a**-pheromone at or above the normal α-pheromone-induced level is equally proficient in courtship. It would be interesting to determine whether there is a saturating concentration of **a**-pheromone above which discrimination is no longer possible. Presumably, this would occur at a concentration of **a**-pheromone at which the receptors on α cells are fully occupied. The concentration of α-pheromone at which receptors on **a** cells are fully occupied has been determined (26, 27), but a similar analysis has not been done for **a**-pheromone.

How does an α cell choose the **a** cell producing the highest level of **a**-pheromone? One model is that an α cell detects a gradient of **a**-pheromone in the surrounding medium and orients its morphogenesis in the direction of highest **a**-pheromone concentration. **a**-Pheromone is a hydrophobic peptide with the hydrophobic farnesyl group attached to its C-terminal cysteine residue (1), and it is not secreted into culture medium at high levels (51). Thus, **a**-pheromone is not highly soluble in the aqueous environment of yeast cells and so perhaps a substantial portion of the **a**-pheromone produced by an **a** cell remains cell associated. The hydrophobic nature of **a**-pheromone suggests an alternative to the gradient of diffusible **a**-pheromone as a model for partner selection. **a**-Pheromone bound to an **a** cell (presumably to the cell membrane) is transmitted directly to the α cell through the juxtaposed cell walls. Studies of oriented morphogenesis and courtship of α cells in a gradient of purified **a**-pheromone will help to distinguish these models.

Michaelis and Herskowitz (35) showed that when α cells were challenged to mate with a mixture of wild-type and **a**-pheromoneless **a** cells, the α cell mated only with the wild-type **a** cells. Our results suggest that this is because an α cell chooses the **a** cell producing the highest level of **a**-pheromone as a mating partner. It is interesting that adding exogenous **a**-pheromone to a mixture of α and *mfa1 mfa2* cells does not rescue the mating defect of the sterile *mfa1 mfa2* cells (35). It could be that an α cell is unable to choose a mating partner at all if there is no gradient of **a**-pheromone in its environment. Alternatively, it could that the level of **a**-pheromone added back in these experiments was not sufficient for mating to occur.

This study focused on the mechanism by which an α cell chooses an **a**-cell mating partner. It would be interesting to perform reciprocal experiments to study the mechanism by which an **a** cell chooses an α-cell partner and, in particular, to determine whether production of α-pheromone is important for this process. We attempted to perform the reciprocal competition mating experiments in which a small number of **a** cells were challenged to mate with a mixture of wild-type and sterile α cells. However, an analysis similar to that described here was complicated by the fact that increasing the number of wild-type α cells present in a mating mixture decreased the mating efficiency between wild-type **a** and α cells. These results are consistent with previous reports that a high concentration of α-pheromone inhibits mating (47). Hence, in the reciprocal competition mating experiments, E varies with r, but for the theoretical model to valid, it is essential that E be constant with respect to r. We are now attempting to develop alternative approaches to studying the mechanism by which an **a** cell chooses an α-cell mating partner.

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