Exploratory polarization facilitates mating partner selection in Saccharomyces cerevisiae

Manuella Clark-Cotton, Nicholas Henderson, Michael Pablo, Debraj Ghose, Timothy Elston, and Daniel Lew*

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Review Timeline:

	Submission Date:	2021-02-10
	Editorial Decision:	2021-02-17
	Revision Received:	2021-02-23
	Accepted:	2021-03-01

Editor-in-Chief: Matthew Welch

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

RE: Manuscript #E21-02-0068
TITLE: "Exploratory polarization facilitates mating partner selection in Saccharomyces cerevisiae"

Dear Danny,

Thank you for submitting your interesting paper to MBoC. I have reviewed it and read the reviewers' comments and your rebuttal. I think that you have addressed most of the comments convincingly and will be happy to see a revised version that addresses the few points listed below.

Best wishes,
Sophie

Small data analysis addition:
Line 190: to support the statement that rsr1Δ cdc24-1 mutants exhibit constitutively mobile sites in mating mixes, it would be very helpful to use the same auto-correlation analysis on these mutants as you did on the WT cells.

I would encourage you to add to the manuscript the data presented in the rebuttal letter in response to reviewer 2. I find your findings that exploratory polarization happens also in cells treated with homogeneous low levels of pheromone important. Adding these data to your manuscript would further strengthen the support for the exploratory polarization model by showing that the dynamic behaviour is caused by pheromone and happens irrespective of gradient sensing. It also offers a further similarity to the situation in S. pombe.

Text/figure edits:
Line 53: ... even when surrounded by *many/several* potential partners,...

Line 97: The finding that polarity sites are only unstable at low pheromone levels was already shown in Bendezu and Martin, 2013.

Line 208: It would be helpful to the non-specialist reader to briefly explain the halo assay in the text.

Line 247 and following: I understand the reasoning for treating cdc24-4 ste20ΔCRIB cells with alpha-factor, but I don't understand whether this is what is shown in Fig 6: Fig 6 legend does not state addition of alpha-factor and in the text the paragraph describing Fig 6 is separate from the explanation of addition of alpha-factor. If Fig 6 is without alpha-factor addition, then I am not sure how different it is from Fig S6.

Line 266: I understood from your answer to reviewer 1 why it is necessary to treat the MT-GFP-CDC24-38A with alpha-factor as these will otherwise arrest in G2. However, this reasoning is not apparent in the text. It would be good to explain it to justify the addition of alpha-factor.

Fig 2A: Could you add a Bem1 label to ease figure reading?

Fig 4: You can save on white space in this figure.

Fig 5: Panel A is presented in reverse of the time - the cell appears to shrink !!

Fig S1: In the Ste3-sfGFP example shown, the decision to attribute the -16 timepoint to colocalized but -14 to not colocalized seems rather arbitrary, and similarly for timepoints -32 to -22 in the Ste6-sfGFP example. I feel that, at least in some cases, the lack of colocalization is likely attributable to the very low Ste6 signal rather than its presence elsewhere. I am well aware that these quantifications are difficult and that your main point is that there is colocalization at least some of the time. However, you could add in legends or methods that there is a certain level of subjectivity in deciding whether signals are colocalized or not.

Fig S3: The legend is very confusing. It states the figure focuses on the green WT cells, but the headers of panels A and B state WT cells as MATa BEM1-tdT. There are also two legends for panel B.

Fig S6: Is the imaging at 37C as stated in the legend or 35C as stated in the text?

Sophie Martin

Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Lew,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us immediately at mboc@ascb.org.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Response to Editor Comments:

We thank Dr. Martin for her speedy and thorough comments!

Small data analysis addition:
Line 190: to support the statement that rsr1Δ cdc24-1 mutants exhibit constitutively mobile sites in mating mixes, it would be very helpful to use the same auto-correlation analysis on these mutants as you did on the WT cells.
We added this analysis (because these cells have Bem1-GFP instead of Bem1-tdTomato, we also analyzed control wildtypes with Bem1-GFP: new Fig. 4C,D). As expected, spatial autocorrelation remained low for the constitutively mobile mutant polarity sites. We slightly rearranged the text and supplementary Figures to accommodate this addition.

I would encourage you to add to the manuscript the data presented in the rebuttal letter in response to reviewer 2. I find your findings that exploratory polarization happens also in cells treated with homogeneous low levels of pheromone important. Adding these data to your manuscript would further strengthen the support for the exploratory polarization model by showing that the dynamic behaviour is caused by pheromone and happens irrespective of gradient sensing. It also offers a further similarity to the situation in S. pombe.
We added those data as Fig. S1B, along with new text (lines 130-134).

Text/figure edits:
Line 53: ... even when surrounded by *many/several* potential partners,...
Fixed

Line 97: The finding that polarity sites are only unstable at low pheromone levels was already shown in Bendezu and Martin, 2013.
Fixed

Line 208: It would be helpful to the non-specialist reader to briefly explain the halo assay in the text.
Added (now lines 217-221)

Line 247 and following: I understand the reasoning for treating cdc24-4 ste20ΔCRIB cells with alpha-factor, but I don't understand whether this is what is shown in Fig 6: Fig 6 legend does not state addition of alpha-factor and in the text the paragraph describing Fig 6 is separate from the explanation of addition of alpha-factor. If Fig 6 is without alpha-factor addition, then I am not sure how different it is from Fig S6.
Apologies for the omission. Fig. 6 Legend now states “10 μM α-factor was added to (B) and (C) to sustain G1 arrest of mutant MAT**a** cells.”

Line 266: I understood from your answer to reviewer 1 why it is necessary to treat the MT-GFP-CDC24-38A with alpha-factor as these will otherwise arrest in G2. However, this reasoning is not apparent in the text. It would be good to explain it to justify the addition of alpha-factor.
Added (280-282)

Fig 2A: Could you add a Bem1 label to ease figure reading?
Added

Fig 4: You can save on white space in this figure.
We have added the new spatial autocorrelation data for *cdc24-m1 rsr1* mutants in that white space.

Fig 5: Panel A is presented in reverse of the time - the cell appears to shrink !!
Fixed. Thank you for catching that!!

Fig S1: In the Ste3-sfGFP example shown, the decision to attribute the -16 timepoint to colocalized but -14 to not colocalized seems rather arbitrary, and similarly for timepoints -32 to -22 in the Ste6-sfGFP example. I feel that, at least in some cases, the lack of colocalization is likely attributable to the very low Ste6 signal rather than its presence elsewhere. I am well aware that these quantifications are difficult and that your main point is that there is colocalization at least some of the time. However, you could add in legends or methods that there is a certain level of subjectivity in deciding whether signals are colocalized or not.
Added to Fig. S1 Legend

Fig S3: The legend is very confusing. It states the figure focuses on the green WT cells, but the headers of panels A and B state WT cells as MATa BEM1-tdT. There are also two legends for panel B.
Apologies for the confusion. The Legend has been fixed.

Fig S6: Is the imaging at 37C as stated in the legend or 35C as stated in the text?
It is at 37C as stated. The 35C refers to the experiment with added alpha factor, not the experiment in Fig. S6.

RE: Manuscript #E21-02-0068R
TITLE: "Exploratory polarization facilitates mating partner selection in Saccharomyces cerevisiae"

Dear Danny,

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

Best wishes,
Sophie

Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Lew:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

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