**Author response**

Proceedings B Manuscript ID RSPB-2014-1025

"Stress-Induced Mutagenesis and Complex Adaptation"

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7/17/2014 10:24 AM

We are grateful to the editors and reviewers for their thorough consideration of our manuscript and constructive recommendations for revision.

In response to the comments raised by the editor and the reviewers we have made major changes to the manuscript. Briefly, we discuss model assumptions which were not discussed before: the use of a threshold relationship between fitness and mutation rate; the possible effect of single mutants having intermediate fitness; the plausibility that low fitness can induce mutagenesis. We also compare our results with those of other authors that studied the trade-off between *adaptability* and *adaptedness*.

Following is the review, complete with our responses to each of the issues (line numbers in the form of L# refer to the original manuscript, the form M# refer to the revised manuscript):

**Response to Editor comments:**

*First, I'd like to see one or two sentences outlining that it is reasonable to consider low fitness -- arising from a maladapted genotype (in reality, aren't almost all genotypes maladapted?) rather than a potentially mutagenic environmental stress like starvation -- as a stress that might induce SIM. I see one reference on this point, but it would be nice to note explicitly any evidence that comes from a bacterial system.*

We added a few sentences to the model section (L132):

"Evidence shows that numerous stress responses induce mutagenesis in bacteria [16,32]. These responses can be activated due to deteriorating environmental conditions (see section 3.5) or due to mutations that impair important cell functions, thereby reducing fitness and inducing a stress response. For example, a frameshift mutation in the lac gene causes cells to starve on lactose, thus inducing mutagenesis via a stress response [53]."

*Second, and related, I'd like to see some justification for modeling SIM as 'all or nothing' rather than as a function of a genotype's fitness decrement from the optimum. Lenski's group recently published a paper predicting that fitness may never be reached, even during adaptation to a simple constant environment (Wiser, M. J., N. Ribeck, and R. E. Lenski. 2013. Long-term dynamics of adaptation in asexual populations. Science 342:1364–1367). If this result is at all general, does it mean we should think that SIM will always be induced?*

First, SIM is modeled as an 'all or nothing', but the threshold doesn't occur at the optimum but rather at the wildtype fitness. We changed the definition of SIM to reflect this (L138):

"The main analysis assumes that the SIM induces mutagenesis in individuals less fit than the wildtype, that is, the mutation rate *U* of individuals with fitness *ω* is…"

Therefore, if adaptation proceeds in a constant environment then we expect that mutagenesis will be induced in individuals that have below average fitness.

Second, we already relaxed the assumption of a threshold strategy in the previous submission in Appendix E but unfortunately forgot to refer to it in the main text. Briefly, we modeled continuous relationships between fitness and mutation rate based on those proposed by Agrawal (2002) and more recently Shaw & Baer (2011). We found that because the most important effects on the adaptation rate are the mutation rates of wildtype and single mutants, the continuous SIM is very similar to a threshold SIM with comparable mutation rates in single mutants. Therefore, even if threshold relationships ('all or nothing') are not realistic, they are a good approximation. We now refer to these continuous SIM in the model section (L144):

"The assumption of a threshold relationship between fitness and the mutation rate in eq. 1 is relaxed in Appendix E in which we explore continuous relationships between fitness and the mutation rate. The results are robust to this relaxation (see section 3.3)."

And also in the results section (L265):

"The adaptation rate of SIM with continuous relationships between fitness and mutation rate (Online Appendix E) is comparable to that of SIM with threshold relationships (Figure E2). This is because the main factor determining the adaptation rate is the mutation rates of the wildtype and the single mutants (ab, aB, and Ab), as individuals with more than a single mutation do not have a significant contribution to adaptation. Therefore, our results are robust to the choice of the relationship between fitness and the mutation rate. "

We also revised the text based on all minor comments:

* Refs in numbers in L50 and L31
* Changed to "…simulation results…" in L228
* Fig. 1 legend: note on x-axis jitter
* Fig. 1 x-label: changed to "Number of mutations"
* Figs. 2,3: fixed typo in x-label

**Response to Reviewer II comments:**

*\* What happens in the case where s<0 (that is, the intermediate mutant has a slight fitness advantage)? This interesting case can be quite different and should be discussed separately.*

This is indeed a different scenario than the one we studied in this manuscript and we plan to analyze the adaptation rate with SIM on smooth landscapes in future work. We added this remark to the model section (L96):

"We do not consider smooth fitness landscapes in which single mutants have intermediate fitness (sH<s<0). We have already shown that SIM is favored by selection on these landscapes [37]; however, analysis of the effect of SIM on the adaptation rate on smooth landscapes will be the subject of future work."

*\* The questions of the tradeoff between adaptability and adaptedness were discussed in Komarova, Wodarz. "The optimal rate of chromosome loss for the inactivation of tumor suppressor genes in cancer." PNAS 101.18 (2004): 7017-7021, and also Komarova, Sadovsky, and Wan. "Selective pressures for and against genetic instability in cancer: a time-dependent problem." Journal of The Royal Society Interface 5.18 (2008): 105-121. It can be interesting to compare the approach of the authors with the approach and the results of these papers.*

Our many thanks to reviewer II for pointing out the very interesting work by Komarova and colleagues. We added a new paragraph to the discussion section (L455) which discusses these papers, along with other models that optimize mutation rates to balance the trade-off between *adaptability* and *adaptedness*. We conclude that SIM can be seen as a strategy to break this trade-off rather than to optimally balance the trade-off:

"Several authors have suggested that the mutation rate must balance between adaptability and adaptedness: Kimura found a mutation rate that balances between mutational and substitutional load [6]; Johnson and Barton found an optimal mutation rate that balances the generation of beneficial and deleterious mutations during adaptation [73]; Leigh found an optimal mutation rate that balances the generation of deleterious mutations and maintenance of standing variation in a fluctuating environment [74]; Komarova and Wodartz found an optimal rate of chromosome loss that balances the unmasking of recessive alleles and genetic load during carcinogenesis [75]; Komarova et al. and Agur et al. found a time-dependent mutation rate strategy that optimizes carcinogenesis [76] and adaptive immune response [77], respectively. In contrast, we find that SIM breaks, rather than balances, the trade-off between *adaptability* and *adaptedness*: it allows individuals to switch between rates optimized for stressful and benign conditions according to the circumstances."

*\* How do the results fit into the debate about the role of mutations (and genetic instabiliy) in carcinogenesis (see papers by Loeb on the one hand, and by Bodmer and Tomlinson, on the other)?*

This is an interesting question. We added a new paragraph to the discussion section (L469) which suggests that SIM may be relevant to evolution of cells within tumors because it induces mutator phenotypes, which have been suggested to promote cancer:

"Mutators have been suggested to play a role in cancer [78–80]. Furthermore, there is evidence that cancer cells increase their mutation rate in response to stresses such as hypoxia [81,82]. Our results show that such increases can have an important effect on the emergence of drug resistance, progression, and metastasis of tumors [80,83]."

**Unsolicited changes:**

* Corrected Figure in Appendix D to Fig. D1
* Changed reference to "Appendix 2" to "Appendix B" in Appendix C
* Moved part of the paragraph on adaptive peak shift from the discussion to the introduction (L67).

We hope that our response will meet your approval.

Sincerely,

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