**Author response**

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"Stress-Induced Mutagenesis Breaks the Trade-Off Between Adaptability and Adaptedness"

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We are grateful to the reviewers and editors for their thorough consideration of our manuscript and constructive recommendations for revision. In response to comments made in the general assessment of the manuscript, our revisions include (line numbers in the form of L# -refer to the original manuscript):

Main things:

1. Competitions
2. SIMk
3. All deleterious mutations induce stress
4. Correction of *q* equations

Detailed responses:

Associate Editor

1. *"There should be an explicit function that gives the mutation rate for every genotype or relates mutation rate to fitness":* We now explicitly define the mutation rate function in the model overview – see eqs. 1, 9, and 12 in the revised ms.
2. *"Ln 95 says that it requires N(u/s)^2 < 1.  However, that ignores the “background mutations”.  Don’t you requires N(e^-U/s)(u/s)^2 < 1.  A similar issue applies to Ln 96":* We corrected the relevant expressions.
3. *"Eqn 1 RHS seems to ignore selection occurring within that generation (prior to mutation producing the possible double mutant offspring)"*: Eq. 1 doesn't ignore selection - we use the MSB frequencies of *ab*, *Ab*, and *Ab*, which already include the effect of selection before mutation*.* The effect of selection after mutation is considered in the calculation of the fixation probability.
4. *"In the Discussion, the authors should be clear about which results are likely to be limited to asexual taxa"*: We emphasized that our model is strictly asexual without recombination or segregation and discuss this limitation in the discussion.
5. *"Please define explicitly (with an equation) “adaptedness” and “adaptability” as used in Figs. 3-4":* We added the definitions of adaptedness and adaptability to the legend of figure 4.
6. *"A revision should also include an explicit model of the evolution of the mutational strategies"*: We simulated direct competitions between NM, CM, and SIM. The results favor SIM and are presented in section 3.5 and Figure 5.

Reviewer 1

1. *"Out of the cited papers (P2 L30, L34–35), which ones are theoretical and which present empirical evidence for mutator alleles?":* We separated the evidence of evolution of mutator alleles into empirical and theoretical results. Also, we marked references to reviews as such (Sniegowski et al. 2000, de Visser 2002, Denamur & Matic 2006
2. *"What evidence exists for the possible presence of stress-induced mutator alleles?":* We added more references and clarified the section on evidence of SIM.
3. *"This exposition is important in explaining to the reader if the authors are explaining the evolution of an observed mechanism, or whether this is a more conceptual paper to fill a theoretical gap":* We emphasized the main question of the manuscript in the introduction - How does SIM affect complex adaptation? Also, we added a recap of our previous results on the evolution of SIM (Ram & Hadany 2012) to the introduction and expanded on them in the discussion. We also added results of direct competitions between the different mutation strategies that suggest that SIM is favored by selection.
4. *"The effect of different loci really needs to be made explicit":* We have revised the section defining how mutation operates in our model in order to clarify that individuals are defined by the alleles they have in the focus loci (*A/a* and *B/b*) together with the number of deleterious mutations they have in the non-specific loci. Also, we clarified that stress is determined by fitness which is determined by all loci, not only by the specific focus loci.
5. *"The authors have also not discussed if their mechanism can be valid in sexual species":* We emphasized that our model is strictly asexual without recombination or segregation and discuss this limitation in the discussion.
6. "Approximations and production of emergence probabilities": Appendix 1: we added a comment about the assumptions made on the model parameters; added the missing factor of 2 to the left-hand-side of the first equation in L407; added missing << in L414; added an explicit *sH*<<1 note in L432; added a comment at L111 that all the simplifications can be found in the appendix and a reference to Fig. S1 that shows a comparison of the analytic results and the simulation results.
7. "Simulations: Some details in the description of the simulations are also lacking": We added the number of bootstrap samples and the number of simulation replicates in the legends of Fig 2, Fig. S1 and Fig. S2; we added the number of simulations per parameter set in the methods at the end of section 2.5; we revised section 2.5 so that the simulations description will be clearer.
8. "Figure 3: Why include figure 3 if it's not going to be discussed?": We expanded on Fig. 3 in the text, at the beginning of section 3.2, and added a note in L182 about the source of the small advantage of SIM in adaptedness.
9. "General Formatting: The formatting for some sections is a bit messy; the references section does not conform to that expected for Evolution, and there are numerous errors in the supporting material": We checked and fixed formatting issues in the supporting information and the references section.
10. "L50: What's the difference between the findings of the Agrawal 2002, and Shaw and Baer 2011 studies?": We added a note about the difference between Agrawal 2002 and Shaw & Baer 2011 in L50.
11. "L133: “We use Wright-Fisher simulations...” To do what?" We revised L133 to complete the sentence on what the simulations were used for.
12. "L121: “...but see an exception below.” In which section? Same for L160": We replaced "see below" with a reference to section 3.3 in L 121 and L160.
13. "L66: I take it that each mutation has the same selection coefficient, s?": Indeed, all mutations have the same effect on fitness. We added a note in the model overview to avoid confusion.
14. "L250: “....other mechanisms were proposed”. What were they? Also explain in the context of line 253 when you say “SIM can resolve this problem” (what is 'this' problem?)": We included more details on proposed solutions to the "adaptive peak shifts" problem; We rephrased the last sentence of the paragraph to explain which problem SIM may help resolve.
15. "L427: It is pointless and confusing to state 𝛽 = 0 here": We removed the β=0 note to avoid confusion.
16. "Table 1: If this isn't too onerous, maybe it would be worth adding an extra row on s/µ, (s/µ)^2 ranges as well to show the reader the range of N that is permissible? I am happy to leave this to the author's discretion.": we considered the suggestion and decided to leave the table in its current form.
17. "L6: Is Liliach's email written correctly?" Lilach's email was indeed missing an 'a' after the 'd'.
18. "I have a list of typographical errors…": We fixed typos and grammar in L40, L41, L75, L78, L108, L126-127. We didn't change L52 because we prefer the active voice rather than the passive one. We left 'constraints' in L101.
19. "L17: This abstract should be expanded upon, especially in explaining the traditional assumption of the adaptedness/adaptability trade-off… L36–38: As with the abstract, the introduction here could do with more detail to explain the traditional assumption of the adaptedness/adaptability trade-off, and its impact on biological theory": We revised the abstract and the first two paragraphs of the introduction to give more details about the literature on mutation rate evolution and the trade-off between *adaptability* and *adaptedness.*
20. "P.P. 3–4: The last paragraph of introduction needs expanding upon. What did you set out to test, since there's more to this work than just creating models of different mutagenesis mechanisms? Also, what did you find that was specifically different from previous work?": We revised the last paragraph of the introduction to make our research goal clearer – " we explore the effect of stress-induced mutagenesis on the rate of fitness valley crossing… We show that stress-induced mutagenesis breaks the trade-off between *adaptability* and *adaptedness*".

Reviewer 2

1. "There seems to be an artificial distinction in the paper that maladaptation due to a mismatch in genotype with environment (i.e., *ab* vs. *AB*) is distinct from maladaptation due to non-specific “deleterious mutations.”": As specified above (7) we revised the model overview to emphasize that all deleterious mutations (in the *A/a* and *B/b* loci or in the non-specific loci) are equal in regard to their effect both on fitness and on the mutation rate. This is because with SIM, the mutation rate is a function of the fitness and not of the number of deleterious mutations – the mutation rate is *U* if the fitness is ≥ 1 and *τU* otherwise (in section 3.3 it is *U* if fitness is > 1 and *τU* otherwise).
2. "the presence of a single mutation at the *A/a* or *B/b* loci leads to the full *τ*‐fold increase in mutation rate, whereas the number of nonspecific “deleterious mutations” has no effect on increasing mutation rate. Lastly, the number of deleterious mutations at the *A/a* and iloci and at non-specific loci has no effect on the magnitude of *τ*.": We ran simulations in which the mutation rate is a continuous function of the mean fitness. We used the functions suggested by Agrawal (2002): *U(ω)= τU-(τU - U)ωk* where *k* is a curvature parameter (we used *k*=1/10, 1, 10, and 100), *U* is the baseline mutation rate used in normal mutagenesis, *ω* is the fitness,and *τ* is the maximum fold-increase in mutation rate. We present the results in section 3.4 and Fig. 2B.
3. "In my opinion, a more robust paper would [a] allow for non-­‐specific deleterious mutations to affect mutation rate, [b] have SIM be a function of the number of deleterious mutations (both specific to an environment and non-­‐specific) and [c] allow for the possibility that a population is extinct or at very low population size at the time of an environmental shift": we respond to [a] in (‎22) and to [b] in (‎23). We respond here to [c]" If SIM is already extinct at the environmental change then it will not achieve adaptation; however, before the environmental change SIM is as likely to reach extinction as NM, because the mutation rate of the fittest individuals is the same as in the case of NM and the population mean fitness is slightly higher. To account for the possibility that a SIM population can go to extinction while waiting for adaption in a non-constant sized population, we ran competitions between NM and SIM in which SIM can suffer from the increased mutation load and lose to NM due to a decrease of the SIM sub-population size. Our simulation results show that SIM is advantageous over NM – see new Fig. 5 and section 3.5. This was also done in our previous work (Ram & Hadany 2012) with a smooth fitness landscape - SIM and NM competed over multiple environmental changes until one of them went to extinction. In that work, SIM was introduced at 5% and was still significantly advantageous over NM and CM.
4. "L85: Add “with mean U/s” to “. . . MSB is Poisson distributed": We added the mean of the Poisson distribution to L85.
5. "L95: The assertion about *N*(*μ*2/*s*2) >1 with respect to double mutants does not seem correct.": The frequency of individuals with a mutant (uppercase) allele in either the *A/a* or *B/b* locus at the MSB is *µ/s*. assuming independence between the loci, the frequency of a double mutant *ab* is *(µ/s)2*. Multiplied by *N* we get the expected number of double mutants at the MSB, *N(µ/s)2*. The number of mutations per individual is Poisson distributed with mean *U/s*. If we define *g*=*U/*µ, then the frequency of any double mutants is *(U/s)2e-U/s/2*. For some double mutant to be the double mutant *ab* we need both mutations to be at the right locus, with common probability *~1/g2*. So we get (*U/s)2e-U/s/2g2 = (µ/s)2 e-U/s / 2 ≈ (µ/s)2*. The last approximation is discussed above in (1).
6. "L95 – 96, the caveat of “on average” should be added": We added "expected" in L95 and L96.
7. "L105: It is not clear how you are conditioning to get the frequency of mutation free genotypes.": Thank you for finding the mistake in eq. 1 and 2 and in Appendix 1 in the calculation of *q* the appearance probability. We corrected the expressions, which led also to corrections in eqs. 5-7 (in the original ms). We reproduced Figs. 2, 4 and S1. The fit with the simulations results is now slightly better. There is no qualitative change in the conclusions.
8. "It seems like the probability that a genotype is ab AND mutation free is exp(-(2*μ*+*U*)/*s*) and the probability that a genotype is *aB* or *Ab* and mutation free is (2*μ*/*s*)exp(-(2*μ*+*U*)/*s*) , assuming *μ* is small.": We added a note about *U+2µ≈U* because *U>>µ* to appendix 1. Therefore the frequency of mutation-free *ab* can be written as *e-U/s* rather than *e-(U+2µ)/s*. The same goes for the frequency of mutation free *aB* which can be written as *µ/s e-U/s* with the additional factor of *(1-µ/s)*.
9. "Figure 2: The SIMe case is poorly motivated": Section 3.3 on SIMe was revised. We hope that the motivation for this extension is clearer now.
10. "Figure 4: It is not clear what to make of the values in this figure. In principle the level of adaptedness could approach zero and adaptability could approach a very large number for the SIMe and CM cases. Similarly there seems to be no limit to the SIM case in terms of adaptability.": Figure 4: We changed the axes labels, the legend, and the text so that it would be clearer that the figure axes are relative measures in comparison to NM; added a note about limit the limit on τ (*τU*<<1) to the figure legend – for CM this is equivalent to the constraint *U*<<1 used throughout the manuscript, for SIM this is a constraint that ensures that single mutants don't become rare due to mutational load.
11. "L223: Up until about this point in the paper, it was not clear whether the authors think SIM is an adaptive strategy, such that there can be selection for SIM… there can be direct competition between NM, CM and SIM strategies.": We added a paragraph to the introduction and also expanded in the discussion about our former findings (Ram & Hadany 2012) in which we've shown that SIM can be selected for (see also (‎6) above). Also, we added the results of competitions between the different mutational strategies to show that indeed SIM can be selected for in this model – section 3.5 and Figure 5.
12. "The principle that there is no cost to SIM is more a consequence of assumptions than added insight… should the author’s consider an allocation of resources to this process and therefore the potential for a cost of the SIM process besides higher deleterious mutation rate?": We added a paragraph on direct fitness costs to the discussion section. In short, we agree that assessing the individual's condition is costly. However, organisms constantly asses their condition for other purposes – bacteria, for example, have a number of stress responses such as the SOS response and the general stress response. Once these mechanisms already exist, they can be recruited to regulate the mutation rate. One doesn't need to consider their cost for SIM because these mechanisms operate in NM and CM and are essential for viable organisms (see Foster 2007 for details on SIM and bacterial stress responses).
13. "Figure 1: The process of hypermutation is not clear in the figure. Where is *τ*?": We emphasized in the figure legend that genotypes with ellipses are stressed and genotypes wit squares are not, and that with SIM only stressed individuals hypermutate. We didn't include *τ* in the figure because the figure shows the baseline mutation rates (those used by NM).
14. "Figure 3: this could go in the supplementary information": we chose to leave Fig. 3 in the main text and add more information on the figure, see also (‎11) above.