**Author response**

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

Yoav Ram and Lilach Hadany

12/15/2013

We are grateful to the reviewers and editors for their thorough consideration of our manuscript and constructive recommendations for revision. Regarding the major issues raised by the editor and the reviewers:

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

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):

***Associate Editor Comments to Author:*** *AE Recommendation for Ram and Hadany  
  
This manuscript has been reviewed by two reviewers and I have read it myself.  The authors tackle an interesting topic addressing how stress-induced mutagenesis (SIM) affects the adaptability and adaptedness of asexual populations.  Unfortunately, there are some substantial problems, and neither reviewer was positive about this work.  The authors are not explicit about some basic details of the model (as stated by Reviewer 1), and so we are left to infer what is going on.  In doing so, I have reached a similar conclusion to Reviewer 2, namely that the authors seem to have made some odd decisions with respect to how stress increases mutation.  As R2 points out, some deleterious (“stressful”) alleles increase mutation rate but others do not and this appears to be done in a way that is biased towards increasing the evolutionary advantage of SIM.  Because the relationships between “stress”, fitness, genotypes, and mutation rate are so essential to this work, I cannot recommend a version of this manuscript unless it makes explicit and sensible choices in this regard.  I do think the topic is very interesting, and a revised paper (which I think requires a new model) would be worthwhile.  A revision should also include an explicit model of the evolution of the mutational strategies (at a minimum, by simulation and involving the 3 pure types NM, CM, SIM, which should be quite straightforward).*

1. We simulated direct competitions between NM, CM, and SIM. The results favor SIM and are presented in section 3.5 and Figure 5. *A few other comments:  
     
   There should be an explicit function that gives the mutation rate for every genotype or relates mutation rate to fitness.*
2. We now explicitly define the mutation rate function in the model overview – see eqs. 1, 9, and 12 in the revised ms.

*In the main model, it is unclear to me what the fitness is of the single mutants Ab and aB and why these mutants should have higher mutation rates than the ab genotype which carries FEWER adaptive alleles?  
  
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*

1. We corrected the relevant expressions.

*Eqn 1 RHS seems to ignore selection occurring within that generation (prior to mutation producing the possible double mutant offspring).  Maybe this doesn’t matter in the leading order approximation.*

1. 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.

*Please define explicitly (with an equation) “adaptedness” and “adaptability” as used in Figs. 3-4.  How is it measured?*

1. We added the definitions of adaptedness and adaptability to the legend of figure 4.

*In the Discussion, the authors should be clear about which results are likely to be limited to asexual taxa.*

1. We emphasized that our model is strictly asexual without recombination or segregation and discuss this limitation in the discussion.

*Reviewer(s)' Comments to Author:***Reviewer 1** *Review of Manuscript: “Stress-induced mutagenesis breaks the trade-off between adaptability and adaptedness”  
Authors: Yoav Ram and Lilach Hadany  
Journal: Evolution  
Manuscript number: 13-0825  
  
This manuscript demonstrates how some relatively simple stochastic results from population genetics can be combined to determine the likelihood that a rescue mutation can be formed from an unfit gene combination. This framework is used to compare the effect of three kind of mutation mechanisms; normal mutagenesis, constitutive mutagenesis and stress-induced mutagenesis. The main finding of this paper is that if stress-induced mutagenesis is the mechanism present, then organisms are able to retain the ability to adapt to a new environment, without any loss in existing fitness. That is, the trade-off between “adaptedness” and “adaptability” is removed.  
  
There certainly seems to be some insightful theoretical results here; the mathematical derivations are pretty straightforward yet lead to clear predictions that allow the comparison of multiple mechanisms. I am also sure that the model predictions advances the knowledge of adaptation processes, especially given the current interest in how species, especially bacterial populations, are able to react in stressful environs (e.g. in the presence of drugs). However, I feel impeded in making a firm decision on this manuscript since it is lacking in sufficient detail in places, especially regarding the biological background of the model, as well as the analysis of the mathematics used. The preparation of the manuscript also seems rather rushed, with many points not explained in sufficient detail. If these issues are addressed in a subsequent revision, then I would be more able to judge the thoroughness of the research.  
  
Major points that I feel need addressing are as follows.  
  
Introduction and context: The different kind of evidence behind mutator alleles, and their evolutionary mechanisms, need to be made clearer. For example, out of the cited papers (P2 L30, L34–35), which ones are theoretical and which present empirical evidence for mutator alleles?*

1. 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)

*In addition, what evidence exists for the possible presence of stress-induced mutator alleles?*

1. We added more references and clarified the section on evidence of SIM.

*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. Both approaches have their merits, but it nevertheless needs to be outlined.*

1. 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.

*Description of the model: The effect of different loci really needs to be made explicit. I was initially confused by the introduction of the genome-wide mutation rate U when it was previously mentioned that the per-site rate at loci A and B was µ. After re-reading the manuscript several times, I think I understood the model; as well as focal loci A and B, mutation also arises at other (background) loci, with rate U, that contribute to an ongoing fitness decline. Therefore, there is no need to explicitly account for these other sites. Is this correct?*

1. 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.

*If so the authors also need to state assumptions pertaining to how the sites are linked. I also take it that all sites are assumed to be linked (or the organism is asexual), so one does not have to consider how recombination complicates matters? (I believe this is also an assumption needed for mutation-accumulation to act.)  
  
Related to this note, the authors have also not discussed if their mechanism can be valid in sexual species, since it is easily imagined that recombination can (a) arrest the buildup of deleterious mutations, so producing a rescue mutation is less important to aid the population, and (b) produce the double mutant instead of stepwise mutation. This really needs to be explored further in the manuscript, probably in the discussion.*

1. We emphasized that our model is strictly asexual without recombination or segregation and discuss this limitation in the discussion.

*Approximations and production of emergence probabilities: It is not entirely clear how some of these probability terms are derived. To give some examples: how is the first equation in Appendix A approximated? (I think this assumes U/s << 1 – is this correct? It also appears that the authors have left out a factor of 2 in the µ^2/s term in the very first equation of Appendix A.) For lines 411 and 413, are these inequalities as they stand or meant to be “a lot less than” (i.e. Us << U and µ/s << 𝜏 U)? Finally, I take it that it is also assumed that sH << 1? In addition, these simplifications should really be stated briefly when referring to the appendices (e.g. at P6 L111).*

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.

*Simulations: Some details in the description of the simulations are also lacking. Why did the authors not decide to replicate several environmental changes from the same starting backgrounds, as in other papers? How many replicates were ran per point in the figures (this is listed in some figure legends, but this really should be in the methods description)? If bootstraps were performed, how many per point?*

1. 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.

*Figure 3: Why include figure 3 if it's not going to be discussed? Please expand on the inference one obtains from this figure, or move it to the supplementary material if it's not an important result. This also ties into your explanation on lines 181–182 (explain what is the slight advantage of SIM over NM).*

1. 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.

*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 relating to cross-referencing other sections. Please thoroughly check for these errors and correct where needed.*

1. We checked and fixed formatting issues in the supporting information and the references section.

*Some minor concerns are below (P indicates page number, L is line number).  
P2 L17: This abstract should be expanded upon, especially in explaining the traditional assumption of the adaptedness/adaptability trade-off.  
P3 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 it's impact on biological theory.*

1. 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.*

*P3 L50: What's the difference between the findings of the Agrawal 2002, and Shaw and Baer 2011 studies?*

1. We added a note about the difference between Agrawal 2002 and Shaw & Baer 2011 in L50.

*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?*

1. 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*".

*P7 L133: “We use Wright-Fisher simulations...” To do what?*

1. We revised L133 to complete the sentence on what the simulations were used for.

*P7 L121: “...but see an exception below.” In which section? Same for P9 L160.*

1. We replaced "see below" with a reference to section 3.3 in L 121 and L160.

*P4 L66: I take it that each mutation has the same selection coefficient, s?*

1. Indeed, all mutations have the same effect on fitness. We added a note in the model overview to avoid confusion.

*P13 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?)*

1. 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.

*P20 L427: It is pointless and confusing to state 𝛽 = 0 here, since the 𝛽 parameter is only introduced in the supplement. Please discard this or rewrite.*

1. We removed the β=0 note to avoid confusion.

*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.*

1. we considered the suggestion and decided to leave the table in its current R

*Finally I have a list of typographical errors that, if changed, will improve the general clarity of the manuscript. While the standard of English throughout is very good, the exposition can be muddled in places. I suggest the authors take time to carefully check that their arguments are made as clear as possible before resubmitting this manuscript.  
P1 L6: Is Liliach's email written correctly?P3 L40: “has been demonstrated” instead of “was demonstrated”.  
P3 L41: “including both prokaryotes and eukaryotes”.  
P3 L52: “More recently, it was shown...” instead of “we showed...”.  
P4 L75: “it undergoes adaptive evolution” instead of “goes through adaptive evolution...”.  
P5 L78: “Into two distinct changes” instead of “To two distinct changes”.  
P6 L101: Write “Combining these two limitations” perhaps, instead of “Combining these two constraints”?  
P6 L108: “...given there are no double mutants in the current generation”, instead of “this generation”.  
P7 L126–127: General error in the sentence here.*

1. Lilach's email was indeed missing an 'a' after the 'd'. 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.

***Reviewer 2***

*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.” In the model, non-­‐specific “deleterious mutations” have no effect on mutation rate. This seems like a questionable assumption sense in terms of fitness, maladaptation is maladaptation, whether it comes from a mismatch with environment or non-­‐specific deleterious effects. In the paper, deleterious effects at the A/a and B/b loci have the same selection coefficient as at non-­‐specific loci, which reinforces the questionable approach to modeling SIM.*

1. 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).

*Furthermore, 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 B/b loci and at non-specific loci has no effect on*

*the magnitude of τ .*

1. 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.

*Being a theoretician, I am open to simplifying assumptions, but the assumptions in this paper seem to miss the mark and are biased in favor of SIM. It seems an equally possible scenario for SIM is “stressed-induced mutational meltdown”, where individuals with non-specific deleterious mutations have higher mutation rates, which increases the level of non-specific deleterious mutations, which in a constant or slowly varying environment leads to extinction. In principle, the paper could have explored this possibility in the framework of adaptability and adaptiveness by evolving a population under mutation-selection-drift balance in a population in which population size is a function of mean fitness, in contrast to the Wright-Fisher model that assumes a constant population size. 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. In SIM cases, the population may be extinct at the time of environmental change and cannot adapt, or be at low population size and the population-level mutation rate so low (despite an elevated τ) that the rate of adaptation is compromised.*

1. We respond to [a] and [b] above. We respond to [c] here: 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.

*It could be argued that this paper investigates stress-induced mutation caused only by the “environment”, but this is questionable because in the introduction, lines 39-­‐40, they define SIM as “. . . the increase of mutation rates in stressed or maladapted individuals . . .” Maladapted individuals can come about through non-specific deleterious mutations that occur at baseline rate U in the paper’s model.*

*Other comments:*

*line 85: Add “with mean U/s” to “. . . MSB is Poisson distributed . . .” Otherwise, terms in equations (1) and (2) seem to come from nowhere.*

1. We added the mean of the Poisson distribution to L85.

*line 95: The assertion about N (µ 2 / s2 ) > 1 with respect to double mutants does not seem correct. Should this be ~ N (µ 2 / (2s))?*

1. 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).

*More generally, for lines 95 – 96, the caveat of “on average” should be added, I think.*

1. We added "expected" in L95 and L96.

*Line 105: It is not clear how you are conditioning to get the frequency of mutation free genotypes. In one case you seem to be conditioning on the genotype being ab and then asking if it is mutation free and in the second you seem to calculate the probability a genotype is aB or Ab and do not consider whether these are then mutation free.*

1. 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.

*It seems like the probability that a genotype is ab AND mutation free is*

*exp(!(2µ +U ) / s) and the probability that a genotype is aB or Ab and mutation free is*

*(2µ / s)exp(!(2µ +U ) / s), assuming µ is small. If these changes are correct, then Appendix 1 may have to be revised, but the general logic of App. 1 looks correct, otherwise.*

1. 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)*.

*Figure 1: The process of hypermutation is not clear in the figure. Where is τ?*

1. 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).

*Figure 2: The SIMe case is poorly motivated and explained in the main text in the lead*

*up to figure 2.*

1. Section 3.3 on SIMe was revised. We hope that the motivation for this extension is clearer now.

*Figure 3: This could go in the supplementary materials.*

1. We chose to leave Fig. 3 in the main text and add more information on the figure, see also (‎11) above.

*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. None of this seems under any realistic constraint. The principle that there is no cost to SIM is more a consequence of assumptions than added insight. The combination of τ SIM and τCM that give a particular adaptability is more interesting.*

1. 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.

*line 223: 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. At this point the authors indicate that individuals are assessing their condition and adjusting mutation rate accordingly. If this is the case, then in principle there will be variation within a population for the ability of individuals to assess and adjust mutation rate according to condition and that there can be direct competition between NM, CM and SIM strategies. In this context, the present paper is an intermediate step in the ultimate question of what is the evolutionary stable strategy in terms of SIM.*

1. 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.

*Furthermore, if individuals assess condition and adjust mutation accordingly, then 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?*

1. 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).

*Given my comments at the beginning of the review, I am concerned that the main conclusions coming from this paper are not generally true. The question whether SIM or SIMe can persist in direct competition with other strategies seems to be the fundamental question. Does the theoretical approach presented here help answer this question? It is not clear that the analytical approach can be extended to answer the direct competition question. If it can, then this would make the paper more compelling.*

*Extraneous comment: In bacteria frequency dependent processes have been commonly reported in experimental systems. How would SIM play out under frequency-­‐dependent selection, where stress is caused by intraspecific competition?*