**Author response**

Evolution Manuscript ID 13-0825

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

Yoav Ram and Lilach Hadany

12/24/2013 6:23 PM

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

In response to the comments and concerns raised by the editor and the reviewers we have made major changes to the manuscript. Briefly, (i) we emphasize that all deleterious mutations can induce mutagenesis – a point that was unclear in the original manuscript; (ii) we added the results of direct competitions between the different mutational strategies; (iii) we added results of adaptation with continuous relationship between fitness and mutation rate; and (iv) we corrected the approximation of *q* the probability of appearance of a double mutants, following a remark by reviewer 2.

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):

***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.*

This unclarity of the original manuscript has been a major cause of misunderstanding, and we thank the reviewer and the editor for pointing it out. In our model, the mutation rate is affected by deleterious mutations in all loci, not only in the specific loci. We made a major revision of our model overview to make sure that the relationship between fitness and mutation rate is clear. This includes the addition of new equations (1, 9, 12, 13).

*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 added simulations of the evolution of mutational strategies (direct competitions between NM, CM, and SIM). The results show that SIM is favored 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.*

1. We added eq. 1 to the model overview:

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*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?*

1. We modified the model overview to make the fitness landscape more clear: " the fitness of the wildtype *(ab/0*)is 1, of the single mutants (*Ab*/0 and *aB*/0) is 1-*s*, and the double mutant (*AB/0*)has the highest fitness 1+*sH*, where *s* is the selection coefficient and *H* is the relative advantage of the double mutant. This is the simplest case of a "rugged fitness landscape" - the single mutants *Ab* and *aB* are fitness "valleys" between the local and global fitness peaks *ab*/0 and *AB/0*" *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*
2. We corrected the relevant expressions per the editor's remarks.

*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. Eqn. 1 includes 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: *"Adaptedness* is defined as the population mean fitness at the MSB, (Figure 3). *Adaptability* is defined by the adaptation rate, (eqs. 6-8).". The legend of Figure 3 doesn't refer to adaptedness or adaptability.

*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 in the model overview: "We consider the effects of mutation, selection, and genetic drift but ignore population structure effects (*i.e.* migration, group selection) and sex-related mechanisms (*i.e.* recombination, segregation)."

We also added a discussion of this limitation to the discussion: "Our model focuses on asexual populations, ignoring recombination, segregation, and sexual reproduction. These mechanisms are important for adaptation on a rugged fitness landscape both because they help to cope with deleterious mutations and because they allow two different single mutants to produce a double mutant without an increased mutation rate. We expect that recombination will reduce the advantage of SIM over NM in population mean fitness (Agrawal, 2002), direct competitions (Tenaillon, Le Nagard, Godelle, & Taddei, 2000), and adaptation rate (due to the Fisher-Muller effect)."

*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 (Sniegowski, Gerrish, & Lenski, 1997; Wielgoss et al., 2012) and theoretical results (Taddei et al. 1997; Kessler and Levine 1998; Tenaillon et al. 1999). We marked references to reviews as such (Sniegowski et al. 2000, de Visser 2002, Denamur & Matic 2006). We also added more information on the classic problem of mutation rate evolution to the introduction: "constitutive mutators become associated with poor genetic backgrounds due to increased accumulation of deleterious mutations – as evidenced both in the lab (Funchain et al. 2000) and in the clinic (Montanari et al. 2007). Models of mutator alleles support the "reduction principle" (Liberman and Feldman 1986) which suggests that natural selection reduces the mutation rate in a stable environment. But many adaptations may require new beneficial mutations, especially in asexual populations. This tension between the effects of beneficial and deleterious mutations leads to "the rise and fall of the mutator allele" dynamics, where mutator alleles increase in frequency in a maladapted population, only to be eliminated by selection when the population is well-adapted."

*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: "SIM was observed in lab strains (Foster 2007; Rosenberg et al. 2012) and in natural populations of *Escherichia coli* (Bjedov et al. 2003), and in other species of bacteria such as Pseudomonads (Kivisaar 2010), *Helicobacter pylori* (Kang et al. 2006), and *Streptococcus pneumonia* (Henderson-Begg et al. 2006). SIM was also observed in yeast (Heidenreich 2007; Rodriguez et al. 2012), algae (Goho and Bell 2000), Caenorhabditis (Matsuba et al. 2012), flies (Sharp and Agrawal 2012), and human cancer cells (Bristow and Hill 2008)."

*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: "Here, we explore the effect of stress-induced mutagenesis on the rate of fitness valley crossing by analyzing population genetic models of adaptive evolution." We added a recap of our previous results to the introduction: "In a previous work, we showed that SIM can evolve in asexual populations due to association with the beneficial mutations it generates (Ram and Hadany 2012). This evolutionary advantage was shown at the population- and individual-level, in constant environments and changing ones."

We also expanded on our previous results in the discussion: "In a previous work we demonstrated that 2nd order selection can lead to the evolution of SIM (Ram and Hadany 2012): In an asexual population evolving on a smooth fitness landscape, selection favored SIM over both NM and CM. SIM was favored both in a constant environment and in a constantly changing environment. Here we showed that selection also favors SIM on a rugged fitness landscape (Figure 5)."

We also added results of direct competitions between the different mutation strategies that suggest that SIM is favored by selection (Figure 5, section 3.5).

*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. In the model overview (section 2.1) we have revised the definition of how mutation operates in our model 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.)*

1. We added remarks to the model overview to make it clear that we only model asexual populations with complete linkage: "We consider a population of *N* haploid asexual individuals with an infinite number of loci in full linkage. We consider the effects of mutation, selection, and genetic drift but ignore population structure effects (*i.e.* migration, group selection) and sex-related mechanisms (*i.e.* recombination, segregation)."

*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 (previous response) and discuss this limitation in the discussion: "Our model focuses on asexual populations, ignoring recombination, segregation, and sexual reproduction. These mechanisms are important for adaptation on a rugged fitness landscape both because they help to cope with deleterious mutations and because they allow two different single mutants to produce a double mutant without an increased mutation rate. We expect that recombination will reduce the advantage of SIM over NM in population mean fitness (Agrawal 2002), direct competitions (Tenaillon et al. 2000), and adaptation rate (due to the Fisher-Muller effect)."

*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. Thanks! 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: "We used Wright-Fisher simulations to study the evolution of a finite asexual population under selection, mutation and drift (Figure 1B). We divide the individuals to classes according to their genotypes (*ab/x*, *Ab/x*, *aB/x*,and *AB/x*, where *x*≥0 is the number of deleterious mutations), and track the number of individuals in each class. The simulations start with a mutation-free population (all individuals start in the *ab/0* class) that accumulates deleterious mutations over the first 500 generations of the simulation. With *s*=0.05, 500 generations are enough to get the average number of deleterious mutations per individual to 99.3% of its MSB value, *U/s*"

*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: "If the mutation rate is constant and uniform across the population, the population mean fitness – the *adaptedness* – only depends on the fitness and mutation rate of the fittest individuals. Therefore, the mean fitness decreases when the mutation rate increases, due to the increased generation of deleterious mutations in the fittest individuals. However, if mutation rates are not uniform across the population, increased mutation rates in unfit individuals increase the population mean fitness, as long as beneficial (compensatory) mutations are allowed (Ram and Hadany 2012). Figure 3 shows this advantage of SIM over NM in terms of the differential population mean fitness.".

We added a note in L182 about the source of the small advantage of SIM in adaptedness: " The highest mean fitness will always be attained with SIM, which has a small advantage over NM (that cannot be seen in this figure, but see Figure 3) due to the increased generation of beneficial (compensatory) mutations in individuals with deleterious mutations."

We added a note about the difference from the previous model (Ram & Hadany 2012) – mainly the opportunity for multiple mutations in the same individuals at the same generation and a formal protocol on how to calculate the mean fitness: "Our extended model allows beneficial mutations and for multiple mutations to occur in reproduction. In the *supporting information* we demonstrate how to use this model to calculate the mean fitness of an asexual population."

*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. To give a better exposition to mutation rate evolution and the trade-off between *adaptability* and *adaptedness* we revised the abstract: " Because mutations are mostly deleterious, mutation rates should be reduced by natural selection. However, mutations also provide the raw material for adaptation. Therefore, evolutionary theory suggests that the mutation rate must balance between *adaptability* – the ability to adapt – and *adaptedness* – the ability to remain adapted. We model an asexual population crossing a fitness valley and analyze the adaptation rate with and without stress-induced mutagenesis – the increase of mutation rates in response to stress or maladaptation. We show that stress-induced mutagenesis breaks the evolutionary trade-off between *adaptability* and *adaptedness*, increasing one without reducing the other. Our theoretical results support the hypothesis that stress-induced mutagenesis promotes adaptation and provide quantitative predictions for the adaptation rate with different mutational strategies.*"*

We also revised the first two paragraphs of the introduction: "There is experimental, clinical and theoretical evidence that high mutation rates increase the rate of adaptation and that during adaptive evolution, constitutive mutators - alleles that constitutively increase the mutation rate - can rise in frequency because of the beneficial mutations they generate (reviewed in Sniegowski et al. 2000; de Visser 2002; Denamur and Matic 2006). However, during evolution in a stable environment, constitutive mutators become associated with poor genetic backgrounds due to increased accumulation of deleterious mutations – as evidenced both in the lab (Funchain et al. 2000) and in the clinic (Montanari et al. 2007). Models of mutator alleles support the "reduction principle" (Liberman and Feldman 1986) which suggests that natural selection reduces the mutation rate in a stable environment. But many adaptations may require new beneficial mutations, especially in asexual populations. This tension between the effects of beneficial and deleterious mutations leads to "the rise and fall of the mutator allele" dynamics, where mutator alleles increase in frequency in a maladapted population, only to be eliminated by selection when the population is well-adapted. This dynamic was studied using experimental evolution (Sniegowski et al. 1997; Wielgoss et al. 2012), mathematical analysis, and simulations (Taddei et al. 1997; Kessler and Levine 1998; Tenaillon et al. 1999).

Leigh (1970) suggested that the mutation rate must balance between two evolutionary traits: *adaptability* – the capacity to adapt to new environmental conditions – and *adaptedness* – the capacity to remain adapted to existing conditions. Because mutation is fundamental to every biological system, and mutation rates vary significantly between species, spanning from roughly 10-4 (bacteria) to over 10 (humans) mutations per genome per generation (Sung et al. 2012), it is important to understand this 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 rephrased L50: "The effect of SIM on *adaptedness* was studied with deterministic (Agrawal 2002) and stochastic (Shaw and Baer 2011) models"

*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: *"*We used Wright-Fisher simulations to study the evolution of a finite asexual population under selection, mutation and drift"

*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 in the non-specific loci have the same effect on fitness. A mutation in just one of the specific loci also has the same effect on fitness. We added a note in the model overview to avoid confusion: "The effects of these deleterious mutations on fitness are independent (*i.e.*, multiplicative) and identical, such that the fitness of an individual with *x* deleterious mutations is *ω*=(1-*s*)*x*, where *s* is the selection coefficient… Mutations at these loci change *a* to *A* and *b* to *B* at reproduction with probability *µ* (without back-mutations) and their effect is initially deleterious, reducing the fitness by 1-*s* (similar to deleterious mutations in other loci)".

*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: "other mechanisms were proposed: increased phenotypic variance after population bottlenecks (Whitlock 1995); environmental fluctuations (Whitlock 1997); environmental heterogeneity (Hadany 2003); fitness-associated recombination (Hadany and Beker 2003); and intermediate recombination rates (Weissman et al. 2010)*"*

We rephrased the last sentence of the paragraph to explain which problem SIM may help resolve: "Our results (Figure 2) suggest that SIM can help resolve the problem of fitness valley crossing by reducing the time required for a population to shift an adaptive peak."

*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 as it is.

*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 extended our model, and 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] in (28) above. We respond to [b] in (29). Our response to [c]: SIM is as likely to reach extinction as NM even in a smaller population, 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.

If the population size is small then the adaptation rate will be compromised because there will not be enough single mutants, and the wildtype doesn't hypermutate with SIM. Our constraint on the population size – s/µ<N – appears in section 2.2 and deals with this case.

*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.*

*31. We hope that we clarified this confusion of our original manuscript. In our model the rate of mutation is a function of fitness, affected by all loci. We consider two models differing in… so "environmental SIM" means…*

*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: "the number of deleterious mutations per individual at the MSB is Poisson distributed with mean *U/s"*

*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 good when *U*<<*s*.

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

1. We added "expected" in L95 and L96: "double mutants are already expected at the MSB", "no single mutants are expected at the MSB"

*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. We thank the reviewer for his careful reading. We corrected the expressions in eqs. 1 and 2 and in Appendix 1, 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 that *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 with 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.*

Section 3.3 on SIMe was revised. We hope that the motivation for this extension is clearer now: " So far, we considered the case where the environmental change creates an opportunity for adaptation without affecting the absolute fitness of the population – for example, a new ecological niche can be favorable without affecting the well-being of the current population. In that scenario, the wildtype *ab* wasn't stressed and did not hypermutate.

Next, we consider a different scenario in which an environmental change affects the well-being of the entire population - for example, exposure to an antibiotic drug or a host's immune response. In this case the environmental change doesn't just create an opportunity for adaptation but also causes stress in the entire population. As before the double mutant *AB* is resistant to the stress (*i.e.* the drug or immune response) and therefore has a higher fitness than either the wildtype or the non-resistant single mutant*s*. However, in this scenario the wildtype *ab* is also stressed and therefore hypermutates with SIM – compare with eq. 1: *"*

*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 (‎15) 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 to "relative adaptability" and "relative adaptedness"; We changed the legend of Figure 4 and the main 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 (*τ*<<s/*U*) to the figure legend.

*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 expanded on our previous work about the evolution of SIM. In the introduction: " In a previous work, we showed that SIM can evolve in asexual populations due to association with the beneficial mutations it generates (Ram and Hadany 2012). This evolutionary advantage was shown at the population- and individual-level, in constant environments and changing ones."

In the discussion: "In a previous work we demonstrated that 2nd order selection can lead to the evolution of SIM (Ram and Hadany 2012): In an asexual population evolving on a smooth fitness landscape, selection favored SIM over both NM and CM. SIM was favored both in a constant environment and in a constantly changing environment. Here we showed that selection also favors SIM on a rugged fitness landscape (Figure 5).*"*

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 about direct fitness costs to the discussion section: "Our model does not assume direct fitness costs for any of the mutational strategies. A "cost of DNA replication fidelity" (Dawson 1998) – the energy and time expended in order to maintain a low mutation rate – could make both CM and SIM more successful. The "cost of fidelity" might require further study, but empirical evidence suggest that it doesn't play an important role in the evolution of the mutation rate (Giraud et al. 2001; Loh et al. 2010; Gentile et al. 2011; Shee et al. 2011). Another fitness cost might be associated with the regulation of the mutation rate: for individuals to determine if their condition calls for the induction of mutagenesis, they must invest resources and energy in costly sensory mechanisms. However, such mechanisms already exist for various unrelated purposes, such as the maintenance of cell cycle and homeostasis. Therefore, we consider these mechanisms as "free" in terms of fitness costs. Moreover, in *E. coli* stress is induced by several stress responses that serve other cellular functions (Foster 2007; Al Mamun et al. 2012), and this is probably also the case is other organisms*."*

*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.*

1. We used the stochastic simulations approach to show that SIM can persist – and even outcompete – in direct competition with other strategies.

*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?*

1. This is an interesting direction for future research.

We hope that our response will meet your approval.

Sincerely,

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