

Revision of the manuscript entitled “**Is adaptation limited by mutation? A timescale dependent effect of genetic diversity on the adaptive substitution rate in animals**”, by Rousselle et al.

**Dear Editor,**

**Thank you for the time you devoted to this manuscript entitled “Is adaptation limited by mutation? A timescale dependent effect of genetic diversity on the adaptive substitution rate in animals”, by Rousselle et al., and please find enclosed the revised manuscript. We thank the reviewers for insightful comments and suggestions. We have addressed the issues they raised and modified our manuscript accordingly. Find below our replies to each referees' comments. Please also note that we had the text reviewed by a professional and native english speaker, hence the many grammatical and language adjustments. We hope that this revision improved the quality and the clarity of the manuscript and that it can be deemed suitable for a PCI Evol Biol recommendation.**

**Best regards,**

**Marjolaine Rousselle, Paul Simion, Marie-Ka Tilak, Emeric Figuet, Benoit Nabholz, Nicolas Galtier.**

**Reviewer 1 : (Konstantin Popadin)**

In the manuscript authors aim to address the fundamental question about the relationship between the rate of adaptation and the supply of new mutations.

The supply of new mutations is expected to be higher in species with large-sized populations because of (i) the higher rate of origin of new alleles, (ii) increased frequency of already existing alleles and (iii) increased probability of the beneficial alleles to be fixed. However, the “necessity” in beneficial mutations might be higher in species with low-sized populations: they are expected to be further away from the optimum and/or being more complex (living in the phenotypic space with high dimensionality) meaning that the fraction of beneficial mutations in such species might be higher as compared to high-population-size species.

To distinguish between these two opposite expectations authors derived a dataset of 50 species from 10 distant groups of animals. For each group and each species they estimated  $W_a$  (the adaptive substitution rate) and  $\Pi_s$  (synonymous polymorphism - a proxy of the population mutation rate). The main result is clearly visualized on Figure 2: global negative correlation between the rate of adaptive substitutions and mutation rate which consists of many group-specific positive correlations. Authors explained this result through the time-scale dependent effect: the global negative trend might be driven by the increased necessity in beneficial mutations in low-population-size species (primates) as compared to high-population-size specie (mussels); the local positive within-group correlations might be explained by the fact, that positive selection indeed, is limited by the supply of new mutations: in species with increased mutation rate ( $\Pi_s$ ), the rate of positive selection ( $W_a$ ) is faster.

The manuscript is very interesting, well-written and has several provocative ideas and suggestions. It was a pleasure to read it.

**Thanks much for this comment.**

I have several comments / questions:

**Comment 1 :**

=> Transition from local positive trends to the global negative one: how and when? I have a problem understanding the proposed time-scale dependent scenario: at which moment and how the trends are changing? I think the manuscript will benefit from a potential mechanism of shifting from one scenario to another.

**Answer 1 : Thanks for this pertinent comment. We do not think that there is a clear divergence time threshold that would make the relationship shift from a positive to a negative relationship – rather a more gradual process. One should keep in mind that our sampling scheme is highly stratified: species within a group in our sample share essentially the same genome content and organization, whereas species from distinct groups are quite different. Our results suggest that when species share similar traits such as life history traits and genome structure so that they do not show a significantly different DFE, then the relationship between  $\theta$  and  $\omega_a$  will be driven by the mutation limitation mechanism. On the contrary, if two species are divergent enough to show different DFE, and in particular to show a different proportion of adaptive mutations, then other factors such as their long-term population size seem to be the main determinants of the relationship between  $\theta$  and  $\omega_a$ . Depending on the species one is looking at, the divergence time needed to show significantly different DFE might vary. Methods aiming at testing for invariance of the distribution of fitness effects across species (such as polyDFEv2.0 (Tataru and Bataillon 2018)) might actually help to answer this question which we think will be the subject of a future study.**

**Comment 2 :**

=> Can the local positive trends be driven by the sampling bias(es): individuals, genes? Despite the fact that authors provide a lot of analyses and controls in the manuscript, I would still propose several potential reasons which may lead to non-biologically meaningful within-group positive correlations.

==> Population structure. Probably in vertebrates and other species with steep positive slopes (primates, ants, rodents) the populations are more structured (there are more deep sub-population separations in the within-species phylogeny) as compared to more panmictic species (mussels, butterflies). This may lead to the fact that 4-6 individuals from each species will represent well enough mussels, but not primates. In primates, for example, one individual, sequenced from distant population may significantly increase both:  $\theta$ s and  $w_a$ , leading to a positive correlation: from less population structured to more population structured species within each group.

==> Public data versus exome data: subsets of analyzed genes. We can imagine that exome data of non-model invertebrate species is shifted towards more evolutionary constrained genes (easier to map, annotate, etc). If, so, such genes are expected to have decreased rate of adaptive substitutions as compared to less-evolutionary constrained genes, available in whole-genome data of publicly available (mainly vertebrate) species.

**Answer 2 : As for population structure, two elements comfort us with the idea that it does not introduce a bias in the results:**

-First, we incorporated the so-called  $r_i$ 's nuisance parameters (Eyre-Walker et al. 2006), to be optimized along side with DFE parameters. These parameters are intended to capture a wide range of effect that would distort the shape of SFS, including orientation errors, demography or population structure. We clarified the Material and Method section of the manuscript where these nuisance parameters are mentioned (lines 584-587).

-Second, we estimated the  $F_{is}$  statistics for each species. A positive  $F_{is}$  indicates population structure.  $F_{is}$  values are actually lower for low- $\pi_s$  species (i.e., ants, rodents, primates, passerines) than for high- $\pi_s$  groups (non-vertebrates excluding ants) (average  $F_{is}$  for low- $\pi_s$  species: 0.068 vs. average  $F_{is}$  for high- $\pi_s$  species: 0.16). Second, we observe no correlation between  $F_{is}$  and  $\omega_a$  (regression test p-value=5.9e-01) or  $F_{is}$  and  $\pi_s$  (regression test p-value=2.9e-01), and so even when focusing only on groups with steep positive slopes (ants, rodents, primates, passerines: regression test p-value=8.9e-01 and 2.1e-01 respectively), suggesting that population structure is not likely to explain the observed pattern. We added this additional result at the end of the Results section (lines 266-268).

As for public data vs. exome data : there is no reason exome data from non-model invertebrates would be biased towards more constrained genes, as the captured genes were chosen randomly from *de novo* assemblies of transcriptome data and not by homology with distantly related species (Material & Methods, section 4 of discussion). Additionally, we have a "control" group as we included *Drosophila*, an invertebrate group with public data available.

**Comment 3 :**

=> Authors mentioned that both  $W_a$  and  $W_{na}$  (non-adaptive substitutions) globally demonstrate negative correlations with  $\pi_s$ . If so, do we see a positive correlation between both of them:  $W_a$  and  $W_{na}$ ? I think it is an interesting analysis to discuss (partially covered by Fig S5).

**Answer 3 : Analyzing all species, we did not detect any correlation between  $\omega_a$  and  $\omega_{na}$ , either considering all mutations or only the GC-conservative ones.**

**Comment 4 :**

=> in the chapter 5 in the result section authors mentioned that correlation between  $W_a$  and life-history traits disappeared after the control for phylogenetic inertia. What about the control for phylogenetic inertia between  $W_{na}$  and life-history traits? See also the related question below.

**Answer 4 : Here please see the answer to comment 9 of the second reviewer.**

**Comment 5 :**

=> at the end of the chapter 5 (lines 253-260) authors describe positive relationships between  $\omega_{na}$  (non-adaptive substitutions) and fecundity, and negative relationships between  $\omega_{na}$  and body mass, longevity and propagule size. It means that  $\omega_{na}$  is higher in species with high effective population size ( $N_e$ ) which is wrong, according to my knowledge.

**Answer 5 : This is a mistake in the text: as shown in figures S4, there is a negative relationship between  $\omega_{na}$  and fecundity, and a positive relationship between  $\omega_{na}$  and body mass, longevity and propagule size. This is now corrected in the manuscript (lines 214-219). Thanks for spotting this.**

**Comment 6 :**

=> line 61: 'not particularly long-lived'. I think it is not a clear statement.

**Answer 6 : We now simply state “Adaptive mutations are expected to contribute negligibly to the pool of segregating alleles.” (line 61).**

**Reviewer 2 :(anonymous)**

The study by Rousselle et al. investigates the relationship between the rate of adaptation ( $\omega_a$ ) and the effective population size across different time-scales. For this purpose, the authors collate newly generated and publicly available protein coding sequence resequencing data across 50 species belonging to ten divergent groups of animals. Based on this data set, the authors then estimate the rate of adaptation in these 50 species. Subsequent analysis of the relationship between the rate of adaptation and different proxies of the effective population size suggests a positive relationship at short time-scales, and no or a negative relationship at large time-scales.

The authors address a relevant question based on an impressive data set. Their findings are interesting, and are discussed from different angles. I have only a few major concerns with respect to data analysis, presentation and interpretation of results. In addition, I think the writing of the Introduction and Discussion as well as the order of the Results should be improved to better guide the reader.

Major remarks:

**Comment 8 :**

1) My biggest concern with respect to the presentation and interpretation of results is the inconsistency between Figures 1 and 2. Figure 1 reports a negative relationship between  $\omega_a$  and  $\pi_S$  at large time-scales, and Figure 2 reports positive relationships between  $\omega_a$  and  $\pi_S$

at short time-scales. However, while Figure 1 is based on all mutations, Figure 2 is based on GC-conservative mutations only. Looking up the respective of Figure 1 A and B for GC-conservative changes in the Supplementary Material, I once find a slightly positive and once a negative relationship between  $\omega_a$  and  $\pi_S$  at large time-scales, Figure S2 and S3, respectively. This leaves a somewhat dubious impression. Given that the main conclusion of the study is based on the contrast between relationships shown in Figures 1 and 2, I suggest the authors to consistently report results based on GC-conservative changes in the main text, and report results based on all mutations in the Supplementary Material.

Moreover, significance levels of relationships should be reported throughout the main text and the Supplementary Material. It seems the authors intended to report significance levels by a star, but stars are absent throughout Figures S2 and S3. If this means all relationships are not significant, this should in addition be spelled out. If stars instead have been forgotten to add, they should be added. At present the statement starting on page 9, line 179, “Here again, the correlations, even if not significant, were in line with ...” seems not well supported.

**Answer 8 : Right. We now chose to display both the results with all mutations and only GC-conservative mutations on figure 1 and 3 (previously figure 2): indeed, even if we can trust more the estimates using only GC-conservative mutations because we remove the potential influence of gBGC, this approach also strongly reduces the size of the data set, which probably increases the sampling variance (see figure 1 and 3, and several modifications in Results section 4 of the manuscript).**

**We also made clearer the presence or absence of significance level in figures: we now indicate in black dotted lines the significant regressions, and in grey dotted lines the non-significant regressions.**

**Comment 9 :**

2) The authors seem to control for phylogenetic inertia in some of their analyses but in others not. It is not entirely clear to me why the authors choose to do so. I suggest the authors to consistently control for phylogenetic inertia.

**Answer 9: We actually consistently controlled for phylogenetic inertia, even if this was indeed not clear in the previous version of the manuscript. In the revised version of the manuscript, we chose to simplify the management of phylogenetic inertia: due to our stratified sampling scheme, which includes sets of closely related species from distantly related taxonomic groups, we actually control for phylogenetic inertia when performing group-level analyses (i.e. in section 2 and part of the section 3 of Results). On the contrary, for all correlations performed at the species level, phylogenetic inertia is not controlled for, and p-values are not calculated. We hope this appears more clearly in the manuscript now (lines 204-209 for instance).**

**Comment 10 :**

3) In the discussion of their results, the authors state on page 17, line 355, “We do not see any particular reason why the gene sample would be biased with respect to virus interacting proteins in

some specific groups, ...” I suggest the authors to back up this statement by actually examining if the gene sample is biased with respect to virus interacting proteins in some specific groups.

**Answer 10 :** This would be a good control analysis, but it is actually very hard to achieve this efficiently: indeed, if there is a good database of VIPs (viruses interacting proteins) in human (Enard et al. 2016; Castellano et al. 2019), there is, to our knowledge, no database in any of the invertebrate species we used in this study. Based on Castellano et al. 2019’ s list provided in supplementary material, we identified 2100 orthogroups over 8700 that are defined as VIPs in the great ape group, i.e. 24% of the great ape dataset. But we have no way to compare this quantity with other groups, preventing us from identifying a bias in gene content between large- vs. low- $N_e$  species.

**Comment 11:**

4) In order to better guide the reader through the results, I suggest the authors to re-order results sections, and present section 5 directly after section 2. Sections 2 and 5 both address the relationship between  $\omega_a$  and the effective population size at large time-scales, and are both suggestive of a negative relationship. I think it would be nice to first address the relationship between  $\omega_a$  and the effective population size at large time-scales from all different angles, and afterwards resolve the puzzle by the ANCOVA currently presented in section 3. Thus, my suggested order is 1, 2, 5, 3, 4.

**Answer 11 :** We changed the order of the sections as suggested.

**Comment 12 :**

5) In the opening of the Introduction, the authors explain that different theoretical models can predict either a positive or a negative relationship between  $\omega_a$  and the effective population size. This is a very nice opening of the Introduction. However, I think it is important that underlying assumptions of different models are stated more explicitly. Specifically, instead of stating “one would intuitively expect” (page 2, line 33), the authors should clearly state, “under the assumption of a constant DFE one would expect”. The assumption of a constant DFE is crucial to the positive relationship between  $\omega_a$  and the effective population size, and is in clear contrast to other models discussed in the same paragraph (page 3, line 45). This is only one example. More generally, differences in the underlying assumptions of the different models should be stated more clearly. In addition, the authors mention that if  $s \gg 1/N_e$ , then mutations should accumulate roughly at rate  $4N_e \mu_a s$ . It would be more accurate to say, if  $s$  is small and  $N_e s \gg 1$ , then mutations should accumulate roughly at rate  $4N_e \mu_a s$ . Besides, I also suggest shortening sentences throughout the Introduction. Some of the sentences span up to six lines, and could easily be split into two or three separate sentences in order to improve readability.

**Answer 12 :** We added details concerning the underlying assumptions in the introduction (lines 35, 44-45). We also split some sentences to make the text more readable (for instance in lines 77-80). Most importantly, the whole manuscript has been reviewed by an English proofreading and correcting service.

**Comment 13 :**

6) In the opening of the Discussion (section 1), the authors seem to emphasize that data have been generated as part of the present study. I don't think it is necessary to "sell" the study by emphasizing data generation. In my opinion, the value of the study rather lies in their interesting observations. I therefore suggest the authors to reduce the emphasis on data generation, but instead directly start by a summary of their main findings. Moreover, I think it is important to also in the opening of the Discussion clearly state that a fixed DFE across divergent taxa would be necessary in order to expect the same relationship across taxa. Section 2 of the Discussion seems rather technical and lengthy. Most of its content is actually already mentioned in the Results section. I suggest the authors to radically shorten this section. I think it would be more valuable to instead focus the Discussion on sections 3 and 4, and also strengthen the respective sections.

**Answer 13 :** We removed the short paragraph emphasizing data generation in the opening section of the discussion. We also now add a comment on the fixed DFE across taxa assumption in this part at line 274-275. We also slightly shortened section 2 of the Discussion, particularly sentences repetitive of the Results section. We strengthened section 3, especially with elements suggested by the third reviewer (see line 343 to 350).

**Minor remarks:****Comment 14 :**

7) Page 3, line 63, the authors mention near-neutrality together with the original MK test. Note that the original MK test is based on the Neutral theory of molecular evolution not on the Nearly neutral theory of molecular evolution. This should be corrected.

**Answer 14 :** We modified the manuscript from line 62 to 65.

**Comment 15 :**

8) Page 5, line 97, the sentence "Of note, the species sampled in this study ..." comes a bit out of the blue, and might better be introduced in context of the next paragraph starting line 100. Besides, the wording "in this study" gives the impression the authors refer to the present study and should be replaced with "Galtier (18)".

**Answer 15 :** Right. We actually removed this sentence.

**Comment 16 :**

9) Page 5, line 110, "In this study, we propose to test the effect of evolutionary scale on ..." should be replaced with "In this study, we test the effect of evolutionary time-scale on ...".

**Answer 16 :** Done.

**Comment 17 :**

10) Could the author please comment on why the percentage of recovered among targeted transcripts was noticeably low in two of the earth worms?

**Answer 17:** The group in which the *de novo* transcriptome and exome were the most fragmented and in which we recovered the more non-targeted DNA is earth worms, for a reason we do not fully understand. This may be linked to the globally lower percentage of recovered among targeted transcripts compared to other groups.

Additionally, *Lumbricus terrestris* is the species within our exon capture experiment that have the highest divergence with the species used to design the baits (i.e. *Allobophora chlorotica L1*), with a synonymous substitution divergence of 0.2 subst./site (a divergence that we did not know before performing the experiment). The recommendations of MYbaits were to use a maximum divergence of 15% between the species used to design the baits and the targeted species, so we think that the low the proportion of recovered exons for this species is due to the low performance of the capture experiment for such divergent sequences.

As for *Allobophora chlorotica L4*, we have no explanation regarding the low percentage of recovered among targeted transcripts.

We added a brief explanation for the lowest value of the table in the text (see line 133-134).

**Comment 18:**

11) Page 8, line 147, “ands called the diploid genotypes of individuals ar every coding position.” should be replaced with “and called the diploid genotypes of individuals for every coding position.”.

**Answer 18: Done.**

**Comment 19 :**

12) Page 8, line 148, “summed up” should be replaced with “summarized”.

**Answer 19: Done**

**Comment 20 :**

13) Table S3, the same number of decimal digits should be reported throughout the table. Besides, a precision of 6 decimal digits seems not necessary.

**Answer 20: Right, we modified Table S3 accordingly.**

**Comment 21 :**

14) The caption of Table S3 provides an explanation why #SNPs are not integers. Reading this explanation several times, I am still not able to understand it. I suggest to replace with a simpler explanation.

**Answer 21: The reason why SNP numbers are not integers in our raw SFS data has to do with missing data. In essence an SFS has a unique sample size, but in actual data sets many positions are affected by one or a few individuals missing a genotype. This is a practical problem. Our (and others') solution to this problem is:**

**- arbitrarily decide on an SFS sample size, k, which must be lower than (or equal to) the true sample size**



- discard any SNP at which the number of non-NA genotypes is below  $k$   
- for SNPs at which the number of non-NA genotypes,  $k'$ , is above  $k$ , perform hypergeometric projection, i.e., calculate the probability of obtaining 1, 2, ...etc variant states by subsampling  $k$  genotypes out of  $k'$ , then alter the SFS accordingly (see Hernandez et al. 2007 MBE 24:2196, Gayral et al. 2013 PLoS Genetics e1003457). This last step is why SNP numbers are not integer.

This is very technical. Plus, it happens our program for estimating  $\omega_a$  rounds SNP numbers before fitting the model to data. For this reason, and for the sake of simplicity, we now provide round SNP numbers in Table S3.

**Comment 22 :**

15) Page 12, line 223, “We were concerned that the correlation ...” should be replaced with “We were concerned that the positive correlation ...”.

**Answer 22: Done.**

**Comment 23 :**

16) Figure 3, the same legend is presented in each of the panels. However, not all of the species groups are represented in each of the panels. The legends should be updated accordingly.

**Answer 23: This is right. We modified the legends accordingly in Figure 3, as well as Figure S2 and S3.**

**Comment 24 :**

17) Formatting of p-values should be consistent throughout the manuscript. I suggest consistent formatting as scientific numbers.

**Answer 24: Right. Corrections have been done throughout the manuscript.**

**Reviewer 3 : (D. Enard)**

In their manuscript, the authors explore the complex relationship between rates of protein adaptation and population size. The authors provide evidence that a positive correlation between the rate of adaptation and population size is visible only within the lowest range of population sizes where evolution is mutation-limited. This is indeed shown very clearly by figure 2. The authors also suggest that there might be a negative correlation between the rate of adaptation and group-level, overall population size that is compatible with a Fisher’s geometric model of protein adaptation where proteins in small populations tend to be further away from their optimum, thus leaving more space for adaptive steps.

Despite some limitations that are well acknowledged by the authors themselves, the manuscript represents an important milestone for the understanding of how adaptation is influenced by

population size. The main limitation of the manuscript is the “small” number of data points in figure 1 for the group-level analysis. Despite the impressive sampling and sequencing effort, the ten data points do not make it possible to conclude firmly that there is a negative correlation between adaptation rate and population size at this scale. But the sampling effort that would be required is unrealistically large and cannot be asked of the authors. This preliminary evidence is still extremely valuable and will certainly pave the way for future studies. I could see other reviewers pointing out that the small number of data points does not provide enough power, but this would be missing the point of the message of the paper. It is a first foray with an impressive, yet still inconclusive sample size, but that shows the way to the field for making progress.

This is what I like the most about this study. It really puts some order to the former literature mess, and really shows a clear path toward understanding the problem at hand. The introduction is also an excellent recap of the recent progress made.

**Thanks much for such a positive assessment of our work.**

**Comment 25 :**

In addition to the reasonable explanation of Fisher’s Geometric model, the authors could also discuss the possibility that adaptation itself could have decreased  $p_s$ , which could contribute to the group-level negative correlation. The authors should also discuss the possibility that the effect of adaptation itself on diversity might hide a group-level positive correlation, and that at the very least, future simulations will be needed to see the selective/population size regime where this could, or could not happen.

**Answer 25 : Thanks for this suggestions. We added a paragraph addressing the effect of linked selection at the end of section 3 of the Discussion (lines 343-350).**

**Comment 26:**

Always about figure 1, the authors mention that for figure 1B,  $\omega_a$  is unbiased estimate of the adaptive rate. However, the authors do not specify if for figure 1A,  $\omega_a$  is also an unbiased estimate. This should be specified because as it is, the reader is left wondering.

**Answer 26 : Being an ML estimator, Eyre-Walker's estimator of the adaptive rate  $\omega_a$  must in principle be unbiased under its model assumptions - i.e., constant  $N_e$ , panmixy, neutral synonymous mutations, Gamma-distributed deleterious effects of non-synonymous mutations. We here similarly show (Supp Mat Box 1) that under the assumption of rare, sudden changes in  $N_e$ , such that the sampled species have reached the mutation/selection/drift equilibrium, the  $\omega_a[A]$  multi-species estimator is unbiased.**

**This however does not account for fluctuations of  $N_e$  at a short timescale. Eyre-Walker et al (2006, 2009) cleverly introduced the  $r_i$ 's nuisance parameters to control for these effects, something we reproduce here. The  $r_i$ 's are not part of any explicit model, so there is no formal proof, as far as we know, that  $\omega_a$  estimators using  $r_i$ 's are unbiased in case of short-term fluctuations of  $N_e$ . This is true of both the  $\omega_a[A]$  and  $\omega_a[P]$  estimators. The latter combines SFS from different species into a single one, so, also heavily relies on the  $r_i$ 's. What we have,**

however, are simulations suggesting that  $r_i$ -based estimators are reasonably accurate under a variety of conditions (e.g. Galtier 2016).

In summary, the matter is a bit complex due to our use of the  $r_i$ 's. To avoid any confusion while addressing the reviewer's concern, we removed the word "unbiased" from the main text, and rather indicate that  $\omega_a[A]$  was intended to account for long-term fluctuations in  $N_e$  (modifications in lines 168-169 and 190-192).

**Comment 27 :**

My main remaining concern has to do with weakly advantageous mutations that do not fix fast enough that they can be neglected in the estimation of the number of nonsynonymous polymorphism. If there are more weakly advantageous mutations with a smaller intensity of selection ( $2Ns$ ) in smaller populations, and if the still-segregating adaptive variants bias the estimate of  $\omega_a$  downwards, then this could also explain the within-group positive correlation particularly visible in groups with small  $p$  s . This possibility really depends on the ability of Grapes to deal properly with weakly advantageous mutations. From Galtier Plos Genetics 2016S1 text, it looks like weakly advantageous mutations that still segregate are well taken into account, with a simulated intensity of  $2Ns=20$ . However, it would be great to see the performance of Grapes across a wider range of selection intensities, and also when coding sequences experience a mix of weak and strong selection intensities. This would lift a small remaining doubt I have about the robustness of Grapes relative to selection intensity, and how this could influence the results presented in the manuscript. Maybe the authors just need to provide more information about Grapes in their manuscript to address this.

**Answer 27 :** To account for the presence of weakly adaptive polymorphisms, and especially, for the discrepancies in terms of contribution of such mutations to the SFS across species, we used an estimates of  $\omega_a$  that is an average weighted by the AIC of three estimates obtained by three DFE models:

- the so-called "GammaZero" that models a continuous, negative Gamma distribution.
- the so-called "GammaExpo" that models a continuous, negative Gamma distribution and a proportion of weakly advantageous mutations, assumed to be exponentially distributed.
- the so-called "ScaledBeta" that models a discrete DFE, with a class of neutral mutations and a class of strongly deleterious mutations, which do not contribute to polymorphism or divergence, and a Beta shaped distribution of weak-effect mutations (both positive and negative) (Galtier 2016)

The two last models take into account the presence of weakly adaptive polymorphisms in two different ways, and the models that fits best the data will contribute more to the final estimation of  $\omega_a$  due to the AIC-weighted averaging approach we used. We think that this approach allows to deal correctly with species discrepancies in terms of influence of weakly adaptive polymorphisms in the SFS. This was a bit cryptic in the first version of the manuscript, and is now more explicit (line 160-162, 579-580).

**Comment 28 :**

Finally, in order to make the manuscript even more thorough than it already is, the authors could add a paragraph of discussion about how interference between nearby advantageous mutations could potentially decrease the rate of adaptation when  $p_s$  is high.

**Answer 28: Good point. Now covered by the additional paragraph we wrote in section 3 of the Discussion (see from line 339 to 340).**

Overall, this manuscript represents a very solid contribution. Most limitations are well acknowledged already, and the few things left unanswered are easy to address. It should also be pointed out that this manuscript adds to a growing body of work that highlights the relevance of Fisher's geometric model regarding protein evolution (for example, recent papers from the Lohmueller lab). It is reassuring to see that different labs and approaches are converging to a similar conclusion that Fisher's geometric model may explain differences in both adaptive and deleterious rates across species with distinct complexity and population sizes.

**Other comment:**

**-We added two tables in Supplementary data to provide the population genetics estimates of all species (Table S6) and all groups (Table S7).**

**-We added a "Conflict of interest disclosure" paragraph (see lines 631-633).**

**References :**

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