Supporting information for:

The inflated significance of neutral genetic diversity in conservation genetics

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Text S1. Estimating and interpreting mutation load.

Mutation load is a reduction in fitness of individuals in a population caused by the constant pressure of deleterious mutations appearing in their genomes, which was first theoretically conceptualized by Haldane in 1937 (1, 2). Recent advances in genome sequencing technology have allowed us to empirically investigate mutation load and test theoretical predictions using genomic data from humans (3–5), gorillas (6), dogs (7), horses (8), Alpine ibex (9), pandas (10), and several other species (11). In these studies, the estimation of load relies on identifying deleterious mutations in the genome, which is usually achieved using measures of sequence conservation. If a mutation within an individual appears at a site that is conserved (i.e. is the same nucleotide across many different phylogenetically diverged species) then it can be presumed that this mutation has a negative effect on that individual's fitness. For each individual within a population, the total number of mutations at phylogenetically constrained sites can be added up to arrive at a statistic that is proportional to mutation load (12, 13). However, it is important to note that this score makes multiple assumptions: 1) it is possible to accurately identify deleterious mutations and their selection coefficient; 2) deleterious mutations act additively; 3) there is no epistasis. However, available comparative genomic methods do not reliably detect the majority of selected sites (14) and do not differentiate between mutations with minor effects on fitness from those with drastic effects on fitness (14, 15). Furthermore, we do not have a good understanding of the distribution of mutational fitness parameters such as dominance and epistasis (13, 16–18). Thus, it is not yet entirely clear how much interspecies

comparisons of estimated mutation load truly reflect underlying differences in fitness.

Apart from the difficulty of measuring mutation load, arguably the biggest obstacle for understanding its relevance in conservation genetics is the question of what mutation load actually means for the size and viability of a population. Mutation load is a population genetics concept regarding relative fitness to an idealized individual that does not carry any deleterious mutations, i.e. to the perfect genotype. However, it was mathematically shown that the perfect genotype is exceedingly unlikely to exist (19). Thus, individuals in a population never directly compete with the perfect genotype. Furthermore, the viability of a population in its ecological and environmental context, i.e. its *absolute* mean fitness, is more relevant for conservation genetic purposes than the relative fitness of its individuals (2, 20). Agrawal and Whitlock have investigated models that try to close the gap between population genetic and ecological consequences of deleterious mutations (2). In their model, mutation load can affect birth and death rates, and an individual's rate of resource acquisition. Whereas birth and death rates affect the equilibrium population size, the rate of resource acquisition does not. Individuals that can acquire more resources produce more offspring and, in case mutation load reduces the rate of resource acquisition in some individuals, others can gather more resources and produce more offspring, leading to an unchanging equilibrium population size. Similarly, if mutation load leads to an individual's early death, e.g. in the zygote state, then this individual does not exhaust resources and the population size is, again, not affected (2). On the other hand, in models where two species compete for the same resources, mutation load on the rate of resource acquisition does indeed have a strong effect on equilibrium population size and can lead to population extinction (2). Although these models are agnostic to the many complex ecological dependencies between individuals, populations, and species, they emphasise the importance of ecological

relationships for gaining an understanding of how mutation load affects the persistence of a population.

In short, mutation load does not necessarily have a simple relationship with population persistence, and the idea that species cannot persist with high loads, independent of other assumptions, is incorrect (2). At what life-stages deleterious mutations act, and which life-history traits they affect, becomes relevant for predicting the extinction risk of the population—an aspect that is not captured by the plain selection coefficients of deleterious mutations as measured by population genetic methods.

References

1. J. B. S. Haldane, The effect of variation of fitness. *The American Naturalist* **71**, 337–349 (1937).
2. A. F. Agrawal, M. C. Whitlock, Mutation load: The fitness of individuals in populations where deleterious alleles are abundant. *Annual Review of Ecology, Evolution, and Systematics* **43**, 115–135 (2012).
3. K. E. Lohmueller, *et al.*, Proportionally more deleterious genetic variation in European than in African populations. *Nature* **451**, 994–997 (2008).
4. Y. B. Simons, M. C. Turchin, J. K. Pritchard, G. Sella, The deleterious mutation load is insensitive to recent population history. *Nat. Genet.* **46**, 220–224 (2014).
5. B. M. Henn, *et al.*, Distance from sub-Saharan Africa predicts mutational load in diverse human genomes. *Proc. Natl. Acad. Sci. U. S. A.* **113**, E440–E449 (2016).
6. T. van der Valk, D. Díez-Del-Molino, T. Marques-Bonet, K. Guschanski, L. Dalén, Historical genomes reveal the genomic consequences of recent population decline in Eastern Gorillas. *Curr. Biol.* **29**, 165–170.e6 (2019).
7. C. D. Marsden, *et al.*, Bottlenecks and selective sweeps during domestication have increased deleterious genetic variation in dogs. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 152–157 (2016).
8. M. Schubert, *et al.*, Prehistoric genomes reveal the genetic foundation and cost of horse

domestication. *Proc. Natl. Acad. Sci. U. S. A.* **111**, E5661–9 (2014).

9. C. Grossen, F. Guillaume, L. F. Keller, D. Croll, Purging of highly deleterious mutations through severe bottlenecks in Alpine ibex. *Nat. Commun.* **11**, 1001 (2020).

10. Y. Hu, *et al.*, Genomic evidence for two phylogenetic species and long-term population bottlenecks in red pandas. *Sci Adv* **6**, eaax5751 (2020).

11. T. van der Valk, T. van der Valk, M. de Manuel, T. Marques-Bonet, K. Guschanski, Estimates of genetic load in small populations suggest extensive purging of deleterious alleles. *biorxiv* (2019) https:/doi.org/10.1101/696831.

12. Y. B. Simons, G. Sella, The impact of recent population history on the deleterious mutation load in humans and close evolutionary relatives. *Curr. Opin. Genet. Dev.* **41**, 150–158 (2016).

13. B. M. Henn, L. R. Botigué, C. D. Bustamante, A. G. Clark, S. Gravel, Estimating the mutation load in human genomes. *Nat. Rev. Genet.* **16**, 333–343 (2015).

14. C. D. Huber, B. Y. Kim, K. E. Lohmueller, Population genetic models of GERP scores suggest pervasive turnover of constrained sites across mammalian evolution. *PLoS Genet.* **16**, e1008827 (2020).

15. D. S. Lawrie, D. A. Petrov, Comparative population genomics: power and principles for the inference of functionality. *Trends Genet.* **30**, 133–139 (2014).

16. C. D. Huber, A. Durvasula, A. M. Hancock, K. E. Lohmueller, Gene expression drives the evolution of dominance. *Nat. Commun.* **9**, 2750 (2018).

17. D. J. Balick, R. Do, C. A. Cassa, D. Reich, S. R. Sunyaev, Dominance of deleterious alleles controls the response to a population bottleneck. *PLoS Genet.* **11** (2015).

18. A. J. Dagilis, M. Kirkpatrick, D. I. Bolnick, The evolution of hybrid fitness during speciation. *PLoS Genet.* **15**, e1008125 (2019).

19. B. Galeota-Sprung, P. Sniegowski, W. Ewens, Mutational load and the functional fraction of the human genome. *Genome Biol. Evol.* **12**, 273–281 (2020).

20. B. Wallace, *Fifty Years of Genetic Load: An Odyssey* (Cornell University Press, 1991).