

COMMENT



Some hope and many concerns on the future of the vaquita

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Recently, Robinson et al. (2022) concluded that “The critically endangered vaquita is not doomed to extinction by inbreeding depression”. This stems from the assumption that, because of its low amount of deleterious variation, the inbreeding load B (i.e., the fitness load ascribed to recessive deleterious components that is hidden in heterozygotes and which fuels future fitness inbreeding depression), should be negligible in this extremely endangered porpoise, represented by about 10 individuals living in the northern-most part of the Gulf of California, Mexico. Like Robinson et al. (2022), we do not think the vaquita is doomed and we are in favor of encouraging conservation efforts in critically endangered populations (García-Dorado 2015; Hedrick and García-Dorado 2016). However, we think the optimistic view of Robinson et al. (2022) is unsupported by the evidence and their approach can be dangerous to the conservation of the vaquita and other endangered species.

DELETERIOUS VARIATION AND INBREEDING LOAD UNDER DIFFERENT EFFECTIVE POPULATION SIZES

Deleterious variation is expected to be smaller in endangered species because of their history of reduced effective population size (N_e), that results in both purging and genetic drift. It has been estimated that the vaquita suffered an ancient reduction of its effective size by about one order of magnitude about 30,000 generations ago (roughly from $N_e = 3 \times 10^4$ to $N_e = 5 \times 10^3$; Morin et al. 2021) followed by a modest reduction from $N_e = 4485$ to $N_e = 2807$ about 2162 generations ago and then a dramatic reduction for the last 40 years down to about 10 individuals, which suggests a present effective size well below 10 (Robinson et al. 2022). For a perspective on N_e values in endangered species, a recent estimate (Garner et al. 2020) suggested that 25% of the 170 endangered species they examined had a current effective size $N_e < 50$. The estimated historical effective population sizes of the vaquita are therefore relatively large, and do not suggest that the species has been historically endangered.

For all the above historical N_e values estimated in the vaquita (down to 2807), the genetic diversity due to alleles with deleterious effects large enough to be relevant in the conservation context should be mainly constrained by natural selection instead of by drift (Kimura et al. 1963, García-Dorado 2007). Therefore, a substantial reduction of that deleterious diversity compared to the ancestral population with $N_e = 3 \times 10^4$ should be mainly ascribed to purging, as suggested by Morin et al. (2021). However, as illustrated by the predictions

given in the next section, computed considering the demographic history of the species, purging is not expected to have been able to reduce the inbreeding load to the point that inbreeding depression can be assumed of no concern for the survival of the present critically endangered extant population. As explained below, the low gene diversity reported for the vaquita can be consistent with a relatively large and threatening inbreeding load.

The low genetic diversity for neutral and deleterious categories reported in the vaquita should be mainly ascribed to the modest effective size during the last 30,000 generations (including the $N_e = 4485$ period more than 2162 generations ago), or even to some ancient transient bottleneck that could have passed undetected in the demographic history analysis. Most of the later decline down to the present census seems to have occurred during a short 40-year period equivalent to three generations with population sizes about 500, 200 and 10, according to Figure 1A in Robinson et al. (2022), and only small recent inbreeding has been detected ($F_{ROH} = 0.05$). Therefore, this recent dramatic population shrinkage is unlikely to have caused a relevant decline on gene diversity, either due to genetic drift or to purging. It is important to note that, for the range of historical N_e values estimated in vaquita, the gene diversity contributed at equilibrium by alleles with moderate or severe deleterious effects is much less sensitive to N_e than that contributed by mildly deleterious or neutral alleles. Even in the Loss of Function (LoF) category, deleterious alleles with smaller effects (or smaller recessive deleterious components) are expected to make a larger contribution to the larger gene diversity of other cetacean species compared to the vaquita. Thus, the low putatively deleterious genetic diversity of vaquitas could contain a larger proportion of relatively severe deleterious alleles than that for other cetaceans, which could contribute significant inbreeding load. Also, ancient transient undetected bottlenecks should impact current gene diversity for severely deleterious alleles less than that for neutral or mildly deleterious alleles because the mutation-drift (MD) balance for neutral variants is likely not to have been attained yet, while the mutation-selection-drift (MSD) balance for more deleterious alleles builds up much more quickly (Kimura 1980).

Therefore, the low gene diversity for deleterious categories reported by Robinson et al. (2022), is not a guarantee of small inbreeding load. The inbreeding load is unknown basically because the fitness effects of the segregating putatively deleterious alleles are unknown. This is illustrated by the theoretical equilibrium predictions given in the next section,

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which account for both inbreeding and purging and give important inbreeding load for all the effective sizes in the history of vaquita demography. Robinson et al. (2022) performed simulations based on the inferred demographic history of the vaquita and found a small inbreeding load ($B = 0.47$) by the time of the recent dramatic reduction in census numbers. Below we discuss how the mutational model used in those simulations can lead to an underestimation of B and, therefore, of the extinction risk.

MUTATIONAL MODEL AND PREDICTIONS

The per genome deleterious mutation rate used in Robinson et al. (2022) simulations was computed using the rate of mutation per base pair in just the coding sites that can produce nonsynonymous mutations in the vaquita genome. The distribution of deleterious effects (s) was inferred from the site frequency spectra by assuming additive gene action. Then, arbitrarily decreasing fixed values of the coefficient of dominance (h) were assigned to classes of increasingly deleterious effects. Robinson et al. (2022) performed a sensitivity analysis showing that the extinction risk could in fact be twice that reported. However, the robustness of the results might have been overestimated because these genomic inferences of the distribution of deleterious effects tend to severely underestimate the rate of mutation for the relatively severe deleterious effects that are more relevant for conservation (say, $s > 0.05$). As explained below, a mutational model that accounts for such severely deleterious mutations predicts a substantial inbreeding load ($B = 3.8$) for an equilibrium population with the smaller recent historical effective size reported for vaquita ($N_e = 2807$) that can jeopardize the survival of the critically endangered vaquita population.

To illustrate this, we have considered the mutational model used and discussed by Pérez-Pereira et al. (2021), designed to account for the mutational deleterious effects revealed by mutation accumulation experiments as well as for the more relevant fraction of the mildly deleterious mutations detected in genomic analysis. The advantage of this model (our model hereafter) is that mutation accumulation experiments give detailed information about the distribution of deleterious effects with moderately large effect (Davies et al. 1999, Eyre-Walker and Keightley 2007; Eyre-Walker and Keightley 2013) that are relevant for the short time periods and small population sizes usually involved in conservation issues. This model will underestimate the mutation rate for the fraction of mutations with very small deleterious effects that is detected in genomic analysis, but these are of little conservation relevance.

To highlight the comparison between the models used in Robinson et al. (2022) and Pérez-Pereira et al. (2021), note that both models give a 0.12 mutation rate for homozygous deleterious effects for $s < 0.1$, but the one used in Pérez-Pereira et al. (2021) gives a 7.5-fold larger mutation rate for $s > 0.1$ (0.0733 vs. 0.0098). In addition, Pérez-Pereira et al. (2021) assumed that the average degree of dominance is a random variable whose expected value decreases with increasing homozygous deleterious effects, as deduced from mutation accumulation experiments, instead of assigning arbitrary fixed h values as in Robinson et al. (2022).

It is convenient to note that the mutational model used by Robinson et al. (2022) and the one we use here (Pérez-Pereira et al. (2021) have been recently compared by Wade et al. (2022) where they have inferred that a proportion of 0.5 and 1% of the total number of nonsynonymous mutations are lethal in humans and *Drosophila*, respectively. Then they compare these values to that of our model, where the rate of quasi lethal recessive mutation amounts 3.5% of the model's total deleterious mutation rate. We note that this direct comparison is not informative because, as

explained above, our deleterious mutation rate does not correspond to the rate of nonsynonymous mutation. In fact, our lethal mutation rate (7.7×10^{-3}) accounts just for half the classical estimates for *Drosophila* and contributes just 28% of our prediction for the vaquita inbreeding load (see below). As for Wade et al. (2022) estimates, they imply extremely small lethal mutation rates (1.5×10^{-3} for humans and 5×10^{-4} for *Drosophila*), compared to classical estimates (0.015 in *Drosophila* or even twice that, Mukai 1964, Fry et al. 1999). Thus, the quasi-lethal mutation rate in our model, despite being much larger than that obtained by Wade et al. (2022), is likely an underestimate of the true rate, and is not expected to produce upward biased predictions of the inbreeding load, but maybe some downward bias.

Using our mutational model (Pérez-Pereira et al. 2021) we obtain predictions for B at the mutation-selection-drift (MSD) balance using Eq. 13 in García-Dorado (2007) integrated over the joint distribution of deleterious effects and degrees of dominance. We obtain $B = 9.3$ for the ancient $N_e = 3 \times 10^4$ population size, and smaller but roughly similar values for the population sizes corresponding to the two more recent historical periods considered in Robinson et al. (2022): $B = 4.6$ for $N_e = 4485$ and $B = 3.8$ for $N_e = 2807$. With an $N_e \approx 5000$ maintained since 30,000 generations ago, the population should have virtually attained the MSD balance (and even the MD balance for neutral variation) with the corresponding $B \approx 4.6$ value when, 2000 generations ago, N_e dropped down to 2807. Then, after about 2000 additional generations with $N_e = 2807$ the species should be approaching the new MSD balance leading to an expected inbreeding load $3.8 \leq B < 4.6$ by the time of the last drastic reduction of N_e . No substantial additional reduction is expected during the last three generations of dramatic shrinkage. All the above predictions of B are on the order of those usually obtained in wild populations, for which a mean value $B \approx 6$ was proposed by O'Grady et al. (2006) after a meta-analysis (see also Hedrick and García-Dorado 2016) and which is consistent with a more recent survey finding that the mean inbreeding load for survival until sexual maturity was $B = 3.5$ among wild vertebrate populations (Nietlisbach et al. 2019) (note that the inbreeding load for lifetime fitness, which also includes reproductive traits, is expected to be substantially higher).

Our prediction for the current inbreeding load in vaquita (at least $B = 3.8$) is 8 times larger than the $B = 0.47$ value obtained in the simulations by Robinson et al. (2022). This suggests that the vaquita population was historically sustainably large, in agreement with the reported demographic history, and is now in the first few generations of a dramatic decline where inbreeding load can substantially contribute to the extinction risk. According to our theoretical predictions, the two historical reductions in size estimated for the vaquitas about 30,000 and 2000 generations ago are expected to have promoted a reduction of the inbreeding load, which should mainly be ascribed to purging as discussed above. That reduction of the inbreeding load can be important and even crucial for the persistence of the critically endangered population, as has been appreciated by Morin et al. (2021), particularly under appropriate conservation surveillance. However, the remaining inbreeding load can still be large enough to contribute substantial extinction risk after the dramatic population shrinkage recently experienced by the vaquita.

OTHER CONCERNS ABOUT THE SIMULATIONS

According to Robinson et al. (2022) simulation results, vaquitas are expected to recover if bycatch is immediately and completely suppressed. In fact, since census recovery implies both a slower future inbreeding and more efficient (though

slower) purging (García-Dorado 2007), the positive outcomes predicted by these simulations could extend forward in time according to theory. However, several factors challenge the reliability of these simulation results. To mention a few: a) the inbreeding load can be much higher than assumed, as discussed above; b) purging under the present extremely small population size could be inefficient and, furthermore, is not expected until some inbreeding has accumulated, often after several generations of unopposed inbreeding depression (García-Dorado 2012); c) since life history parameter are unknown for the vaquita, values inspired in the closely-related harbor porpoise are used instead in the simulations which implies a adventurous extrapolation, particularly considering that this species is considered of minor conservation concern. These life -history parameter determine the species reproductive potential which critically affects the Minimum Viable Population size (García-Dorado 2015; Khan et al. 2021; Pérez-Pereira et al. 2022); and d) stochastic environmental and demographic factors, not considered in simulations, will increase the extinction risk.

In addition, although there might be exceptions to the association between adaptive potential and gene diversity, the low genetic diversity of the species suggest that its adaptive potential could be seriously impaired (García-Dorado and Caballero 2021; Kardos et al. 2021; Teixeira and Huber 2021). Also, even in the case that present adaptive potential would not be a concern, it is being lost rapidly. Robinson et al. (2022) argued that the risk from reduced adaptive potential “is challenging to quantify and should not preclude recovery efforts in the short term”. We agree with this view, but that is not a reason to ignore that a threat exists and that present and near future environmental changes might be more challenging and necessitate more adaptive variation than that remaining in the vaquita.

Demographic predictions of the effects of inbreeding depression on population dynamics requires demographic estimates of inbreeding depression. Some predictions could be made about how strong inbreeding depression might be in a population using simulations with assumptions regarding the demographic history, mutation parameters, recombination rate (none of which are understood well in the vaquita). However, these are only useful for making very rough assessments and not for making specific predictions for a particular population. Another issue is that even if these parameters are known exactly, such predictions are probably not very reliable because of the stochasticity in all the factors that lead to the observed real inbreeding load and drift load in a given population.

Thus, a major concern is that there is no direct demographic estimate of inbreeding depression in the vaquita. This is understandable because there are very few individuals where fitness could be assayed, and because there are no individuals that have been examined that are the result of close inbreeding (their genomic estimate of average inbreeding in 10 individuals is 0.05). This lack of more highly inbred individuals could be because the bottleneck is too recent or because these individuals have died because of low viability. In other words, there is no ground truth for the prediction of no inbreeding depression in individuals that are actually inbred. Obviously, if the species survive into the future there will be close inbreeding because the current numbers are so low which could lead to some fitness component being quite low due to inbreeding depression.

GENERAL CONCLUSION

Hope for recovery in the vaquita is qualitatively supported by theory, which predicts that a) a modest historical effective population size (N_e) reduces the inbreeding load ascribed to (partially) recessive deleterious alleles, increasing the resilience

of the populations to future inbreeding (García-Dorado 2007) and b) after a reduction of the population size, purging tends to reduce and partially revert inbreeding depression as long as the population is not so small that natural selection becomes inefficient (García-Dorado 2012). Therefore, we agree with the message given by Robinson et al. (2022) that it is worthwhile and necessary to completely halt bycatch at once, and we think there would be ground for this message even if only on the basis of theoretical predictions, but there is no evidence that the inbreeding load of the extant population is irrelevant, and the optimistic rate of increase in population size observed in the simulations may not be a reliable representation of the future. Vaquitas might not be fatally doomed to extinction, but their prospects are dismal. If the conclusions in the vaquita paper are naïvely accepted and those methods used as a blueprint for simulation of viability and conservation policy on other small, endangered populations, it will likely lead to misguided management, and then to extinctions.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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