



## Simulation Suggests that Repeated Supplementations from the Wild into Captive Population Reduce but Cannot Eliminate Inbreeding

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### Abstract

Captive population can suffer from inbreeding due to founder's effect and supplementation from the wild has been considered to increase genetic diversity and reduce inbreeding. However, a recent simulation study suggests that one-off naïve supplementation from the wild cannot increase genetic diversity; thereby, suggesting more complicated supplementation regimes. Hence, we hypothesize that that repeated supplementations can better increase genetic diversity compared to single supplementation. Our simulations show that repeated 10% supplementations results in significantly higher genetic diversity ( $p$ -value  $\leq 1.48E-03$ ) compared to one-off 10% supplementation, and increasing the supplementation ratio of repeated supplementations results in higher genetic diversity ( $p$ -value  $\leq 2.35E-04$ ) compared to repeated 10% supplementations. However, increasing repeated supplementation ratios above 100% may not further increase genetic diversity. This implies that repeated supplementations have the potential to reduce but not eliminate inbreeding.

**Keywords:** Captive Population; Inbreeding; Supplementation; Simulation; Genetic Diversity

### Introduction

Inbreeding is the result of successful mating between two organisms which are closely related to each other, such as consanguineous unions between parents, siblings and first and second cousins [1]. It has been used as a breeding strategy used by breeders for the aim of enhanced performance [2] and is useful in genetics research as inbred populations tend to express recessive traits and diseases [3]. Artificial breeding is known to result in inbreeding [4]. Another causes of inbreeding are population bottleneck or founder's effect, which are common in captive populations, where there is reduced diversity due to the lack of mating options for the organisms [5,6]. Inbreeding can result in reduced genetic fitness and performance of off-springs, leading to increased risk of extinction [7-10].

Supplementing captive population from the wild [11,12] to increase genetic diversity, leading to lowered inbreeding, have been suggested; even though inbreeding is also possible in wild populations [1,13,14]. A recent simulation study by Johny, *et al.* [15] suggests that one-off naïve supplementation is unlikely to be able to increase genetic diversity; thereby, suggesting more complicated supplementation regimes. In this study, we extend the work of Johny, *et al.* [15] by examining the effects of genetic diversity from repeated simple supplementations from the wild into captive population using computer simulations, which are commonly used in the study of inbreeding [16-18] and evolution [19-22]. We hypothesize that repeated supplementations can better increase genetic diversity compared to single supplementation as previously studied [15]. Our results that repeated supplementations with supplementation ratios of up to 100% may increase genetic diversity in captive population.

## Methods

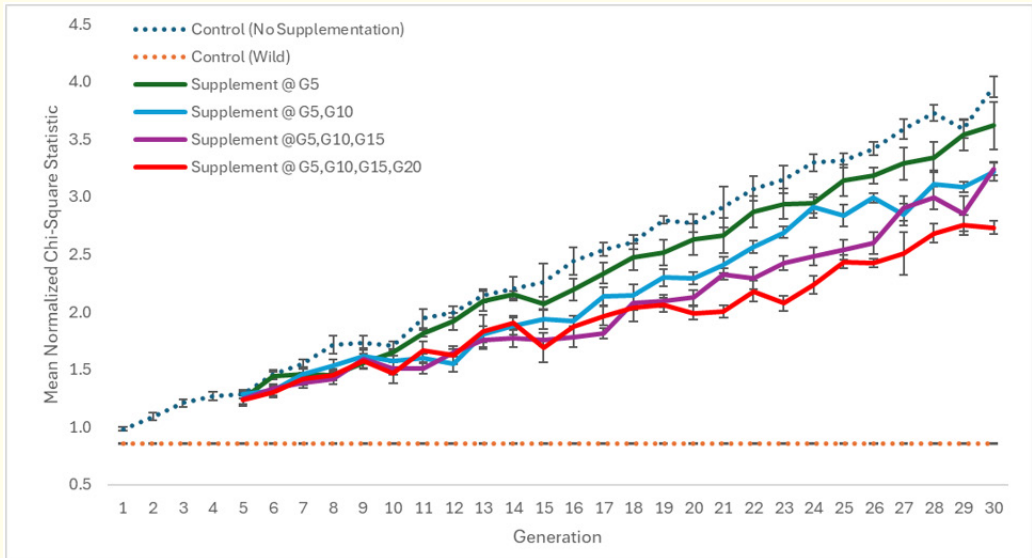
The simulation setup was based on Johny, *et al.* [15] using Island [23], which had been used in a previous studies [14,15]. A population of one million organisms, representing wild population, was generated with 50 genetic markers where each marker had 10 alleles of equal proportion. Five replicates of 500 organisms each, representing five captive populations, were randomly selected from wild population, and simulated for 30 generations with captive population size kept constant throughout the simulation. In the first set of simulation (one-off versus multiple supplementation), 50 organisms (10% supplementation) were randomly selected from the wild and supplemented at (a) generation 5; (b) generations 5, and 10; (c) generations 5, 10, and 15; and (d) generations 5, 10, 15, and 20. In the second set of simulation (different supplementation ratios), a sample equivalent to 33%, 55%, 77%, or 100% of the captive sample size were randomly selected from the wild and supplemented to each captive population at generations 5, 10, 15, and 20. In the third set of simulation (supplementation to absurdity), a sample equivalent to 300%, 500%, 700%, or 900% of the captive sample size were randomly selected from the wild and supplemented to each captive population at generations 5, 10, 15, and 20. Chi-square statistic was calculated for each generation using the uniform allelic frequency as expected while the allelic frequencies in the population for each generation were used as observed values [24-26]. To compare results from different simulations, normalized Chi-square value was calculated from Chi-square statistic as a quotient of Chi-square statistic and the degrees of freedom.

## Results and Discussion

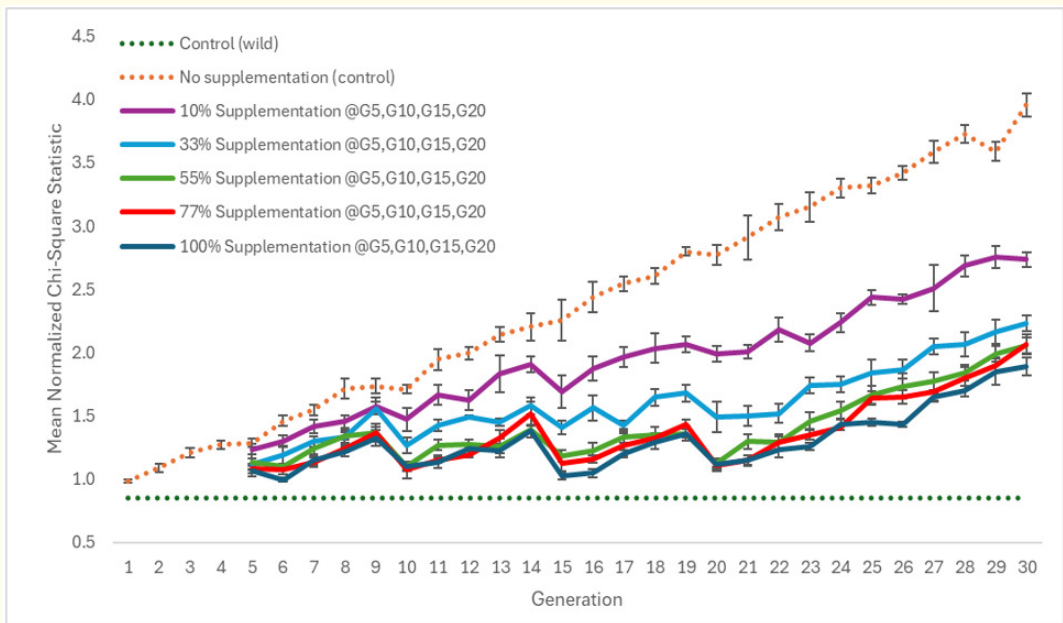
Johny, *et al.* [15] suggests that one-off naïve supplementation at generation 5 is unlikely to be able to increase genetic diversity; thereby, suggesting more complicated supplementation regimes. In this study, we replicate Johny, *et al.* [15]'s supplementation at generation 5; and repeated the supplementation at generations 10, 15, and 20 (Figure 1). Our results show that there are no significant differences between no supplementation and single supplementation in generation 5 at generation 20 (p-value = 0.391) or 30 (p-value = 0.182). However, there are significant differences between no supplementation and (a) repeated supplementations in gen-

erations 5, and 10 at generation 20 (p-value = 1.48E-03) or 30 (p-value = 3.17E-04); (b) repeated supplementations in generations 5, 10, and 15 at generation 20 (p-value = 2.76E-04) or 30 (p-value = 3.68E-04); and (c) repeated supplementations in generations 5, 10, 15, and 20 at generation 20 (p-value = 6.97E-05) or 30 (p-value = 1.13E-05). More importantly, repeated supplementations in generations 5, 10, and 15, is significantly different (p-value = 2.94E-04) to repeated supplementations in generations 5, 10, 15, and 20 at generation 30. This suggests that the number of supplementations is proportional to genetic diversity. Hence, repeated supplementations are likely more effective than one-off supplementation as constant introduction of new alleles into the captive population can improve the fitness of the population [27,28].

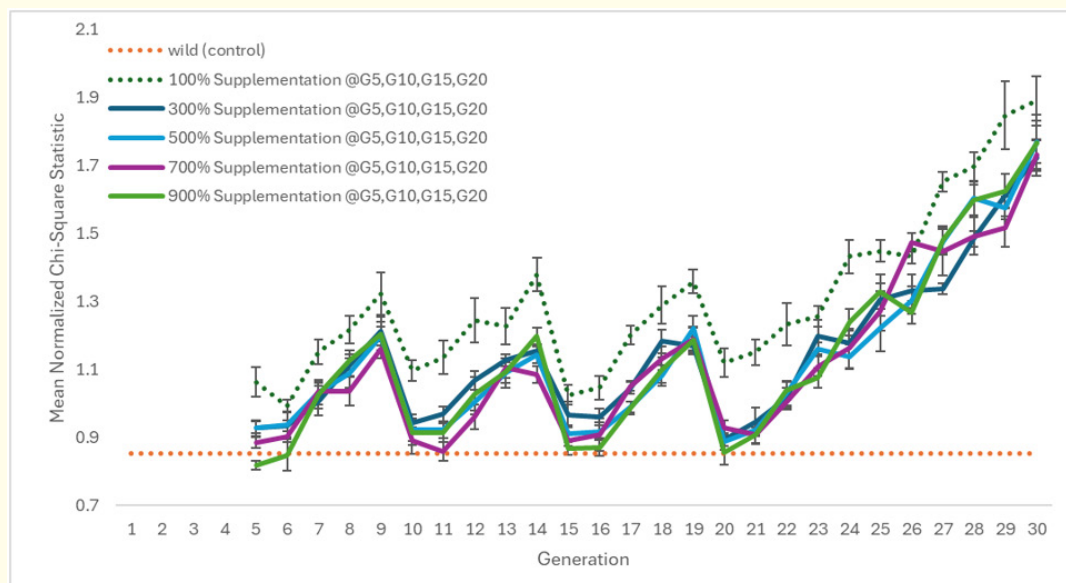
Since multiple 10% supplementations are likely to increase genetic diversity (Figure 1), it is plausible that repeated supplementations at higher supplementation ratios can increase genetic diversity more than multiple 10% supplementations. Hence, 33%, 55%, 77%, or 100% supplementation ratios were simulated. Our results (Figure 2) show significant differences with 33%, 55%, 77%, or 100% when compared to 10% repeated supplementations at generation 20 (10% vs 33%, p-value = 1.64E-05; 10% vs 55%, p-value = 1.64E-05; 10% vs 77%, p-value = 4.91E-06; or 10% vs 100%, p-value = 6.00E-06) and 30 (10% vs 33%, p-value = 6.18E-05; 10% vs 55%, p-value = 6.18E-05; 10% vs 77%, p-value = 2.35E-04; or 10% vs 100%, p-value = 2.34E-05). However, supplementations to absurdity (300%, 500%, 700%, or 900% supplementation ratios) are not statistically significant between each other (p-value  $\geq$  0.103) (Figure 3). This suggests that increasing supplementation ratios can increase genetic variability up to a certain point. These results also show the effectiveness of multiple supplementation method as previously suggested [15]. Repeated supplementations with sufficient diversity may reduce the chances for inbreeding [5,28]. However, our results also suggest that repeated supplementations are not likely to eliminate potential inbreeding. Hence, the effects of larger supplementation ratios and multiple generations need to be investigated as there needs to more research on long term effects of supplementations [12,29].



**Figure 1:** Single versus multiple 10% supplementation. Four supplementation schemes were evaluated – (a) single supplementation at generation 5 (G5); (b) repeated supplementations at generations 5, and 10 (G5,G10); (c) repeated supplementations at generations 5, 10, and 15 (G5,G10,G15); and (d) repeated supplementations at generations 5, 10, 15, and 20 (G5,G10,G15,G20).



**Figure 2:** Repeated supplementations at generations 5, 10, 15, and 20; with supplementation ratio from 10% to 100%.



**Figure 3:** Repeated supplementations at generations 5, 10, 15, and 20; with supplementation ratio from 100% to 900%.

## Conclusion

This study suggests that repeated supplementations with high supplementation ratio (up to 100% supplementation) have the potential to increase genetic diversity but not eliminate inbreeding in captive population.

## Supplementary Materials

Data files for this study can be downloaded at <https://bit.ly/GeneticDriftC>.

## Conflict of Interest

The authors declare no conflict of interest.

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