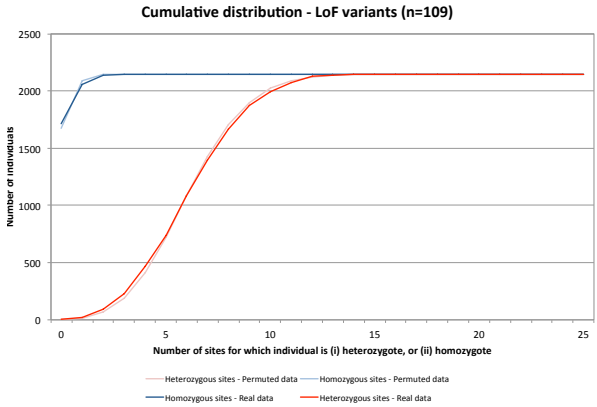
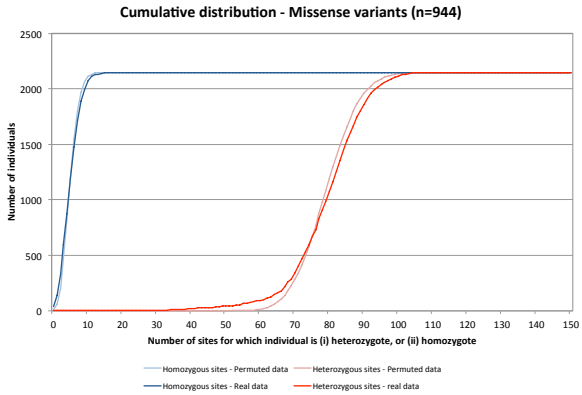
Supplemental Material S3 – Lack of evidence for synergistic epistasis

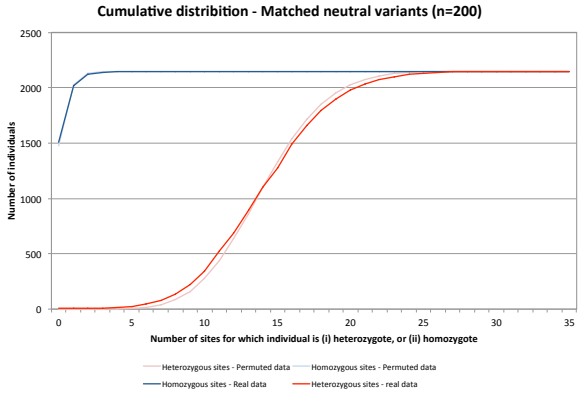
It has been hypothesized that deleterious variants might be purged from the population by synergistic epistasis, i.e. the fact that multiple deleterious variants have a larger cost on fitness than predicted from their multiplicative effect[1]. This hypothesis predicts that (healthy) individuals carrying multiple deleterious variants will be fewer than expected assuming random assortment.

To test this hypothesis we look at the distribution of the number of individuals that were (i) heterozygote, and (ii) homozygote for *i* genetic variants, where *i* ranged from 0 to the *n* (i.e. the number of genotyped variants). The analysis was conducted separately for "loss-of-function" variants (stop gains, splice site and frame shift variants), missense variants considered by SIFT/POLYPHENE2 to be deleterious/damaging, and matched neutral variants. We used the genotypes of 2,147 BBCB animals generated as part of this study. The distribution for the real genotypes was compared with that obtained by permuting the labels of the individuals (separately for each variant), i.e. by randomizing the genotypes 100 times. For the permuted genotypes, the graphs show the average number of individuals that are heterozygote/homozygote for *i* variants across the 100 permutations.

There was no evidence for a reduction in the number of individuals with the higher number of heterozygous/homozygous sites, whether considering LoF or missense variants, on the contrary (i.e. there were more individuals with large number of heterozygous/homozygous sites with the real than with the permuted data). We observed a slight but significant ($p<0.01$) increase in the variance of the distribution for the real when compared to the permuted data (i.e. there were more individuals on both tails of the distribution with the real versus permuted data). This was observed for the three types of tested variants, including the matched neutral variants. The reason for this systematic increase in variance remains unknown.







We conclude that our data do not provide evidence for synergistic epistasis in this population.

[1] For instance : Keightley PD (2012) Rates and fitness consequences of new mutations in humans. Genetics 190 :295-304.