

Supplemental file S2: Supplementary file for wood degradation capacity and supplementary Figures S3-S10

Supplementary Methods

In addition to the mycelium growth rate, we also measured the capacity to degrade spruce wood (wood weight loss, hereafter WWL) as a fitness proxy in both homokaryons and heterokaryons. The capacity to degrade dead wood is indeed considered as an important fitness component in *Heterobasidion* spp. (Olson *et al.* 2012).

Estimation of wood weight loss in homokaryons and heterokaryons

To estimate WWL for each of the 16 homokaryons and 198 heterokaryons, spruce wood blocks were autoclaved three consecutive times at 121°C during 15 minutes, dried at 60°C for two days and weighed to the nearest 0.1 mg with a microbalance (Precisa ES 120A, Northern Balance, Gateshead, UK). Two sterile wood blocks were placed on a sterile metal grid and put on top of Hagem agar medium cast in a Petri dish. The middle of the Petri dish was inoculated with a 4 mm diameter plug taken from the margin of an actively growing mycelium culture (three different plate replicates per homokaryon or heterokaryon isolate). Wood blocks were colonized within one or two days. Plates were incubated at 20°C in the dark. After six months, each wood block was dried at 60°C for two days and weighed again as described previously. For each wood block, the amount of weight loss was computed by subtracting the final weight from the initial weight (94 and 1186 wood weight loss estimates for homokaryons and heterokaryons respectively). This experiment was conducted in a single assay for each homokaryon or heterokaryon isolate.

Estimation of c_{nucb} , c_{mit} and the average level of dominance of a trait

To comply with our statistical model, WWL was log-transformed prior to the analyses. Homokaryon genetic effects were estimated by averaging the values measured across different replicates (n=16 non-senescent homokaryon average WWL estimates). For each heterokaryon, the average genetic effect was computed by averaging the values measured across different replicates (n=198 heterokaryon average estimates).

As we used a single assay, we used a full model such as the one presented in Eq. 33 and 34 in Supplementary File S1, but omitting u_{assay} . In addition to those described, fixed effects in \mathbf{b} also comprised a covariate that accounts for the potential long-lasting effect of the initial biomass of wood block inoculated (*wood block initial weight*). Random effect and variance-covariance matrices are the same as those described in the main text, except for the dimension of the identity matrix used for random acceptor x donor genetic effects and plate effects ($V[\mathbf{u}_{acc \times don}] = \sigma_{acc \times don}^2 \mathbf{I}_{198}$, and $V[\mathbf{u}_{plate}] = \sigma_{plate}^2 \mathbf{I}_{640}$). As we used a single assay for WWL, random acceptor x donor genetic effects potentially comprised some uncontrolled environmental effects.

Supplementary results

The WWL of heterokaryon isolates (mean=0.25 mg, $sd^2=0.006$ mg) was higher on average and less variable than the WWL of homokaryon isolates (mean=0.17 mg, $sd^2=0.010$ mg, Figure S6B). The average MGR and WWL were positively correlated for heterokaryons (P -value=0.002) but not for homokaryons (P -value=0.12, Figure S7), potentially due to the lower number of homokaryons tested.

The models that included a covariance between nuclear genetic effects and between mitochondrial genetic effects were not supported ($\Delta AICc > 2.1$ and $\Delta AICc > 4.1$ respectively, Table S7A, Figure S9). As there was no genetic (nuclear or mitochondrial) covariance between

homokaryon and heterokaryon WWL, we could not estimate the parameters c_{nucl} and c_{mit} . Support for a covariance between acceptor and donor genetic effects was low ($\Delta AICc = 0.15$ for the model with $\sigma_{accdon}^2 = 0$, Table S7A). Furthermore, the log-likelihoods of the models *ii*, *iii* and *iv*, that included an effect of genetic distance and/or different average WWL for homokaryons and heterokaryons was the same as model *i* without these effects (Table S7B, Figure S10), so this effect had no support. Similar to MGR, mitochondrial effects accounted for a very large proportion of the phenotypic variation in WWL among homokaryons, but not among heterokaryons (78% vs. 0.5% respectively, Table S9). The proportion of phenotypic variance explained by acceptor and donor nuclear genetic effects was higher than that explained by homokaryon genetic effects (51% and 38% vs. 15% respectively, Table S9). WWL was estimated in heterokaryons from a single synthesis measured in a single assay, which can spuriously inflate the relative importance of genetic effects.

Supplementary discussion

We found that c_{nucl} is undefined (no correlation between nuclear genetic effects in homokaryons and heterokaryons). This result suggests that loci that are responsible for wood degradation in heterokaryons are different from those in homokaryons. Let us consider mutations that are deleterious in homokaryons but neutral in heterokaryons. We expect selection to favor an increase of the heterokaryotic phase proportionally to their selection coefficient (Rescan *et al.* 2016). However, we would expect fitness to be slightly higher in heterokaryons than in homokaryons if this explanation was true. An alternative explanation is that we did not have enough replication to accurately estimate c_{nucl} , c_{mit} and H. Finally, WWL assayed in the laboratory might not be a good proxy for fitness in the field. For example, the capacity to degrade dead wood might trade-off with the capacity to infect living trees (Olson *et*

al. 2012). Further experiments investigating this trait in the field with sufficient replication (e.g. using tree root inoculation, Garbelotto *et al.* 1997) would help validate this interpretation.

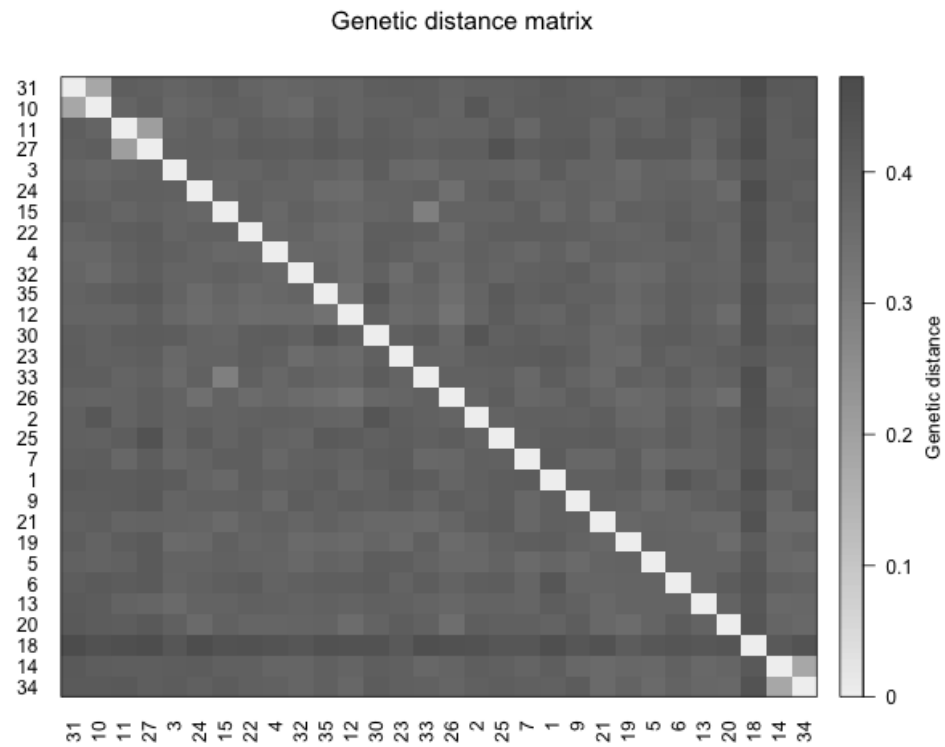


Figure S3 Pairwise nuclear genetic distance matrix between each isolate.

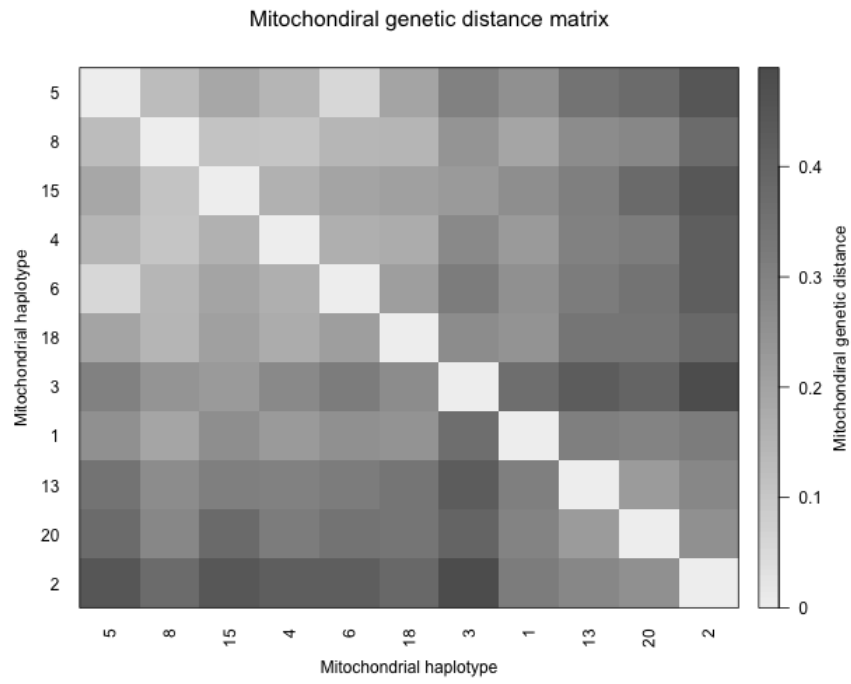


Figure S4 Pairwise mitochondrial genetic distance matrix between each isolate.

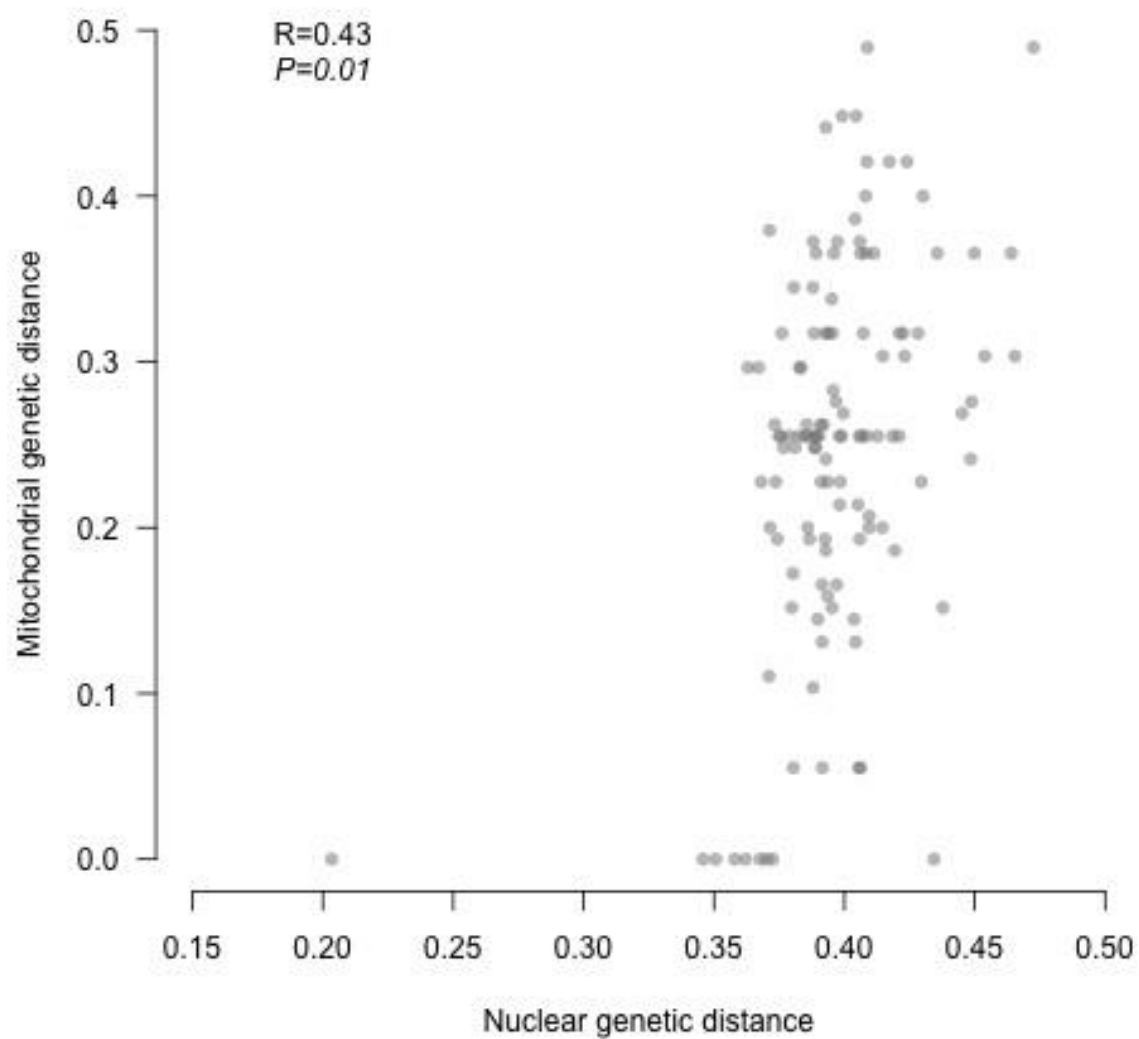


Figure S5 Correlation between the matrix of pairwise mitochondrial genetic distances and the matrix of pairwise nuclear genetic distances for the 16 non-senescent homokaryons. Mantel test: $R=0.43$, $P=0.01$, based on 100 permutation replicates.

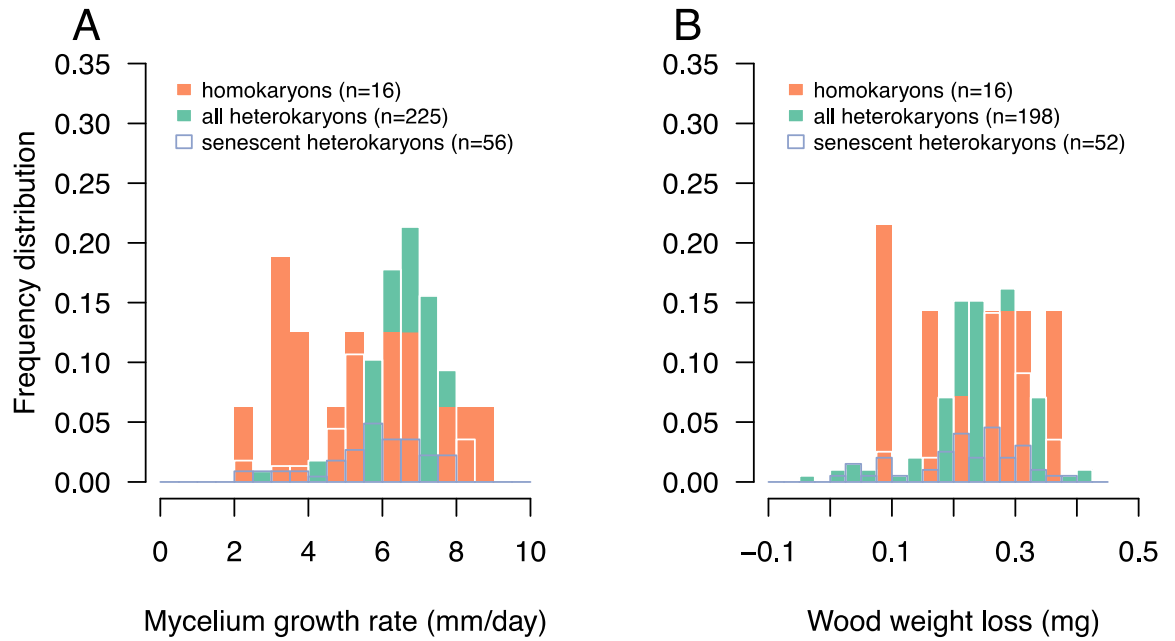


Figure S6 Distribution (A) mycelium growth rate and (B) wood weight loss for homokaryon and heterokaryon isolates. Senescent homokaryons had lower mycelium growth rates and wood weight loss compared to non-senescent heterokaryons.

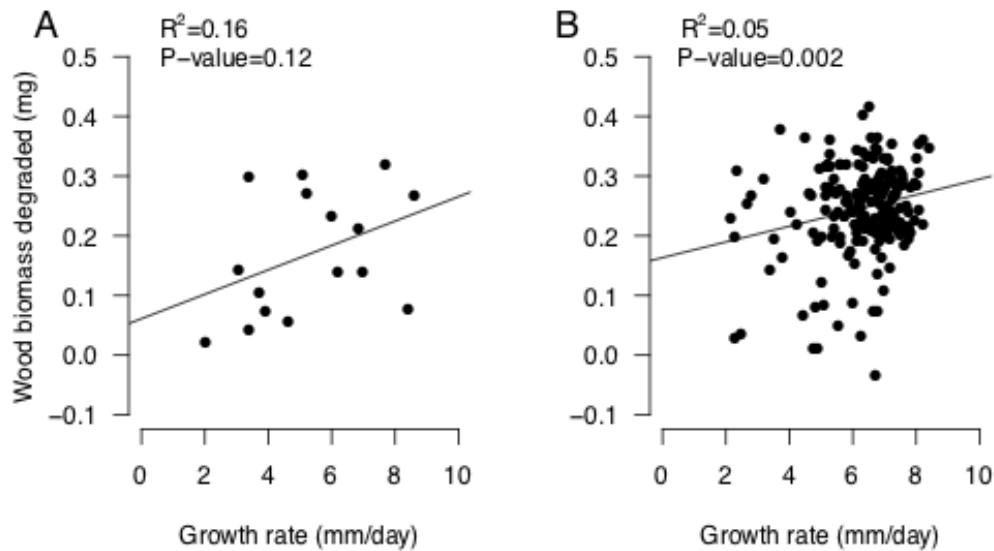


Figure S7 Correlation between the average mycelium growth rate and average wood biomass degraded by (A) homokaryons and (B) heterokaryons.

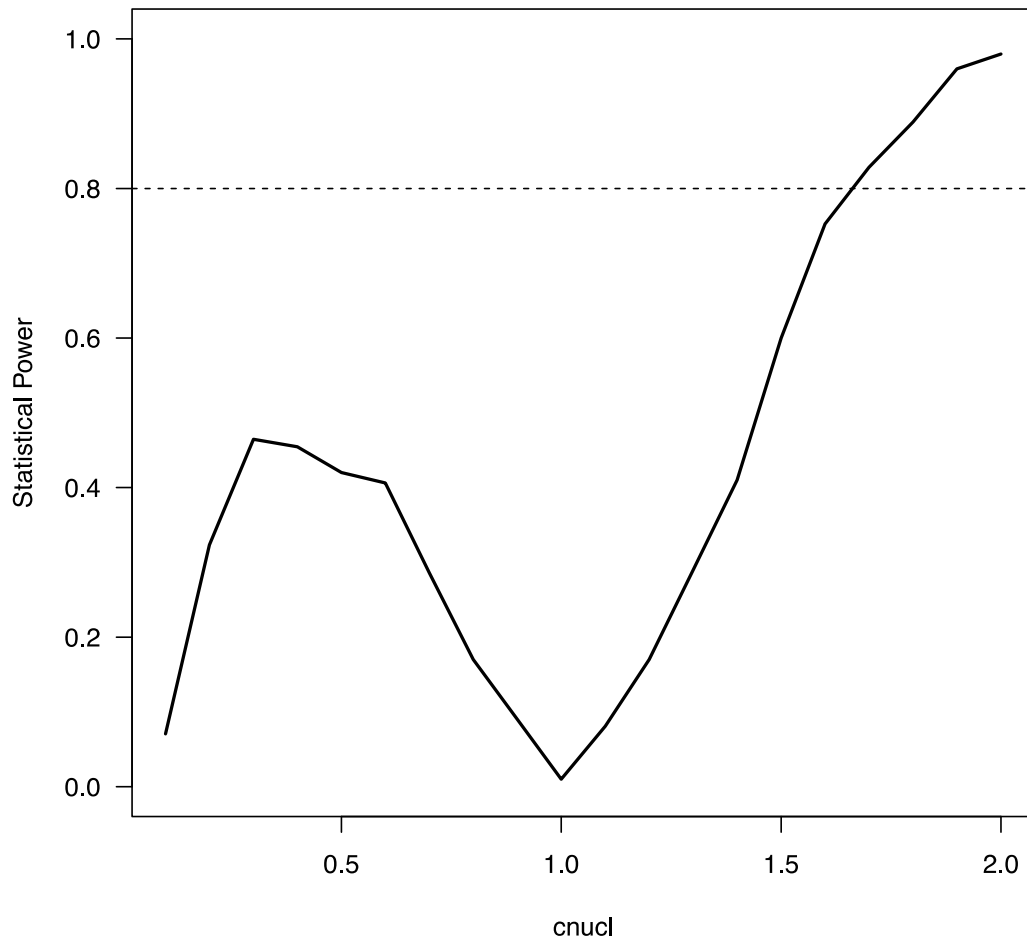


Figure S8 Statistical power to detect that c_{nucl} differed from 1 for different values of c_{nucl} . Power was estimated based on 100 simulated datasets for each value of c_{nucl} . The dashed horizontal line represents a threshold of 80%.

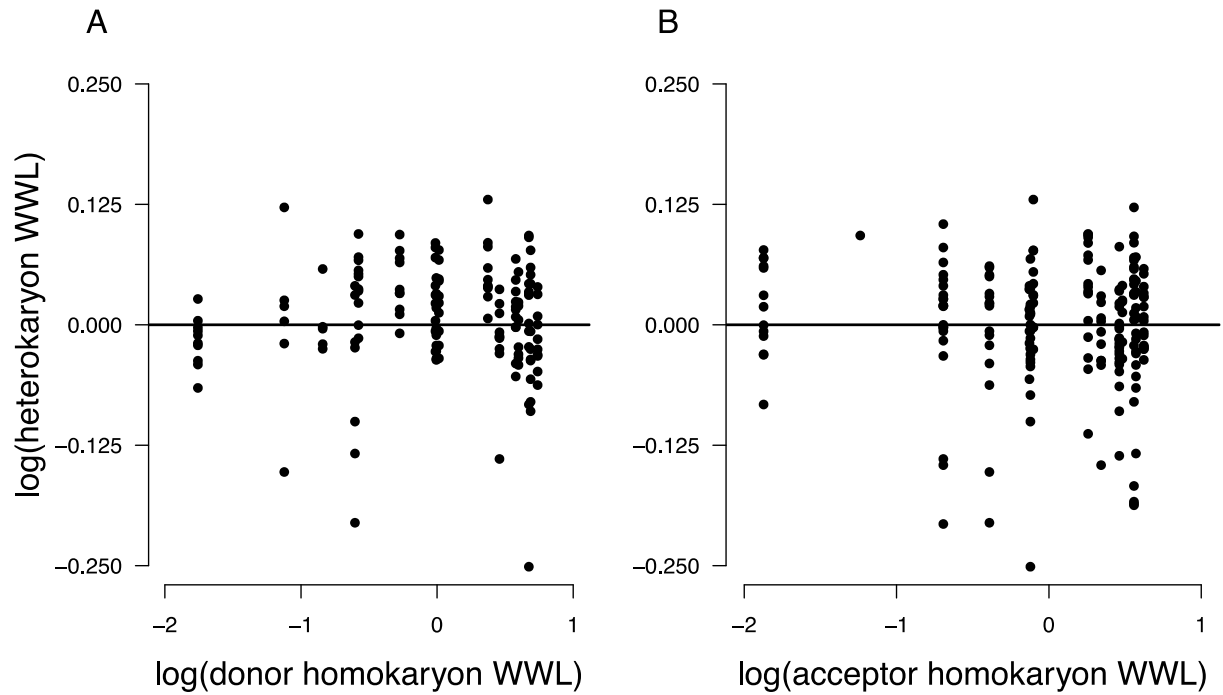


Figure S9 Relationship between the genetic values of wood weight loss of heterokaryon offspring and either their (A) donor or (B) acceptor homokaryon parent. As the variance due to nuclear and mitochondrial loci with fitness effects in both homokaryons and heterokaryons was zero, the slope (solid line) in A and B is zero. Genetic values are represented on a log scale and mean-centered ($n=151$ and $n=197$ heterokaryons with donor and acceptor homokaryon MGR data respectively).

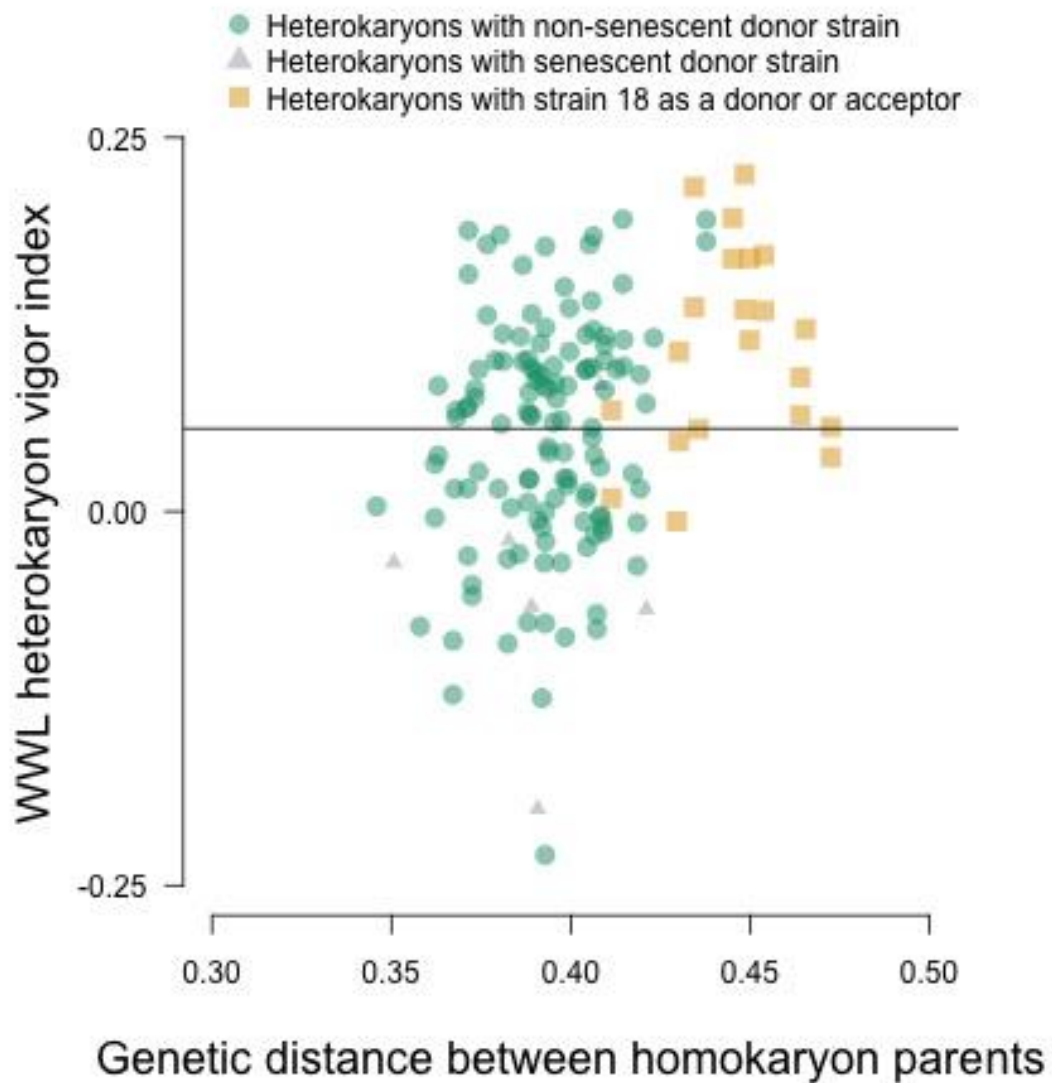


Figure S10 Relationship between WWL heterokaryon vigor index and genetic distance between homokaryon. Strain 18 is more genetically distant compared to the other strains and heterokaryon synthesized using this strain have higher trait values on average, creating a spurious correlation between heterokaryon and genetic distance when the non-independence between heterokaryons is not accounted for. Heterokaryon vigor is computed as the difference between $\log(\text{heterokaryons value})$ and $\log(\text{homokaryon mid-parent value})$. Solid lines represent the average heterokaryon vigor value (n=151 estimates).