Supplementary Information for

Mitonuclear interactions may contribute to fitness of fungal hybrids

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Supplementary tables

Table S1. Radial growth (average measure in mm ± SD) of the four different genotypes (ten replicate per genotype).

Genotype	**Replicate #**	**3 days**	**5 days**	**7 days**	**9 days**	**11 days**	**13 days**	**15 days**
A,a	1	5.5±2.12	12.5±0.71	18.5±0.71	26.5±2.12	29.5±2.12	34.5±0.71	40±0
A,a	2	3.5±0.71	9.5±0.71	17±1.41	23.5±2.12	27±1.41	32.5±0.71	35±0
A,a	3	2.5±0.71	8.5±0.71	17±0	25.5±0.71	29±1.41	32.5±0.71	34±1.41
A,a	4	4.5±0.71	10.5±0.71	19.5±0.71	27±0	30±0	35±1.41	37±0
A,a	5	4±0	9.5±0.71	17±0	24.5±0.71	27.5±0.71	33.5±0.71	36±0
A,a	6	5±1.41	11.5±0.71	20±1.41	28.5±0.71	31.5±0.71	35.5±0.71	37.5±0.71
A,a	7	6±2.83	12±1.41	22.5±0.71	28.5±0.71	31.5±0.71	36±0	40±0
A,a	8	4.5±0.71	9.5±0.71	17±0	25±1.41	28±1.41	34±1.41	36.5±0.71
A,a	9	4.5±0.71	11.5±0.71	19±1.41	26±1.41	29±0	33.5±0.71	35.5±0.71
A,a	10	4.5±0.71	10.5±0.71	15.5±0.71	21±1.41	25.5±2.12	31±2.83	34.5±2.12
I,i	1	6.5±0.71	12±0	16±1.41	24±1.41	31±1.41	35.5±0.71	40±0
I,i	2	6±0	11.5±0.71	16.5±0.71	25.5±2.12	31±1.41	36.5±0.71	40±0
I,i	3	8±0	14±0	18.5±0.71	26.5±0.71	32±1.41	36.5±2.12	40±0
I,i	4	6±0	12±0	17±0	25.5±0.71	31.5±2.12	36.5±2.12	40±0
I,i	5	5±0	10±0	15±0	21.5±0.71	27.5±0.71	32.5±2.12	36±0
I,i	6	7±1.41	12±0	17.5±0.71	26±1.41	30.5±2.12	34±1.41	40±0
I,i	7	4.5±0.71	9.5±0.71	15.5±0.71	23.5±0.71	29±0	34±0	40±0
I,i	8	5±0	10±0	15±1.41	23.5±0.71	28±0	33.5±0.71	38±0
I,i	9	3.5±0.71	9±1.41	13.5±0.71	21.5±0.71	26.5±0.71	32.5±0.71	37.5±0.71
I,i	10	0±0	7.5±0.71	13±0	20±0	25±0	31±1.41	36±1.41
IA,a	1	7±1.41	13±1.41	17.5±2.12	21±1.41	24±0	30±1.41	32.5±2.12
IA,a	2	8±0	13±0	17±0	20±0	23±0	29±0	33±0
IA,a	3	8±0	12±0	17±1.41	21±1.41	28±1.41	35±0	37±0
IA,a	4	0±0	8.5±0.71	14.5±0.71	19.5±0.71	23.5±2.12	32±1.41	34.5±2.12
IA,a	5	7.5±0.71	13.5±0.71	16±0	21.5±0.71	26±1.41	32.5±0.71	34.5±0.71
IA,a	6	6±0	10±1.41	14.5±0.71	19±1.41	22±2.83	30.5±0.71	33.5±0.71
IA,a	7	8±0	13.5±0.71	19±0	22.5±0.71	24.5±0.71	32.5±0.71	35.5±0.71
IA,a	8	3.5±2.12	9±1.41	14±0	18.5±0.71	21.5±0.71	28.5±2.12	32±2.83
IA,a	9	7.5±0.71	10.5±0.71	16.5±2.12	22±1.41	24±1.41	31±1.41	35.5±2.12
IA,a	10	7±0	11.5±0.71	17±0	22±0	25.5±0.71	28±0	30.5±0.71
IA,i	1	6.5±0.71	11±1.41	17.5±0.71	25.5±2.12	31±1.41	36±1.41	40±0
IA,i	2	6±0	11.5±0.71	17.5±2.12	25.5±0.71	29.5±0.71	33.5±0.71	40±0
IA,i	3	7.5±0.71	13±0	20±0	26.5±2.12	31±2.83	35±4.24	40±0
IA,i	4	9.5±0.71	16.5±2.12	21±1.41	28±0	31±0	34.5±2.12	40±0
IA,i	5	7±0	12±0	17±1.41	27.5±0.71	32±0	35.5±0.71	40±0
IA,i	6	7±0	11.5±0.71	16±0	23.5±0.71	28±1.41	32±1.41	40±0
IA,i	7	5.5±0.71	12±1.41	19.5±3.54	27±4.24	30±4.24	33.5±3.54	35.5±2.12
IA,i	8	9±0	14.5±0.71	21.5±0.71	30.5±0.71	34±0	37.5±0.71	40±0
IA,i	9	8.5±0.71	13.5±0.71	20±0	26.5±2.12	30.5±2.12	34.5±2.12	40±0
IA,i	10	5±0	10.5±0.71	19.5±0.71	26±0	29.5±0.71	33.5±0.71	40±0

Table S2. Expression levels of genes of *IA,a* compared to the parent *A,a* (control). The hypothesis test performed by REST2009 and based on 1000 iterations provides a *p-value* which represents the probability of the alternate hypothesis that the difference between *IA,a* and *A,a* is due only to chance.

Gene ID	Fold change (expression level)	*p-value*
46054	3.64	0.05
59167	0.27	0.00
147699	1.17	0.52
143314	2.75	0.00
33584	89.06	0.00
109183	0.29	0.00
148283	32.87	0.00
108968	0.22	0.00
46250	0.62	0.30

Table S3. Expression levels of genes of *IA,i* compared to the parent *I,i* (control). The hypothesis test performed by REST2009 and based on 1000 iterations provides a *p-value* which represents the probability of the alternate hypothesis that the difference between *IA,i* and *I,i* is due only to chance.

Gene ID	Fold change (expression level)	*p-value*
46054	1.44	0.22
59167	1.70	0.17
147699	0.28	0.00
143314	0.96	0.93
33584	0.27	0.06
109183	0.59	0.23
148283	0.02	0.00
108968	0.57	0.08
46250	0.82	0.58

Table S4. Expression levels of genes of hybrid *IA,i* compared to the hybrid *IA,a*. The hypothesis test performed by REST2009 and based on 1000 iterations provides a *p-value* which represents the probability of the alternate hypothesis that the difference between *IA,i* and *IA,a* is due only to chance.

Gene ID	Fold change (expression level)	*p-value*
46054	2.335	0.01
59167	1.330	0.49
147699	0.959	0.92
143314	1.404	0.41
33584	2.170	0.01
109183	0.815	0.63
148283	4.993	0.01
108968	0.720	0.41

Table S5. List of isolates used in the work and their Mycotheca Universitatis Taurinensis (MUT) accession numbers.

Genotype	Species	MUT accession N.
I,i	*H. irregulare*	MUT00005666
A,a	*H. annosum* s.s.	MUT00003656
IA,i	Artificial heterokaryotic hybrid (with *H. irregulare* mitochondria)	MUT00005668
IA,a	Artificial heterokaryotic hybrid (with *H. annosum* s.s mitochondria)	MUT00005669

Table S6. List of the *Heterobasidion* spp. primers used in the current work and relative Temperature of annealing (Ta).

Sequence	Name	Ta (°C)
GGCAGTTTGGTGCACTTAC	*Het_Trypt_metF*	***60***
CCACCAGCCTGCGTACTT	*Het_Trypt_metR*	
AGTGCTGCAACTCTGTCCAG	*Het46054f*	***60***
CAATTGATGCCAACCATGGTG	*Het46054r*	
AAACGGGACGCTGGTTGAT	*Het108968Af*	***60***
GTGGGCAAAGGCGAAGTAT	*Het108968Ar*	
CGGAGCCTATCACGACTGA	*Het436250Af*	***60***
AGTGCCGGAGACCTGAGAT	*Het436250Ar*	
GATGTCGTGAAGCGGGAAGG	*Het109183Af*	***60***
TATTTCGCCAGATCGCTCGC	*Het109183Ar*	
GACGGCGGCATTGTGTTCTA	*Het148283Af*	***60***
GACTTCAACCAGCTCCTGCC	*Het148283Ar*	
GCGCCAAGACTCATCCTAGC	*Het143314Af*	***60***
TCATAGTGCGACTCCTCGCT	*Het143314Ar*	
AACTCCGTGATTTTCGGCCC	*Het147699Af*	***62***
ATCAGGGTGCCGATCCAGAA	*Het147699Ar*	
ATTCTACTCACGCCGACCGA	*Het33584Af*	***60***
CGAAGGCCGAGCACAACATA	*Het33584Ar*	
ATGTCGTAGAGGAGGGCGTC	*Het59167Af*	***62***
TTCACTTGCCGTGTACGGTG	*Het59167Ar*	

Table S7. Average Ct values of the nine selected genes in the four different genotypes (Ct values of each of the three biological replicates ± SD of technical replicates).

	Biological replicate	***Aa1***	***Aa2***	***Aa3***	***Ii1***	***Ii2***	***Ii3***	***IA,i1***	***IA,i2***	***IA,i3***	***IA,a1***	***IA,a2***	***IA,a3***
Gene ID													
Trypt_met		19.51	18.56	23.35	22.77	22.70	18.11	24.89	24.17	23.21	21.26	20.62	22.50
SD		0.32	0.27	0.66	0.24	0.28	0.32	0.84	0.46	0.21	0.26	0.43	0.47
46054		15.27	14.58	23.90	20.42	19.41	15.74	21.63	21.51	19.54	17.38	16.27	17.47
SD		0.25	0.37	0.63	0.24	0.12	0.19	0.21	0.21	0.12	0.16	0.37	0.15
59167mit		16.63	16.24	21.21	21.95	21.85	18.69	25.13	24.34	22.43	20.50	19.65	22.63
SD		0.01	0.09	0.10	0.81	0.25	0.08	0.96	0.44	0.79	0.05	0.33	0.67
147699		16.45	14.75	20.09	17.32	16.44	13.39	20.26	20.75	20.27	17.32	16.90	19.35
SD		0.86	0.25	0.38	0.32	0.28	0.30	0.15	0.07	0.12	0.28	0.18	0.11
143314		18.23	18.77	19.66	17.89	17.62	19.22	20.10	21.75	21.77	19.13	19.95	19.18
SD		0.07	0.07	0.08	0.88	0.16	0.15	0.04	0.10	0.07	0.08	0.06	0.11
33584mit		20.32	18.65	23.48	22.71	22.68	20.41	25.64	25.28	24.31	19.83	19.72	20.46
SD		0.69	0.50	0.82	0.27	0.44	0.07	0.27	0.10	0.17	0.13	0.22	0.03
109183		17.38	16.73	22.55	21.61	20.45	18.95	23.71	24.83	23.44	21.25	20.51	23.22
SD		0.16	0.20	0.03	0.35	0.40	0.01	0.29	0.24	0.45	0.26	0.17	0.24
148283		25.45	23.93	26.84	20.35	17.65	15.38	25.60	24.29	25.03	23.53	22.35	23.19
SD		0.67	0.32	0.20	0.05	0.12	0.24	0.18	2.03	0.70	0.29	0.31	0.23
108968		19.29	18.40	21.56	22.52	22.23	19.49	25.39	25.58	24.37	21.11	23.37	24.39
SD		0.22	0.08	0.36	0.37	0.27	0.38	0.92	0.68	0.29	0.13	0.34	0.23
46250		21.14	20.43	25.53	24.54	23.61	19.66	26.34	25.20	26.22	21.71	24.21	26.22
SD		1.50	1.74	1.00	0.06	0.82	0.47	0.58	0.10	0.40	0.15	0.93	0.40