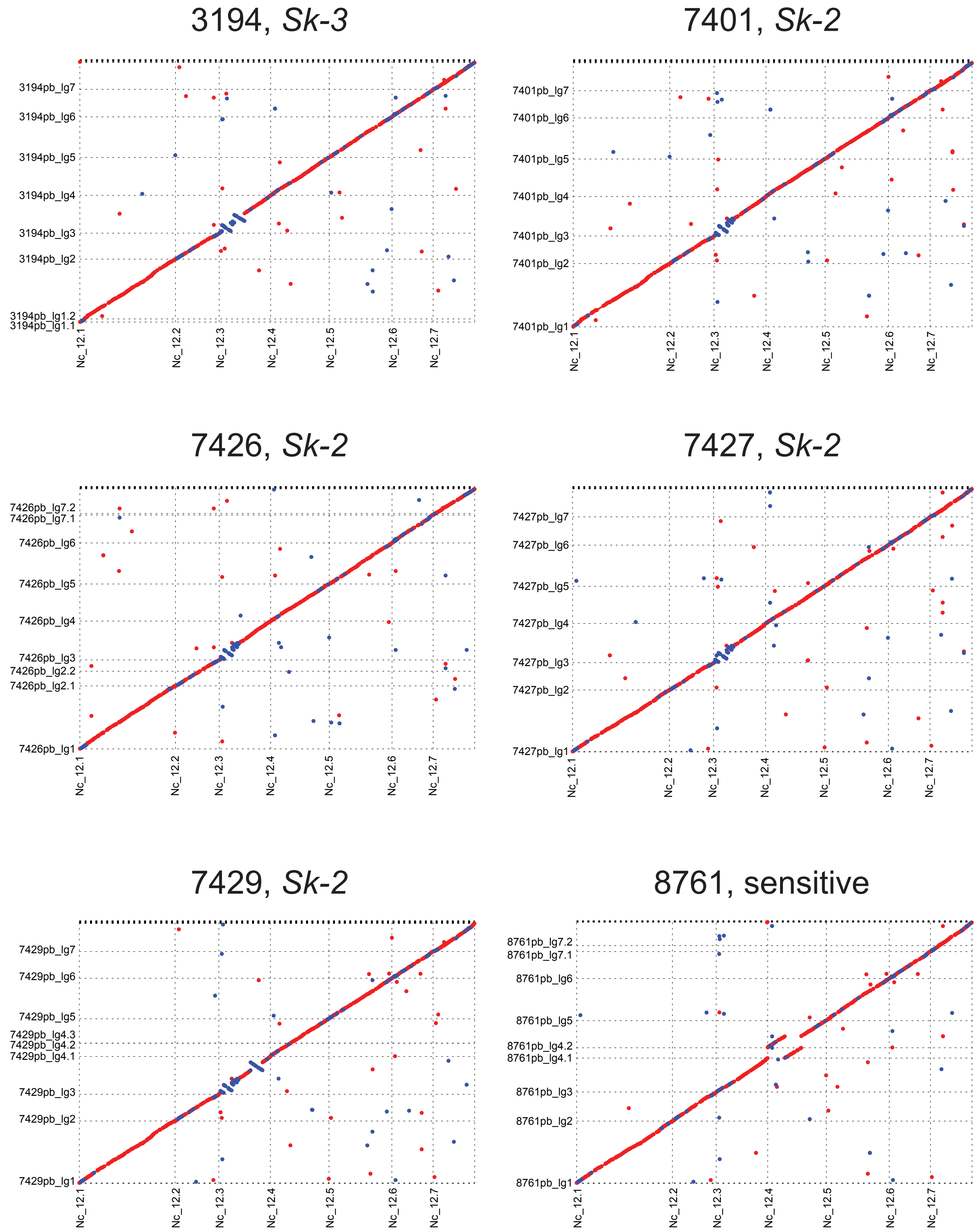
Supplementary Information

Convergent evolution of complex genomic rearrangements in two fungal meiotic drive elements

Svedberg et al.

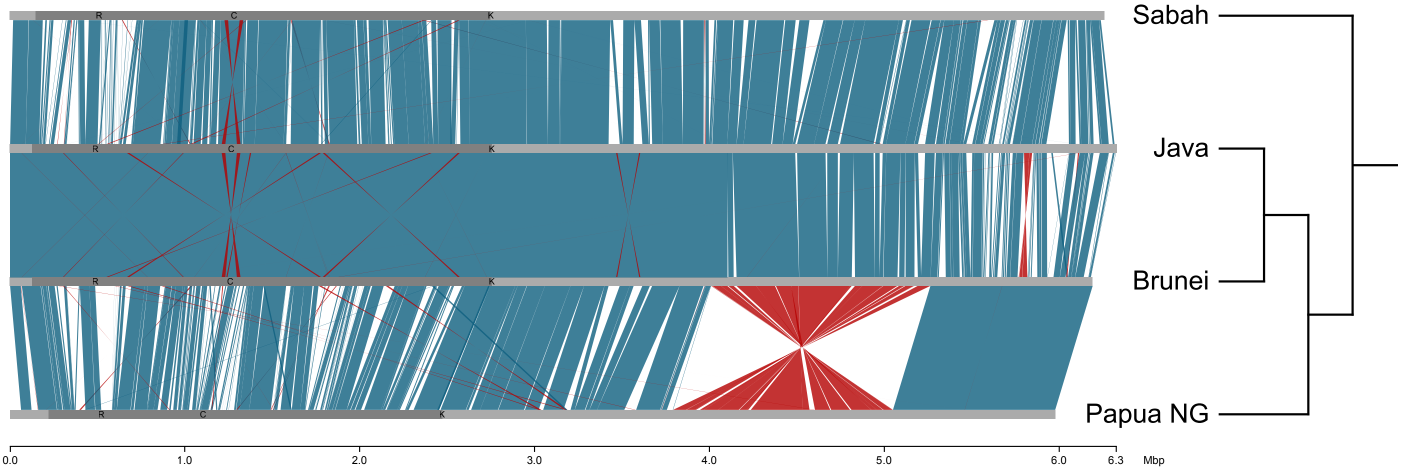
Supplementary information includes:

Supplementary figure 1-9
Supplementary table 1-14
Supplementary references



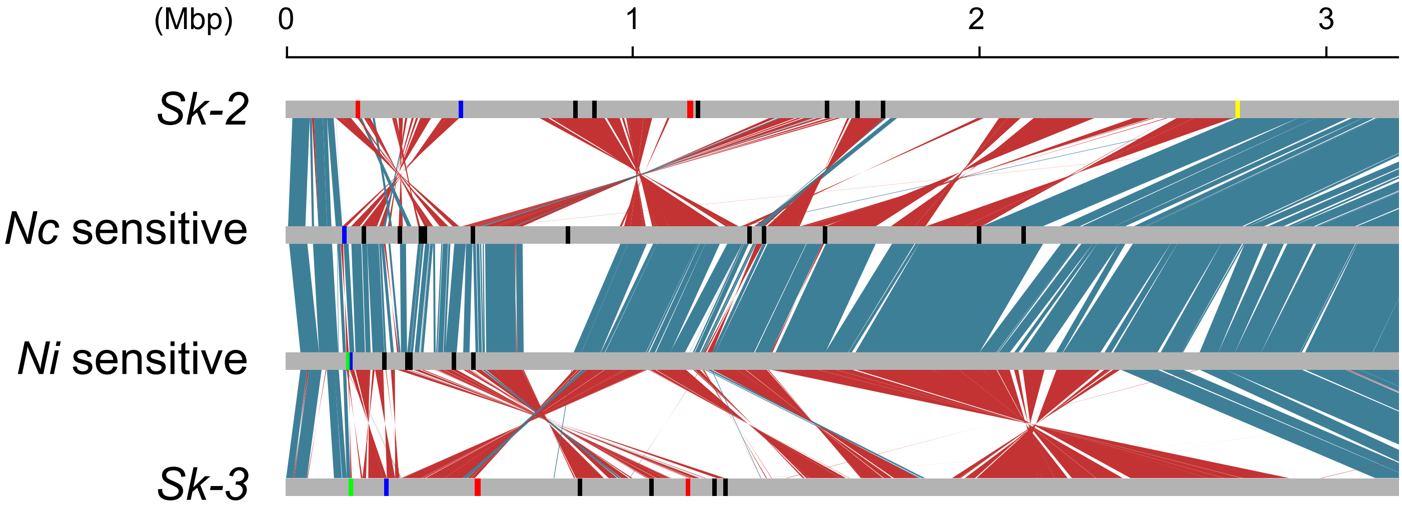
Supplementary Figure 1

Dotplots of the six PacBio assemblies aligned to the *N. crassa* OR74 genome. PacBio contigs are plotted vertically, and the *N. crassa* chromosomes horizontally.

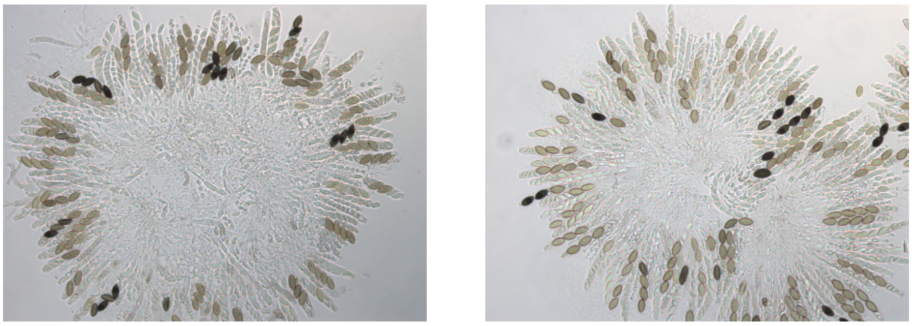


Supplementary Figure 2

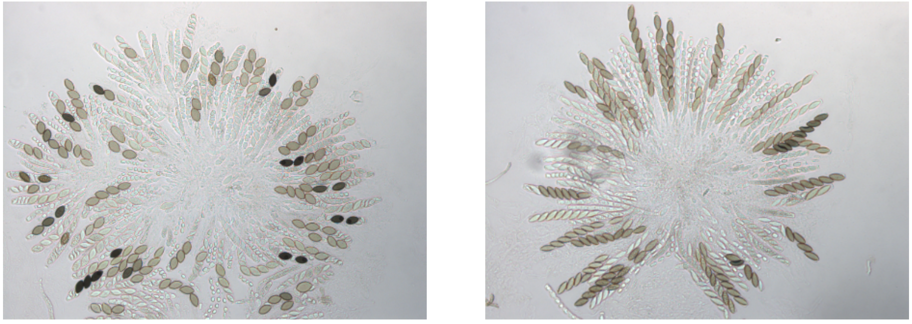
Chromosome 3 of all four known *Sk-2* isolates were aligned to each other. Green blocks show collinear alignments and red blocks show inverted alignments. The dark grey bars indicate the region where the four *Sk-2* isolates all carries inversions relative to sensitive strains. The tree to the right shows their phylogenetic relationship. The inverted region of the Brunei and Java isolates are very closely related and show no structural differences. Comparing to the other isolates variation in repeat regions is clearly visible. The inverted region of the Papua New Guinea isolate is smaller and also carries an extra, large inversion to the right. Strains: Brunei = 7401, Sabah = 7426, Java = 7427, Papua New Guinea = 7429.



Supplementary Figure 3
Selected and unique genes in the *Sk* region. The genome assemblies of one *Sk-2* (7426), one *Sk-3* (3194) and one sensitive ("*Ni* sensitive", strain 8761) *N. intermedia* strain were annotated using transcriptomic data. The gene content of these assemblies were compared to each other and to the well-annotated sensitive *N. crassa* OR74 reference genome ("*Nc* sensitive") in order to identify genes in the *Sk* region that were unique for each strain (black vertical lines). Genes that were only found in the Sk-2 and Sk-3 genome assemblies and not in any of sensitive strains were also identified (red vertical lines). For reference, the *rsk* resistance locus is marked in blue and the *Sk-2 rfk-1* killer locus is marked in yellow. The sequence which showed homology to *rfk-1*, that was deleted in *Sk-3* is also found in the *N. intermedia* sensitive strain and is marked in green.



a *Sk*^{S} × *Sk-3* **b** *Sk*^{S} × *Sk-3 v207*$^{\Delta}$



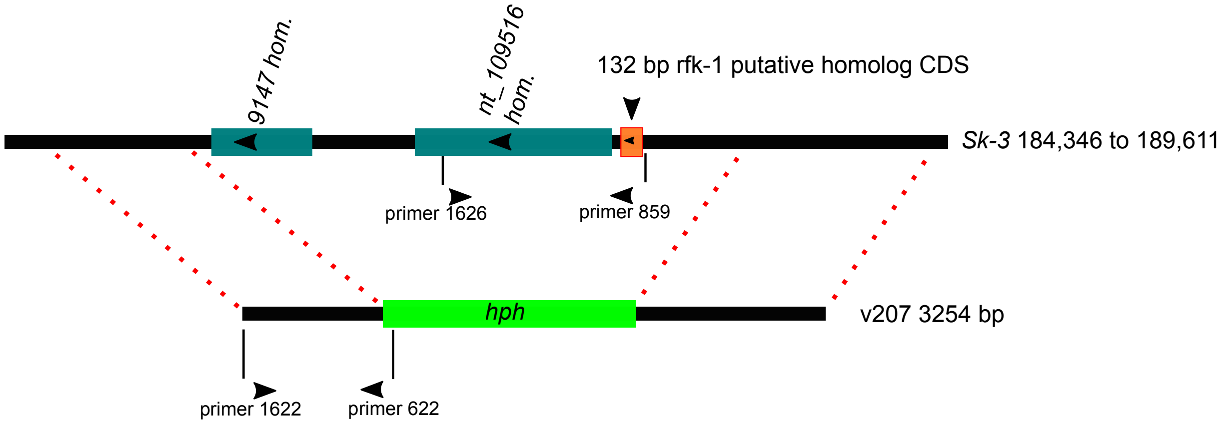
c *Sk*^{S} × *Sk-3 v208*$^{\Delta}$ **d** *Sk*^{S} × *Sk*^{S}

Supplementary Figure 4

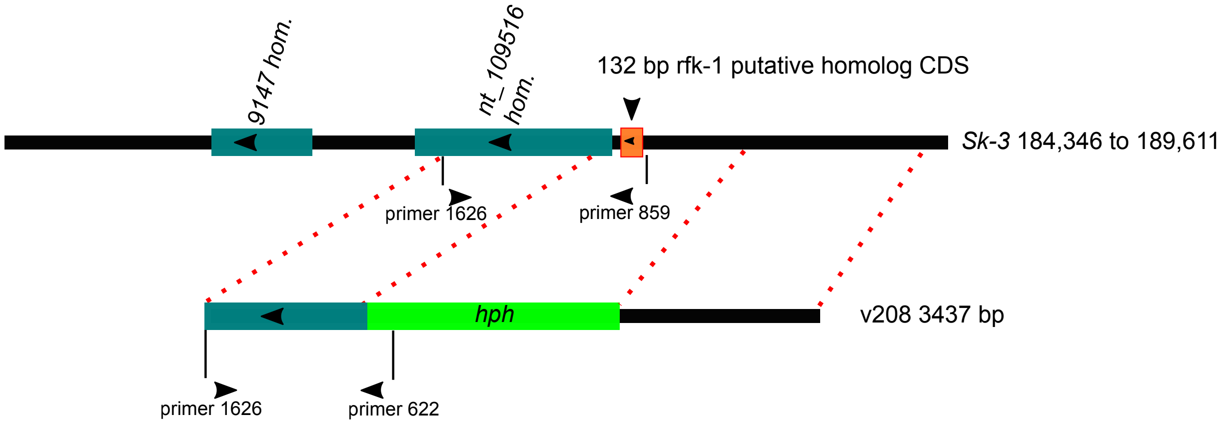
Asci were dissected from perithecia 12 days post fertilization. Crosses are as follows: **a)** RTH1005.2 × RDGR170.3; **b)** RTH1005.2 × HAA207.6.1; **c)** RTH1005.2 × HAA208.4.1; **d)** RTH1005.2 × RTH1005.1.

Genotypes:
RTH1005.2: rid; fl; a
RTH1005.1: rid; fl; A
RDGR170.3: rid; Sk-3; mus-51Δ::bar A
HAA207.6.1: rid; Sk-3 v207Δ::hph; mus-51Δ::bar A
HAA208.4.1: rid; Sk-3 v208Δ::hph; mus-51Δ::bar A

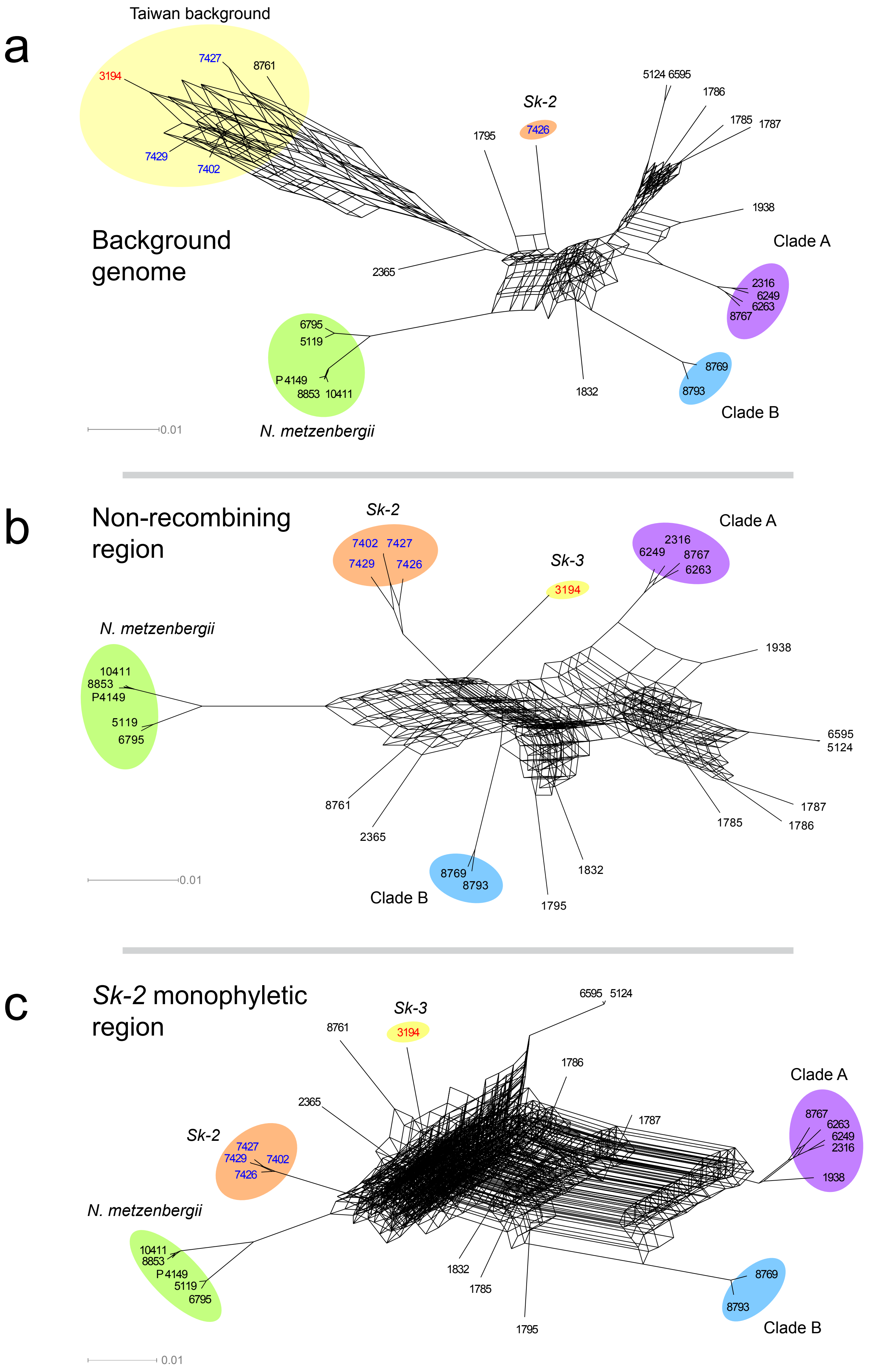
Sk-3 v207Δ::hph deletion vector



Sk-3 v208Δ::hph deletion vector

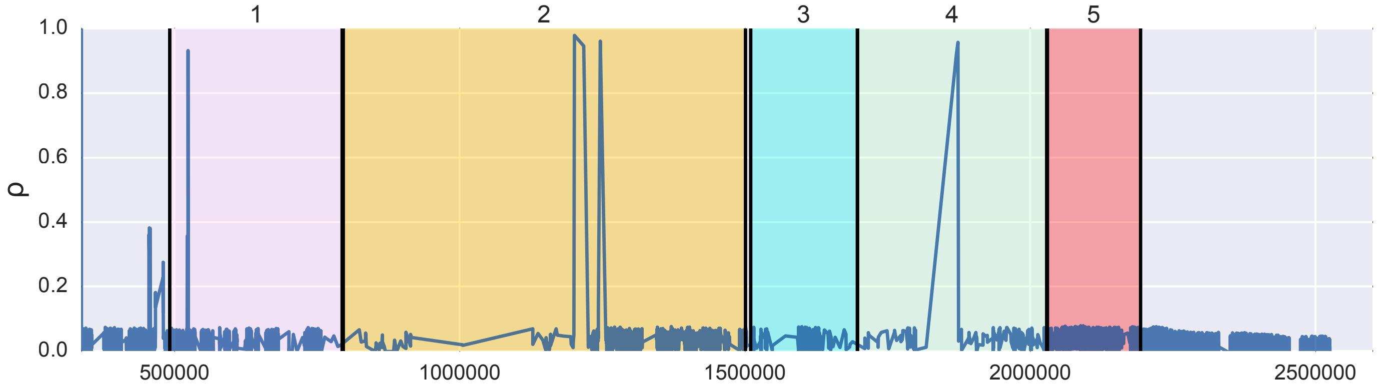


Supplementary Figure 5
Schematic of the two vectors used to delete the fragment in *Sk-3* which shows homology to the *rfk-1* killer gene in *Sk-2*.

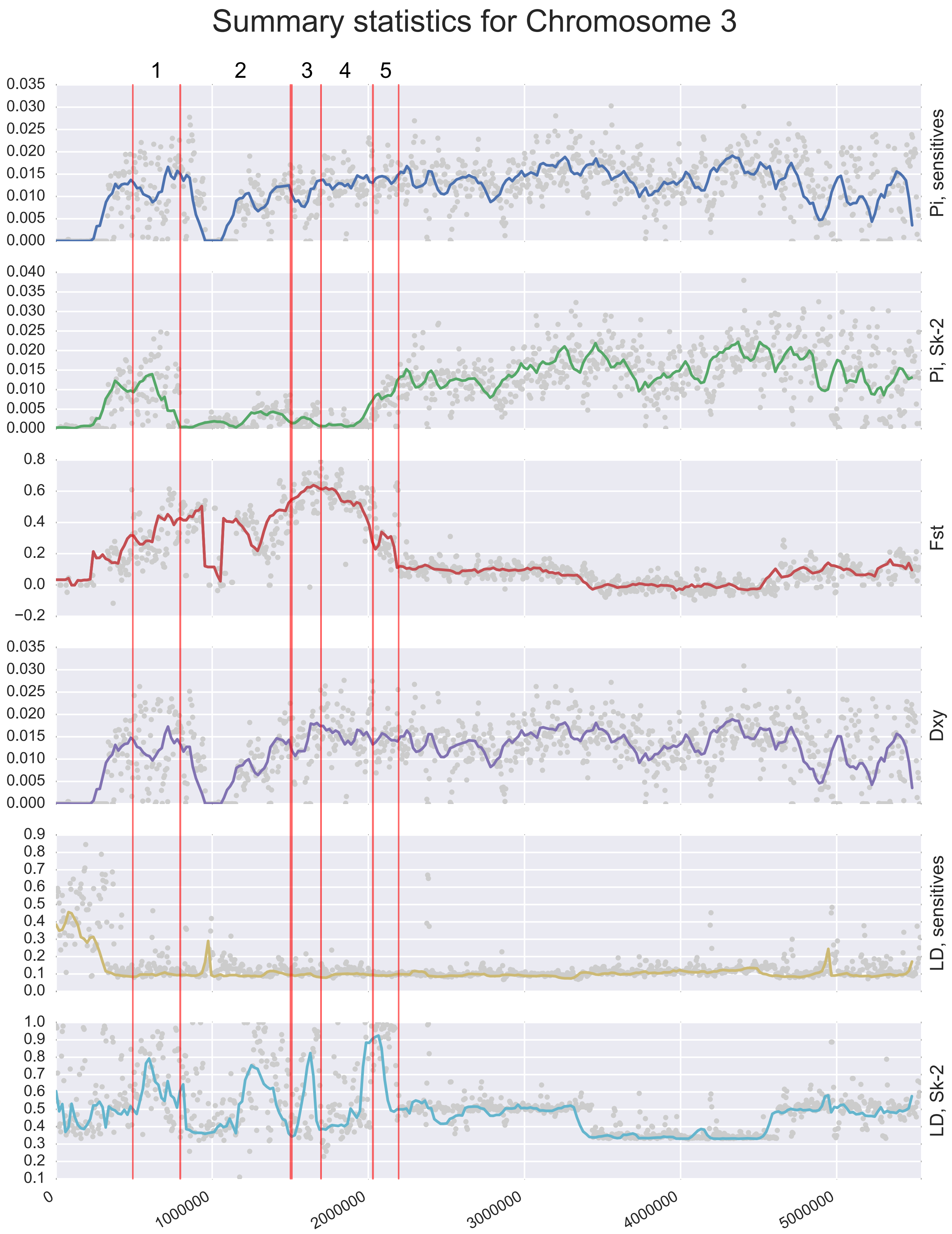

a
Taiwan background
3194
7427
8761
7429
7402
Background genome
Sk-2
7426
1795
5124 6595
1786
1785
1787
1938
Clade A
2365
2316
6249
6263
8767
6795
5119
P4149
8853 10411
1832
8769
8793
Clade B
0.01
N. metzenbergii
b
Non-recombining region
Sk-2
7402 7427
7429 7426
Sk-3
3194
Clade A
2316
6249 8767
6263
N. metzenbergii
10411
8853
P4149
5119
6795
1938
6595
5124
8761
2365
1787
1785
1786
8769
8793
1832
Clade B
1795
0.01
c
Sk-2 monophyletic region
Sk-3
3194
8761
6595 5124
1786
2365
1787
Clade A
Sk-2
7427
7429
7402
7426
8767
6263
6249
2316
1938
N. metzenbergii
10411
8853
P4149
5119
6795
1832
1785
8769
8793
Clade B
1795
0.01

Supplementary Figure 6

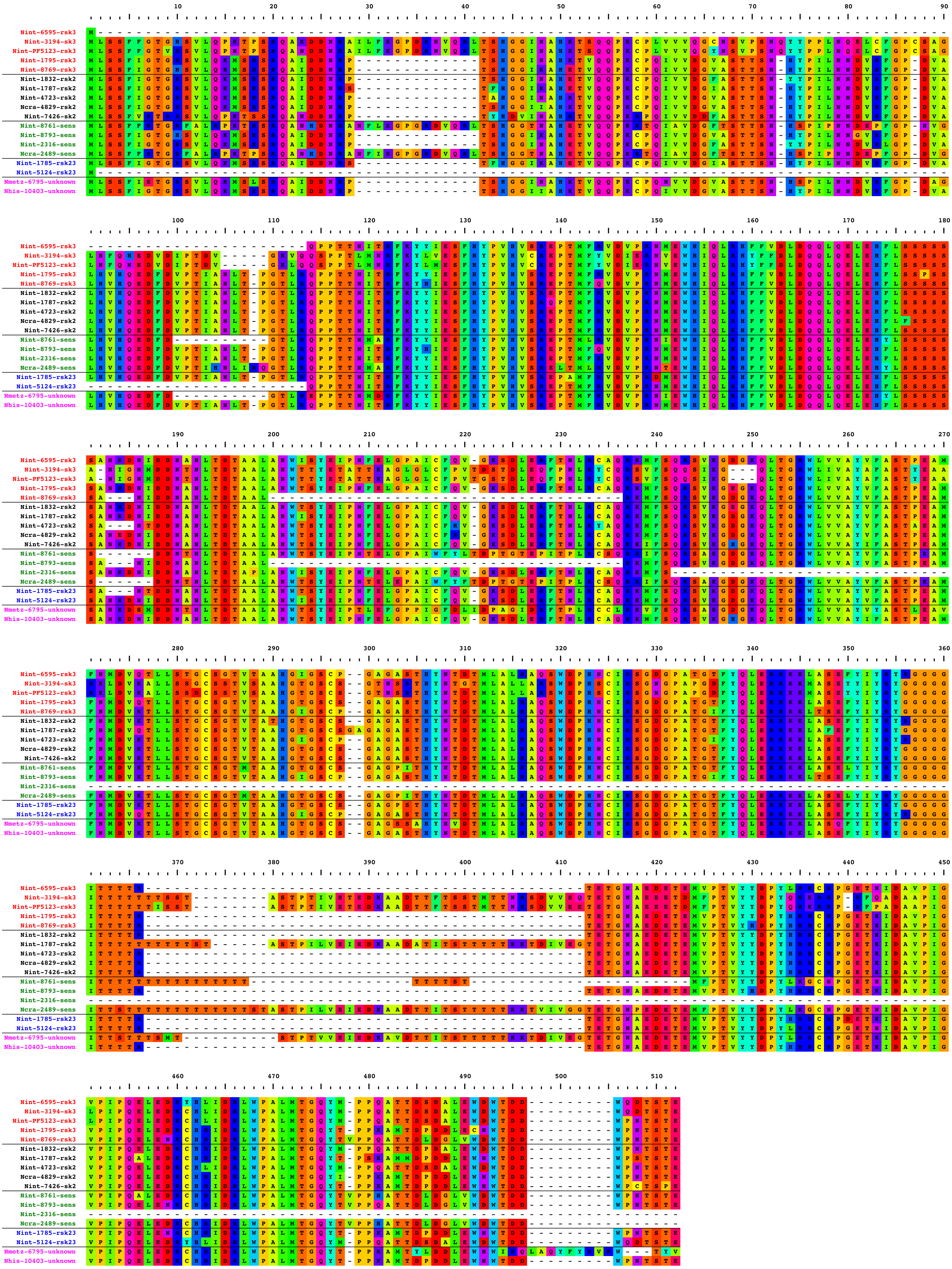
a) Network of *N. intermedia* and *N. metzenbergii* based on SNPs from all chromosomes, except chromosome 3. The network is constructed from phylogenetic trees generated using RAxML of each chromosome independently, the four spore killer strains clustering with the Taiwan strain 8761 are highlighted, showing the signal of the backcrossing into the Taiwan background, as are the ones grouping together with either of *N. intermedia* clades A and B, and of the outgroup, *N. metzenbergii*. **b)** Network based on SNPs in the nonrecombining region. The network is constructed using phylogenetic trees independently generated using RAxML from the five inversions in *Sk-2*. All *Sk-2* strains cluster together, but *Sk-3* is placed elsewhere in the network. **c)** Network based on SNPs from the regions where the four *Sk-2* strains show a signal of monophyly. In all networks, *Sk-2* strains are marked with red text, and the *Sk-3* strain is marked with blue text.



Supplementary Figure 7
Population scaled recombination rate (ρ) for the four *Sk-2* strains was estimated using LDhat plotted over the *Sk* region of chromosome 3. The five inversions are marked in color and numbered. There is little evidence for recombination between the *Sk-2* strains, besides four narrow peaks. They could potentially be due to gene conversion, but mutation or mapping error cannot be excluded.



Supplementary Figure 8
Diversity, divergence and linkage disequilibrium plotted over chromosome 3. F_{ST} and D_{XY} is calculated by comparing *Sk-2* strains to sensitive *N. intermedia* strains. The non-recombining spore killer region is located between ~300 kbp and ~2.3 Mbp and inversion break-points are marked with red lines.



Supplementary Figure 9

Amino acid alignment of *rsk*. Strain colors: resistance against *Sk-3*, red; resistance against *Sk-2*, black; resistance against both Sk-2 and Sk-3, blue; sensitive strains, green and unknown phenotype, magenta. No sites show fixed differences between any of the phenotypes.

Supplementary Table 1: Types of data generated for *N. intermedia* strains

Strain [1]	Phenotype [2]	Location	Illumina	PacBio	BS-seq	ChIP-seq	RNA-seq
1785	*r(Sk-2) + r(Sk-3)* [3]	Papua New Guinea	X				
1786	*r(Sk-3)*	Papua New Guinea	X				
1787	*r(Sk-2)*	Papua New Guinea	X				
1795	*r(Sk-3)*	Indonesia	X				
1832	*r(Sk-2)*	Australia	X				
1938	Sensitive	Papua New Guinea	X				
2316	Sensitive	USA	X				
2365	Sensitive	Hawaii	X				
3194 [4]	*Sk-3*	Papua New Guinea	X	X	X	X	X
5124	*r(Sk-2) + r(Sk-3)* [3]	Tahiti	X				
6249	Sensitive	Haiti	X				
6263	Sensitive	Ivory coast	X				
6595	*r(Sk-3)*	Tahiti	X				
7401 [4, 5]	*Sk-2*	Brunei		X			
7402 [4, 5]	*Sk-2*	Brunei	X				
7426	*Sk-2*	Sabah	X	X	X	X	X
7427 [4]	*Sk-2*	Java	X	X			
7429 [4]	*Sk-2*	Papua New Guinea	X	X			
8761	Sensitive	Taiwan	X	X	X	X	X
8762	Sensitive	Taiwan			X		X
8767	*r(Sk-2)*	Indonesia	X				
8769	*r(Sk-3)*	Indonesia	v				
8793	Sensitive	Papua New Guinea	X				
8807 [6]	Sensitive	Indonesia		X			

[1] Strains are referred to by their accession number at the Fungal Genetics Stock Center, see http://www.fgsc.net

[2] *r(Sk-2)* indicates resistant to *Sk-2* and *r(Sk-3)* resistant to *Sk-3*.

[3] Strain 1785 and 5124 show partial resistance to both *Sk-2* and *Sk-3*.

[4] These strains have been backcrossed 1-3 times to the “Taiwan” *N. intermedia* tester strains (FGSC strains 8761 and 8762).

[5] 7401 and 7402 are the two mating types isolated from a single backcross to the “Taiwan” background.

[6] Strain 8807 was sequenced together with the other strains in this study, but has been released in an earlier study[1].

Supplementary Table 2: Location and size of the *Sk* region in 7426 and 3194, and of inversions within it.

Strain		Start coordinate [1]	End coordinate [1]	Size (bp)	*N. crassa* start [2]	*N. crassa* end [2]
***Sk-2*, 7426**						
Whole region		146,604	2,744,839	2,598,235	167,108	2,011,073
Inversion	1	146,604	505,422	358,818	167,222	503,812
	2	735,396	1,601,169	865,773	506,357	1,310,671
	3	1,621,721	2,016,643	394,922	1,318,300	1,486,497
	4	2,057,118	2,549,759	492,641	1,487,028	1,845,202
	5	2,551,222	2,733,837	182,615	1,846,558	2,010,416
***Sk-3*, 3194**						
Whole region		218,817	3144,883	2,926,066	162,630	2,594,373
Inversion	1	220,519	291,562	71,043	167,437	241,256
	2	291,595	315,284	23,689	241,099	264,955
	3	318,455	327,952	9,497	273,483	282,880
	4	333,059	1,267,606	934,547	283,021	1,192,260
	5	1,370,044	1,627,213	257,169	1,192,579	1,359,520
	6	1,663,134	1,845,276	182,142	1,361,585	1,544,816
	7	1,922,683	2,895,612	972,929	1,548,552	2,594,215

[1] Start and end coordinates are the coordinates for the breakpoints in *Sk-2* strain 7426 and *Sk-3* strain 3194.

[2] *N. crassa* start and end are the coordinates for the breakpoints when aligning *Sk-2* and *Sk-3* to the *N. crassa* OR74 genome[2].

Supplementary Table 3. The 20 most common repeat families as defined by total sequence length.

Sk-2, 7426					
Whole genome			**Spore killer region**		
Type [1]	**Counts**	**Total (bp)**	**Type [1]**	**Counts**	**Total (bp)**
ncra_Tad1_01	136	494796	ncra_Tad1_01	21	99170
ncra_LTR_40	42	353924	ncra_LTR_48	15	82023
ncra_LTR_49	78	335496	ncra_LTR_40	12	75155
ncra_LTR_48	69	333100	ndisc_LTR_48	15	33476
ncra_Gypsy_08	34	154516	ntet_LTR_14	12	32515
ndisc_LTR_48	47	148203	ncra_Gypsy_01	11	27959
ncra_LTR_44	27	144423	ncra_LTR_44	5	26124
ncra_Tad1_06	58	108439	ncra_LTR_82	5	23795
ncra_Gypsy_01	34	104891	ncra_Tad1_06	8	22913
ntet_LTR_27	64	103611	ncra_LTR_86	7	22692
ncra_LTR_86	50	95745	ncra_Gypsy_08	5	21377
ncra_Gypsy_10	16	90772	ntet_LTR_27	9	19032
ncra_LTR_111	173	87862	ncra_LTR_42	7	17323
ncra_LTR_82	17	79224	ncra_Gypsy_10	2	16450
ncra_RPTFAM_02	54	73894	ncra_LTR_49	8	16026
ncra_LTR_34	90	71248	ntet_LTR_16	5	14275
ncra_LTR_93	29	71184	ncra_LTR_34	11	12468
ncra_Gypsy_13	36	70232	ndis_RPTFAM_105	13	12277
ntet_LTR_16	36	69150	ncra_RPTFAM_02	9	11973
ncra_LTR_33	56	67843	ncra_LTR_111	19	11426

Sk-3, 3194					
Whole genome			**Spore killer region**		
Type [1]	**Counts**	**Total (bp)**	**Type [1]**	**Counts**	**Total (bp)**
ncra_Tad1_01	128	445762	ncra_Tad1_01	23	99425
ncra_Gypsy_08	49	254516	ncra_LTR_40	7	56498
ncra_LTR_48	88	243225	ncra_Gypsy_01	10	34910
ncra_LTR_40	22	223803	ncra_LTR_86	14	31598
ncra_Gypsy_01	57	200126	ncra_LTR_48	7	27225
ncra_LTR_86	80	169734	ncra_LTR_05	10	24234
ncra_LTR_49	51	143704	ndisc_LTR_48	7	24108
ntet_LTR_16	56	141634	ncra_LTR_49	7	19828
ndisc_LTR_48	35	123693	ndisc_LTR_58	10	18402
ncra_LTR_93	39	101865	ncra_Tad1_06	9	16600
ncra_LTR_05	40	100834	ntet_LTR_14	5	15452
ncra_Tad1_06	50	98002	ncra_LTR_69	2	14975
ncra_LTR_75	22	94813	ncra_Gypsy_08	2	14352
ncra_Gypsy_10	12	81946	ncra_LTR_122	3	13929
ntet_LTR_27	57	76058	ncra_LTR_111	27	12176
ncra_LTR_111	152	73566	ncra_LTR_34	6	12095
ncra_LTR_69	13	69297	ndis_Gypsy_03	6	11166
ncra_LTR_04	21	68337	ncra_Gypsy_13	9	10696

ncra_Gypsy_13	38	68187	ncra_LTR_45	10	10509
ncra_LTR_44	20	63517	ndisc_nonLTR_61	9	10416

Sensitive, 8761					
Whole genome			**Spore killer region**		
Type [1]	**Counts**	**Total (bp)**	**Type [1]**	**Counts**	**Total (bp)**
ncra_Tad1_01	110	375887	ntet_LTR_14	6	13030
ncra_Gypsy_01	77	281007	ncra_LTR_86	6	12332
ncra_LTR_40	21	249242	ncra_LTR_04	3	12190
ncra_LTR_86	103	229409	ncra_LTR_40	1	12149
ncra_Gypsy_08	44	205203	ncra_Gypsy_01	4	10466
ncra_LTR_48	68	197506	ncra_LTR_34	9	8216
ncra_LTR_49	50	161543	ncra_LTR_36	1	8061
ncra_LTR_75	33	150945	ncra_Tad1_01	2	7781
ncra_LTR_05	30	93292	ncra_LTR_69	1	7516
ntet_LTR_16	41	86784	ncra_LTR_48	2	7236
ncra_LTR_93	33	84967	ncra_LTR_75	2	7164
ndisc_LTR_48	20	82389	ndisc_LTR_44	3	6417
ncra_LTR_34	91	75223	ndis_Copia_01	1	5401
ncra_LTR_33	55	73303	ncra_RPTFAM_171	5	5177
ncra_Tad1_06	42	72608	GA-rich	61	3618
ncra_LTR_63	11	68558	ncra_LTR_72	1	3545
ncra_LTR_21	12	67224	nsub_Gypsy_05	1	2901
ncra_RPTFAM_02	47	64518	ncra_Tad1_06	1	2330
ncra_LTR_111	124	61505	ncra_LTR_16	1	2311
ntet_LTR_27	43	58414	ncra_RPTFAM_10	9	2279

N. crassa					
Whole genome			**Spore killer region**		
Type [1]	**Counts**	**Total (bp)**	**Type [1]**	**Counts**	**Total (bp)**
ncra_Tad1_01	198	514976	ncra_Tad1_01	33	97465
ncra_Gypsy_01	95	249139	ncra_Gypsy_01	9	29693
ncra_LTR_86	90	224242	ncra_LTR_86	8	21062
ncra_RPTFAM_02	81	150143	ncra_LTR_49	4	18418
ncra_Gypsy_08	26	92483	ncra_LTR_40	4	14829
ncra_LTR_04	24	87427	ncra_LTR_56	3	11470
ncra_LTR_40	19	84761	ncra_nonLTR_55	2	11334
ncra_LTR_44	13	84511	ncra_LTR_34	7	10939
ncra_LTR_33	63	82717	ncra_LTR_53	4	10556
ncra_LTR_93	25	73040	ncra_Gypsy_08	2	10251
ncra_RPTFAM_16	32	70961	ncra_RPTFAM_188	6	9390
ncra_LTR_05	31	70784	ncra_LTR_112	1	8604
ncra_Gypsy_13	26	63481	ncra_LTR_05	4	8533
ncra_RPTFAM_05	40	60701	ncra_LTR_62	1	7922
GA-rich	961	53916	ncra_LTR_48	2	7763
ncra_LTR_06	12	52298	ncra_LTR_58	1	7566

ncra_LTR_21	12	51976	ncra_LTR_33	6	7525
ncra_LTR_75	9	50191	ncra_LTR_50	1	7325
ncra_LTR_83	7	48929	ncra_LTR_75	1	6922
ncra_Gypsy_10	7	48237	ncra_RPTFAM_16	4	6739

[1] Transposable family names are taken from the repeat library generated by Gioti et al. (2012)[3].

Supplementary Table 4: Repeats enriched in Spore killer region

		Whole genome		Spore killer region		Enrichment		Total number of repeats			
Strain	**Repeat type [1]**	**Copies**	**Base pairs**	**Copies**	**Base pairs**	**Copies**	**Bases**	**Whole genome**	**Sk region**	**p-value**	**Bonferroni corrected significance**
3194	ndisc_LTR_58	15	34473	10	18402	8.08	6.47	26157	2158	2.97E-08	5.44E-05
3194	ncra_LTR_45	16	19125	10	10509	7.58	6.66	26157	2158	7.33E-08	5.44E-05
3194	ncra_LTR_53	52	26534	17	7323	3.96	3.35	26157	2158	4.95E-07	5.44E-05
3194	(TTA)n	76	3737	18	914	2.87	2.96	26157	2158	3.64E-05	5.44E-05
3194	(TAA)n	106	5251	22	1105	2.52	2.55	26157	2158	4.75E-05	5.44E-05
7426	(TTA)n	94	4652	26	1393	3.58	3.87	26539	2053	7.26E-09	5.73E-05
7426	ncra_LTR_45	29	40562	12	10616	5.35	3.38	26539	2053	6.80E-07	5.73E-05
7426	ndisc_LTR_48	47	148203	15	33476	4.13	2.92	26539	2053	1.46E-06	5.73E-05
7426	(TAA)n	96	5121	22	1248	2.96	3.15	26539	2053	3.30E-06	5.73E-05
7426	(TAT)n	84	3773	20	999	3.08	3.42	26539	2053	4.88E-06	5.73E-05
7426	ntet_LTR_14	34	65726	12	32515	4.56	6.40	26539	2053	4.98E-06	5.73E-05
7426	(AAT)n	74	3807	18	837	3.14	2.84	26539	2053	1.04E-05	5.73E-05
7426	(ATAATT)n	9	486	6	303	8.62	8.06	26539	2053	1.47E-05	5.73E-05
7426	ncra_Gypsy_01	34	104891	11	27959	4.18	3.45	26539	2053	3.16E-05	5.73E-05
7426	ncra_LTR_42	14	37796	7	17323	6.46	5.92	26539	2053	3.49E-05	5.73E-05
7426	ntet_LTR_13	4	4145	4	4145	12.93	12.93	26539	2053	3.58E-05	5.73E-05
7426	ncra_LTR_40	42	353924	12	75155	3.69	2.75	26539	2053	5.59E-05	5.73E-05
7426	ncra_LTR_101	55	19677	14	5364	3.29	3.52	26539	2053	5.68E-05	5.73E-05
N. crassa	ncra_Tad1_01	198	514976	33	97465	2.51	2.85	25908	1718	9.99E-07	6.47E-05
N. crassa	ncra_LTR_34	15	37026	7	10939	7.04	4.46	25908	1718	2.25E-05	6.47E-05

[1] Transposable family names are taken from the repeat library generated by Gioti et al. (2012)[3].

Supplementary Table 5: Bisulfite sequencing statistics

Strain	Phenotype	Mating type	Sample name	Number of reads	Read length	Total data (bp)	Coverage
3194	*Sk-3*	mat A	SK3-1	17,662,450	126	4,450,937,400	106.7
			SK3-2	16,514,315	126	4,161,607,380	99.8
			SK3-3	19,851,533	126	5,002,586,316	120.0
7426	*Sk-2*	mat A	SK2-1	18,622,707	126	4,692,922,164	111.2
			SK2-2	20,000,164	126	5,040,041,328	119.4
			SK2-3	20,167,691	126	5,082,258,132	120.4
8761	Sensitive	mat A	SenA-1	19,217,691	126	4,842,858,132	118.1
			SenA-2	18,868,363	126	4,754,827,476	116.0
			SenA-3	20,112,790	126	5,068,423,080	123.6
8762	Sensitive	mat a	Sena-1	19,759,188	126	4,979,315,376	121.4
			Sena-2	18,538,934	126	4,671,811,368	113.9
			Sena-3	20,112,790	126	5,068,423,080	123.6

Supplementary Table 6: ChIP-seq sequencing statistics

Strain	Phenotype	Data type	Sample name	Number of reads	Read length	Total data (bp)	Coverage
8761	Sensitive	H3	senA-H3-1	2,391,286	126	602,604,072	14.7
			senA-H3-2	3,176,333	126	800,435,916	19.5
			senA-H3-3	3,287,804	126	828,526,608	20.2
8761	Sensitive	input	senA-input-1	2,786,779	126	702,268,308	17.1
			senA-input-2	6,091,738	126	1,535,117,976	37.4
			senA-input-3	5,861,906	126	1,477,200,312	36.0
8761	Sensitive	H3K27me	senA-K27-1	4,539,789	126	1,144,026,828	27.9
			senA-K27-2	5,614,113	126	1,414,756,476	34.5
			senA-K27-3	3,431,904	126	864,839,808	21.1
8761	Sensitive	H3K4me2	senA-K4-1	1,939,960	126	488,869,920	11.9
			senA-K4-2	5,623,058	126	1,417,010,616	34.6
			senA-K4-3	5,177,248	126	1,304,666,496	31.8
8761	Sensitive	H3K9me3	senA-K9-1	5,532,061	126	1,394,079,372	34.0
			senA-K9-2	7,274,107	126	1,833,074,964	44.7
			senA-K9-3	3,484,261	126	878,033,772	21.4
7426	*Sk-2*	H3	SK2-H3-1	3,153,238	126	794,615,976	18.8
			SK2-H3-2	3,850,303	126	970,276,356	23.0
			SK2-H3-3	2,216,851	126	558,646,452	13.2
7426	*Sk-2*	input	SK2-input-1	4,550,749	126	1,146,788,748	27.2
			SK2-input-2	3,891,064	126	980,548,128	23.2
			SK2-input-3	5,008,086	126	1,262,037,672	29.9
7426	*Sk-2*	H3K27me	SK2-K27-1	4,610,774	126	1,161,915,048	27.5
			SK2-K27-2	3,255,844	126	820,472,688	19.4
			SK2-K27-3	2,947,638	126	742,804,776	17.6
7426	*Sk-2*	H3K4me2	SK2-K4-1	3,688,828	126	929,584,656	22.0
			SK2-K4-2	4,434,644	126	1,117,530,288	26.5
			SK2-K4-3	3,407,310	126	858,642,120	20.3
7426	*Sk-2*	H3K9me3	SK2-K9-1	7,226,248	126	1,821,014,496	43.2
			SK2-K9-2	4,527,742	126	1,140,990,984	27.0
			SK2-K9-3	4,247,728	126	1,070,427,456	25.4
3194	*Sk-3*	H3	SK3-H3-1	3,112,293	126	784,297,836	18.8
			SK3-H3-2	4,821,281	126	1,214,962,812	29.1
			SK3-H3-3	3,100,894	126	781,425,288	18.7
3194	*Sk-3*	input	SK3-input-1	5,029,166	126	1,267,349,832	30.4
			SK3-input-2	6,036,475	126	1,521,191,700	36.5
			SK3-input-3	4,302,365	126	1,084,195,980	26.0
3194	*Sk-3*	H3K27me	SK3-K27-1	3,404,053	126	857,821,356	20.6
			SK3-K27-2	4,273,854	126	1,077,011,208	25.8
			SK3-K27-3	2,893,884	126	729,258,768	17.5

Strain	Phenotype	Data type	Sample name	Number of reads	Read length	Total data (bp)	Coverage
3194	*Sk-3*	H3K4me2	SK3-K4-1	3,936,471	126	991,990,692	23.8
			SK3-K4-2	4,723,153	126	1,190,234,556	28.5
			SK3-K4-3	3,076,918	126	775,383,336	18.6
3194	*Sk-3*	H3K9me3	SK3-K9-1	4,653,903	126	1,172,783,556	28.1
			SK3-K9-2	5,651,398	126	1,424,152,296	34.2
			SK3-K9-3	3,914,395	126	986,427,540	23.7

Supplementary Table 7: RNA-seq sequencing statistics for crosses

Strain	Phenotype	Sample name	Number of reads	Read length (bp)	Total data (bp)
8761 x 8762	Sens x sens	CrossA-1	52,471,010	126	13,222,694,520
8761 x 8762	Sens x sens	CrossA-2	20,993,267	126	5,290,303,284
8761 x 8762	Sens x sens	CrossA-3	28,147,157	126	7,093,083,564
7426 x 8762	*Sk-2* x sens	CrossB-1	20,736,078	126	5,225,491,656
7426 x 8762	*Sk-2* x sens	CrossB-2	22,255,907	126	5,608,488,564
7426 x 8762	*Sk-2* x sens	CrossB-3	18,808,203	126	4,739,667,156
3194 x 8762	*Sk-3* x sens	CrossC-1	26,837,792	126	6,763,123,584
3194 x 8762	*Sk-3* x sens	CrossC-2	35,555,809	126	8,960,063,868
3194 x 8762	*Sk-3* x sens	CrossC-3	36,769,552	126	9,265,927,104

Supplementary Table 8: RNA-seq sequencing statistics for vegetative samples

Strain	Phenotype	Mating type	Sample name	Number of reads	Read length (bp)	Total data (bp)	Coverage
7426	*Sk-2*	*mat A*	SK2-1	18,422,104	126	4,642,370,208	110.0
7426	*Sk-2*	*mat A*	SK2-2	24,658,673	126	6,213,985,596	147.3
7426	*Sk-2*	*mat A*	SK2-3	15,867,499	126	3,998,609,748	94.8
3194	*Sk-3*	*mat A*	SK3-1	22,771,549	126	5,738,430,348	137.6
3194	*Sk-3*	*mat A*	SK3-2	15,605,292	126	3,932,533,584	94.3
3194	*Sk-3*	*mat A*	SK3-3	27,762,217	126	6,996,078,684	167.8
8761	Sensitive	*mat A*	SenA-1	29,809,029	126	7,511,875,308	183.2
8761	Sensitive	*mat A*	SenA-2	38,073,840	126	9,594,607,680	234.0
8761	Sensitive	*mat A*	SenA-3	15,273,227	126	3,848,853,204	93.9
8762	Sensitive	*mat a*	Sena-1	18,594,466	126	4,685,805,432	114.3
8762	Sensitive	*mat a*	Sena-2	18,133,803	126	4,569,718,356	111.5
8762	Sensitive	*mat a*	Sena-3	15,521,625	126	3,911,449,500	95.4

Supplementary Table 9: Unique transcripts in the Spore killer region

Strain	Phenotype	Gene	Annotation	Chromosome	Start coordinate	End coordinate	Protein size
3194	*Sk-3*	maker-3194pb_lg3-snap-gene-0.220-mRNA-1	-	lg3	848,540	849,297	197
3194	*Sk-3*	snap_masked-3194pb_lg3-processed-gene-1.380-mRNA-1	-	lg3	1,054,612	1,055,785	108
3194	*Sk-3*	maker-3194pb_lg3-snap-gene-1.156-mRNA-1	-	lg3	1,236,639	1,237,847	110
3194	*Sk-3*	maker-3194pb_lg3-snap-gene-1.160-mRNA-1	-	lg3	1,267,791	1,268,487	193
7426	*Sk-2*	maker-7426pb_lg3-est_gff_Cufflinks-gene-0.212-mRNA-1	-	lg3	834,238	835,468	166
7426	*Sk-2*	maker-7426pb_lg3-snap-gene-0.47-mRNA-1	-	lg3	887,375	892,546	59
7426	*Sk-2*	maker-7426pb_lg3-snap-gene-1.7-mRNA-1	annexin A7-like	lg3	1,188,494	1,189,250	107
7426	*Sk-2*	maker-7426pb_lg3-snap-gene-1.24-mRNA-1	Peptidase_S8	lg3	1,557,848	1,561,995	1028
7426	*Sk-2*	maker-7426pb_lg3-snap-gene-1.30-mRNA-1	-	lg3	1,648,309	1,649,385	60
7426	*Sk-2*	maker-7426pb_lg3-est_gff_Cufflinks-gene-1.165-mRNA-1	-	lg3	1,721,388	1,722,228	140
8761	Sensitive	maker-8761pb_lg3.1-snap-gene-0.477-mRNA-1	-	lg3	284,264	284,823	161
8761	Sensitive	maker-8761pb_lg3.1-snap-gene-0.487-mRNA-1	-	lg3	350,887	351,224	106
8761	Sensitive	snap-8761pb_lg3.1-processed-gene-0.52-mRNA-1	-	lg3	358,752	359,078	100
8761	Sensitive	maker-8761pb_lg3.1-snap-gene-0.500-mRNA-1	-	lg3	483,926	484,259	97
8761	Sensitive	snap-8761pb_lg3.1-processed-gene-0.70-mRNA-1	Procollagen type XV	lg3	540,460	540,852	74
N. crassa	Sensitive	NCU09164T0	-	lg3	224,366	225,339	105
N. crassa	Sensitive	NCU10629T0	-	lg3	328,150	328,634	133
N. crassa	Sensitive	NCU05657T0	-	lg3	388,933	390,336	264
N. crassa	Sensitive	NCU05662T0	-	lg3	401,171	401,661	84

Strain	**Phenotype**	**Gene**	**Annotation**	**Chromosome**	**Start coordinate**	**End coordinate**	**Protein size**
N. crassa	Sensitive	NCU05742T0	-	lg3	813,119	813,977	103
N. crassa	Sensitive	NCU16630T0	Has domain(s) with predicted RNA binding, RNA-directed DNA polymerase activity, nucleic acid binding activity and role in DNA integration, RNA-dependent DNA replication	lg3	1,336,781	1,337,245	155
N. crassa	Sensitive	NCU06427T0	-	lg3	1,377,695	1,378,620	128
N. crassa	Sensitive	NCU06480T0	-	lg3	1,554,709	1,555,852	213
N. crassa	Sensitive	NCU06234T0	-	lg3	1,998,310	1,999,669	114
N. crassa	Sensitive	NCU10274T0	-	lg3	2,126,140	2,127,451	156

Supplementary Table 10: Genes in the *Sk* region in *Sk-2* and *Sk-3* but not sensitive strains

	***Sk-2* gene**				***Sk-3* gene**				
Ortholog group	**Name**	**Start coordinate**	**End coordinate**	**Protein size**	**Name**	**Start coordinate**	**End coordinate**	**Protein size**	**Annotation**
g8661 [1]	snap-7426pb_lg3-processed-gene-1.183-mRNA-1	1166491	1171390	1359	maker-3194pb_lg3-snap-gene-0.141-mRNA-1	553888	558728	1298	Unknown function.
g8662 [1]	maker-7426pb_lg3-snap-gene-1.61-mRNA-1	1160146	1165796	1249	maker-3194pb_lg3-snap-gene-0.215-mRNA-1	548640	553430	1316	Putative DNA transposon similar to Sly1-1.
g8741	snap-7426pb_lg3-processed-gene-0.248-mRNA-1	206934	207542	202	snap-3194pb_lg3-processed-gene-1.241-mRNA-1	1160484	1161293	269	Unknown function.

[1] The genes from ortholog group g8661 and g8662 are found next to each other in both the *Sk-2* and *Sk-3* genomes, but at different locations within the inverted regions in each genome. Potentially together both genes form a DNA transposon similar to *Sly1-1*.

Supplementary Table 11: Illumina HiSeq sequencing and assembly statistics

Species	Phenotype	Location	Strain	Contigs	Assembly size (bp)	N90	N80	N50	N20	Largest contig	Number of reads	Read length	Total data (Gbp)	Coverage
N. intermedia	*r(Sk-3)*	Papua New Guinea	1785	1427	41,090,782	22,979	36,251	79,388	139,516	385,06	7,243,053	124	1.80	43.7
N. intermedia	*r(Sk-3)*	Papua New Guinea	1786	2006	41,240,449	21,417	35,582	74,694	141,476	299,644	7,986,704	124	1.98	48.0
N. intermedia	*r(Sk-2)*	Papua New Guinea	1787	2310	41,013,218	17,942	28,138	59,684	112,607	302,963	7,552,672	124	1.87	45.7
N. intermedia	*r(Sk-3)*	Indonesia	1795	1158	40,300,591	30,2	52,525	100,035	179,165	458,907	9,630,836	124	2.39	59.3
N. intermedia	*r(Sk-2)*	Australia	1832	1461	40,871,442	25,71	39,38	84,475	152,06	381,938	7,691,056	124	1.91	46.7
N. intermedia	Sensitive	Papua New Guinea	1938	1495	39,275,068	23,946	42,525	85,994	166,362	580,129	10,554,317	124	2.62	66.6
N. intermedia	Sensitive	USA	2316	1640	38,668,777	22,506	37,398	75,639	145,963	333,43	13,342,846	124	3.31	85.6
N. intermedia	Sensitive	Hawaii	2365	1566	41,437,638	31,559	58,355	109,578	221,442	495,884	12,762,957	124	3.17	76.4
N. intermedia	*Sk-3*	Papua New Guinea	3194	1284	41,405,991	30,312	46,192	93,356	172,444	412,602	9,595,575	124	2.38	57.5
N. intermedia	Unknown	New Zealand	5119	2264	42,735,677	22,091	39,298	86,059	160,525	368,397	11,135,305	124	2.76	64.6
N. intermedia	*r(Sk-2) + r(Sk-3)*	Tahiti	5124	4066	43,829,016	19,411	34,816	71,135	137,27	422,048	10,623,131	124	2.63	60.1
N. intermedia	Sensitive	Haiti	6249	1579	38,828,937	18,734	29,08	64,597	122,293	273,718	10,569,810	124	2.62	67.5
N. intermedia	Sensitive	Ivory coast	6263	1570	38,666,853	21,835	34,269	71,773	138,977	458,262	10,157,083	124	2.52	65.1
N. intermedia	*r(Sk-3)*	Tahiti	6595	2704	43,159,925	21,518	35,593	77,075	127,477	322,32	9,473,377	124	2.35	54.4
N. intermedia	Unknown	New Zealand	6795	2337	42,540,341	21,601	34,31	75,613	133,21	362,532	9,022,418	124	2.24	52.6
N. intermedia	*Sk-2*	Brunei	7402	1293	42,076,821	25,495	40,122	90,389	165,648	309,97	9,342,724	124	2.32	55.1
N. intermedia	*Sk-2*	Sabah	7426	1757	41,922,822	24,97	42,414	89,659	163,579	388,017	9,218,243	124	2.29	54.5
N. intermedia	*Sk-2*	Java	7427	1709	42,477,818	26,412	45,112	94,222	166,322	334,189	9,547,705	124	2.37	55.7
N. intermedia	*Sk-2*	Papua New Guinea	7429	1357	41,087,187	29,068	48,296	90,077	156,631	318,967	9,373,713	124	2.32	56.6
N. intermedia	Sensitive	Taiwan	8761	1762	41,071,115	23,212	37,929	75,851	147,727	326,559	9,459,755	124	2.35	57.1
N. intermedia	*r(Sk-2)*	Indonesia	8767	1676	37,775,537	15,552	24,292	52,492	99,573	220,91	6,921,303	124	1.72	45.4
N. intermedia	*r(Sk-3)*	Indonesia	8769	1194	39,799,185	23,381	37,153	78,057	160,447	440,184	9,175,022	124	2.28	57.2
N. intermedia	Sensitive	Papua New Guinea	8793	1147	39,486,933	21,7	34,444	70,635	132,168	348,704	9,462,259	124	2.35	59.4
N. intermedia	Unknown	Mexico	P4149	4116	44,075,354	15,375	24,436	50,905	95,065	279,828	10,211,750	124	2.53	57.5
N. metzenbergii	Unknown	Mexico	8853	3291	43,176,579	16,611	25,566	57,213	100,504	273,505	9,143,136	124	2.27	52.5

Species	Phenotype	Location	Strain	Contigs	Assembly size (bp)	N90	N80	N50	N20	Largest contig	Number of reads	Read length	Total data (Gbp)	Coverage
N. metzenbergii	Unknown	Mexico	10411	3540	43,486,000	16,788	27,102	55,515	105,517	262,589	10,229,242	124	2.54	58.3
N. crassa	*r(Sk-2)*	Malaya	4723	1621	43,069,651	35,89	58,892	136,267	250,436	598,204	10,359,829	124	2.57	59.7
N. crassa	*r(Sk-2)*	Ivory coast	4829	1601	42,013,408	27,506	45,162	102,13	200,119	439,843	11,185,181	124	2.77	66.0
N. crassa	Unknown	India	8863	1279	40,248,786	27,037	41,9	86,864	166,428	349,317	9,596,092	124	2.38	59.1

Supplementary Table 12: Age estimates of the non-recombining region of the *Sk-2* spore killers

Strain	Ds	Low age estimate (years) [1]	High age estimate (years) [2]
7402	0.00512	256,411	430,081
7426	0.00505	252,794	424,014
7427	0.00510	255,472	428,505
7429	0.00510	255,370	428,334
Mean	0.00509	255,012	427,734

[1] Calculated using mutation rate of 9.98e-9 synonymous substitutions per site per year.

[2] Calculated using mutation rate of 5.95e-9 synonymous substitutions per site per year.

Mutation rates have been taken from Kasuga et al. (2002)[4].

Table 13: Nucleotide diversity and divergence of two *N. intermedia* sensitive strains

***N. intermedia* sensitive [1]**	π_{total}	π_N	π_S	$\pi_{N/}\ \pi_S$	P_N	P_S	D_N	D_S	P_N / P_S	D_N / D_S	**NI**
Total	0.014	0.0076	0.040	0.191	52135	131114	63279	145860	0.40	0.43	0.92
Chr1	0.013	0.0063	0.033	0.191	11040	27292	15037	33353	0.40	0.45	0.90
Chr2	0.012	0.0075	0.038	0.194	5594	13658	6514	15853	0.41	0.41	1.00
Chr3 [2]	0.016	0.0077	0.038	0.201	4162	9315	5499	12668	0.45	0.43	1.03
Chr4	0.014	0.0081	0.044	0.183	8384	22627	10157	22678	0.37	0.45	0.83
Chr5	0.017	0.0071	0.040	0.179	7914	20999	8908	21767	0.38	0.41	0.92
Chr6	0.013	0.0088	0.047	0.187	6325	16314	7101	16583	0.39	0.43	0.91
Chr7	0.012	0.0092	0.045	0.206	6851	16456	7741	17170	0.42	0.45	0.92
Sk reg.	0.014	0.0075	0.037	0.202	1865	4453	2322	5788	0.42	0.40	1.04
Bootstrap mean (95%) [3]	/	0.0076 (0.0065, 0.0086)	0.04 (0.037, 0.043)	0.191 (0.16, 0.21)	1990 (1646, 2284)	4955 (4449, 5472)	2381 (2113, 2678)	5467 (5135, 5905)	0.40 (0.35, 0.45)	0.44 (0.39, 0.48)	1.09 (0.96, 1.25)

[1] Two haplotypes were selected for calculation of diversity and divergence.

[2] Chromosome 3 with Spore killer region excluded.

[3] Bootstrap confidence intervals were calculated by randomly picking the same number of genes as the number of genes in the Spore killer region. For bootstrap in total number of polymorphism and divergence, we also rescaled the number by the ratio of the total bootstrapped gene length and the length of the Spore killer region.

Supplementary Table 14: Nucleotide diversity and divergence of nine *N. intermedia* sensitive strains

***N. intermedia* sensitive [1]**	π_{total}	π_N	π_S	$\pi_{N/}\ \pi_S$	P_N	P_S	D_N	D_S	**NI**
Total	0.014	0.0076	0.040	0.191	162822	418779	34189	68886	0.78
Chr 1	0.013	0.0063	0.033	0.191	35709	87856	9190	17787	0.79
Chr 2	0.012	0.0075	0.038	0.194	17093	44453	3435	7239	0.81
Chr 3 [2]	0.016	0.0077	0.038	0.201	13870	35545	2753	5467	0.77
Chr 4	0.014	0.0081	0.044	0.183	26533	69961	4941	10097	0.78
Chr 5	0.017	0.0071	0.040	0.179	25320	67771	4658	9972	0.80
Chr 6	0.013	0.0088	0.047	0.187	18795	48628	3866	7712	0.77
Chr 7	0.012	0.0092	0.045	0.206	19943	49764	4107	7834	0.76
Sk region	0.014	0.0075	0.037	0.202	5559	14801	1239	2778	0.84

[1] Nine haplotypes were selected for calculation of diversity and divergence

[2] Chromosome 3 with Spore killer region excluded

Supplementary references

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