

Supporting information

Table S1. Primers used in this study

| Primers | Sequence (5' to 3') | Comment |
|-----------|--|------------------------------|
| JOHE9212 | GGCCCTAGGTAAGTCTACTCT | <i>SXI1α</i> Deletion |
| JOHE9213 | AGCTCACATCCTCGCAGCTATTTGATCAGCTGCTTATTGTTT | <i>SXI1α</i> Deletion |
| JOHE9214 | CCGTGTTAATACAGATAAACCGCAAACGCACTCATGATGGTTCGAG | <i>SXI1α</i> Deletion |
| JOHE9215 | TTCGAAACCGGAAGACCTCAG | <i>SXI1α</i> Deletion |
| JOHE10020 | ATGTGCGGATCCGAGGAATGA | <i>SXI2a</i> Deletion |
| JOHE10021 | AGCTCACATCCTCGCAGCGGTGACGATAGAACTGTTGATAGA | <i>SXI2a</i> Deletion |
| JOHE10022 | CCGTGTTAATACAGATAAACCGACCAAACCTTCTTTGCCCTCCAG | <i>SXI2a</i> Deletion |
| JOHE10023 | GCCTTGCTCGCACTTTAAGTC | <i>SXI2a</i> Deletion |
| JOHE9052 | GACCGAGAAAACGCCAAAAAT | <i>SXI1α::URA5</i> screening |
| JOHE9624 | TTGGGTCAACTCTAATAAGGTGGT | <i>SXI1α::URA5</i> screening |
| JOHE16835 | TTGGGTCAACTCTAATAAGGT | <i>SXI2a::URA5</i> screening |
| JOHE16836 | TCTATGGCGAACGTGCTCATT | <i>SXI2a::URA5</i> screening |
| Da3 | GCAATAGCATATACCATCCCG | Mitochondrial DNA |
| Da20 | GACACTACACAAGATGCCTC | Mitochondrial DNA |

Supplementary Figures

Fig. S1. Serotype A (*sxi1α* + *SXI2a*) x “a” or (*sxi1α* + *SXI2a*) x **a** crosses can produce basidiospore chains. Serotype A strains of the indicated genotypes were crossed on MS mating medium in the dark at 25°C. After 1 week incubation, the photos of the mating colonies were taken at 100x magnification. The arrows indicate the spore chains produced at the edge of the mating colony.

Fig. S2. Evolution of the tetrapolar mating system. A proposed model depicting how a tetrapolar system may have evolved from an ancestral bipolar mating system. Arrows between or within the alleles indicate compatible recognition.