Note

Evolutionary Strata in a Small Mating-Type-Specific Region of the Smut Fungus *Microbotryum violaceum*

Antonina A. Votintseva and Dmitry A. Filatov¹

Department of Plant Sciences, University of Oxford, Oxford OX1 3RB, United Kingdom

Manuscript received March 24, 2009

Accepted for publication May 13, 2009

ABSTRACT

DNA sequence analysis and genetic mapping of loci from mating-type-specific chromosomes of the smut fungus *Microbotryum violaceum* demonstrated that the nonrecombining mating-type-specific region in this species comprises ~25% (~1 Mb) of the chromosome length. Divergence between homologous mating-type-linked genes in this region varies between 0 and 8.6%, resembling the evolutionary strata of vertebrate and plant sex chromosomes.

EVOOLUTION of mating types or sex-determining systems often involves the suppression of recombination around the primary sex-determining or mating-type-determining locus. In animals and plants, it is often an entire or almost entire chromosome (Y or W in male or female heterogametic species, respectively) that ceases to recombine with its homologous (X or Z) chromosome (CHARLESWORTH and CHARLESWORTH 2000; CHARLESWORTH 2008). Self-incompatibility loci in plants are also thought to be located in regions of suppressed recombination (CHARLESWORTH *et al.* 2005; KAMAU and CHARLESWORTH 2005; KAMAU *et al.* 2007; LI *et al.* 2007; YANG *et al.* 2007). Regardless of the phylogenetic position of a species, such nonrecombining regions are known to follow similar evolutionary trajectories. The nonrecombining region on the sex-specific chromosome expands in several steps, forming evolutionary strata—regions of different X/Y (or Z/W) divergence (LAHN and PAGE 1999; HANDLEY *et al.* 2004; SANDSTEDT and TUCKER 2004; NICOLAS *et al.* 2005)—and genes in the nonrecombining regions gradually accumulate deleterious mutations that eventually render them dysfunctional (CHARLESWORTH and CHARLESWORTH 2005; CHARLESWORTH 2008).

Fungal mating-type systems are very diverse, with the number of mating types varying from two to several

hundred (CASSELTON 2002). Like sex chromosomes in several animals and plants, suppressed recombination has evolved in regions near fungal mating-type loci, including in *Ustilago hordei* (LEE *et al.* 1999), *Cryptococcus neoformans* (LENLEGER *et al.* 2002), and *Neurospora tetrasperma* (MENKIS *et al.* 2008). These species have two mating types, but no morphologically distinct sexes. The mating-type locus (the region of suppressed recombination) of *C. neoformans* is small (~100 kb) compared with known sex chromosomes and contains only ~20 genes that, unlike many sex chromosomes (Y or W chromosomes), show no obvious signs of genetic degeneration (LENLEGER *et al.* 2002; FRASER *et al.* 2004). Judging from the divergence between the homologous genes on the two mating-type-specific chromosomes, *C. neoformans* started to evolve sex chromosomes a long time ago because silent divergence between the two mating types in the most ancient region exceeds 100% (FRASER *et al.* 2004). Genes in the younger mating-type-specific region are much less diverged between the two sex chromosomes, suggesting that the evolution of the sex locus in *C. neoformans* might have proceeded through several steps. The nonrecombining region around the mating-type locus of *N. tetrasperma* is much larger than in *C. neoformans* (at least 6.6 Mb), and silent divergence between homologous genes on the mating-type-specific chromosomes ranges from zero to 9%, demonstrating that these mating-type-specific chromosomes evolved recently (MENKIS *et al.* 2008).

M. violaceum, which causes anther smut disease in *Silene latifolia* and other species in the family Caryophyllaceae, has two mating types, A1 and A2 (reviewed by GIRAUD *et al.* 2008), which are determined by the presence of mating-type-specific chromosomes (here-

Supporting information is available online at <http://www.genetics.org/cgi/content/full/genetics.109.103192/DC1>.

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession nos. F1855822–F1856001.

¹Corresponding author: Department of Plant Sciences, University of Oxford, South Parks Rd., Oxford OX1 3RB, United Kingdom.
E-mail: dmitry.filatov@plants.ox.ac.uk

FIGURE 1.—Testing of mating-type specificity for loci isolated from A1 and A2 chromosomes. (a) PCR amplifications from haploid cultures from SI405 using primers designed from six A1-originated loci. Loci in which a PCR product could be amplified only from A1 cultures (boxed) were classified as specific to mating type A1. (b) PCR tests of six A2-originated loci on the same set of haploids as in a. Loci in which a PCR product amplified only from A2 cultures (boxed) were classified as specific to mating type A2. Loci amplified from both A1 and A2 cultures are not mating type specific.

after A1 and A2 chromosomes, or sex chromosomes) in the haploid stage of the life cycle (HOOD 2002; HOOD *et al.* 2004). The A1 and A2 chromosomes are distinguishable by size in pulsed-field electrophoresis, and it is possible to isolate individual chromosomes electrophoretically (HOOD *et al.* 2004). Random fragments of A1 and A2 chromosomes have previously been isolated from mating-type-specific bands of pulsed-field separated chromosomes of *M. violaceum* (HOOD *et al.* 2004). These fragments were assumed to be linked to mating type. The same method was used to isolate fragments of non-mating-type-specific chromosomes. On the basis of the analysis of their sequences, (HOOD *et al.* 2004) proposed that mating-type-specific chromosomes in *M. violaceum* might be degenerate because they contained a lower proportion of protein-coding genes than other chromosomes. However, it was not determined whether the sequences isolated from the mating-type chromosomes originated from the mating-type-specific or from the recombining regions (HOOD *et al.* 2004), and the relative sizes of these regions are not known for these *M. violaceum* chromosomes. We tested the mating-type specificity of 86 of these fragments and demonstrate that fewer than a quarter of these loci are located in the mating-type-specific region, suggesting that the non-recombining region on the A1 and A2 chromosomes is quite small, while the rest of the chromosome probably recombines (like pseudoautosomal regions of sex chromosomes) and is therefore not expected to undergo genetic degeneration. Genetic mapping confirms the presence of two pseudoautosomal regions in the *M. violaceum* mating-type-specific chromosomes.

As these chromosomes are mating type specific in the haploid stage of *M. violaceum*, mating-type-specific loci (or DNA fragments) can be identified by testing whether they are present exclusively in A1 or A2 haploid strains. We therefore prepared haploid A1 and A2 *M. violaceum* cultures from *S. latifolia* plants from two geographically remote locations (accessions SI405 from Sweden and SI127 from the French Pyrenees). Haploid sporidial cultures were isolated by a standard dilution method (KALTZ and SHYKOFF 1997; OUDEMANS and ALEXANDER 1998). Mating types were determined by PCR amplifica-

tion of each culture with primers designed for A1 and A2 pheromone receptor genes linked to A1 and A2 mating types (YOCKTENG *et al.* 2007). The primers were as follows: 5'-TGGCATCCCTCAATGTTTCC-3' and 5'-CACCTTTTGATGAGAGGCCG-3' for the A1 pheromone receptor (GenBank accession no. EF584742) and 5'-TGACGAGAGCATTCTACCG-3' and 5'-GAAGCGGAACCTGCCTTCT-3' for the A2 pheromone receptor (GenBank accession no. EF584741). Cultures with PCR product amplified only from an A1 or A2 pheromone receptor gene were selected for further use. The mating types of the cultures were verified by conjugating them in all combinations.

The GenBank nucleotide database was searched using BLAST for sequences similar to those isolated by HOOD *et al.* (2004). Sequences with similarity to transposable elements (TE) and other repeats were excluded. The resulting set of nonredundant sequences was used to design PCR primers for 98 fragments. Half of these were originally isolated from the A1 and half from the A2 chromosomes and are hereafter called A1-NNN or A2-NNN (where NNN is the locus number; supporting information, Table S1), which does not imply that these loci are A1 or A2 specific, but merely indicates that they were originally isolated from the A1 or A2 chromosomes. Amplification of these regions from new A1 and A2 *M. violaceum* cultures, independently isolated by ourselves, revealed that only 5 of the 49 loci isolated from the A1 chromosome are indeed A1 specific and only 6 of 49 isolated from the A2 chromosome are A2 specific. All other loci amplified from both A1 and A2 cultures. Figure 1 illustrates some of these results from the Swedish sample (SI405).

The fragments that amplified from both A1 and A2 mating types may be in recombining regions, or they could be present in mating-type-specific regions on both A1 and A2 chromosomes. If they are in recombining regions, the A1- and A2-linked homologs should not be diverged from each other, but if they are in nonrecombining, mating-type-specific regions, the divergence of the A1- and A2-linked homologs should be roughly proportional to the time since recombination stopped in the region. We therefore sequenced and compared PCR fragments amplified from the two mating types

TABLE 1
Loci from mating-type-specific chromosomes of *M. violaceum* used for PCR analysis and genetic map construction

Loci	Mating type specific	With nonzero A1/A2 divergence ^b		With zero A1/A2 divergence ^b	Total
		<1%	>1%		
A1 ^a	5	2 (1)	3 (3)	35 (3)	45 (7)
A2 ^a	6	2 (0)	7 (7)	26 (3)	41 (10)
Subtotal		4 (1)	10 (10)		
Total	11	14 (11)		61 (6)	86 (17)

^a A1, loci originated from the A1 sex chromosome; A2, loci originated from the A2 sex chromosome.

^b The number of loci used for genetic map construction is in parentheses.

of SI405 or SI127 cultures (GenBank accession nos. FI855822–FI856001). Sequencing of PCR products showed that 12 (4 A1 and 8 A2) loci have more than one copy, and they were excluded from further analysis. Sequences of 61 loci were identical between the A1 and A2 strains, and four loci demonstrated low total divergence (0.24–0.61%) between the two mating types (Table 1). This level of divergence is similar to the background level of DNA polymorphism in *M. violaceum* populations, which was measured in 16 mating-type-linked, pseudoautosomal and autosomal genes in a sample of 14 *M. violaceum* accessions sampled across Europe (A. VOTINTSEVA and D. FILATOV, unpublished results). Thus, these loci might be located in the recombining part of the mating-type-specific chromosomes. Ten of 75 loci that amplified in both mating types demonstrated multiple polymorphisms fixed between the mating types rather than between the locations. Given that the strains that we used in the analysis originated from two geographically distant locations, it is highly unlikely that multiple polymorphisms distinguishing the A1 and A2 sequences arose purely by chance; thus, these loci are probably located in the nonrecombining mating-type-specific region of the *M. violaceum* A1 and A2 chromosomes.

To confirm the mating-type-specific or pseudoautosomal locations of the loci with and without A1/A2 divergence, we conducted genetic mapping in a family of 99 individuals, 50 of which were of mating type A1 and 49 of mating type A2. The family was generated by a cross between A1 and A2 *M. violaceum* strains from *S. latifolia* accessions SI405 (Sweden) and SI127 (France), respectively. The choice of strains from geographically distant locations was motivated by the hope of maximizing the number of DNA sequence differences between them that can be used as molecular genetic markers in segregation analysis. We inoculated *S. latifolia* seedlings with sporidial cultures of both mating types. For inoculation, petri dishes with 12-day-old seedlings of *S. latifolia* were flooded with 2.5 ml of inoculum suspension. Inoculum suspension consisted of equal volumes of the A1 and A2 sporidial cultures that were mixed and

conjugated overnight at 14° under rotation (BIERE and HONDERS 1996; VAN PUTTEN *et al.* 2003). Seedlings were potted 3 days after inoculation. Two months later, teliospores were collected from the flowers of the infected plant and grown in petri dishes on 3.6% potato dextrose agar medium. Haploid sporidia formed after meiosis were isolated and grown as separate cultures for DNA extraction. The mating types of single sporidia cultures were identified as described above. The loci analyzed in the segregation analysis were sequenced in the two parental haploid strains and in 99 (50 A1 and 49 A2) haploid strains that were generated in the cross. Single nucleotide differences between the parental strains were used as molecular genetic markers for segregation analysis in the progeny. The genetic map was constructed using MAPMAKER/EXP v3.0 (LINCOLN *et al.* 1992) and MapDisto v1.7 (<http://mapdisto.free.fr/>).

The resulting genetic map is shown in Figure 2. As expected, no recombination was observed between the 10 loci with diverged A1- and A2-linked copies. In addition, one marker with no A1/A2 divergence, A2-397, was also completely linked to the loci with significant A1/A2 divergence. This locus either may be very tightly linked to the nonrecombining mating-type-specific region or may have been added to that region more recently than the loci that had already accumulated some divergence between the alleles in the two mating types. The mating-type-specific pheromone receptor locus (DEVIER *et al.* 2009) and 11 mating-type-specific loci are also located in this nonrecombining region (Figure 2). Interestingly, the cluster of nonrecombining markers is flanked on both sides with markers that recombine in meiosis, demonstrating that there are pseudoautosomal regions on both ends of the mating-type-specific chromosomes.

Our results demonstrate that although the loci reported by HOOD *et al.* (2004) were isolated from the A1 and A2 chromosomes, most of these loci are not located in the nonrecombining mating-type-specific regions. In fact, the nonrecombining region might be relatively small: of 86 tested fragments, only 21 appeared to be either mating type specific or linked to the mating-

FIGURE 2.—Genetic map of the mating-type-determining chromosome in *M. violaceum*. Genetic distance (in centimorgans) and the relative positions of the markers are shown to the left and the right of the chromosome, respectively. The position of the nonrecombining region corresponds to the cluster of linked markers shown on the right of the figure. Total A1/A2 divergence is shown in parentheses. Eleven mating-type-specific markers (for which sequences are available from only one mating type), located in the nonrecombining mating-type-specific region, are not shown.

type locus. Assuming that these loci represent a random set of DNA fragments isolated from the A1 and A2 chromosomes, it is possible to estimate the size of the nonrecombining region using the binomial distribution: the nonrecombining region is expected to be 24.4% (95% CI: 16.7–33.6%) of the chromosome length. As the sizes of the A1 and A2 chromosomes are ~3.4 and 4.2 Mb long (HOOD 2002; HOOD *et al.* 2004), the nonrecombining region might be ~1 Mb long.

Interestingly, total A1/A2 divergence for the 11 loci with A1- and A2-linked copies mapped to the nonrecombining region varied from 0% to 8.6% (Table 2 and Figure 2). In addition, 11 loci amplified from only one mating type. These genes could represent degen-

erated genes, some of which degenerated in A1 strains, and some in A2 strains. Alternatively, they might be highly diverged genes, such that the PCR primers amplify only one allele, and not the other. Variation in divergence may be the result of the stepwise cessation of recombination between the A1 and A2 chromosomes in *M. violaceum*, resembling the evolutionary strata reported for human, chicken, and white campion sex chromosomes (LAHN and PAGE 1999; HANDLEY *et al.* 2004; BERGERO *et al.* 2007). However, only the differences between the most and the least diverged loci are statistically significant (Table 3). Nevertheless, taking into account the much higher A1/A2 divergence of the pheromone receptor locus for which the homologs from the two mating types are barely alignable (DEVIER *et al.* 2009), the *M. violaceum* mating-type region has at least three strata: one oldest stratum, including the pheromone receptor locus; a younger stratum with ~5–9% A1/A2 divergence; and the youngest stratum with 1–4% divergence between the two mating types. There may also be an additional very recently evolved stratum containing the locus named A2-397, which is also present in all A1 strains tested, with no fixed differences between the A1 and A2 strains (Table 3).

As most of the loci isolated from the A1 and A2 chromosomes recombine in meiosis, they are not expected to degenerate. Thus, the observation of a higher proportion of TEs in these loci, compared to other chromosomes (HOOD *et al.* 2004), is unlikely to reflect genetic degeneration attributable to a lack of recombination in these loci. A higher abundance of TEs in the sequences isolated from the A1 and A2 chromosomes, as reported by HOOD *et al.* (2004), may

TABLE 2
Loci with nonzero divergence between the A1 and A2 mating types of *M. violaceum*

Loci ^a		No. of sites analyzed	S ^b total	Within A1		Within A2		Fixed differences between A1 and A2	A1/A2 divergence (%)
				S	π (%) ^c	S	π (%) ^c		
A1/A2 divergence <1%	A1-236	456	3	0	0	2	0.44	1	0.44
	A1-045	654	4	0	0	0	0	4	0.61
	A2-568	413	2	2	0.48	2	0.48	0	0.24
	A2-411	480	2	1	0.21	0	0	1	0.31
A1/A2 divergence >1%	A1-217	667	9	0	0	0	0	9	1.35
	A1-128	569	9	0	0	1	0.18	8	1.49
	A1-199	618	13	0	0	1	0.16	12	2.02
	A2-422	344	9	0	0	0	0	9	2.62
	A2-516	470	14	0	0	0	0	14	2.98
	A2-404	508	20	0	0	3	0.59	17	3.64
	A2-435	506	22	2	0.39	2	0.39	18	3.95
	A2-473	457	23	1	0.22	1	0.22	21	4.81
	A2-457	303	17	1	0.33	0	0	16	5.54
	A2-575	503	47	5	0.99	3	0.59	39	8.55

^a A1, loci originated from the A1 sex chromosome; A2, loci originated from the A2 sex chromosome.

^b S, number of polymorphic sites.

^c π (%), average number of differences per 100 nucleotides.

TABLE 3

P-values for the 2 × 2 G-tests for significance of differences in A1/A2 divergence between the loci in the nonrecombining region

<i>L</i> ^a	<i>S</i> ^b	Locus	A2-397	A1-217	A1-128	A1-199	A2-422	A2-516	A2-404	A2-435	A2-473	A2-457
519	0	A2-397										
667	9	A1-217	0.006									
569	8	A1-128	0.006	0.93								
618	12	A1-199	0.0007	0.41	0.48							
344	9	A2-422	0.0003	0.17	0.21	0.51						
470	14	A2-516	0.00003	0.06	0.086	0.28	0.76					
508	17	A2-404	0	0.025	0.038	0.15	0.55	0.75				
506	18	A2-435	0	0.015	0.024	0.104	0.45	0.62	0.86			
457	21	A2-473	0	0.001	0.003	0.0163	0.15	0.21	0.34	0.43		
303	16	A2-457	0	0.0009	0.0017	0.0097	0.09	0.13	0.203	0.26	0.69	
503	39	A2-575	0	0	0	0.00001	0.002	0.002	0.003	0.006	0.055	0.199

P-values <0.05 are in boldface type.

^a *L*, the length of the region compared.

^b *S*, the number of nucleotide differences observed.

simply reflect variation in the TE density across the genome. Thus, it remains to be seen whether *M. violaceum* mating-type-specific regions degenerate, similar to vertebrate Y (or W) chromosomes, or remain largely intact, as in *C. neoformans* (LENGELER *et al.* 2002). If the latter were the case, it may suggest that non-recombining regions in fungi do not necessarily follow the same degenerative path as animal Y and W chromosomes. The analysis of sequences from the *M. violaceum* genome (and perhaps other fungal genomes) will hopefully provide the answer to this question.

The lack of degeneration of mating-type-specific regions in *C. neoformans* may be due to the relatively small size of the nonrecombining regions. The 20 genes present in this region may not be sufficient for the operation of such detrimental population genetic processes as background selection or Muller's ratchet because the speed of these processes depends critically on the number of active genes linked together (CHARLESWORTH 2008). Larger mating-type-specific regions in *M. violaceum* might contain more genes; thus, more active genetic degeneration may be expected in this species. Indeed, many strains of *M. violaceum* show haplolethality linked to one of the mating types (HOOD and ANTONIVICS 2000; THOMAS *et al.* 2003; TELLIER *et al.* 2005), which may reflect the accumulation of deleterious mutations in the nonrecombining regions around the mating-type loci. Mating-type specificity of the markers that amplified in only A1 or A2 strains in this study may also reflect genetic degeneration.

Another factor that may potentially prevent degeneration of genes linked to mating-type loci in fungi is the haploid expression of genes in these regions. In animals, many Y-linked genes have functional homologs on the X chromosome, and loss of the Y-linked gene may be compensated for by expression of the X-linked homologs. The haploid stage in an animal's life cycle is very short, and very few genes are actively expressed in animal gametes (SCHULTZ *et al.* 2003). In plants, on the other

hand, a significant proportion of the genome is expressed in pollen (DA COSTA-NUNES and GROSSNIKLAKS 2003), and so the loss of Y-linked genes expressed in gametes may be more detrimental than in animals. Indeed, most genes isolated from the white campion X chromosome have intact Y-linked copies (FILATOV 2005; BERGERO *et al.* 2007), but due to the small number of genes available, it is still unclear whether genetic degeneration of Y-linked genes is indeed slower in this species (and in plants generally) compared to animal Y chromosomes. Haploid expression could be an even more powerful force in fungi and other organisms with haploid sexes, such as bryophytes, as most genes are expressed in the haploid stage. Further analysis of genetic degeneration in nonrecombining sex- or mating-type-specific regions in fungi and bryophytes will help to shed light on this question.

We thank Lorna Casselton, Deborah Charlesworth, and two anonymous reviewers for critical reading of the manuscript and helpful comments and Christopher Dixon for assistance in teliospore collection and correction of the manuscript. This project was funded by the University of Oxford John Fell Fund and by the Biotechnology and Biological Sciences Research Council.

LITERATURE CITED

- BERGERO, R., A. FORREST, E. KAMAU and D. CHARLESWORTH, 2007 Evolutionary strata on the X chromosomes of the dioecious plant *Silene latifolia*: evidence from new sex-linked genes. *Genetics* **175**: 1945–1954.
- BIERE, A., and S. HONDERS, 1996 Host adaptation in the anther smut fungus *Ustilago violacea* (*Microbotryum violaceum*): infection success, spore production and alteration of floral traits on two host species and their F1-hybrid. *Oecologia* **107**: 307–320.
- CASSELTON, L. A., 2002 Mate recognition in fungi. *Heredity* **88**: 142–147.
- CHARLESWORTH, B., and D. CHARLESWORTH, 2000 The degeneration of Y chromosomes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**: 1563–1572.
- CHARLESWORTH, D., 2008 Sex chromosome origins and evolution, pp. 207–240 in *Evolutionary Genomics and Proteomics*, edited by P. A. PAGEL. Sinauer Associates, Sunderland, MA.
- CHARLESWORTH, D., and B. CHARLESWORTH, 2005 Sex chromosomes: evolution of the weird and wonderful. *Curr. Biol.* **15**: R129–R131.

- CHARLESWORTH, D., X. VEKEMANS, V. CASTRIC and S. GLEMIN, 2005 Plant self-incompatibility systems: a molecular evolutionary perspective. *New Phytol.* **168**: 61–69.
- DA COSTA-NUNES, J. A., and U. GROSSNIKLUS, 2003 Unveiling the gene-expression profile of pollen. *Genome Biol.* **5**: 205.
- DEVIER, B., G. AGUILETA, M. E. HOOD and T. GIRAUD, 2009 Ancient *trans*-specific polymorphism at pheromone receptor genes in basidiomycetes. *Genetics* **181**: 209–223.
- FILATOV, D. A., 2005 Isolation of genes from plant Y chromosomes. *Methods Enzymol.* **395**: 418–442.
- FRASER, J. A., S. DIEZMANN, R. L. SUBARAN, A. ALLEN, K. B. LENGELER *et al.*, 2004 Convergent evolution of chromosomal sex-determining regions in the animal and fungal kingdoms. *PLoS Biol.* **2**: e384.
- GIRAUD, T., R. YOCKTENG, M. LOPEZ-VILLAVICENCIO, G. REFREGIER and M. E. HOOD, 2008 Mating system of the anther smut fungus *Microbotryum violaceum*: selfing under heterothallism. *Eukaryot. Cell* **7**: 765–775.
- HANDLEY, L. J., H. CEPLITIS and H. ELLEGREN, 2004 Evolutionary strata on the chicken Z chromosome: implications for sex chromosome evolution. *Genetics* **167**: 367–376.
- HOOD, M. E., 2002 Dimorphic mating-type chromosomes in the fungus *Microbotryum violaceum*. *Genetics* **160**: 457–461.
- HOOD, M. E., and J. ANTONOVICS, 2000 Intratetrad mating, heterozygosity, and the maintenance of deleterious alleles in *Microbotryum violaceum* (=Ustilago violacea). *Heredity* **85**: 231–241.
- HOOD, M. E., J. ANTONOVICS and B. KOSKELLA, 2004 Shared forces of sex chromosome evolution in haploid-mating and diploid-mating organisms: *Microbotryum violaceum* and other model organisms. *Genetics* **168**: 141–146.
- KALTZ, O., and J. A. SHYKOFF, 1997 Sporidial mating-type ratios of teliospores from natural populations of the anther smut fungus *Microbotryum* (=Ustilago) violaceum. *Int. J. Plant. Sci.* **158**: 575–584.
- KAMAU, E., and D. CHARLESWORTH, 2005 Balancing selection and low recombination affect diversity near the self-incompatibility loci of the plant *Arabidopsis lyrata*. *Curt. Biol.* **15**: 1773–1778.
- KAMAU, E., B. CHARLESWORTH and D. CHARLESWORTH, 2007 Linkage disequilibrium and recombination rate estimates in the self-incompatibility region of *Arabidopsis lyrata*. *Genetics* **176**: 2357–2369.
- LAHN, B. T., and D. C. PAGE, 1999 Four evolutionary strata on the human X chromosome. *Science* **286**: 964–967.
- LEE, N., G. BAKKEREN, K. WONG, J. E. SHERWOOD and J. W. KRONSTAD, 1999 The mating-type and pathogenicity locus of the fungus *Ustilago hordei* spans a 500-kb region. *Proc. Natl. Acad. Sci. USA* **96**: 15026–15031.
- LENGELER, K. B., D. S. FOX, J. A. FRASER, A. ALLEN, K. FORRESTER *et al.*, 2002 Mating-type locus of *Cryptococcus neoformans*: a step in the evolution of sex chromosomes. *Eukaryot. Cell* **1**: 704–718.
- LI, J., M. WEBSTER, M. FURUYA and P. M. GILMARTIN, 2007 Identification and characterization of pin and thrum alleles of two genes that co-segregate with the *Primula* S locus. *Plant J.* **51**: 18–31.
- LINCOLN, S., M. DALY and E. LANDER, 1992 Constructing genetic maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report. Whitehead Institute, Cambridge, MA.
- MENKIS, A., D. J. JACOBSON, T. GUSTAFSSON and H. JOHANNESSON, 2008 The mating-type chromosome in the filamentous ascomycete *Neurospora tetrasperma* represents a model for early evolution of sex chromosomes. *PLoS Genet.* **4**: e1000030.
- NICOLAS, M., G. MARAIS, V. HYKELOVA, B. JANOUSEK, V. LAPORTE *et al.*, 2005 A gradual process of recombination restriction in the evolutionary history of the sex chromosomes in dioecious plants. *PLoS Biol.* **3**: e4.
- OUDEMANS, P. V., and H. M. ALEXANDER, 1998 The distribution of mating-type bias in natural populations of the anther-smut *Ustilago violacea* on *Silene alba* in Virginia. *Mycologia* **90**: 372–381.
- SANDSTEDT, S. A., and P. K. TUCKER, 2004 Evolutionary strata on the mouse X chromosome correspond to strata on the human X chromosome. *Genome Res.* **14**: 267–272.
- SCHULTZ, N., F. K. HAMRA and D. L. GARBERS, 2003 A multitude of genes expressed solely in meiotic or postmeiotic spermatogenic cells offers a myriad of contraceptive targets. *Proc. Natl. Acad. Sci. USA* **100**: 12201–12206.
- TELLIER, A., L. M. VILLAREAL and T. GIRAUD, 2005 Maintenance of sex-linked deleterious alleles by selfing and group selection in metapopulations of the phytopathogenic fungus *Microbotryum violaceum*. *Am. Nat.* **165**: 577–589.
- THOMAS, A. J., J. A. SHYKOFF, O. JONOT and T. GIRAUD, 2003 Mating-type ratio bias in populations of the phytopathogenic fungus *Microbotryum violaceum* from several host species. *Int. J. Plant Sci.* **164**: 641–647.
- VAN PUTTEN, W. F., A. BIERE and J. M. VAN DAMME, 2003 Intra-specific competition and mating between fungal strains of the anther smut *Microbotryum violaceum* from the host plants *Silene latifolia* and *S. dioica*. *Evolution* **57**: 766–776.
- YANG, Q., D. ZHANG, Q. LI, Z. CHENG and Y. XUE, 2007 Heterochromatic and genetic features are consistent with recombination suppression of the self-incompatibility locus in *Antirrhinum*. *Plant J.* **51**: 140–151.
- YOCKTENG, R., S. MARTEY, H. CHIAPELLO, A. GENDRAULT, M. E. HOOD *et al.*, 2007 Expressed sequence tags of the anther smut fungus, *Microbotryum violaceum*, identify mating and pathology genes. *BMC Genomics* **8**: 272.

Communicating editor: D. CHARLESWORTH

GENETICS

Supporting Information

<http://www.genetics.org/cgi/content/full/genetics.109.103192/DC1>

Evolutionary Strata in a Small Mating-Type-Specific Region of the Smut Fungus *Microbotryum violaceum*

Antonina A. Votintseva and Dmitry A. Filatov

Copyright © 2009 by the Genetics Society of America

DOI: 10.1534/genetics.109.103192

TABLE S1**Primers used for PCR amplification and sequencing**

Loci	GenBank accession numbers*	Primers			A1/A2 divergence, %
		Name	5' Sequence 3'	Tm, °C	
A1-077	BZ782077	A1-077f	CCCTCGACGATGTTCTTCTTG	53.8	A1 sex specific
		A1-077r	CCAGTCGAATATTTGGAACCG	53.7	
A1-116	BZ782116	A1-116f	TGAGTGATGGTCGACAGGTCA	53.5	A1 sex specific
		A1-116r	TGACATCAGCTGAGTTCCCAGT	53.6	
A1-202	BZ782202	A1-202f	ACAGGTCATTACACAACGGCT	51.8	A1 sex specific
		A1-202r	TCCTCGACCGTATTTATCGTG	51.9	
A1-276	BZ782276	A1-276f	AGAACAGAGGCCAAGGGAGC	54.8	A1 sex specific
		A1-276r	CCTGAACAACCCCATCTACCC	54.7	
A1-210	BZ782210	A1-210f	GGAATGCAGTGGGTCATGGT	54.3	A1 sex specific
		A1-210r	CGTTGCGATGACGAGGTATCT	54.2	
A1-128	BZ782128	A1-128f	GAGAGCAGATAGGAGAGACGACA	52.0	1.49
		A1-128r	GAGTTGGTGCAGTCTATCCTGAT	51.7	
A1-217	BZ782217	A1-217f	TACACGAGAGCTCGACCCAG	52.7	1.35
		A1-217r	GCGTCTTCTCCGATTAGCATC	53.2	
A1-199	BZ782199	A1-199f	ACTGATGCGCAGTGCTGATG	54.5	2.02
		A1-199r	CGTTCAAAGCTCGCCAAGTT	54.6	
A1-249	BZ782249	A1-249f	TGGAATGACTTCAAGACCAGC	51.7	0.0
		A1-249r	TGAGACGCCTTGTCAACATC	50.7	
A1-052	BZ782052	A1-052f	CAAGTGTCAACTCAACACCCC	51.7	excluded from analysis
		A1-052r	GTTGCAACTTGGCCTGATCT	51.7	
A1-085	BZ782085	A1-085f	TAATTTTCAGTCAGAATACTGCCG	52.5	0.0
		A1-085r	CATCCGCATAAAATCGACTGTA	52.2	
A1-145	BZ782145	A1-145f	AGAGATCGGTGCGAGTCAAA	52.2	0.0
		A1-145r	TTGCATTCTCACCATCAACG	51.7	
A1-254	BZ782254	A1-254f	CAATCGTTCTCTTCTCCTGTGC	53.1	0.0
		A1-254r	GGCAAGGTAGAGGATGAGGGT	53.7	
A1-283	BZ782283	A1-283f	GATCTGGCTCGGAGTTCCAT	53.0	0.0
		A1-283r	TTTGGATGTGGCGAGTGATC	53.1	
A1-064	BZ782064	A1-064f	TGTTTCGCTATCGGTCTCTCG	52.9	0.0
		A1-064r	GCTCTCGCATCGATGAAGAA	52.9	
A1-163	BZ782163	A1-163f	CCTTACTATCCAGGCTGGGC	52.9	0.0
		A1-163r	ACGGTCGATCCTCGGTAAAC	53.0	
A1-213	BZ782213	A1-213f	CCTTGACGCGAGGATATTGA	52.7	0.0
		A1-213r	AAGCTGGGCATCGAGGATAC	53.2	
A1-175	BZ782175	A1-175f	AATACAGCCAAGGGACACCG	53.9	0.0
		A1-175r	AGCTGTCGTCTCTGTTCTGTC	54.0	
A1-045	BZ782045	A1-045f	GAAGGTCAACGAGCCCTCAA	54.1	0.61
		A1-045r	AACGATGTCTCTTGCCCAGC	54.1	
A1-177	BZ782177	A1-177f	ATCGTCGTAAGGCGGATCAT	53.5	0.0
		A1-177r	ACGGGATGAAGATGAGCGAT	53.4	

A1-129	BZ782129	A1-129f	ACCTGCAAGAGGTGTGAGAGAAC	54.0	0.0
		A1-129r	ATCGGTCCCTCGAGCAGACAA	54.5	
A1-138	BZ782138	A1-138f	ACATACACATTTTGTGTCTACCGAG	52.0	0.0
		A1-138r	TTCGTTCCGATGCTTCAATT	52.3	
A1-181	BZ782181	A1-181f	GTTGACGGGTAGCGGTGTAG	52.2	excluded from analysis
		A1-181r	GGACACGGCTCAAGTAATCG	52.2	
A1-225	BZ782225	A1-225f	GCTCAGGACGACAGCATCAT	52.4	0.0
		A1-225r	CCAGAGTTCCGGTTTGATGA	52.3	
A1-274	BZ782274	A1-274f	ATAGATGGGCATCCAAGTGG	51.3	0.0
		A1-274r	ATTCTTCAACTGCACCAGG	51.5	
A1-257	BZ782257	A1-257f	GCCAGGGTAACTTACTGTAGCAT	51.4	excluded from analysis
		A1-257r	CGCCGGACTTTAGATAATCG	51.8	
A1-242	BZ782242	A1-242f	TCCTCGACGCACCTTCATACC	52.5	0.0
		A1-242r	GTGATAGATGTGCAGCGCTG	51.7	
A1-044	BZ782044	A1-044f	GAAAAGTTGTGCTAAATCTGGATTC	52.6	0.0
		A1-044r	TCAGGCATTTTAAGTAGCTATCGA	52.4	
A1-189	BZ782189	A1-189f	CCAGATTGCAAGGTTCCCTT	53.1	0.0
		A1-189r	ATTCTACGCCTGCTCGGTCT	53.1	
A1-275	BZ782275	A1-275f	CAGCACGACGACGAGAACTT	52.8	0.0
		A1-275r	CCCCCTCCTCTCATAAAACACT	53.0	
A1-270	BZ782270	A1-270f	GCCCAGAGCATTTCTCTCT	52.5	0.0
		A1-270r	CTGCCCAGTGCTCTGAATGT	52.5	
A1-269	BZ782269	A1-269f	GATCCAGTATTACGGGTGATGTG	52.7	0.0
		A1-269r	ACAACACGAAATGTGACGCC	53.2	
A1-263	BZ782263	A1-263f	CAGCATCAAGAGCCGATGTT	52.7	0.0
		A1-263r	TGGACAGATCGTCTCGATCG	53.2	
A1-255	BZ782255	A1-255f	ACTACGCGACGCAGATTGAC	52.8	0.0
		A1-255r	GCGGTCACAAAAGCTCGTAA	53.0	
A1-239	BZ782239	A1-239f	AAGATTGGAGTGATTGGGGC	52.9	0.0
		A1-239r	TCAACTGCCCTCAGTGATGC	53.3	
A1-237	BZ782237	A1-237f	TCACTCGTCGGTCTGGAAGA	52.9	0.0
		A1-237r	CGGAGGAAGAGCGAAGAACT	52.8	
A1-233	BZ782233	A1-233f	ACTCAACCTCGGATTGGTCC	52.7	0.0
		A1-233r	CCGTTGACTTTGTTTGCCTG	53.2	
A1-204	BZ782204	A1-204f	ATGTTATAGCACAAACCGACGATC	52.6	0.0
		A1-204r	GGGTTTACTCTTGGTAGATGAGTGA	52.7	
A1-194	BZ782194	A1-194f	TATGTGAGGGTGAGGCTTGG	52.4	0.0
		A1-194r	GAATCGTGAGTTCGTGCGAC	52.9	
A1-273	BZ782273	A1-273f	GGTCCCTTCAGCCAGGAAC	54.5	0.0
		A1-273r	ATAGCCTGCTCGATGATCGC	54.4	
A1-236	BZ782236	A1-236f	CGTGCAATCCAATTGTGCGAG	53.9	0.44
		A1-236r	CGTATTGCTCGAGGTAGGATACC	53.8	
A1-235	BZ782235	A1-235f	CGAGAAATTCGTGCCAAGCTC	54.9	0.0
		A1-235r	CTTGCGCAACTCGTCTACGA	54.1	
A1-232	BZ782232	A1-232f	AGGTTACCATCGATTTGCGG	54.2	0.0

		A1-232r	AGACCATGCGTGGTTGGATT	54.1	
A1-226	BZ782226	A1-226f	CAAGACGCATGTCCTTACTAGACC	53.9	0.0
		A1-226r	AGAAACGATCGAAGCCTTTCG	53.6	
A1-216	BZ782216	A1-216f	TGTCGTGACCAATAGCCTGC	53.4	0.0
		A1-216r	GAGGAACATGAACGACGGGT	53.6	
A1-187	BZ782187	A1-187f	ATCCAAGTCCAGGTGAGCCA	54.2	0.0
		A1-187r	AGTCGCGCTTCCTCAACATC	54.3	
A1-134	BZ782134	A1-134f	GGGCTTTCGGAAGAAATGATC	53.1	0.0
		A1-134r	TCTTCCCTTCCCTCAGATGGC	53.0	
A1-211	BZ782211	A1-211f	ACAGATCTCACAAAGTCGCAGA	53.2	0.0
		A1-211r	CACAGTGGGCTATATTTACACAAAT	53.2	
A1-281	BZ782281	A1-281f	ACATGTTCGTAGGCGATTTCG	52.7	excluded from analysis
		A1-281r	GTAGAGTAAGATGGCGGCCG	53.9	
A2-558	BZ782558	A2-558f	ATTGGAAGCGATTGATTGTCA	52.2	A2 sex specific
		A2-558r	GAAGGAAAAAAGACGCAAAGAC	51.9	
A2-400	BZ782400	A2-400f	TGGATGAGATGAAGTTGCGC	53.5	A2 sex specific
		A2-400r	CATACATTTTGGCGGGTTCA	53.3	
A2-406	BZ782406	A2-406f	CCCGAAGCAGACAAATGGAT	53.9	A2 sex specific
		A2-406r	CGGATCGAGGAGAGAGTGAGTT	54.0	
A2-500	BZ782500	A2-500f	GAAGAGGGGGTTTGTGACGAC	52.5	A2 sex specific
		A2-500r	CCCAACATGACCTCTTTCGA	52.3	
A2-530	BZ782530	A2-530f	CAAGATTGAGGATAGAAGATGGATG	53.0	A2 sex specific
		A2-530r	GAAGTATTCGAGGTTCTTGTGGTT	52.6	
A2-441	BZ782441	A2-441f	GNCTTGAAGCGAAGGCAAAC	54.1	A2 sex specific
		A2-441r	CGAAACTTACGGCTGCCATAG	54.2	
A2-575	BZ782575	A2-575f	AAGGACAACCTCGTTCGAGACA	51.3	8.55
		A2-575r	TCGATGTGATACCACATGACG	51.4	
A2-435	BZ782435	A2-435f	CCGACTTCGACTTCCAGACA	52.5	3.95
		A2-435r	TATCCACCTCCTTGTCTGCG	52.5	
A2-404	BZ782404	A2-404f	CCGAAGCGACCTTGTTCAAC	54.6	3.64
		A2-404r	CACAACAGACGCTCACACAGC	54.1	
A2-516	BZ782516	A2-516f	CGGTTGCAGATGTCGAAAAG	53.2	2.98
		A2-516r	TCCAACCACAATTCGAGGTG	53.1	
A2-473	BZ782473	A2-473f	GGCCAAGGAAGGGTATTTGA	53.1	4.81
		A2-473r	TACAAGTCTCGCCGCAATTC	52.9	
A2-422	BZ782422	A2-422f	ACCTCGTCCCTCGTGTTAGG	53.0	2.62
		A2-422r	TGATGGTCGTTTCGAGTGAGC	53.3	
A2-466	BZ782466	A2-466f	GCTGAGACGCTTGATCCCAT	54.0	excluded from analysis
		A2-466r	AAGAGCCGACGTTATCCGAA	53.9	
A2-389	BZ782389	A2-389f	TTACCACCGTTGCTAGCCTT	51.7	0.0
		A2-389r	TACTCTCATGGCTGCCCTTC	51.8	
A2-388	BZ782388	A2-388f	CCTGATGCGTGAGGTGTAAAT	51.7	0.0
		A2-388r	AAGTAAGAGAGCCGAAGAAGAGAG	51.8	
A2-444	BZ782444	A2-444f	TTGTTTAGATAGGTGAAAGGGCT	51.2	0.0
		A2-444r	ATCACGAAGGTTGGATGAATG	51.4	

A2-397	BZ782397	A2-397f	CGAGCTCGAGATCAAGTTGG	52.4	0.0
		A2-397r	GTTTGATTGCTTCGCCAGTC	52.3	
A2-583	BZ782583	A2-583f	ACCCCAACATTCCTCTTTGC	53.0	0.0
		A2-583r	GTTGGTTGCTTGATCGTTCG	53.1	
A2-457	BZ782457	A2-457f	TTCGACATCTCAGATCGCATG	53.5	5.54
		A2-457r	TGTCACCTTGGCAGGGTAAA	52.9	
A2-468	BZ782468	A2-468f	GAGGCAACCAACGGGTAAAG	54.0	0.0
		A2-468r	GTCATATGCGTGCTTGGCAC	53.9	
A2-562	BZ782562	A2-562f	CGATAGCGTCGACAGCTTCC	54.9	excluded from analysis
		A2-562r	GAGCCGACGCAACTGCTATC	54.9	
A2-443	BZ782443	A2-443f	CCCACCTCGACCAACTAGCA	54.9	0.0
		A2-443r	CAGGTTCTGTGAGGTCGGA	54.9	
A2-418	BZ782418	A2-418f	GCAACTTGCCACCTTCGATC	54.5	0.0
		A2-418r	CGCTCGGGTAACTCGTTCAT	54.2	
A2-472	BZ782472	A2-472f	ACTCACCACCCACGACGAAC	54.4	0.0
		A2-472r	CCCTCAGCCCATCCTTCTCT	54.6	
A2-546	BZ782546	A2-546f	ACACATCAGTTGGGATTGGG	51.8	0.0
		A2-546r	TGAAAATTGACAGTGACCGG	51.8	
A2-544	BZ782544	A2-544f	CATCAAGAGGGGATGAGACG	51.8	0.0
		A2-544r	CAATACTCTATCCCTCGCACG	51.7	
A2-540	BZ782540	A2-540f	TCGTCGGTTCCTTGAGAGTT	51.2	0.0
		A2-540r	AATGAGGTTGTCAAGTCGCC	51.5	
A2-497	BZ782497	A2-497f	CATGATTGCAGAGCAGGATCT	51.8	0.0
		A2-497r	CGGGATCAGTTAGAAAGCGA	52.1	
A2-568	BZ782568	A2-568f	ACAAGCAGCGATTTCGTGTT	52.4	0.24
		A2-568r	TCGATGGTTCGGTCATCTCAT	52.4	
A2-508	BZ782508	A2-508f	GCTGAGGACTGCATCGATGT	52.4	excluded from analysis
		A2-508r	AAAGGCACAAGAGGCGTGTA	52.5	
A2-552	BZ782552	A2-552f	GCTCGAGATTCTCGCAGCTA	52.5	0.0
		A2-552r	GGGGCTGATCAGAACTCTCC	52.5	
A2-411	BZ782411	A2-411f	ACTTGACAAGGACGAGGGGA	53.4	0.31
		A2-411r	TGGCTTGCTCAAAGTTGACC	52.8	
A2-602	BZ782602	A2-602f	CATCCTACGTCAGTGACGCC	52.8	0.0
		A2-602r	CATAGGGACAACGGGGACTC	53.2	
A2-576	BZ782576	A2-576f	CCTGCAGACACACGCAAGAT	53.5	excluded from analysis
		A2-576r	ACGCTTCTCTCCGACTTGCT	53.2	
A2-564	BZ782564	A2-564f	TAGGTGTTGTGAGCGTCGGT	52.9	excluded from analysis
		A2-564r	GCTGTGAGGGCTCAAAAGAGT	52.7	
A2-517	BZ782517	A2-517f	CGGAACTCGGACTCCAAGAT	53.1	0.0
		A2-517r	CGATCGGCATTCTTCTTGTTG	53.1	
A2-496	BZ782496	A2-496f	TGTAATGGAGAGGACGCTCG	52.7	0.0
		A2-496r	CGTAGGCTTCGGTCTCCTTC	53.1	
A2-477	BZ782477	A2-477f	GTAGAACGAACAGCGTGGTCA	52.7	excluded from analysis
		A2-477r	CGTTGCATGTGACTGTCTGC	52.2	
A2-606	BZ782606	A2-606f	TGCTTGTTGCATTTCGAACC	53.6	excluded from analysis

		A2-606r	CCGTCTGCATTAATACCCCTCT	54.0	
A2-597	BZ782597	A2-597f	GATCGATGATCGACCGAGGT	53.6	0.0
		A2-597r	CTTGTCTTCGTTGCTGTCCG	53.3	
A2-589	BZ782589	A2-589f	GTCTCGGAATCTGAGCGGAA	54.2	0.0
		A2-589r	TATCGAACTCTCGCCGACCT	53.9	
A2-578	BZ782578	A2-578f	ACCGAGAACGCAATCATGCT	54.6	0.0
		A2-578r	ATTGACCATCGTCACTCGGG	54.5	
A2-556	BZ782556	A2-556f	CTTCAGCGAGCCGGTTAGAT	53.7	0.0
		A2-556r	GACTCCAAGCCCAAGACGAG	53.7	
A2-551	BZ782551	A2-551f	CGTCTTCCTTTTGCCGACTC	53.9	0.0
		A2-551r	AGTCAGCGAGGAGAGCAAGG	53.6	
A2-542	BZ782542	A2-542f	CTGGGCTCTGGTTGGTCATC	54.2	0.0
		A2-542r	GCCACAGAACAGACACGTCG	53.8	
A2-487	BZ782487	A2-487f	CGGCGGTCTGAATAGATCTGT	54.1	excluded from analysis
		A2-487r	CTGGGGCTGAAGAAGACGAG	53.9	
A2-433	BZ782433	A2-433f	GTCTACCGAGTCAGACTGCACA	53.5	0.0
		A2-433r	GGATCTATGCAGCCTTCGAC	53.4	
A2-461	BZ782461	A2-461f	CAGAAATTGGAAGCGATTGA	53.3	0.0
		A2-461r	ATTCTTGCAGTCTCGACC	53.2	
A2-547	BZ782547	A2-547f	TGGCTGCTTCTTTATGACCA	50.9	0.0
		A2-547r	CAAACAATTTCAATCGATGCAG	52.5	

* - sequences data were provided by Hood, M.E. 2005