

# Concerted Evolution in the Repeats of an Immunomodulating Cell Surface Protein, *SOWgp*, of the Human Pathogenic Fungi *Coccidioides immitis* and *C. posadasii*

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Manuscript received January 14, 2005  
Accepted for publication May 27, 2005

## ABSTRACT

Genome dynamics that allow pathogens to escape host immune responses are fundamental to our understanding of host-pathogen interactions. Here we present the first population-based study of the process of concerted evolution in the repetitive domain of a protein-coding gene. This gene, *SOWgp*, encodes the immunodominant protein in the parasitic phase of the human pathogenic fungi *Coccidioides immitis* and *C. posadasii*. We sequenced the entire gene from strains representing the geographic ranges of the two *Coccidioides* species. By using phylogenetic and genetic distance analyses we discovered that the repetitive part of *SOWgp* evolves by concerted evolution, predominantly by the mechanism of unequal crossing over. We implemented a mathematical model originally developed for multigene families to estimate the rate of homogenization and recombination of the repetitive array, and the results indicate that the pattern of concerted evolution is a result of homogenization of repeat units proceeding at a rate close to the nucleotide point mutation rate. The release of the *SOWgp* molecules by the pathogen during proliferation may mislead the host: we speculate that the pathogen benefits from concerted evolution of repeated domains in *SOWgp* by an enhanced ability to misdirect the host's immune system.

**U**NDERSTANDING the dynamic qualities of genomes that allow pathogens to escape immune response is vital to a deeper knowledge about host-pathogen interactions. We here present an empirical study of the concerted evolution of the repeated region within a gene encoding a protein that elicits a host response when the fungal pathogens *Coccidioides immitis* and *C. posadasii* cause human disease. Concerted evolution is manifested as the interdependent evolution of a repetitive region of the gene, resulting in a sequence similarity of repeat units greater within species than between them (ARNHEIM *et al.* 1980; ZIMMER *et al.* 1980; DOVER 1982). Concerted evolution can be driven by directional or stochastic processes, or both, and the two mechanisms that have been the principal explanations of concerted evolution in nuclear DNA are gene conversion and unequal crossing over (SMITH 1976; OHTA 1980a; HILLIS *et al.* 1991; CHARLESWORTH *et al.* 1994). Studies of the underlying mechanisms of concerted evolution historically have relied on theoretical rather than empirical arguments (SMITH 1974; TARTOF 1974;

OHTA 1980b; SZOSTAK and WU 1980; COEN *et al.* 1982), and the relative importance of these mechanisms remains controversial. Unequal crossing over currently is the favored mechanism to explain the observed sequence homogeneity among repeats in repetitive arrays (KRUGER and VOGEL 1975; SMITH 1976; STEPHAN 1989; CHARLESWORTH *et al.* 1994; ELDER and TURNER 1995). The increases and decreases in repeat number resulting from unequal crossing over lead to turnover among repeats and, in principle, stochastic fixation of a single repeat type. According to this model, unequal crossing over would most commonly occur in central regions of the array where repeats can mispair, and unique sequences flanking the repetitive array would inhibit exchanges involving the edge repeats, *i.e.*, the repeats located at the termini. This bias toward homogenization of central repeats causes identity among repeats to decrease as spatial distance among repeats increases (KIMURA and OHTA 1979; OHTA 1980a; STEPHAN 1989).

The *Coccidioides* spherule outer wall glycoprotein (*SOWgp*) single-copy gene contains tandemly repeated proline- and aspartic acid-rich motifs, ranging from 41 to 47 aa in size. It encodes a glycoprotein that is a component of the parasite cell surface. Not only is the

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coding part of each tandem repeat nearly identical in sequence among repeats, but also each repeat contains an intron nearly identical in sequence among repeats. Human infection by *Coccidioides* spp. possessing this gene leads to the respiratory disease coccidioidomycosis or San Joaquin Valley fever (GALGANI 1999). Two pathogenic species of *Coccidioides* cause infection, *C. immitis* and *C. posadasii* (FISHER *et al.* 2002), and both have a recombining genetic structure (BURT *et al.* 1996; FISHER *et al.* 2000b). The species are dimorphic, living as hyphal saprobes in the desert soil or as single cells that develop into multicellular spherules, which produce and release endospores in the mammalian host. *SOWgp* is expressed in the parasitic phase during growth of the first and second generation of presegmented and segmented spherules prior to endospore formation (HUNG *et al.* 2002).

*SOWgp* appears to contribute to the virulence of *Coccidioides* spp. both by functioning as an adhesin and by modulating the host's immune response (HUNG *et al.* 2000, 2002). The recombinant polypeptide (r*SOWgp*, expressed in *Escherichia coli*) has been shown to bind to mammalian extracellular matrix (ECM) proteins, and deletion of the gene in *C. posadasii* results in a significant reduction in virulence as well as in a partial loss of the ability of spherules to bind ECM proteins (HUNG *et al.* 2002). The glycoprotein elicits both humoral and cellular immune response in patients with coccidioidal infection (HUNG *et al.* 2000) and, intriguingly, the hydrophilic proline-rich repeat array of *SOWgp* is suggested to be the domain containing the B cell-dominant epitopes (HUNG *et al.* 2000). Furthermore, the spherule outer wall fraction produced by a parasitic-phase culture of the  $\Delta$ sowgp82 mutant is not reactive with sera from patients with confirmed, disseminated coccidioidomycosis, suggesting that the *SOWgp* protein is the sole component of this parasitic cell surface layer that is recognized by patients' antibodies. Mice challenged with a lethal inoculum of spores derived from the  $\Delta$ sowgp82 knockout mutant mount a protective Th1 response to infection, while mice infected with the wild-type strain develop disseminated disease characterized by persistent inflammation and a dominant nonprotective Th2 pathway of host immune response (COLE and HUNG 2003). These data suggest that, when spherules release endospores, the pathogen evades immune detection by releasing the immunodominant *SOWgp* from the outer cell wall of the endospores. This process may contribute further to the induction of the Th2 over the Th1 pathway of host immune response. This mechanism is thought to help mitigate the host immune response by misdirecting host defenses to released *SOWgp* and inducing the host to pointlessly damage itself through a fervid inflammatory response (HUNG *et al.* 2002; COLE and HUNG 2003).

By examining variation in *SOWgp* within and between species, the evolutionary processes underlying this

gene's formation, maintenance, and action may be revealed. Here we present the first population-based study of the evolution of a repetitive domain in a protein-coding gene, based on complete sequence of the entire gene from strains selected to represent populations from the whole geographic ranges of the two species that possess it. We used phylogenetic and genetic distance analyses to discover that the repetitive part of *SOWgp* evolves by concerted evolution, principally by the mechanism of unequal crossing over. Further, we used a mathematical model originally developed for multigene families by OHTA (1983), to estimate the rate of homogenization and recombination of the repetitive array. The adaptive significance for the pathogen of having repeated domains evolving in concert in *SOWgp* is speculated to be the enhancement of the immunomodulation such that the pathogen is able to efficiently evade the host immune system.

## MATERIALS AND METHODS

**Fungal material:** Eight strains of *C. immitis* and 16 strains of *C. posadasii* were used in this study (Table 1). All strains were previously genotyped and assigned to species by microsatellite markers (FISHER *et al.* 2002), with the exception of two additional strains that were identified as *C. immitis* by sequencing of diagnostic nuclear gene loci (*GAPDH*, *glnA*, and *hxA*) (JOHANNESON *et al.* 2004).

**DNA manipulations:** Total genomic DNA was extracted from lyophilized material as in BURT *et al.* (1995). All primers were designed manually, on the basis of the GenBank sequence AF308873. The entire coding region of the *SOWgp* gene was amplified from all strains using the primers *SOWgpF5* (GAAGCGCAAGAGAACTGTATG) and *SOWgpR3* (CAAATCCATCTACCCAACTT). Each PCR reaction was performed using the Expand High-Fidelity PCR system (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's recommendation, using an Eppendorf thermal cycler. The amplicons were cloned into pCR4-TOPO vector using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) to facilitate accurate sequencing of an ~30-bp A-T rich segment of one intron in the region flanking the repetitive part of *SOWgp*. To obtain the complete sequence of the repeats, direct sequencing of the PCR products was performed with two additional internal sequencing primers, *SOWgpF2* (ATGAGGAAACGAGGTGCTAC) and *SOWgpR2* (TGGGCTCTGGCA TAATGGTA), after purifying the products using the Qiaquick PCR purification kit (QIAGEN, Valencia, CA). Nucleotide sequences were determined using an Applied Biosystems (Foster City, CA) 3100 sequencer and the Taq DyeDeoxi Terminator cycle system (ABI). The repeat units of *SOWgp* were delimited as in HUNG *et al.* (2002), and sequence alignment of flanking and repetitive parts was performed manually.

**Sequence evolution of *SOWgp*:** Tests of neutral sequence evolution were performed on alignments of both the entire gene and individual repeat units, by using the *D*-statistic of TAJIMA (1989), as implemented in DNAsp3.52 (ROZAS and ROZAS 1999). Furthermore, we calculated the pairwise ratios of nonsynonymous substitutions per nonsynonymous site ( $D_n$ ) to synonymous substitutions per synonymous site ( $D_s$ ) for the aligned repeat units, by using maximum-likelihood estimates in PAML3.13d (GOLDMAN and YANG 1994; YANG 1997). If positive Darwinian selection occurred,  $D_n$  would be greater

than  $D_s$ . Only internal repeats were included in this analysis, and where repeat units were identical, only one representative was used.

**Phylogenetic analyses of sequences of each repeat unit:**

The DNA sequences of all repeats for all strains were used to create a phylogeny for the repeats using maximum-parsimony analyses in PAUP 4.0b (SWOFFORD 2001). Searches were performed using the heuristic search option and support for the branching arrangements was evaluated by bootstrap analyses of 1000 resampled data sets.

**Genetic distance and diversity of the repeats:** A series of likelihood-ratio tests of nested models of DNA substitutions of the data set was completed using Modeltest3.04 (POSADA and CRANDALL 1998). These tests revealed that the best model of substitutions for this data set was a transversional model of substitution with equal base frequencies (TVMef + I + G) (POSADA and CRANDALL 1998). PAUP 4.0b was used to generate distance matrices from the multiple alignments using both this parameter-rich model of substitutions and the uncorrected measurement of distance implemented in the program. Indels were treated as single characters.

Two comparisons of pairwise distances were performed on strains with the same number of repeats for each species. To reveal whether the repeat units evolve in concert, we compared the mean distance between the sequences of different repeat units within each species to the mean distance between the sequences of the corresponding repeat units between the species. To reveal the mechanism of concerted evolution, we determined if the genetic distance of repeat sequences increased with physical distance in the primary sequence for each species. To avoid pseudo-replication in the statistical analyses, the genetic distances of repeat units at different physical distances were analyzed in separate tests, each including only comparisons within strains, and separately for each repeat position; e.g., for each strain with five repeats we calculated separately the genetic distance between 1, 2, 3, 4, and 5 and all other repeats. One-way analyses of variance were performed to test for a significant increase in genetic distance with physical distance, using JMP IN version 4 (SAS 2001).

Diversity of the repeats for each species was calculated as  $D = 1 - \sum p_i^2$ , where the summation is over all repeat sequence types, and  $p_i$  is the frequency in the data set of the  $i$ th repeat sequence type.

**Detection of gene conversion events:** The alignments of the entire repetitive array, from strains of each of the two species harboring the same number of repeats, were analyzed for evidence of gene conversion by using GENECONV version 1.81 (SAWYER 2000). This method detects pairs of sequences that share unusually long stretches of similarity given their overall polymorphism (SAWYER 1989) and is unbiased with regard to individual repeat unit boundaries. We searched for evidence of significant gene conversion events between ancestors of two sequences in the alignments by using global permutation  $P$ -values (SAWYER 2000).

**Estimation of population size and rates of homogenization and recombination:** OHTA (1983) developed an infinite-alleles model for the concerted evolution of a multigene family. Equations 7, 8, and 9 of this model predict the equilibrium values of three summary statistics for population data on DNA containing  $n$  repeated domains:  $f$ , the observed average pairwise identity of corresponding repeats on separate chromosomes;  $C_1$ , the observed average pairwise identity of all repeats within chromosomes; and  $C_2$ , the observed average pairwise identity of all noncorresponding repeats between chromosomes. Ohta's model predicts these statistics given values for  $v$ , the allelic mutation rate;  $N$ , the effective population size;  $\beta$ , the rate at which equal interchromosomal crossover occurs between repeated domains; and  $\lambda$ , the rate at

which a repeat domain is converted to be identical to another of the  $(n - 1)$  homologous domains.

In this study, we implemented Ohta's model to estimate the rate of homogenization and recombination of the repetitive array of *SOWgp*. The summary statistics  $f$ ,  $C_1$ , and  $C_2$  were calculated from the population sample of repeat sequence data for each species, by weighted averaging over groups of strains having the same number of repeats. Statistics  $f$ ,  $C_1$ , and  $C_2$  were set equal to the equilibrium Equations 7, 8, and 9 of OHTA (1983). All sites with INDELS were excluded from the analysis. We assumed an allelic mutation rate,  $v$ , based on the estimated nucleotide mutation rate for the filamentous fungus *Neurospora crassa* (DRAKE 1991), with a number of neutrally evolving sites of either 16 (the number of polymorphic sites within species in this data set,  $v = 7.2 \times 10^{-10}$ ) or 197 (all sites in the repeat domain,  $v = 8.9 \times 10^{-9}$ ). The resulting set of three nonlinear equations could not be algebraically solved. To estimate the parameters  $N$ ,  $\beta$ , and  $\lambda$  from our data, we found approximate solutions to these equations by numerical exploration of the parameter solution space and minimization of the square difference between the predicted and observed values of  $f$ ,  $C_1$ , and  $C_2$ . Random perturbations of the proposed parameter values were drawn from triangular distributions that, at the extremes, halved or doubled each parameter in the current approximate solution, and perturbations were accepted in each generation of the search whenever a uniform variate between zero and one was less than the ratio of the proposed to the current square difference. Each numerical solution was the best result from 5000 iterations of this algorithm. Solutions were examined by eye for fit to observed values and by further manual perturbation to ensure that the solution found was a global rather than a local best fit.

## RESULTS

PCR amplification and sequencing of the *SOWgp* gene from the 24 strains from the two *Coccidioides* species revealed from three to five tandem repeats. In the eight strains of *C. immitis*, 1 strain had three repeats, 5 had four, and 2 had five. In the 16 strains of *C. posadasii*, 1 strain had three repeats, 3 had four, and 12 had five repeats (Table 1).

**Sequence variability in regions flanking the repetitive array:** Sequence variability flanking the repeat array was low. All polymorphisms are presented by using GenBank sequence AF308873 as a reference for *C. posadasii*. In the 447-bp exon sequence, two characters were fixed between the species: one nucleotide replacement (T  $\rightarrow$  A at position 1315) and one 9-bp deletion (between positions 1573 and 1581) were found in *C. immitis*. In the 135-bp intron, four substitutions were fixed between the species: in *C. immitis* we found a T at position 1445, an A at position 1459, a C at position 1618, and a T at position 1632. Intraspecific variation was confined to nucleotide polymorphisms in exons, with three polymorphic sites in *C. immitis* (strain 2018, G  $\rightarrow$  A synonymous substitution at position 1512; strain 2278, G  $\rightarrow$  A synonymous substitution at position 1524; strain 2278, T  $\rightarrow$  C replacement substitution at position 1516) as well as in *C. posadasii* (strain 2345, T  $\rightarrow$  C synonymous substitution at position 1382 and C  $\rightarrow$  T replacement substitution at position 1525; strains 1040, 1049, and 2103, C  $\rightarrow$  T

**TABLE 1**  
**Characteristics of fungal material used in the study**

Species	Source	Origin <sup>a</sup>	RMSCC <sup>b</sup>	No. <i>SOWgp</i> repeat units
<i>C. immitis</i>	R. Talbot	Central California	2012	4
	Unknown	—	2018	4
	T. Kirkland	Southern California	2102	4
	R. Talbot	Central California	2271	4
	R. Talbot	Central California	2275	4
	R. Talbot	Central California	2278	3
	I. Gutierrez	Mexico	3505	5
	Unknown	—	1705	5
<i>C. posadasii</i>	J. Galgiani	Arizona	1038	5
	J. Galgiani	Arizona	1039	5
	J. Galgiani	Arizona	1040	4
	J. Galgiani	Arizona	1049	5
	J. Galgiani	Arizona	1444	4
	T. Kirkland	Southern California	2103	5
	R. Diaz	Mexico	2345	5
	R. Diaz	Mexico	2346	5
	R. Diaz	Mexico	2347	5
	R. Diaz	Mexico	2348	5
	R. Negroni	South America	2377	3
	R. Negroni	South America	2378	5
	R. Negroni	South America	2379	5
	R. Negroni	South America	3272	5
	I. Gutierrez	Mexico	3490	4
	I. Gutierrez	Mexico	3503	5

<sup>a</sup>Origins of the strains of *Coccidioides* spp. follow the classification of populations made by FISHER *et al.* (2001).

<sup>b</sup>Roche Molecular Systems Culture Collection, Alameda, California.

replacement substitution at position 1667). Tajima's *D*-test of neutral sequence evolution for the flanking regions failed to reject neutrality in the region of *SOWgp* flanking the repetitive part.

**Divergence of repeat unit sequences:** Alignment of the 108 repeat units from all strains of *Coccidioides* revealed 34 distinct repeat types (see supplementary Figure S1 at <http://www.genetics.org/supplemental/>). Thirty-one of 197 nucleotide positions were found to be polymorphic (27 SNPs and two 3-bp deletions; at 2 positions both a SNP and a deletion were found). Nine positions were found to be polymorphic in edge and internal repeats in both species, but only 1 of them was polymorphic in the internal repeats of both species. In the coding sequence, 10 SNPs resulted in synonymous substitutions, while 6 resulted in replacement substitutions. Diversity (*D*) of repeats was 0.83 for *C. immitis* and 0.94 for *C. posadasii*. When excluding edge repeats, *D* was 0.75 for *C. immitis* and 0.90 for *C. posadasii*. The evolutionary analyses of repeat unit divergence indicate that the sequence of the repeat units evolves under purifying selection. Tajima's *D*-test of neutral sequence evolution for the aligned repeat units failed to reject neutrality. Furthermore,  $D_n/D_s$  ratios for pairwise sequence comparisons (*cf.* SWANSON and VACQUIER 1998) were found to be very low. In 84% of the comparisons

the ratio was <0.5.  $D_n/D_s$  was <1 for all comparisons except for five cases for which the only polymorphism in the coding region was one replacement substitution between intraspecific sequence types (data not shown).

**Phylogenetic relationship of repeat sequences:** The unrooted maximum-parsimony consensus tree (of 100 retained most parsimonious trees) of the repeat sequences is shown in Figure 1. The 3' edge repeats of the two species cluster together and are each other's closest relatives. The same is true for the 5' edge repeats, with the inclusion of a small cluster of a few *C. posadasii* internal repeats. However, the internal repeat units from the two *Coccidioides* species do not group together by corresponding position. Rather, they group on the basis of species.

**Genetic distance of repeats between and within species:** Using the parameter-rich model of DNA substitution, the mean genetic distance of corresponding repeat units between the species is 6.4%, smaller than the mean genetic distance between sequences of all the different repeat units within *C. immitis* (7.4%), and identical to that in *C. posadasii*. When excluding the two edge repeats, the distance between the corresponding repeats between species is 4.7%, this time higher than the mean genetic distance between repeat units within *C. immitis* (1.8%) and again equal to that within *C. posadasii* (Table 2).

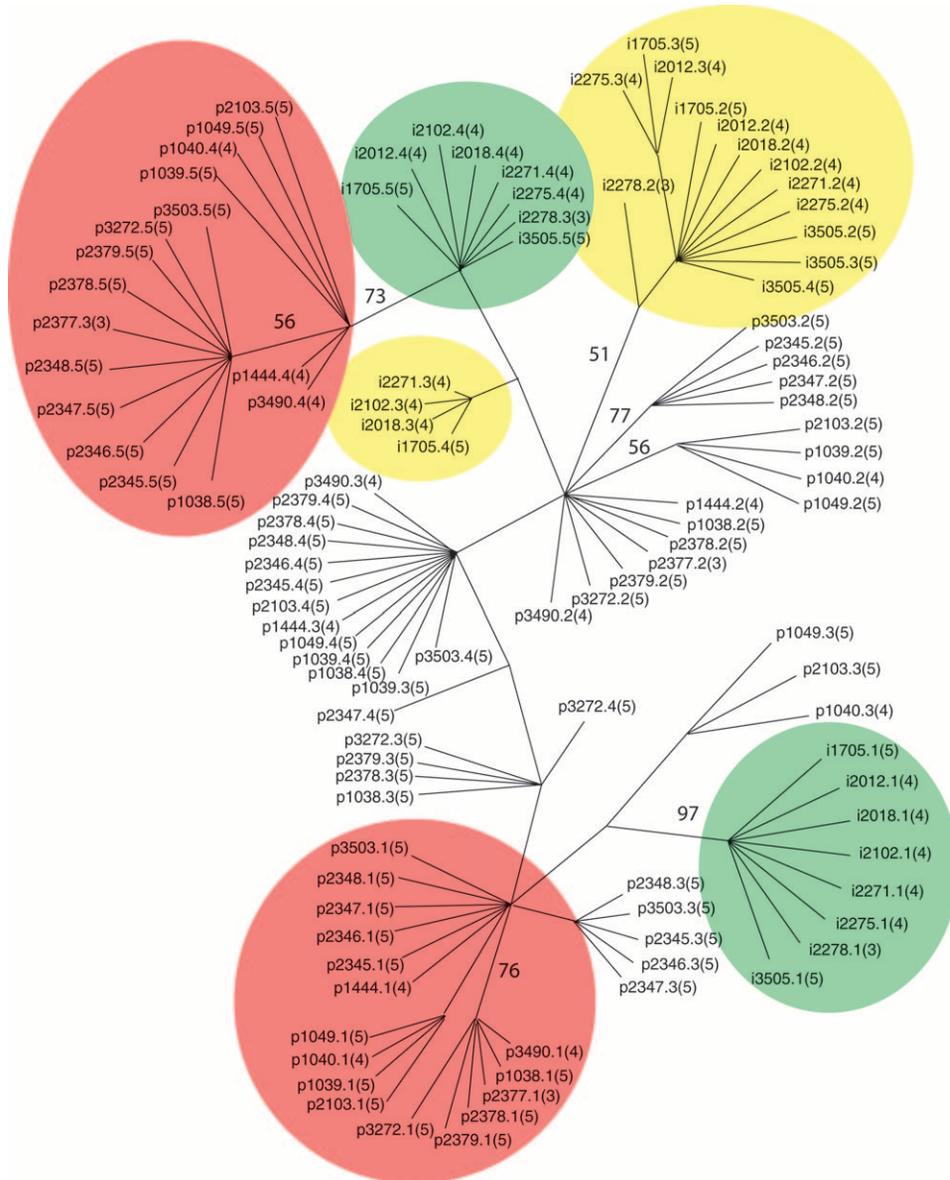


FIGURE 1.—Unrooted consensus tree of repeat sequences from strains of *C. immitis* and *C. posadasii*. Bootstrap values of >50% are given by the branches. Repeats are named as follows: i, immitis; p, posadasii; RMSCC strain number; repeat x out of (y). Edge repeats are encircled with green for *C. immitis* and red for *C. posadasii*. *C. immitis* internal repeats are encircled with yellow.

The average genetic distance between sequences of repeats at different physical distances in the array is shown in Figure 2. The tests of genetic distance of repeat units at different positions revealed that the physical distance in the array significantly affects the genetic distances of the repeats (one-way ANOVAs,  $P < 0.005$ ). However, when the edge repeats were excluded from the analyses, no significant difference was found in any of the data sets. The same results were obtained when using the uncorrected measurement of distance in PAUP 4.0b.

**Gene conversion:** By using global permutation  $P$ -values obtained from GENCONV (SAWYER 2000), we detected one fragment of DNA that had been converted between ancestors of sequences in the alignment. This 311-bp-long fragment was found between the *C. posadasii* strain 2103 and four other strains: 2345, 2346, 2348, and 3503 ( $P = 0.0034$ ). *SOWgp* of all these strains harbors 5

repeat units and the converted fragment spans the last 63 bp on repeat number 3, the entire repeat 4, and the first 51 bp on the last repeat.

**Population size and rate of homogenization and recombination:** The summary statistics of  $f$ ,  $C_1$ , and  $C_2$  and parameter estimates of  $\lambda$ ,  $\beta$ , and  $N$  are given in Table 3 (using a weighted average of  $n = 3, 4$ , and  $5$  and  $v = 7.2 \times 10^{-10}$ ). Estimates for individual parameters when  $n = 3, 4$ , or  $5$  and when  $v$  is assigned a value of either  $7.2 \times 10^{-10}$  or  $8.9 \times 10^{-9}$  are shown in supplementary Table S1 (<http://www.genetics.org/supplemental/>). The observed identity of both corresponding ( $f$ ) and noncorresponding ( $C_1$  and  $C_2$ ) repeat units is greater for *C. immitis* than for *C. posadasii*. For both species, the identity between corresponding repeats decreases when excluding edge repeats from the data set, while the identity between noncorresponding repeats increases.

**TABLE 2**  
**Mean pairwise genetic distance between sequential *SOWgp* repeats within *C. immitis* (*C.i.*) and *C. posadasii* (*C.p.*) and between corresponding repeat sequences between the two species**

Repeats included	Mean pairwise genetic distance (SD)			
	Different repeats within species			Corresponding repeats between species
	<i>C.i.</i>	<i>C.p.</i>	Grouped	
All	0.074 (0.055)	0.064 (0.032)	0.065 (0.036)	0.064 (0.037)
Internal <sup>a</sup>	0.018 (0.012)	0.047 (0.016)	0.045 (0.018)	0.047 (0.023)

<sup>a</sup>The edge repeats are excluded.

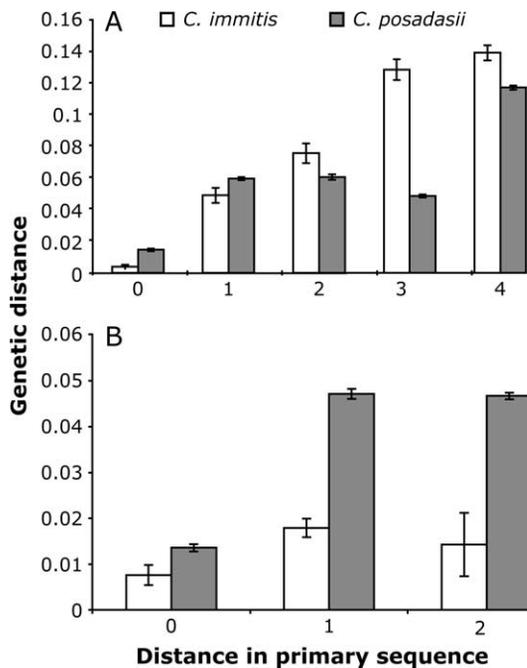
The estimated rates of homogenization ( $\lambda$ ) and recombination ( $\beta$ ) among repeat units were higher for *C. immitis* than for *C. posadasii*, while the estimated population size is smaller for *C. immitis* than for *C. posadasii*. The observed degree of repeat unit identity can be explained by a rate of homogenization events of  $10^{-10}$ – $10^{-11}$ /generation in both *C. immitis* and *C. posadasii*. The recombination rate between adjacent repeat units is estimated to be  $10^{-7}$ – $10^{-8}$ /generation in *C. immitis* and  $10^{-8}$ – $10^{-9}$ /generation in *C. posadasii*.

#### DISCUSSION

Our data show that the repetitive part of the *SOWgp* gene evolves under concerted evolution. Corresponding internal repeat units of the two *Coccidioides* species

do not group together in the phylogenetic tree. Instead, the internal repeats group by species. Furthermore, the mean genetic distance between internal repeats within a species is never larger than the genetic distance of corresponding repeats between the two species and, in *C. immitis*, it is smaller. Thus, internal repeats of *SOWgp* are as similar as, or more similar than, corresponding repeats between the species and do not evolve as independent units. Analysis using the theoretical predictions of OHTA (1983) indicates that the pattern of concerted evolution is a result of homogenization of repeat units proceeding at a rate close to the nucleotide point mutation rate (Table 3).

Our results suggest the rate of homogenization is higher in *C. immitis* than in *C. posadasii*. One plausible explanation for this is that *C. immitis* has a smaller population size than *C. posadasii* (Table 3). This finding is in accordance with what is expected based on the geographic distribution of the two species; while *C. immitis* is confined to northern Mexico, central and southern California, *C. posadasii* has a wider distribution, ranging from southern California and Texas, through Arizona to Argentina. Furthermore, the genetic diversity of microsatellites found in *C. immitis* is much smaller than that found in *C. posadasii* (FISHER



**FIGURE 2.**—Average genetic distance between sequences of repeats at different physical distances in the array. 0, corresponding position; 1, adjacent position; 2–4, separated by one to three repeats. (A) All repeats included; (B) only internal repeats included. The bars represent mean values  $\pm$ SE for all genetic distances at each position.

**TABLE 3**

**Observed identity between repeat units and parameter estimates for the concerted evolution of *SOWgp* in *C. immitis* (*C.i.*) and *C. posadasii* (*C.p.*)**

	All repeats		Internal repeats <sup>a</sup>	
	<i>C.i.</i>	<i>C.p.</i>	<i>C.i.</i>	<i>C.p.</i>
$f$	0.78	0.30	0.61	0.25
$C_1$	0.020	0.0071	0.091	0.026
$C_2$	0.014	0.0022	0.077	0.0075
$\lambda$	$4.3 \times 10^{-11}$	$2.6 \times 10^{-11}$	$1.1 \times 10^{-10}$	$5.3 \times 10^{-11}$
$\beta$	$6.8 \times 10^{-8}$	$6.5 \times 10^{-9}$	$2.8 \times 10^{-8}$	$7.6 \times 10^{-9}$
$N$	$9.3 \times 10^7$	$7.9 \times 10^8$	$2.0 \times 10^8$	$9.9 \times 10^8$

Weighted averages of estimates are given for  $n = 3, 4$ , and 5 repeated domains, assuming an allelic mutation rate of  $7.2 \times 10^{-10}$ .

<sup>a</sup>The edge repeats are excluded.

*et al.* 2001), again consistent with a smaller population size for *C. immitis*.

When comparing the parameters estimated from our data with the parameters estimated from the major histocompatibility complex (MHC) (OHTA 1982), we find that the rate at which repetitive units are made identical to each other is much lower for the repeat units in *SOWgp* in Coccidioides ( $\lambda$  between  $10^{-10}$  and  $10^{-11}$ ) than for the MHC genes in humans ( $\lambda$  between  $10^{-5}$  and  $10^{-6}$  (OHTA 1982)). The difference can be explained in part by considering the role of unequal crossing over in the homogenization process of *SOWgp* repeat units. The estimated recombination rate is substantially higher between the MHC repeats ( $\beta = 10^{-3}$ ) than between the *SOWgp* repeats ( $\beta$  between  $10^{-7}$  and  $10^{-9}$ ). A lower rate of recombination internal to *SOWgp* compared to the rate of recombination internal to MHC is expected, because recombination in *SOWgp* is over a shorter physical distance than recombination in MHC, *i.e.*, between domains of a gene rather than between genes in a multigene family.

We suggest that the most important mechanism of concerted evolution in the *SOWgp* gene is unequal crossing over. By examining the sequences for extended regions of homology, we found evidence for possible gene conversion of only one fragment, a 311-bp-long fragment that has been converted between strains of *C. posadasii*. This fragment corresponds to the fourth repeat unit out of five, with some additional sequence upstream and downstream. However, the test (SAWYER 2000) does not explicitly distinguish between gene conversion and unequal crossing over, and the statistical significance of this test could be a byproduct of homogenization by unequal crossing over of repeat units. Unequal crossing over as a mechanism of concerted evolution in *SOWgp* is supported by the overall high diversity of repeat sequence types in the gene. Furthermore, a positive correlation between genetic distance of repeats and physical distance in the primary sequence was found in the *SOWgp* data set, and the first and last repeat in the *SOWgp* array appear to evolve independently of the internal repeats. Homogenization by unequal crossing over occurs in regions of the array that are free to recombine, while unique sequences flanking a repetitive array inhibit exchanges of edge repeats (OHTA 1980a; STEPHAN 1989). However, these peripheral repeats also are influenced by the presence of unequal exchange, which causes identity among repeats to decrease as physical distance among repeats increases (OHTA 1980a). This pattern has been documented in other systems, such as the  $\alpha$ -satellite arrays in the human genome (COOPER *et al.* 1993), in an array of repeats on the paternal sex ratio chromosome in the parasitic wasp *Nasonia vitripennis* (MCALLISTER and WERREN 1999), and in the egg vitelline envelope receptor for abalone sperm lysin (GALINDO *et al.* 2002). This observed positive correlation of genetic distance

and spatial distance in the array of *SOWgp* repeat units provides further support for the idea that concerted evolution is predominantly by the stochastic mechanism of unequal crossing over. A final line of evidence for unequal crossing over is the observed polymorphism in repeat number in *SOWgp*. It is widely understood that a mechanism such as unequal crossing over is required to generate variation in the number of copies of a repeat (HOOD *et al.* 1975; TARTOF 1975; SMITH 1976).

Our results suggest that the evolutionary process generating length variation at the *SOWgp* locus is occurring more rapidly than that generating sequence variation among repeats within the species. In both species, we found identical *SOWgp* repeat sequences in strains originating from the outskirts of the species geographical range, as well as from repeat arrays of different sizes (data not shown). These results suggest that these repeat sequences were moved across this geographical range very recently and imply that recombination associated with the observed diversity of *SOWgp* must also have been very recent.

The results presented in this study suggest that the sequence of *SOWgp* evolves under purifying selection. However, the persistence of concerted evolution of the repetitive structure of *SOWgp* in both species of Coccidioides suggests that there may be a selective advantage to maintaining a protein comprising multiple identical domains. We suggest that the possible adaptive significance of *SOWgp* consisting of repeat units evolving in concert is to allow the pathogen to generate multiple identical epitopes. The pathogen is believed to misdirect the host's immune system by inducing high titers of antibody production and stimulating a nonprotective Th2 pathway of immune response. The immunodominant *SOWgp* is expressed at the surface of spherules and is released from the outer cell wall of endospores at sporulation. The individual repeat unit of *SOWgp* is the primary antigen-binding site of the antibody and, by having numerous identical epitopes in *SOWgp*, a nonprotective response of the immune system would be multiplied, allowing the pathogen to more efficiently divert the efforts of the immune system. This proposed model for the function of the repeat units of *SOWgp* suggests a selective advantage in having numerous repeat units. However, there appears to be a functional upper limit of *SOWgp* repeat number in Coccidioides, possibly related to its biochemical activity as an adhesin.

Although intriguing, our conjecture of adaptive significance of *SOWgp* consisting of repeat units evolving in concert is speculative. In spite of the two Coccidioides species being deeply divergent, with estimations of the time of genetic isolation ranging from 11 to 12.8 million years (KOUFOPANOY *et al.* 1997; FISHER *et al.* 2000a), it could reasonably be argued that concerted evolution of the repetitive structure of *SOWgp* in both species has been retained as a result of chance alone. The likelihood of losing copy number variation

through stochastic processes will depend not only on the divergence time but also on other factors such as the population rate of unequal crossing over and the degree to which the allelic unequal crossover rate increases with copy number (*e.g.*, TOWNSEND and RAND 2004). Furthermore, lethal threshold selection on copy number, as in the above model, suggests a selective disadvantage to both low and high copy number, and unequal crossing over will tend to destabilize the number of repeats. However, the increases and decreases in repeat number resulting from unequal crossing over could result only in a selection coefficient at the threshold length that is equal to or lesser than the already low rate of unequal crossover. One way to test the proposed model of adaptive significance of multiple SOWgp repeat units would be to use a murine model to investigate the virulence of individuals of *Coccidioides* manipulated to possess a different number of repeat units in the array.

Concerted evolution has previously been suggested to be of biological importance in a diverse range of systems. For example, genes evolving by concerted evolution have been hypothesized to produce a species-specific, selective force on the gene of their cognate interacting protein (DOVER and FLAVELL 1984; DOVER 1993) and to underlie species-specific gamete interaction in free-spawning abalones (SWANSON and VACQUIER 1998). Concerted evolution is thought to be the mechanism behind species-specific transcription of tandemly repeated ribosomal DNA by RNA polymerase I (GRUMMT *et al.* 1982; MIESFELD and ARNHEIM 1984; RUDLOFF *et al.* 1994; HEIX *et al.* 1997), and variation in the number of copies in an array by unequal crossing over can also be a means for varying the rate of synthesis of the product encoded by that gene (SCHIMKE *et al.* 1978; ANDERSON and ROTH 1979; ZIMMER *et al.* 1980). This article provides the first evidence that repeat units proposed to be important for immunomodulation by a pathogen are subjected to concerted evolution and suggests that concerted evolution is important for pathogenicity. The presence in other fungal species of adhesins that are polymorphic for internal repeat number (VERSTREPEN *et al.* 2004) suggests that the concerted evolution seen in *Coccidioides* may be of general significance.

We thank Georgiana May, Anne Pringle, and Willie Swanson for advice and useful discussions. Financial support from the Fulbright Commission and Carl Tryggers Stiftelse för Vetenskaplig Forskning to H.J., from the Miller Institute for Basic Research in Science to J.P.T., and from the National Institutes of Health (AI37232) and the National Science Foundation (0316710) to J.W.T. is gratefully acknowledged.

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Communicating editor: Z. YANG