Supplemental Methods and Results

Principal Components Analyses of Population Structure

Supplemental Fig. 1A illustrates a PC1-3 for all 20 sequenced genomes. The orthogonal distribution of the *C. immitis* isolates relative to the *C.* posadasii isolates indicates that most segregating SNP variants are exclusive to a single species. Supplemental Fig. 1B illustrates the results of an exclusive PC analysis of the ten *C. immitis* isolates. The two *C. immitis* isolates thought to have derived originally from central California (RS, H538.4; red dots) are not cleanly differentiated from the isolates acquired in Southern California/ Northern Mexico (orange dots) by either PC1 or PC2, though H538.4 is an outlier relative to all of the other isolates. Strain H538.4 also exhibits unique phenotypic traits, including reduced endospore formation, large endospore size, and reduced virulence. An exclusive PC analysis of the ten *C. posadasii* isolates (Supplemental Fig. 1C) reveals geographic differentiation within this species in greater detail than the combined 14 isolate analysis. The six Arizona isolates (blue dots) cluster in the upper right quadrant of the plot. The two isolates from Texas and Central and South America (RMSCC_2133 and RMSCC_3700; purple dots) cluster together in the upper left quadrant, along with one of the isolates from southern/central Mexico (RMSCC_3488). PC2 separates the other southern/central Mexico isolate RMSCC_1038 from all other *C. posadasii* isolates. Though sample sizes are small, the Arizona isolates appear to exhibit greater diversity than do the isolates from Texas/S. America, but less diversity than the two isolates from southern/central Mexico.

Sub-population sampling to control for population structure effects in N_e estimation

To compare effective population sizes between populations, we calculated N_e (based on Watterson's Θ) for the *C. immitis* Southern California population (8 sequenced genomes) and *C. posadasii* Arizona population (7 sequenced genomes). The *C. posadasii* Arizona N_e was estimated to be 2.71-fold larger than the *C. immitis* Southern California N_e ($4.07E10^6$ vs. $1.60E10^6$, respectively). This implies that the larger effective population size observed in *C. posadasii* is not simply due to the greater number of subpopulations it exhibits relative to *C. immitis*, and that on a sub-population level *C. posadasii* also exhibits greater diversity.

Methods: Genetic Distance Based Approach for Identifying Introgression

We considered a region contained within a 5kb window to be a candidate for introgression by computing the consensus sequence for each species and then examining the genetic distance between each individual haplotype and the consensus sequences of each species. If the haplotype of a particular isolate exhibited a greater genetic distance to its own consensus than to the consensus of the other species, we consider it to be a candidate region of introgression from the other species. To detect instances of significant introgression, where the consensus haplotype for a species receiving gene flow predominantly reflects the other species, we developed an additional filter. We compared the genetic distance between each individual haplotype and the consensus for its species (*IC*) with the genetic distance between the two species consensus haplotypes (*CC*). If the ratio of *IC* to *CC* was greater than two, we considered the region to be a candidate for major introgression resulting in the loss of the native consensus haplotype for the recipient species. We manually reviewed all windows found to be candidates for introgression and eliminated windows exhibiting anomalous SNP profiles or insufficient data.