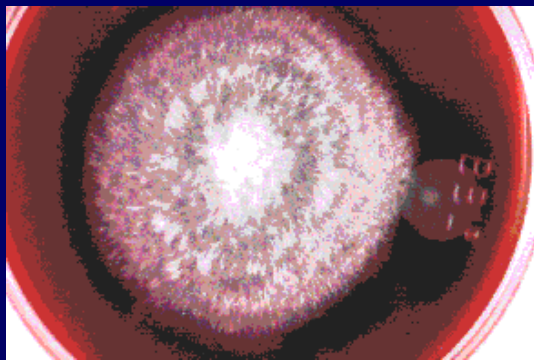
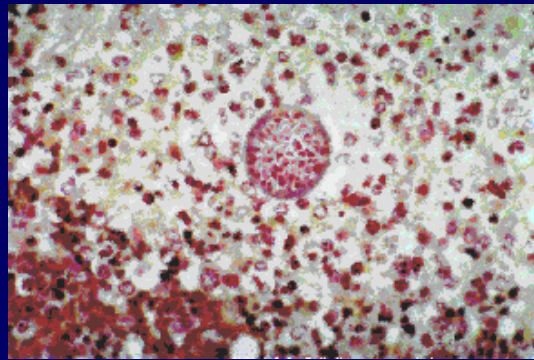


Population genetics and biology of the human fungal pathogen *Coccidioides immitis*



Mycelia



Tissue spherule

**Matthew C. Fisher and John W. Taylor,
University of California at Berkeley**

Distribution of *Coccidioides immitis* in the New World

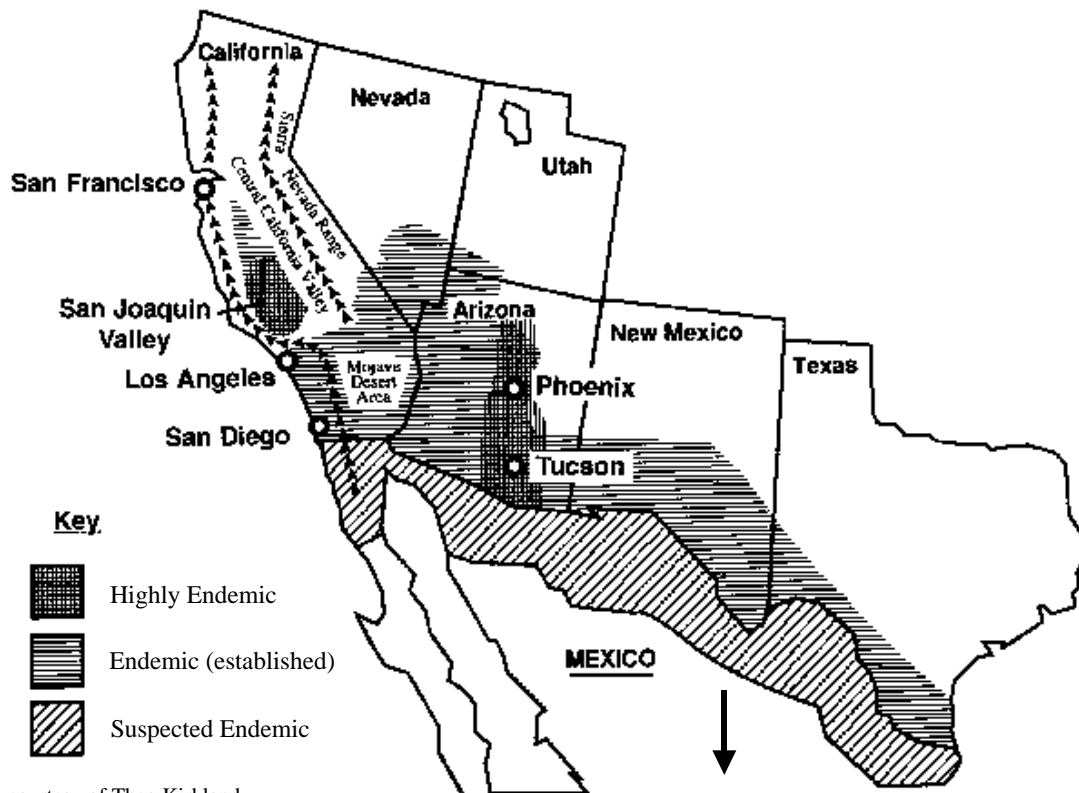


Diagram courtesy of Theo Kirkland,
UC San Diego

Also known from Brazil and Argentina

Coccidioides immitis life cycle

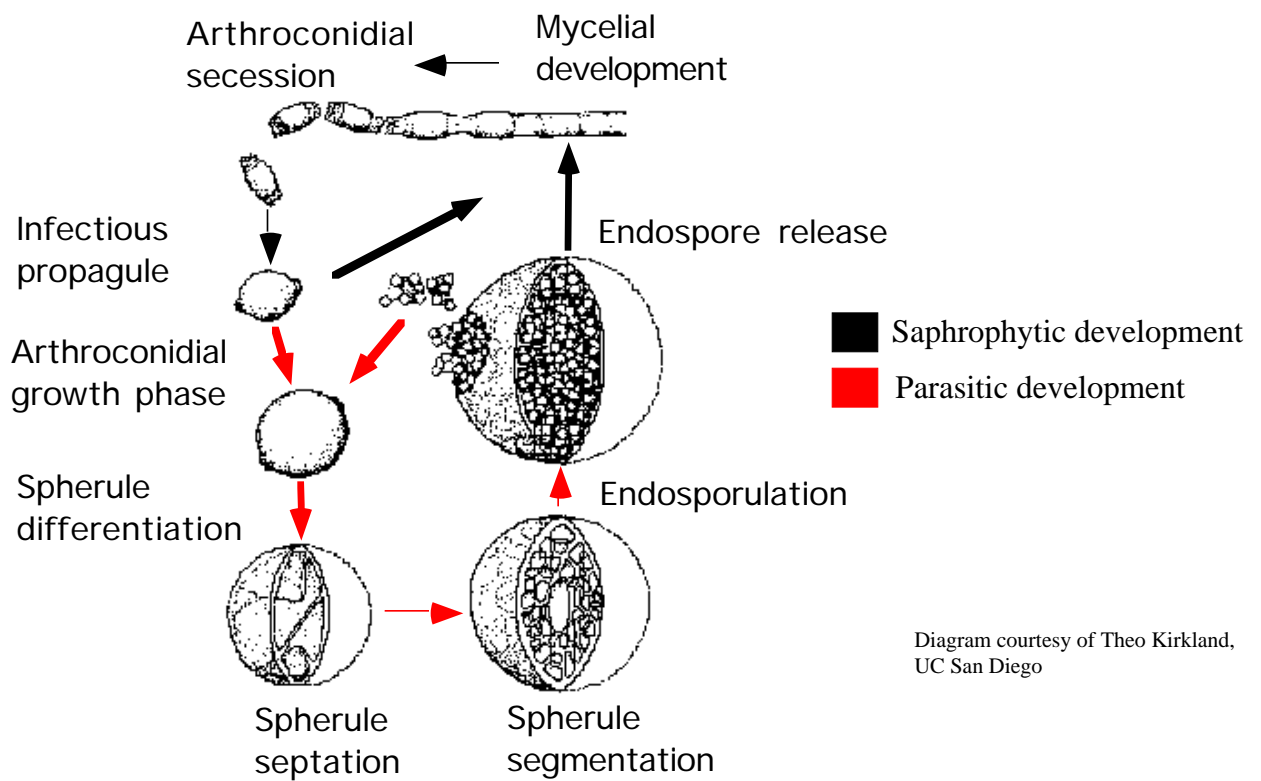


Diagram courtesy of Theo Kirkland, UC San Diego

Aims

- Characterize the population genetic structure

Define limits to gene flow -

PHYLOGENETIC SPECIES

Define geographical barriers to gene flow -

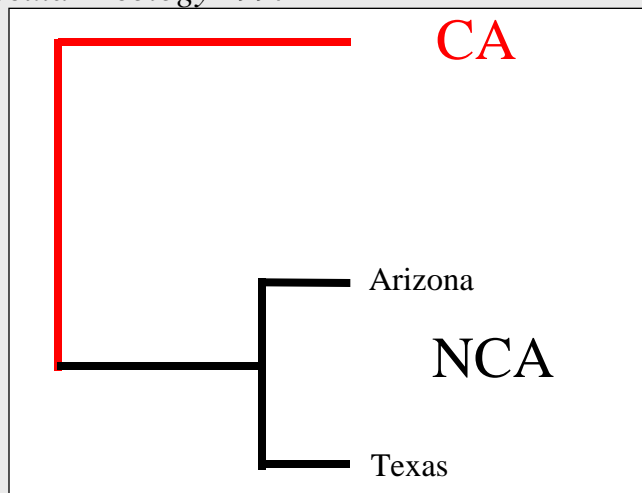
PHYLOGEOGRAPHICAL ISOLATION

- Determine the genetic structure of epidemic populations

RECOMBINING OR CLONAL?

What is known about the population genetics of *C. immitis*?

- Two cryptic species, California (**CA**) and non-California (**NCA**)
Koufopanou et al. PNAS 1997
- NCA has a recombining population structure despite the lack of a known sexual stage *Burt et al. PNAS 1996*
- NCA shows phylogeographical isolation between Arizona and Texas
Burt et al. Molecular Ecology 1997



Two classes of genetic markers used

- Highly polymorphic markers to determine biogeographic populations and species boundaries

Microsatellites (mutation rates $10^{-3} - 10^{-5}$)

- Biallelic, slowly evolving markers within species to characterize reproductive mode and identify clonal populations

Single Nucleotide Polymorphisms (mutation rates 10^{-9})

To isolate (CA)_n and (GA)_n microsatellites

Genomic DNA from both *C. immitis* species



Screen with (CA)₁₅ and (GA)₁₅ probes



Make specific fluorescinated primers to the sequences flanking microsatellites and score size polymorphisms on ABI 377

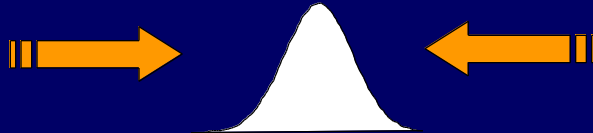
NINE MICROSATELLITES ISOLATED [621.2 by Dee Carter]

Advantages to using microsatellites:

Highly polymorphic and fast to genotype large numbers of isolates

BUT

Homoplasies are possible due stepwise mode of mutation and constraints on allele sizes (Orti et al., *PNAS* 1998)



if this is common then.....

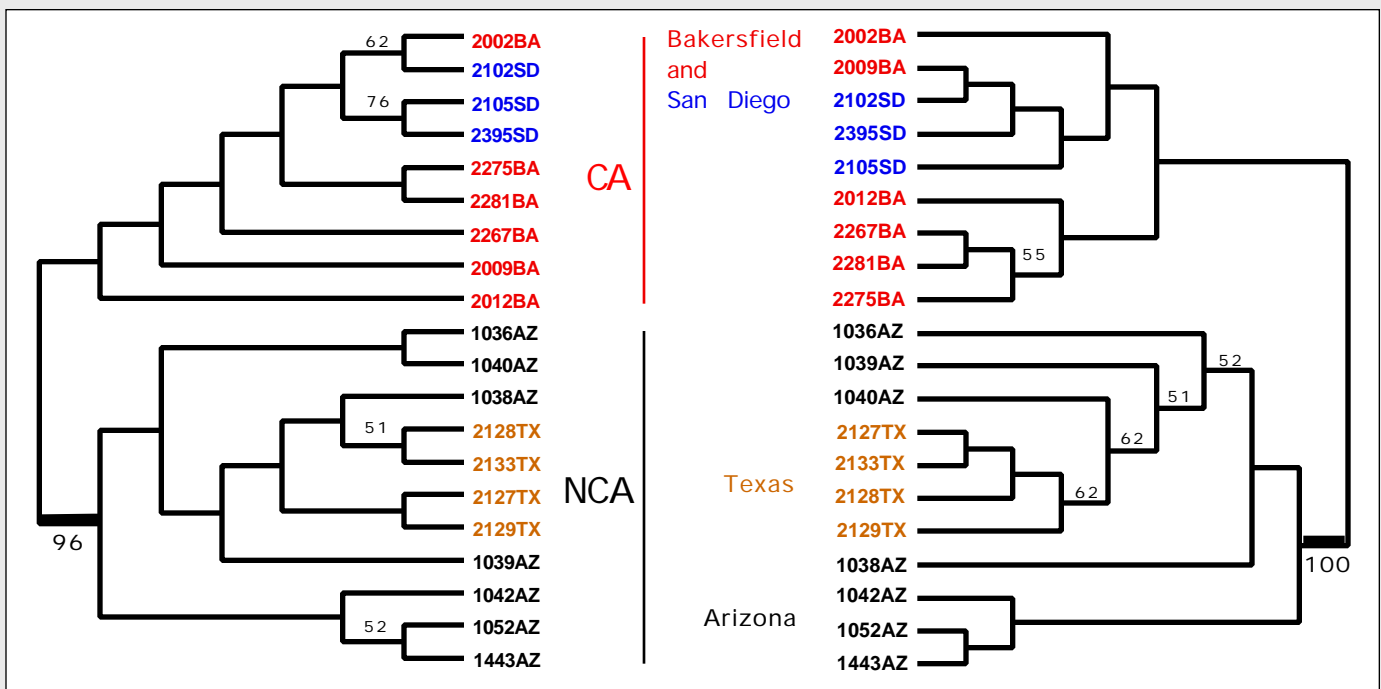
- Genetic distances will not increase linearly with time
- High levels of homoplasy will occur

.....resulting in

INCORRECT PHYLOGENIES

Microsatellite distance

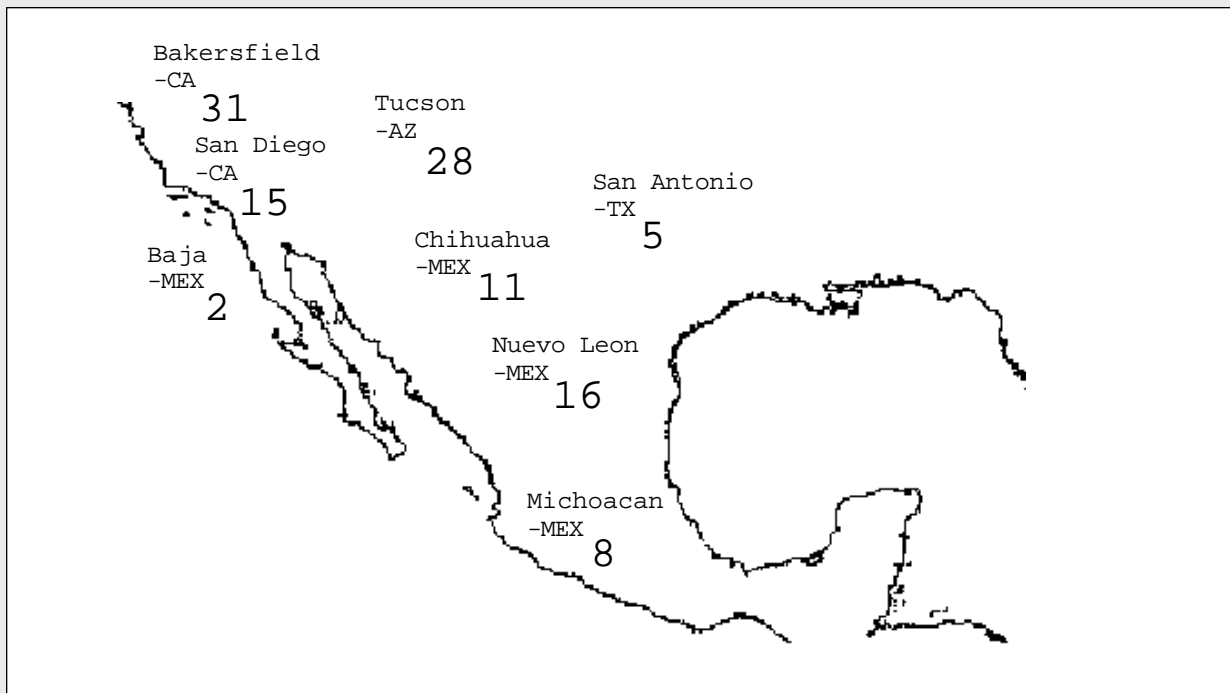
Flanking sequence distance



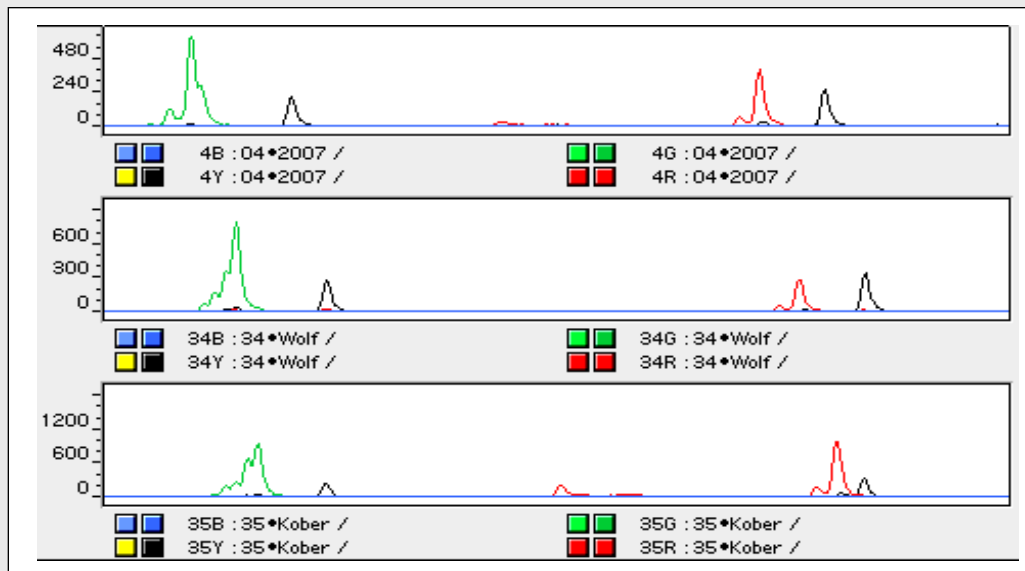
These trees have the same topology (Kishino-Hasegawa test non-significant)

THE MICROSATELLITE MARKERS ARE GOOD

116 clinical isolates collected from North and Central America

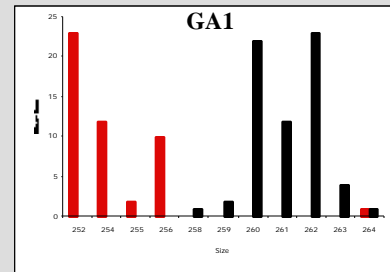
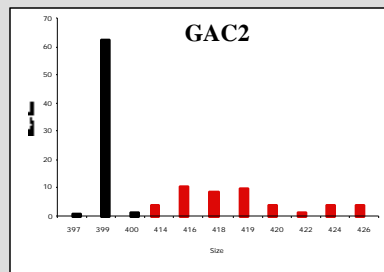
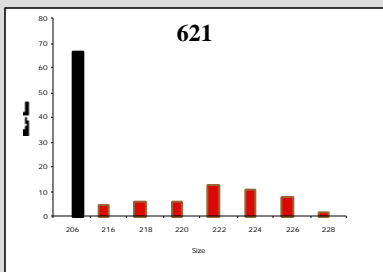


Score microsatellite polymorphisms on an ABI 377

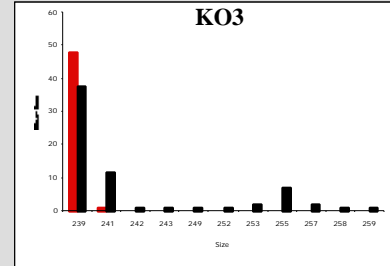
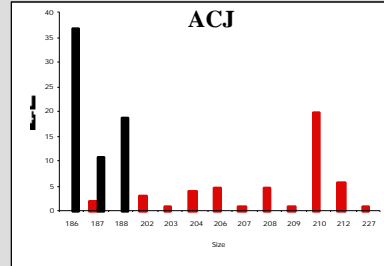
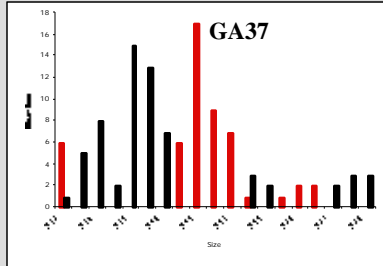


Locus ACJ
Locus KO1

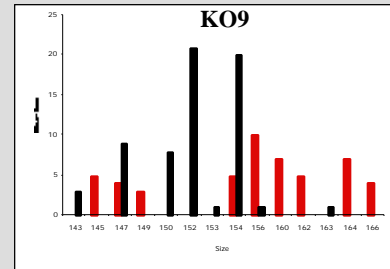
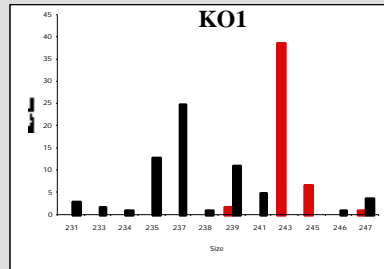
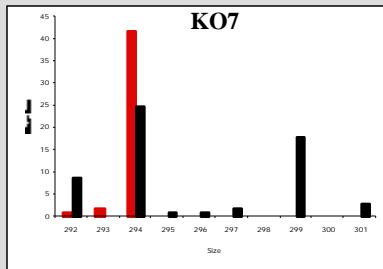
Allele frequencies in the CA (red) and NCA (black) species



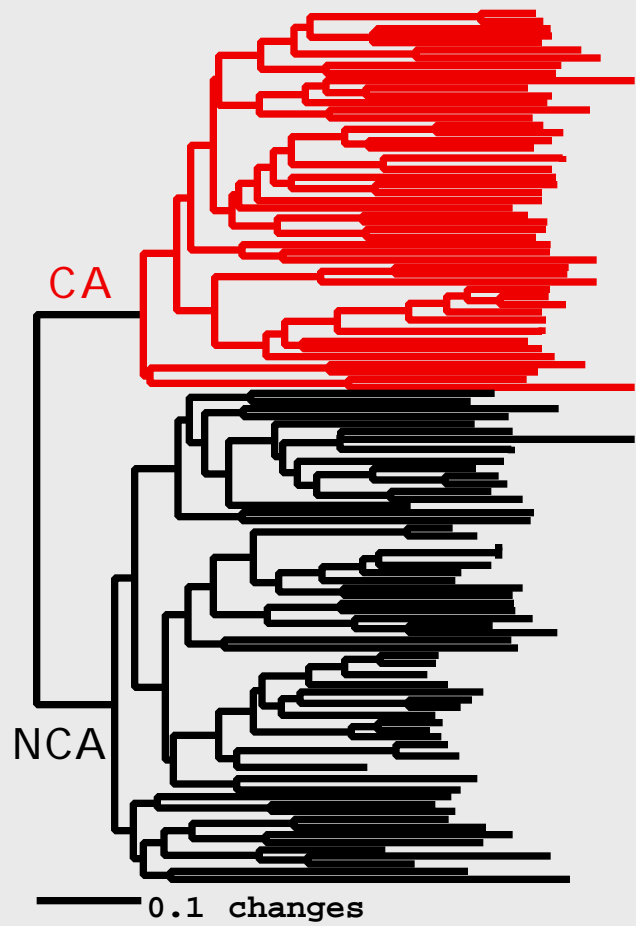
Bimodal distributions



Overlapping distributions

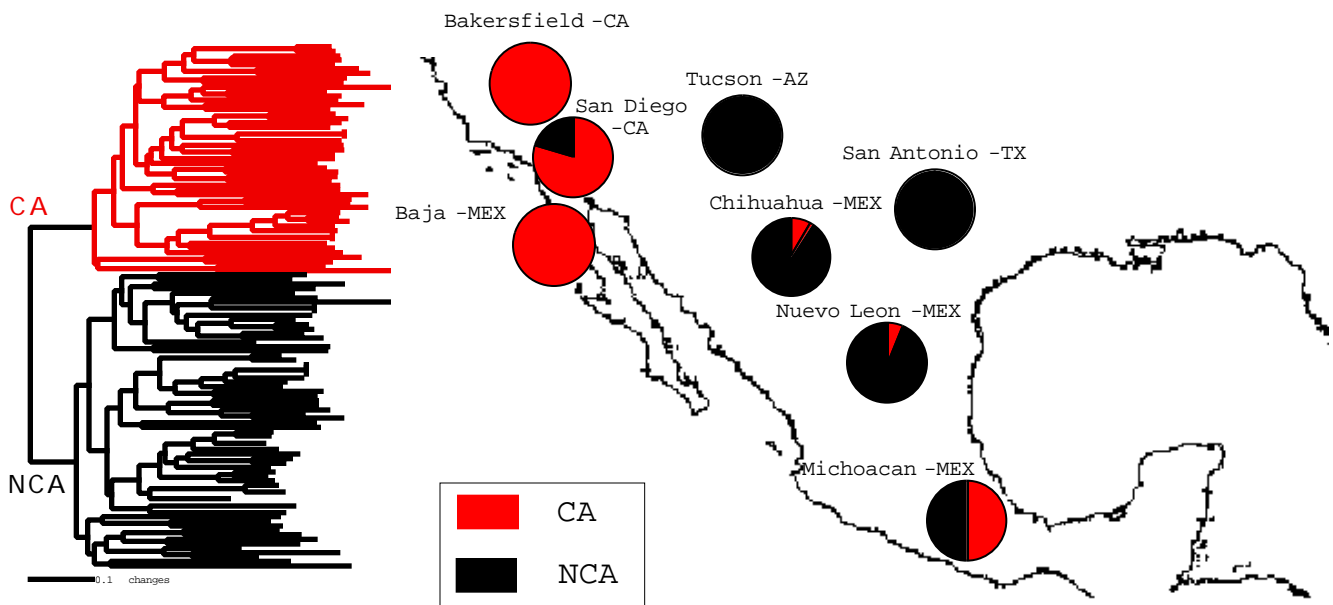


Overlapping distributions



Distance tree calculated from the numbers of alleles shared between isolates support the existence of two *C. immitis* species

Both species are allopatric in Bakersfield, Baja, Tucson and Texas
The species are sympatric in San Diego and Mexico.
NO HYBRIDS WERE OBSERVED



Both the CA and NCA species demonstrate significant phylogeographical structure

BAKERSFIELD
(27 / 27)

**SAN DIEGO/
BAJA**
(9 / 12)

ARIZONA
(27 / 29)

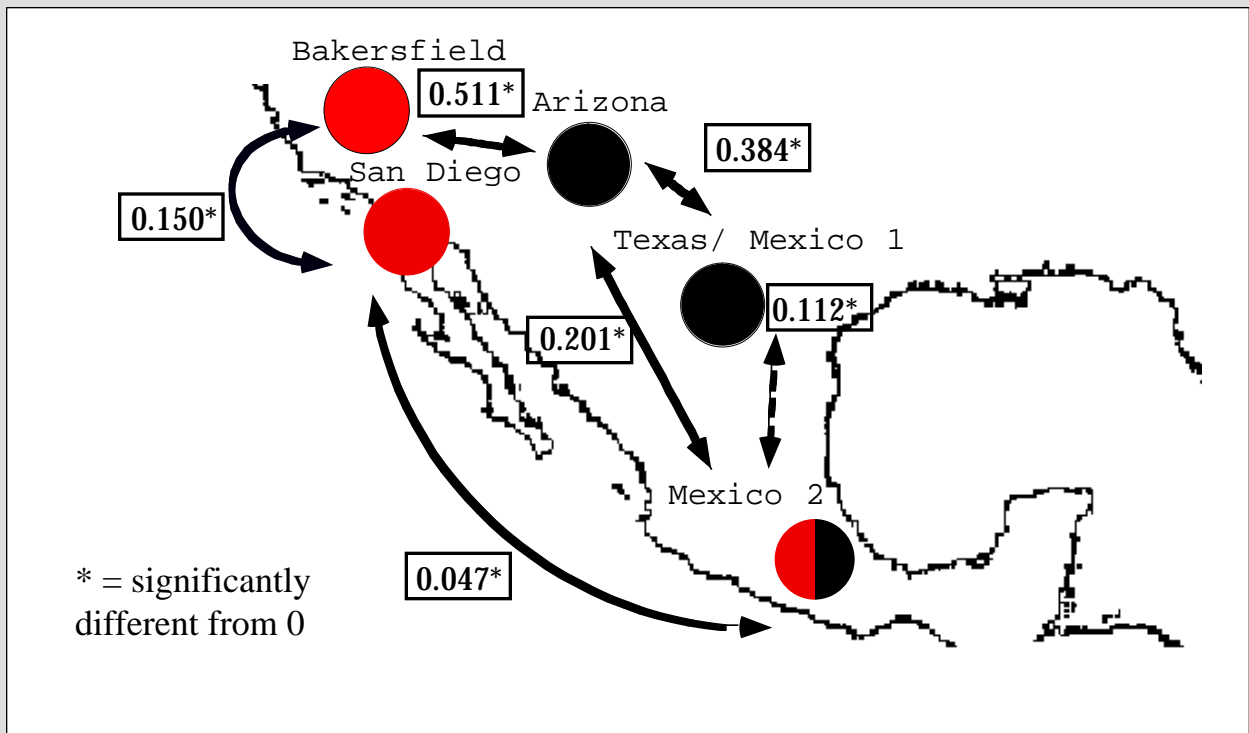
**TEXAS/
MEXICO 1**
(5 / 5)

MEXICO 2
(6 / 10)

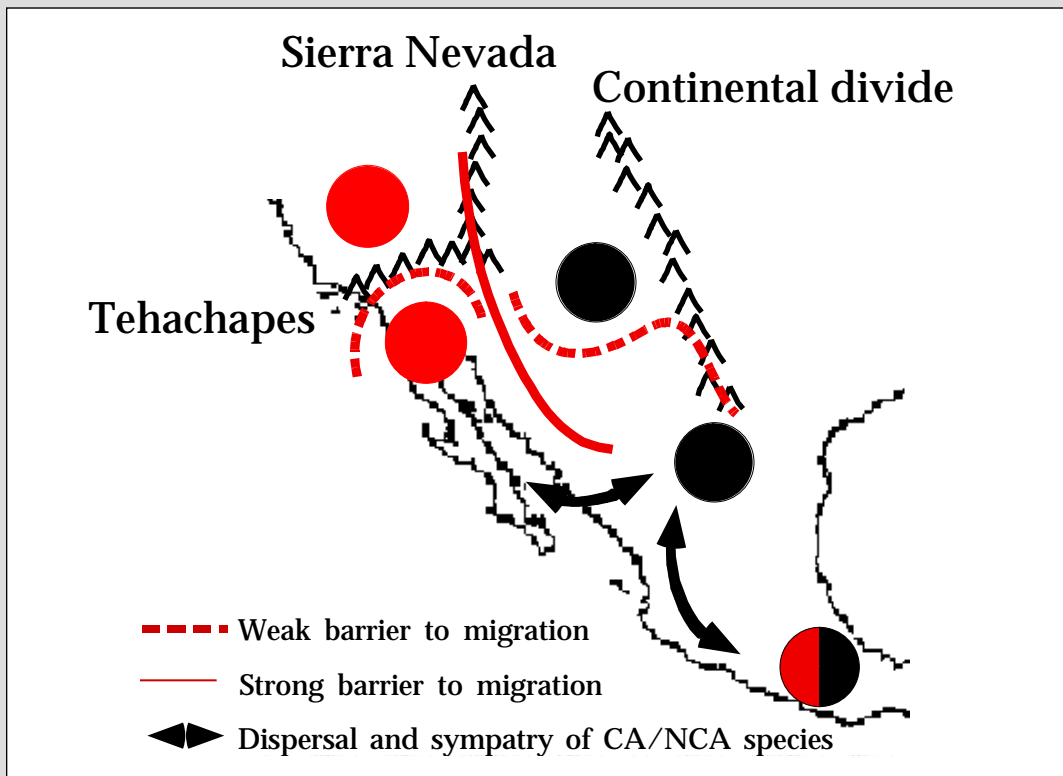


0.1 changes

Fst measures the amount of genetic differentiation between populations.
Ranges from 1 (mutually exclusive allele distributions) to 0 (no genetic differentiation)



Model of *C. immitis* population structure showing major and minor barriers to gene-flow



**Assignment probabilities from allele frequencies:
Identifying **migrant** individuals within each *C. immitis* species**

- Calculate expected frequency of an individual genotype
- Assign to population in which most likely to occur

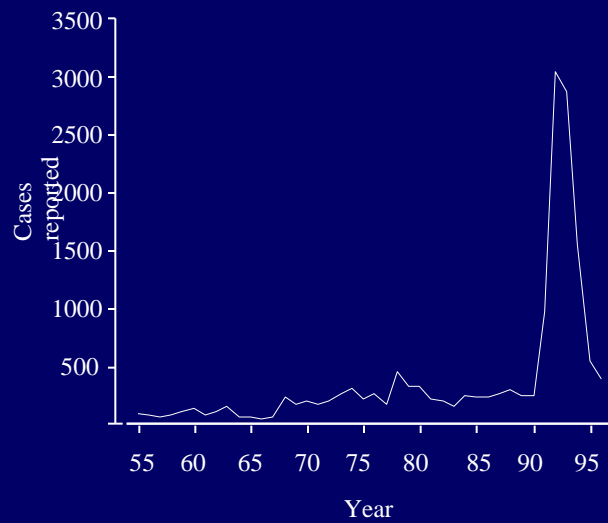
	BAK	SD+M	AZ	TZ+M
BAK	29 / 31	2 / 31	0	0
SD+M	1 / 20	19 / 20	0	0

AZ	0	0	27 / 29	2 / 29
TX+M	0	0	3 / 38	35 / 38

**The genetic structure of epidemic Californian
C. immitis populations**

.....Recombining or Clonal?

1991- 1993 increase in Kern County, California *C. immitis* infection rates



Source: Kern County Health
Department,

EPIDEMIC

Evolution of virulent individual
e.g. Phytophthora Brasier, PNAS 1999

Increases in fungal population size
due to favourable environmental
conditions

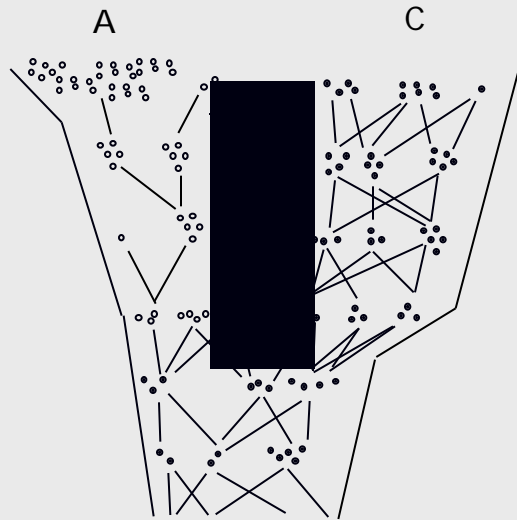
GENETICS

versus

ENVIRONMENT

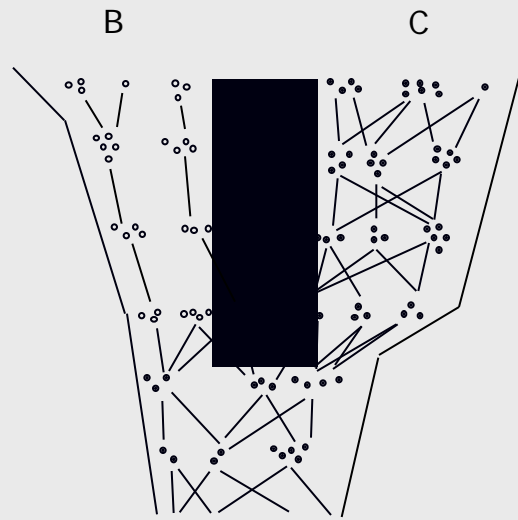
GENETICS

A. Epidemic-clonal population structure



ENVIRONMENT

B. Long term clonal structure



C. Recombining structure

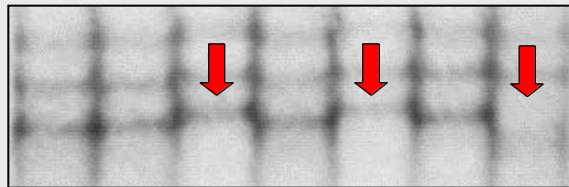
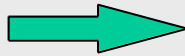
Test A, B and C using phylogenetic analyses of multiple loci

Screen known and anonymous *C. immitis* loci for Single Strand Conformational Polymorphisms amongst isolates from the epidemic

- Amplify a locus by PCR from 7 isolates

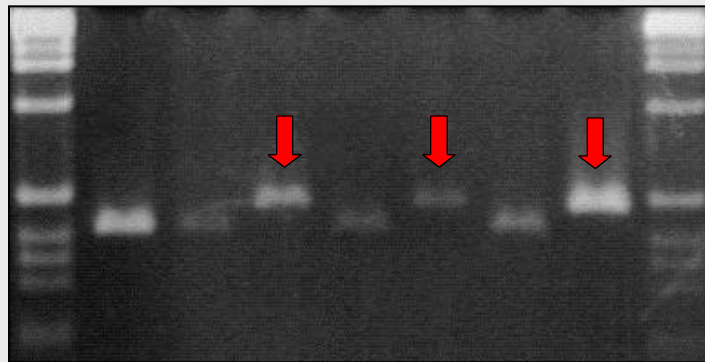
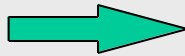
1 2 3 4 5 6 7

- Run out on gel



- Sequence alleles

- Score alleles as RFLPs



Alleles: 1 1 0 1 0 1 0

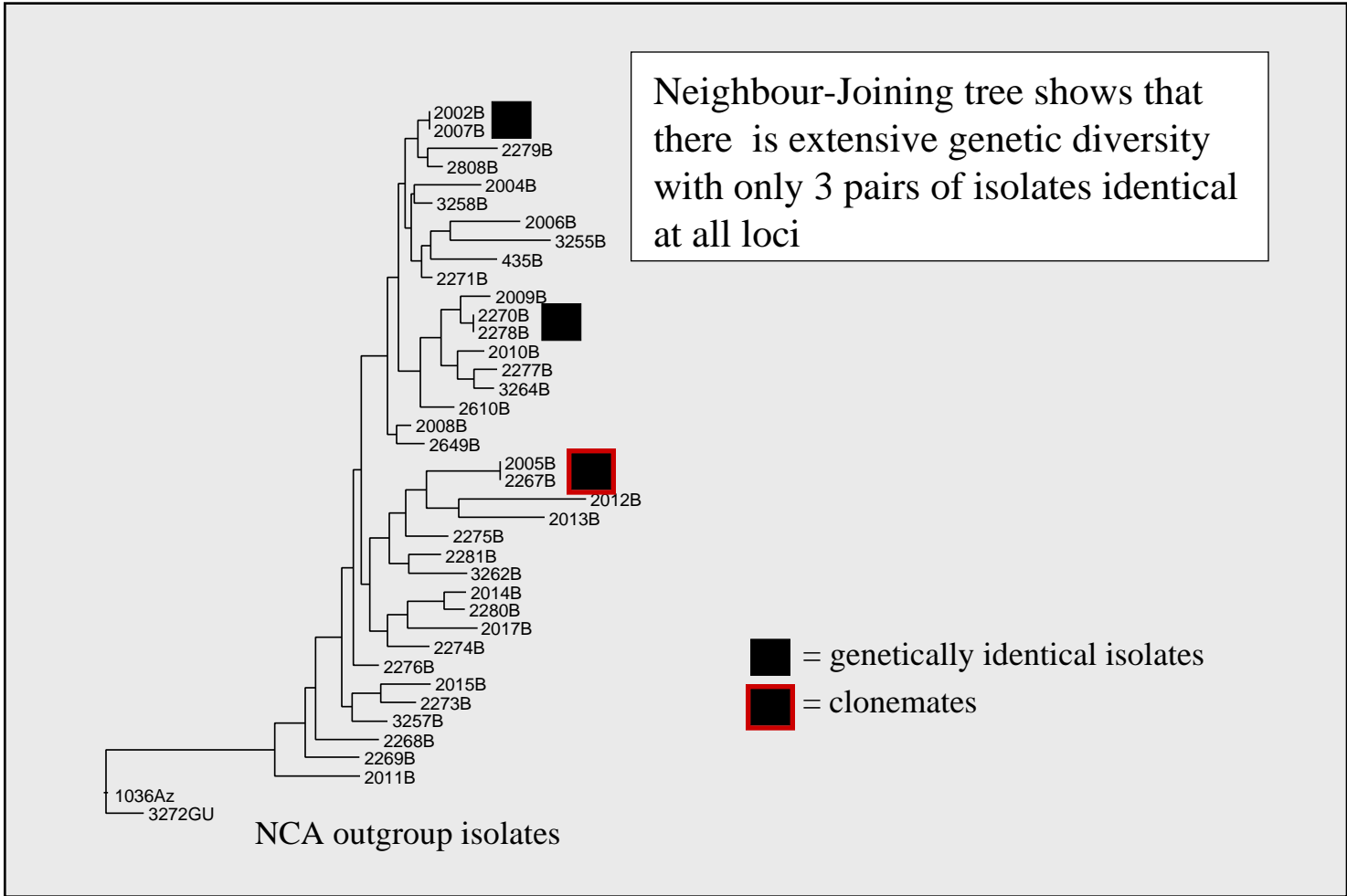
The complete dataset.....37 epidemic isolates and 13 loci

ISOLATES

	isolate																																																
	2002B	2004B	2005B	2006B	2007B	2008B	2009B	2010B	2011B	2012B	2013B	2014B	2015B	2017B	2649B	2267B	2268B	2269B	2270B	2271B	2273B	2274B	2275B	2276B	2277B	2278B	2279B	2280B	2281B	3255B	3257B	3258B	3262B	3264B	2808B	2610B	435B	3272	1036										
VL	0	0	1	0	0	0	0	1	1	1	1	0	1	1	0	1	1	0	0	1	1	1	1	1	1	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	1	1						
CTS1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
OR	1	1	1	0	1	1	1	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0				
ITS	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	1						
VK	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
BL	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0				
CAG	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
CNS	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
HSP	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	0	0	0			
BOR	1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0		
BGL2	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1		
RAN1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
621	1	1	0	1	1	1	1	1	1	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
SP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

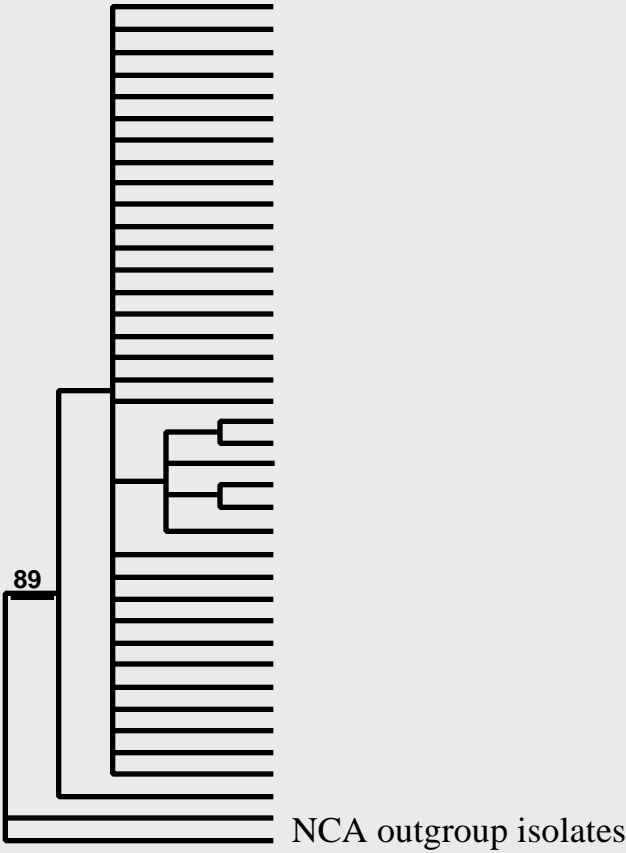
L
O
C
I

= NCA outgroup isolates

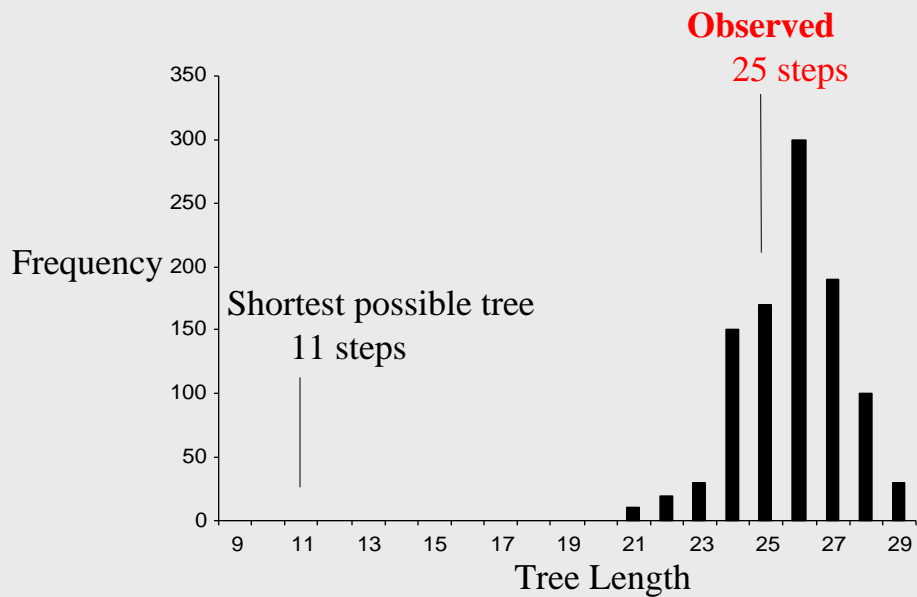


Parsimony analysis of isolates - 4000 equally parsimonious trees

Strict consensus:



Probability that the tree lengths of the observed dataset are shorter than the artificially recombined datasets = 0.68



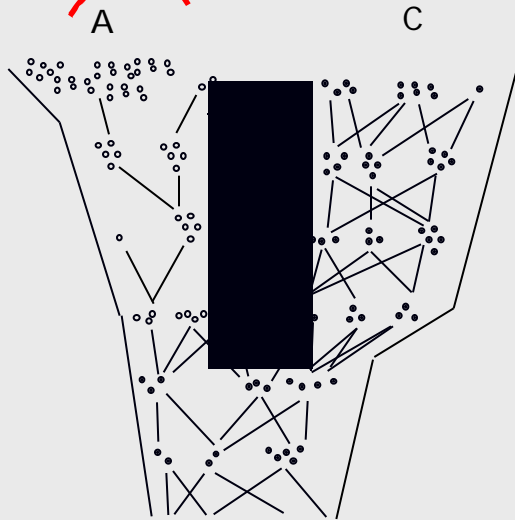
Kern county epidemic *C. immitis* are from a genetically diverse and recombining population

Disequilibrium coefficient calculated between 78 pairs of loci
and Fishers' exact test used to assess significance

4/78 comparisons were significant ($P = 0.05$)

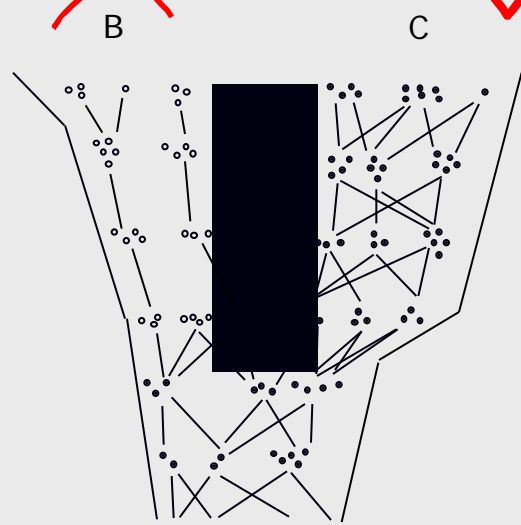
GENETICS

~~A. Epidemic-clonal population structure~~



ENVIRONMENT

~~B. Long term clonal structure~~



C. Recombining structure ✓



So why the epidemic? Epidemiological analyses of key environmental variables influencing fungal growth rates necessary to solve the problem.

Conclusions

- Microsatellite and flanking sequence phylogenies are congruent
- '*Coccidioides immitis*' encompasses a phylogenetic species pair 'CA' and 'NCA' that are sympatric in the South-West/Mexico
- Both CA and NCA show limited intraspecific gene flow resulting in strong patterns of phylogeographical isolation
- CA is genetically recombining and epidemic populations show little evidence of clonal reproduction
 - The Bakersfield epidemic was not due to the recent expansion of a single virulent clone

ACKNOWLEDGMENTS

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Dr. Isidro Guttierrez- Mexico Isolates
Dr. John Galgiani- Arizona Isolates
Dr. Theo Kirkland- San Diego Isolates
Dr. Mike Rinaldi- Texas Isolates
Dr. Rebecca Cox- Texas Isolates