Scoring Microsatellites in *Coccidioides immitis* and *Coccidioides posadasii*

Microsatellites are useful genetic markers due to their multi-allelic nature. Scoring them is usually perfomed by typing the major alleles seen in electrophoretic gels. However, due to slippage of *Taq* polymerase during PCR, multiple amplification products are often present, complicating the accurate scoring of alleles. Fortunately, the patterns seen are locus-specific and, with practice, can be easily scored.

The following pages detail the major patterns seen when running the amplified *Coccidioides* sp. microsatellites described by Fisher et al. (1999, 2000a, 2000b, 2000c) using GENESCAN (Applied Biosystems). The output was calibrated by sequencing alleles as detailed in Fisher et al (1999) and the 'correct' peaks coloured. Black peaks are are the TAMRA-labelled internal size standard (GENESCAN 500). Use of this document will standardize typing *Coccidioides* using these loci.

Fisher MC, Koenig GL, White TJ and Taylor JW. 1999. Primers for genotyping single nucleotide polymorphisms and microsatellites in the pathogenic fungus *Coccidioides immitis*. **Molecular Ecology** 8:1082 - 1084.

Fisher MC, Koenig GL, et al. 2000a. Biogeographic range expansion into South America by the pathogenic fungus *Coccidioides immitis* mirrors human patterns of migration. (submitted)

---. 2000b. A test for concordance between the multilocus genealogies of genes and microsatellites in the pathogenic fungus *Coccidioides immitis*. **Mol. Biol. Evol.** 17: 1164-1174

---. 2000c. Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognized as the *non-California* population of *Coccidioides immitis*. (submitted)
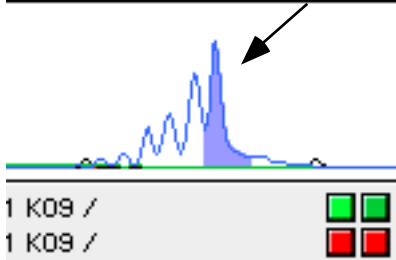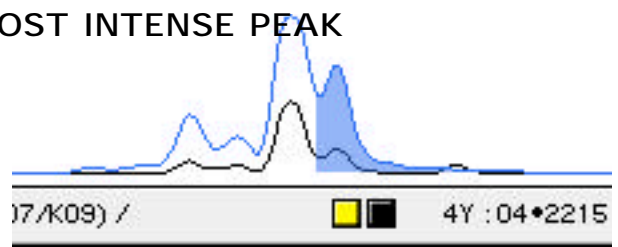
SCORING KO9.fam
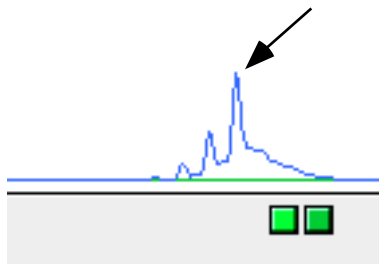Score peak that is furthermost right (both C. immitis and C. posadasii)
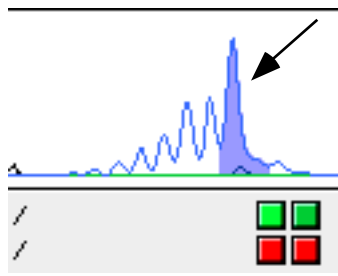
RHS PEAK
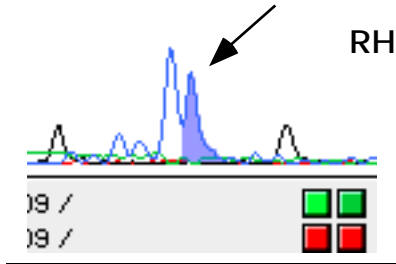
RHS PEAK: IGNORE MOST INTENSE PEAK

07/K09) /   4Y :04•2215

RHS PEAK

35 cycle PCR
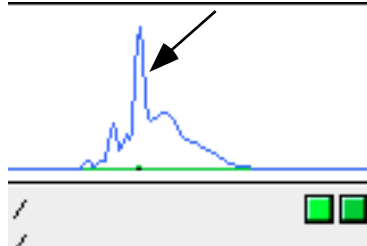- -
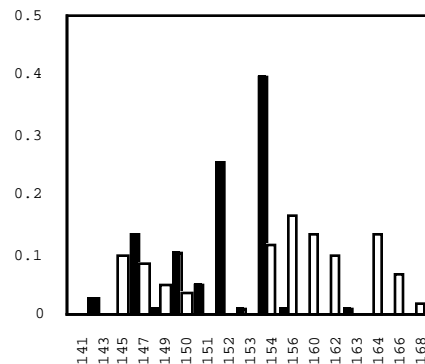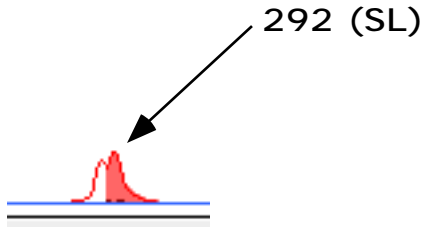28 cycle PCR

Using less cycles dramatically lowers the -1, -3 and -5 peaks

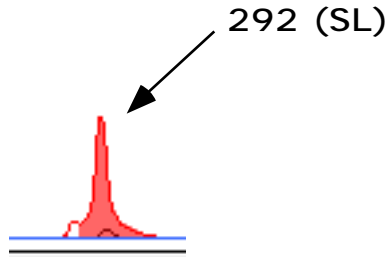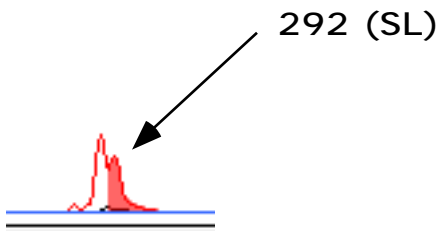Allele sizes seen in C. immitis (white) and C. posadasii (black)

KO9

SCORING LOCUS KO7.rox (C. immitis and C. posadasii)
always score the peak furthermost right.

292 (SL)
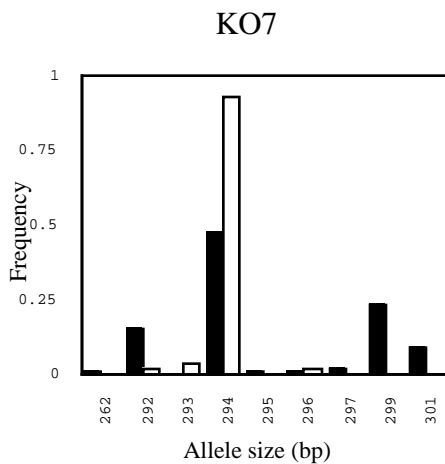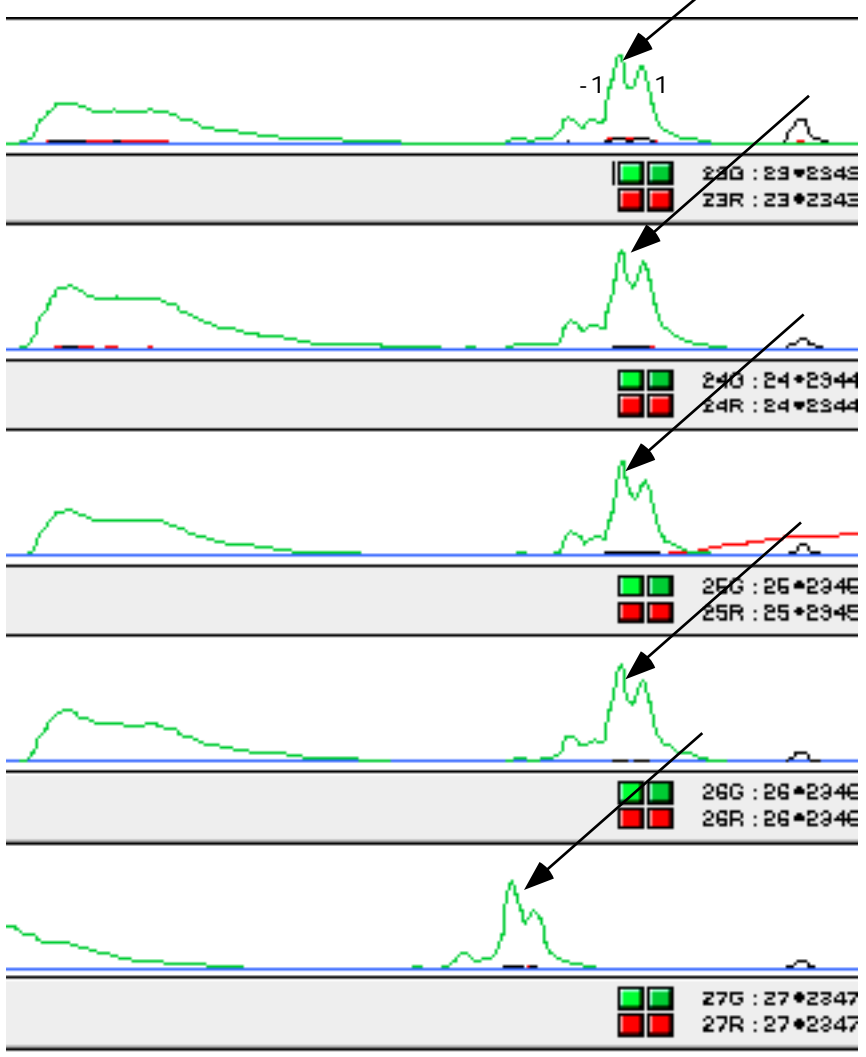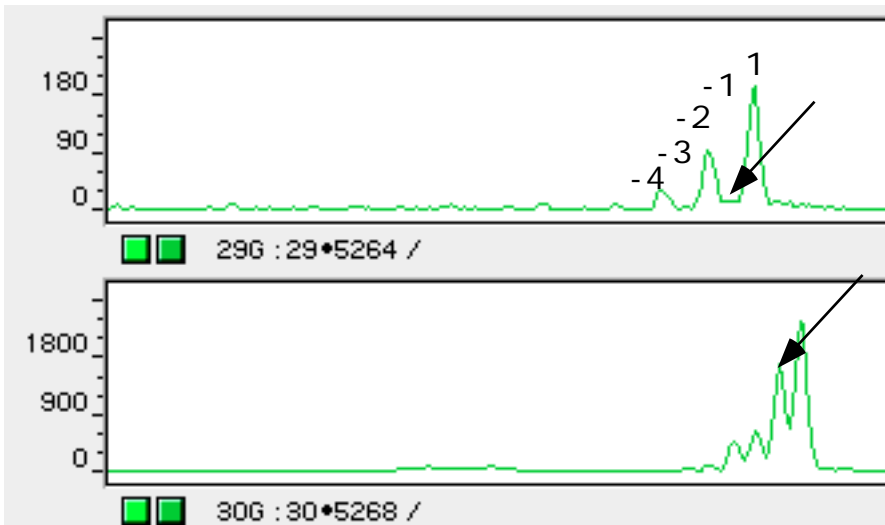
RHS PEAK IS LARGEST: SCORE

292 (SL)

SINGLE PEAK: SCORE

292 (SL)

LHS PEAK IS LARGEST: SCORE RHS PEAK

30 /
30 /

KO7
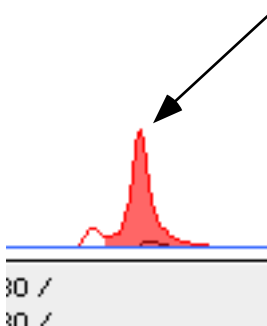
Allele sizes seen in C. immitis
(white) and C. posadasii (black)

Allele size (bp)

Scoring locus KO3 (C. immitis and C. posadasii)

Score the -1 peak

-1 1

23G : 23●2349
23R : 23●2343

24G : 24●2344
24R : 24●2344

25G : 25●2346
25R : 25●2345

26G : 26●2346
26R : 26●2346

27G : 27●2347
27R : 27●2347

occasionally, the -1 peak will be missing (below)-score it anyhow.

1
-1
-2
-3
-4

180
90
0

29G : 29●5264 /

1800
900
0

30G : 30●5268 /

KO3 (continued)

KO3
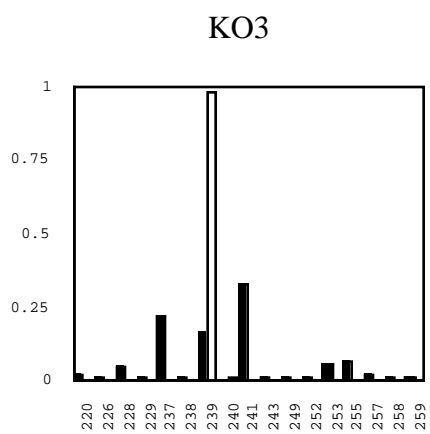
Allele sizes seen in C. immitis
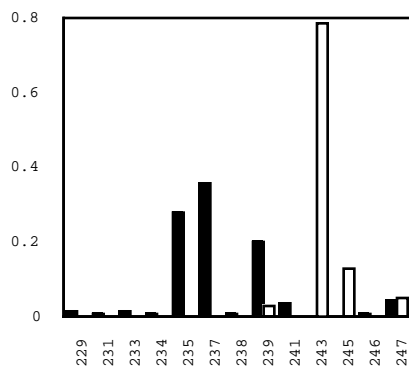(white) and C. posadasii (black)

SCORING LOCUS KO1.hex

C. posadasii. Score as for
C. immitis
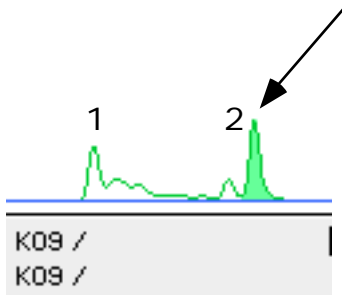
C. immitis. There are often two peaks at this
locus,1 and 2. 1 varies in intensity and is
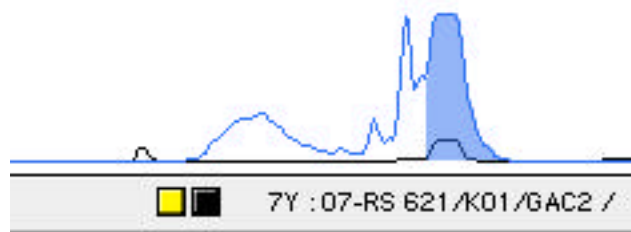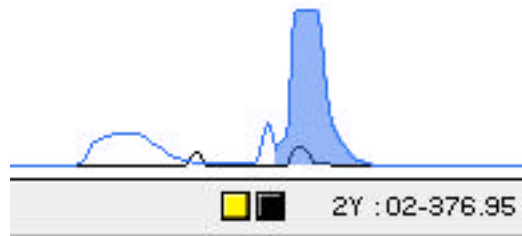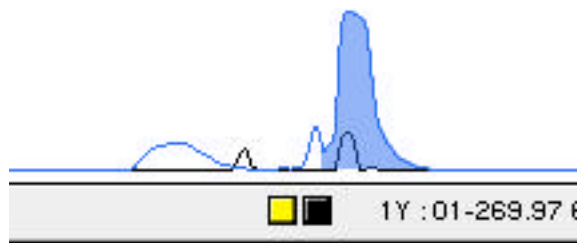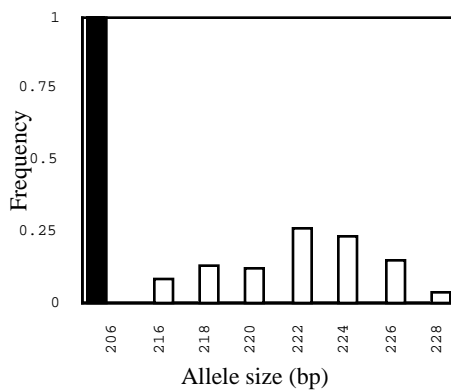spurious (it also co-varies with 2).
Score peak 2.
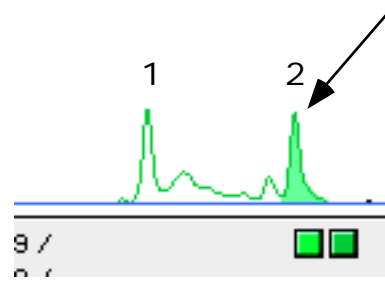
KO1

Allele sizes seen in C. immitis
(white) and C. posadasii (black)

SCORING LOCUS GAC2.fam

Simple locus to score- use largest peak, which is also the rightmost peak. This locus is easy to overamplify (which is why these peaks are truncated somewhat). Dilute before running PCRs on the ABI.
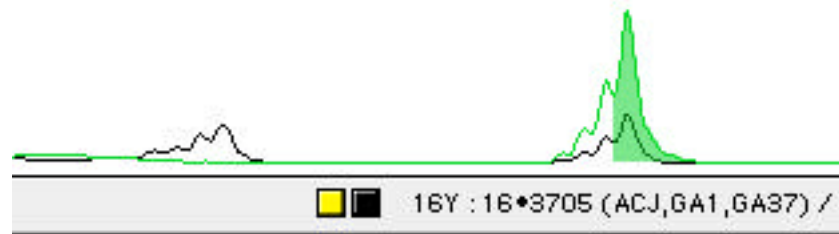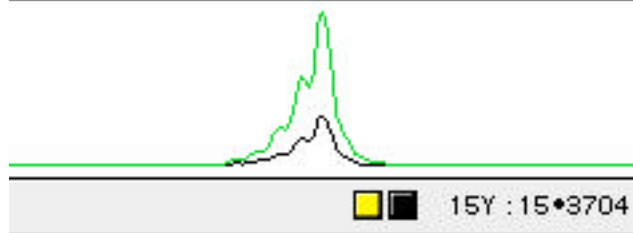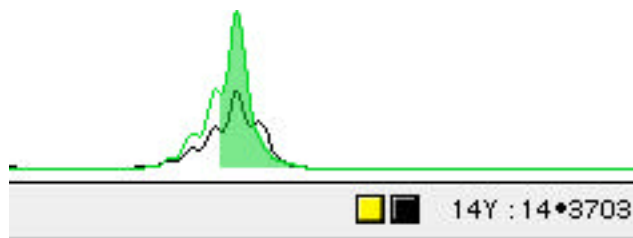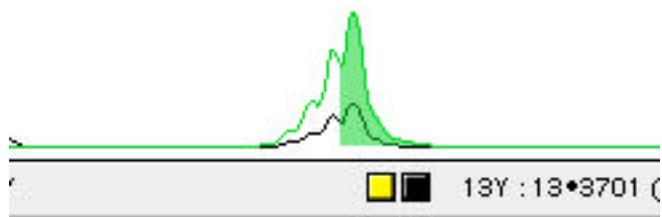

1Y :01-269.97 6
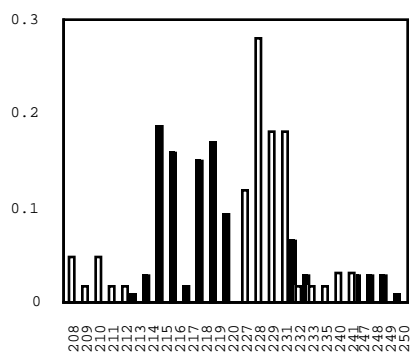

2Y :02-376.95 6

7Y :07-RS 621/K01/GAC2 /

GAC2

Allele sizes seen in C. immitis (white) and C. posadasii (black)

SCORING LOCUS GA37.hex

Simple locus to score- use largest peak, which is also the rightmost peak

13Y : 13•3701 (

14Y : 14•3703

15Y : 15•3704

16Y : 16•3705 (ACJ,GA1,GA37) /

GA37

Allele sizes seen in C. immitis (white) and C. posadasii (black)
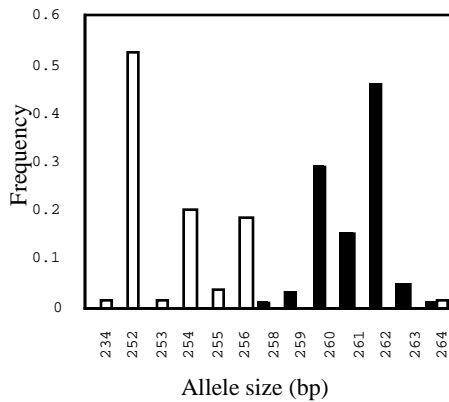
SCORING LOCUS GA1.rox

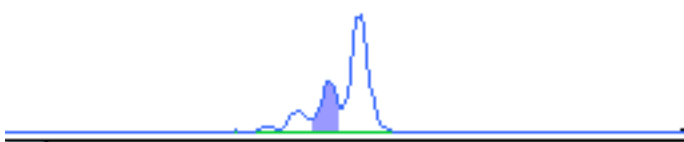## C. posadasii
Score last peak

C. immitis
Score major peak

3R : 03•1038 /

1R : 01•1036 /

5R : 04•2009 /

2R : 02•1037 /

6R : 05•2010 /
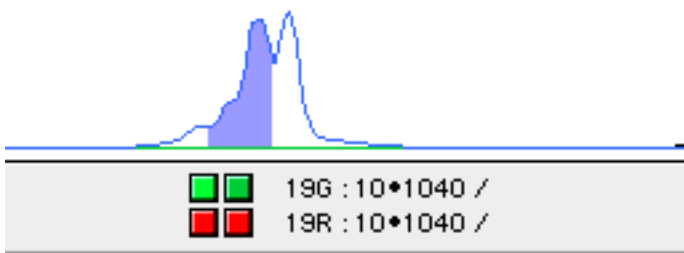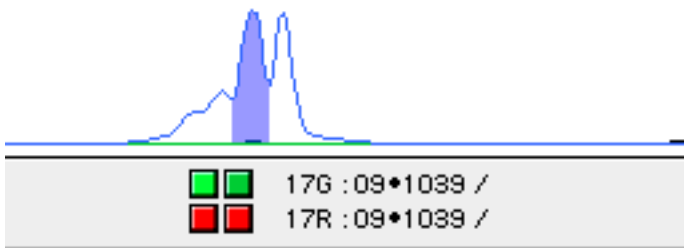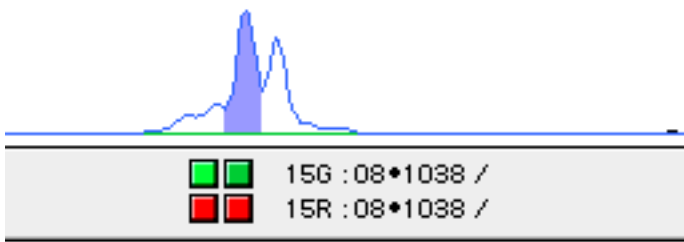
5R : 05•1040 /

7R : 06•2011 /

4R : 04•1039 /

12R : 11•2268 /

GA1

Allele sizes seen in C. immitis (white) and C. posadasii (black)

Frequency
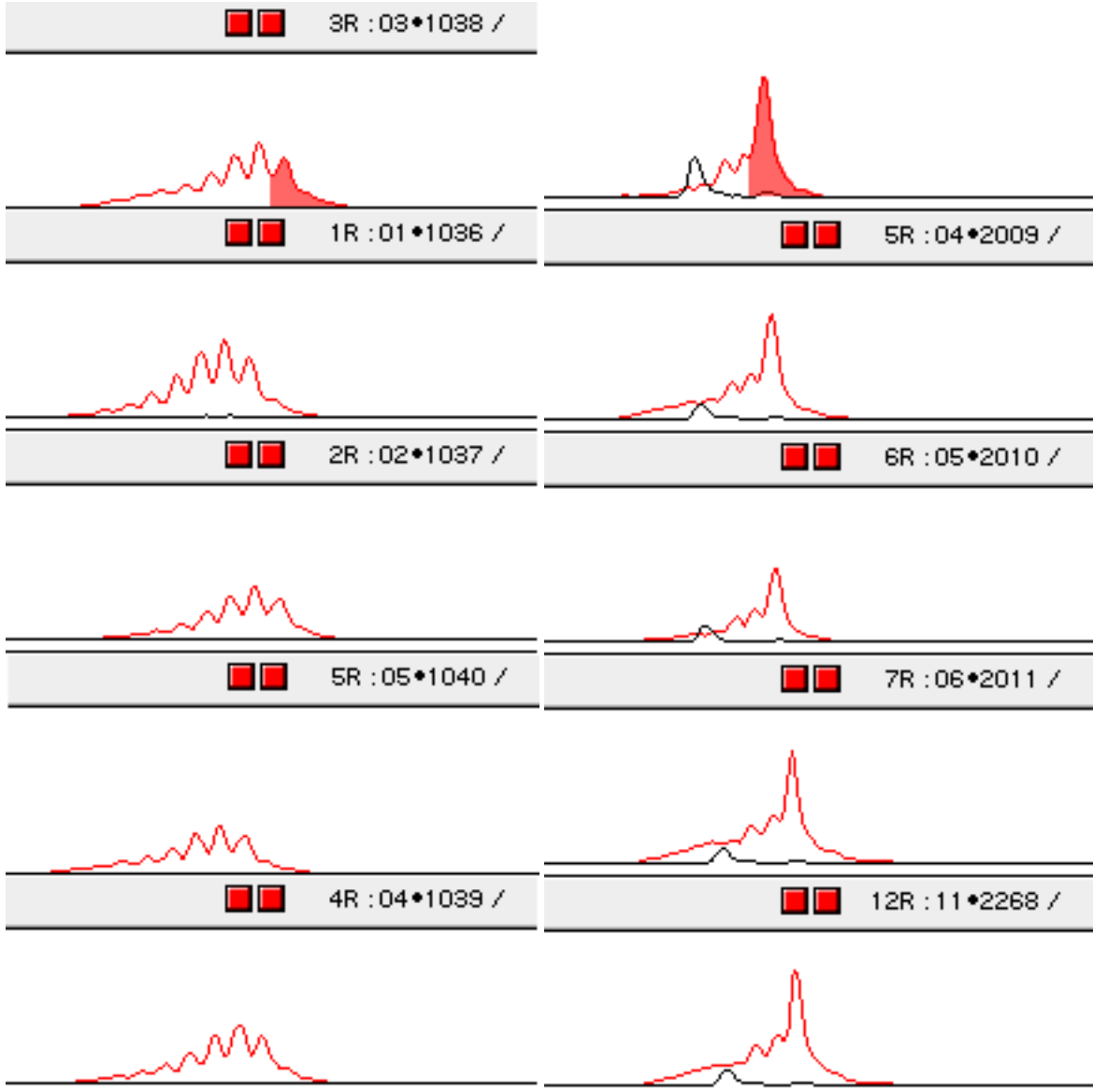
Allele size (bp)

SCORING LOCUS ACJ.fam

ACJ-patterns in C. posadasii

Confusing twin peaks: Always take peak
one to left of last peak

13G : 07•1037 /
13R : 07•1037 /

15G : 08•1038 /
15R : 08•1038 /

17G : 09•1039 /
17R : 09•1039 /

19G : 10•1040 /
19R : 10•1040 /

21G : 11•1042 /
21R : 11•1042 /
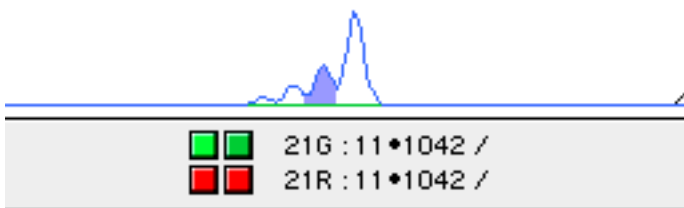
ACJ-patterns in C. immitis

Always take last peak

28Y : 28•2020
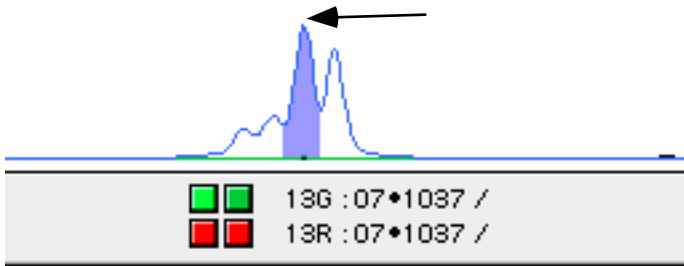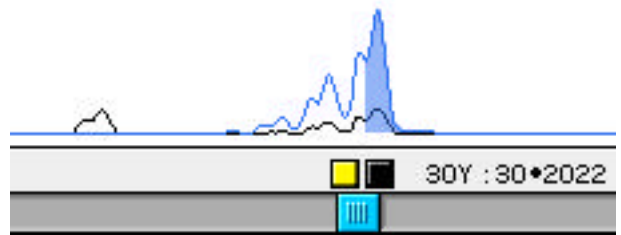
29Y : 29•2021

30Y : 30•2022
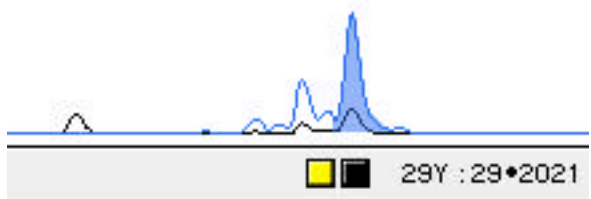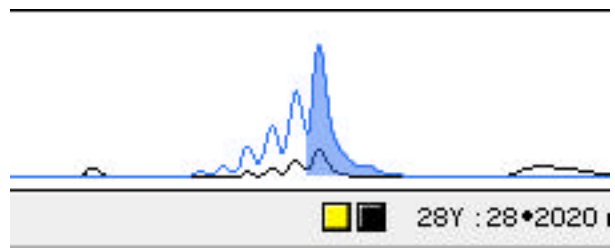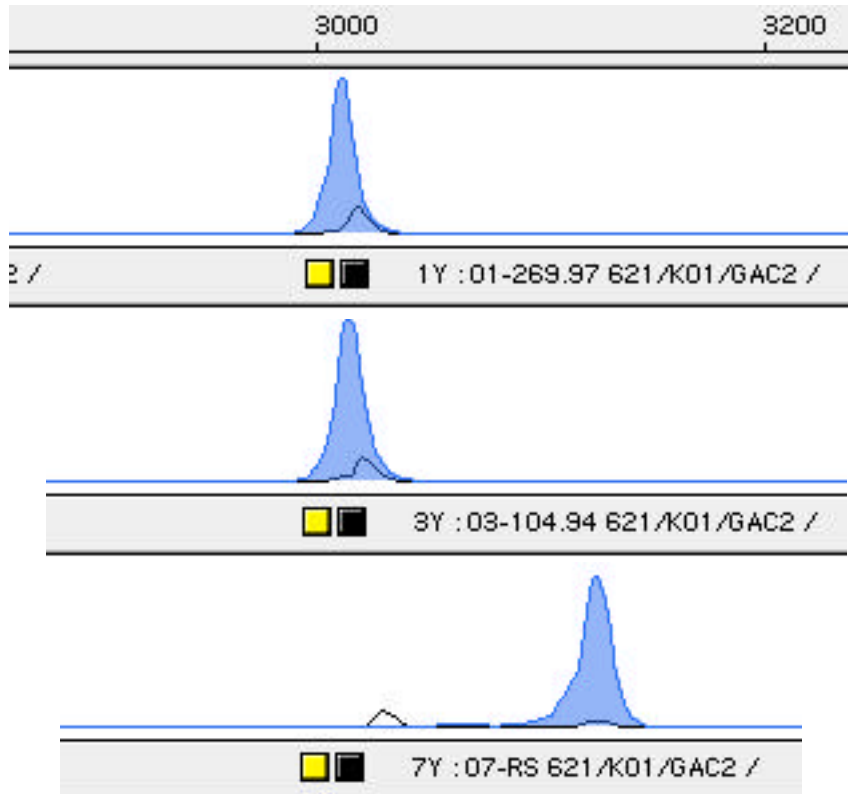
Allele sizes seen in C. immitis
(white) and C. posadasii (black)

ACJ
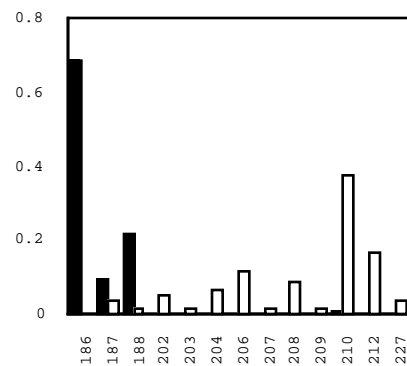
SCORING LOCUS 621.fam

Simple locus to score- use largest peak.

	3000	3200

1Y : 01-269.97 621/K01/GAC2 /

3Y : 03-104.94 621/K01/GAC2 /

7Y : 07-RS 621/K01/GAC2 /

Allele sizes seen in C. immitis (white) and C. posadasii (black)

621