Processing ddRAD for population history inference

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ISU and KU

01-06-2016

ddRAD data

- Reduced-representation genomic method

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- Cheap

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- Lots of data returned

ddRAD data

- Reduced-representation genomic method
- Cheap
- Lots of data returned
- Stable software pipelines for using these data

A Quick Note

Slides that contain ddRAD specific info will be noted. Some steps can be used with multiple data sources.

Our Study

The Edwards Plateau

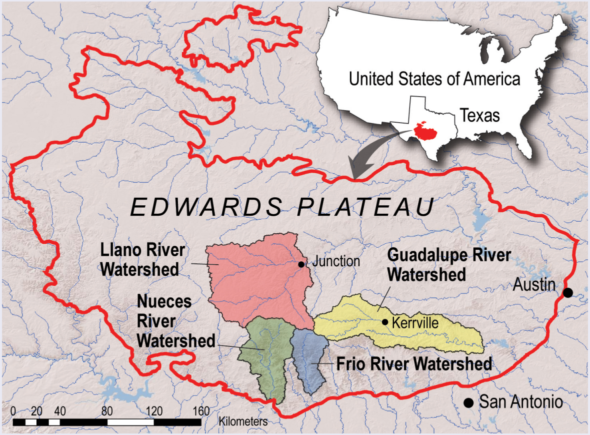


Image: AGU

Our Study

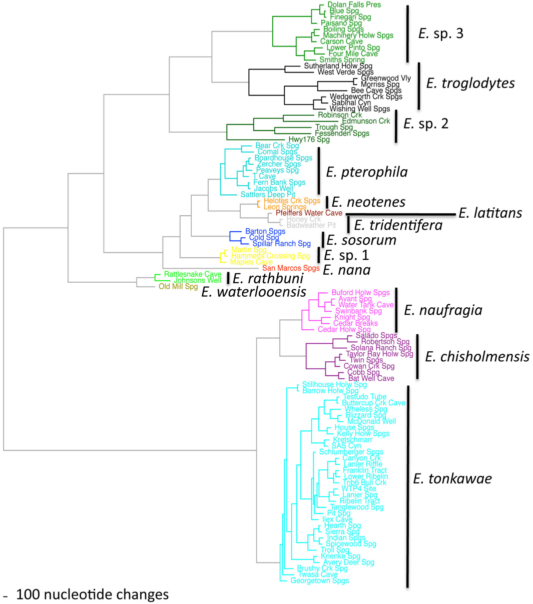
Our Study

13 putative species of *Eurycea*

Our Study

13 putative species of *Eurycea*
All of which are fairly threatened by development

Our Study


Dolan Falls Pres
Blue Spg
Finegan Spg
Paisano Spg
Boiling Spgs
Machinery Holw Spgs
Carson Cave
Lower Pinto Spg
Four Mile Cave
Smiths Spring
E. sp. 3
Sutherland Holw Spg
West Verde Spgs
Greenwood Vly
Morriss Spg
Bee Cave Spgs
Wedgeworth Crk Spgs
Sabinal Cyn
Wishing Well Spgs
E. troglodytes
Robinson Crk
Edmunson Crk
Trough Spg
Fessenden Spgs
Hwy176 Spg
E. sp. 2
Bear Crk Spg
Comal Spgs
Boardhouse Spgs
Zercher Spgs
Peaveys Spg
T Cave
Fern Bank Spgs
Jacobs Well
Sattlers Deep Pit
E. pterophila
Helotes Crk Spgs
Leon Springs
E. neotenes
Pfeiffers Water Cave
E. latitans
Honey Crk
Badweather Pit
E. tridentifera
Barton Spgs
Cold Spg
Spillar Ranch Spg
E. sosorum
Martin Spg
Hammetts Crossing Spg
Maples Cave
E. sp. 1
San Marcos Spgs
E. nana
Rattlesnake Cave
Johnsons Well
E. rathbuni
Old Mill Spg
E. waterlooensis
Buford Holw Spgs
Avant Spg
Water Tank Cave
Swinbank Spg
Knight Spg
Cedar Breaks
Cedar Holw Spg
E. naufragia
Salado Spgs
Robertson Spg
Solana Ranch Spg
Taylor Ray Holw Spg
Twin Spgs
Cowan Crk Spg
Cobb Spg
Bat Well Cave
E. chisholmensis
Stillhouse Holw Spg
Barrow Holw Spg
Testudo Tube
Buttercup Crk Cave
Wheless Spg
Blizzard Spg
McDonald Well
House Spgs
Kelly Holw Spgs
Kretschmarr
SAS Cyn
Schlumberger Spgs
Canyon Crk
Lanier Riffle
Franklin Tract
Lower Ribelin
Trib6 Bull Crk
WTP4 Site
Lanier Spg
Ribelin Tract
Tanglewood Spg
Pit Spg
Ilex Cave
Hearth Spg
Sierra Spg
Indian Spgs
Spicewood Spg
Troll Spg
Krienke Spg
Avery Deer Spg
Brushy Crk Spg
Twasa Cave
Georgetown Spgs
E. tonkawae
100 nucleotide changes

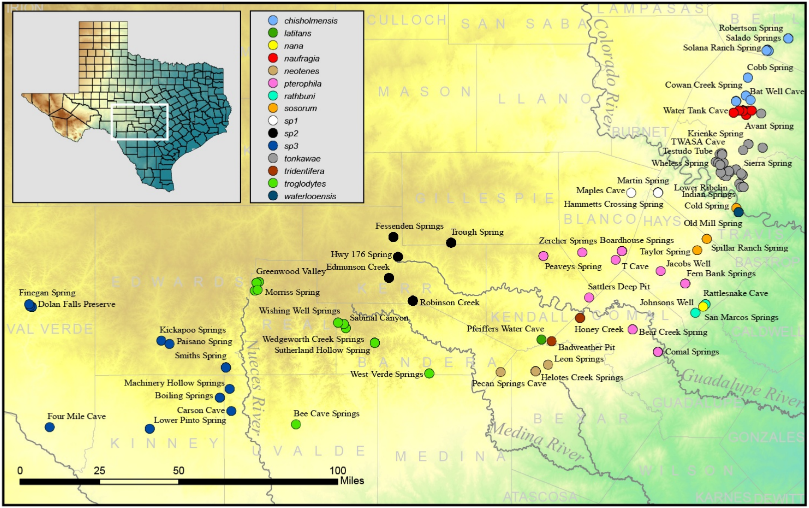
Our Study

How many species of *Eurycea* are there, really?

Our Study

How many species of *Eurycea* are there, really?
And is there introgression between them?

Our Study


chisholmensis
latitans
nana
naufragia
neotenes
pterophila
rathbuni
sosorum
sp1
sp2
sp3
tonkawae
tridentifera
troglodytes
waterlooensis
IRION
CULLOCH
SAN SABA
LAMPASAS
BELL
MASON
LLANO
Colorado River
BURNET
GILLESPIE
BLANCO
HAYS
TRAVIS
BASTROP
EDWARDS
KERR
KENDALL
COMAL
CALDWELL
VAL VERDE
REAL
BANDERA
GUADALUPE
Guadalupe River
Nueces River
BEXAR
Medina River
GONZALES
KINNEY
UVALDE
MEDINA
WILSON
ATASCOSA
KARNES
DEWITT
Robertson Spring
Salado Springs
Solana Ranch Spring
Cobb Spring
Cowan Creek Spring
Bat Well Cave
Water Tank Cave
Avant Spring
Krienke Spring
TWASA Cave
Testudo Tube
Wheless Spring
Sierra Spring
Martin Spring
Maples Cave
Lower Ribelin
Indian Springs
Hammetts Crossing Spring
Cold Spring
Old Mill Spring
Fessenden Springs
Trough Spring
Zercher Springs
Boardhouse Springs
Taylor Spring
Spillar Ranch Spring
Hwy 176 Spring
Edmunson Creek
Greenwood Valley
Peaveys Spring
T Cave
Jacobs Well
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Morriss Spring
Sattlers Deep Pit
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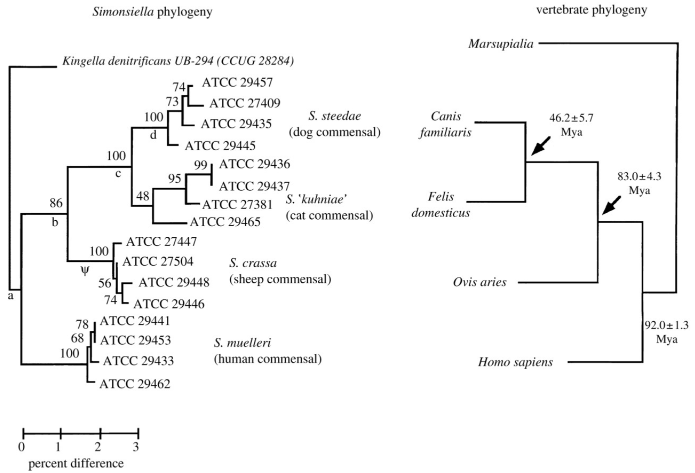
Wedgeworth Creek Springs
Sutherland Hollow Spring
Badweather Pit
Smiths Spring
Leon Springs
Comal Springs
West Verde Springs
Helotes Creek Springs
Pecan Springs Cave
Machinery Hollow Springs
Boiling Springs
Carson Cave
Lower Pinto Spring
Four Mile Cave
Bee Cave Springs
0
25
50
100
Miles

Phylogenetics



JP Staley, 2006. Figure 2

Maximum likelihood

Phylogenetics

Maximum likelihood is a framework for estimating phylogeny by modeling the process of evolution that generated our sequence data

Phylogenetics

- **Maximum likelihood** is a framework for estimating phylogeny by modeling the process of evolution that generated our sequence data
- Model-based

Phylogenetics

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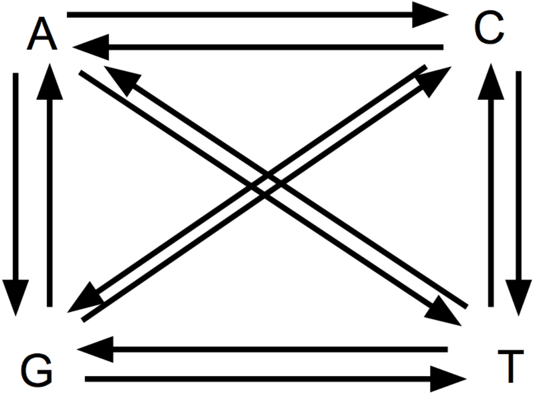
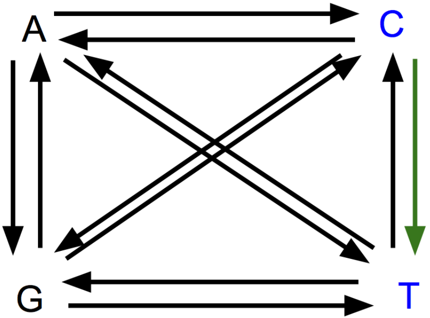
- Model-based: We make mathematically explicit assumptions

Phylogenetics

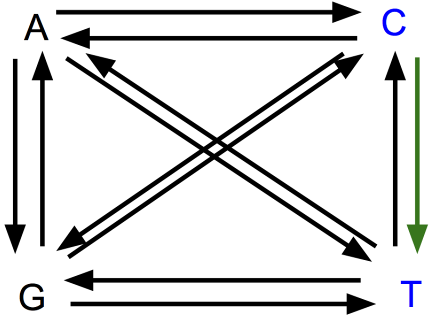


Phylogenetics



Probability of C to T change
Equilibrium frequency of C
0 * .25 = 0

Phylogenetics



Probability of C to T change
Equilibrium frequency of C
.75 * .25 = .1875

Phylogenetics

- **Maximum likelihood** is a framework for estimating phylogeny by modeling the process of evolution that generated our sequence data
- Model-based: We make mathematically explicit assumptions
- Statistically consistent

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Phylogenetics

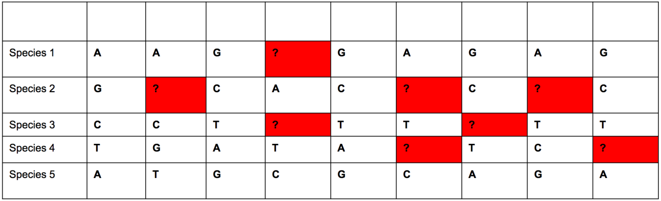
- **Problems**

Phylogenetics

- **Problems**
- Missing data

Phylogenetics

- **Problems**
- **Biased** Missing data



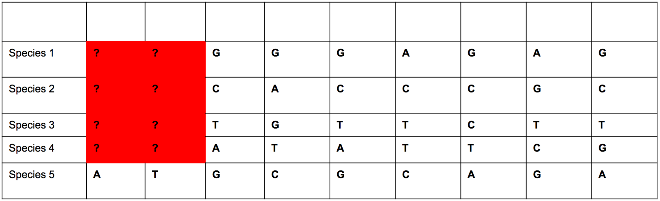
Species 1	A	A	G	?	G	A	G	A	G
Species 2	G	?	C	A	C	?	C	?	C
Species 3	C	C	T	?	T	T	?	T	T
Species 4	T	G	A	T	A	?	T	C	?
Species 5	A	T	G	C	G	C	A	G	A

Phylogenetics

- **Problems**
- **Biased** Missing data

Phylogenetics

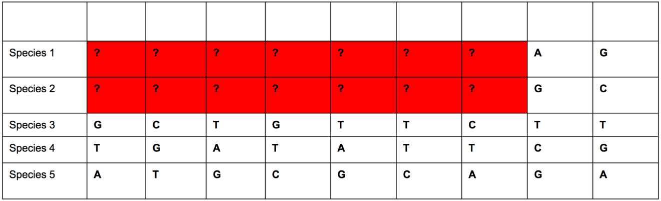
- Missing data concentrated in specific individuals



Species 1	?	?	G	G	G	A	G	A	G
Species 2	?	?	C	A	C	C	C	G	C
Species 3	?	?	T	G	T	T	C	T	T
Species 4	?	?	A	T	A	T	T	C	G
Species 5	A	T	G	C	G	C	A	G	A

Phylogenetics

- Missing data concentrated in specific individuals
- Missing data concentrated in certain loci in your data matrix



Species 1	?	?	?	?	?	?	?	A	G
Species 2	?	?	?	?	?	?	?	G	C
Species 3	G	C	T	G	T	T	C	T	T
Species 4	T	G	A	T	A	T	T	C	G
Species 5	A	T	G	C	G	C	A	G	A

Phylogenetics

- **Problems**
- Model misspecification

Phylogenetics

- **Problems**
- Model misspecification: when your data are not adequately described by your model

Phylogenetics

Today, we'll be visualizing our data at every step to try and minimize a bias in which individuals have missing data

Phylogenetics

We'll also look at ways to make sure we aren't overly-conservative in our choosing of SNPs (i.e., biasing our collection towards sites that exhibit little change)

The Demultiplex

One of the things that makes RADseq, and especially ddRADseq, so cheap is the pooling of samples

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One of the things that makes RADseq, and especially ddRADseq, so cheap is the pooling of samples

The way we recover individual samples is via demultiplexing

The Demultiplex

This allows for the cost-saving properties of batching, without the cost-increasing properties of synthesizing oligonucleotides.

The Demultiplex

The STACKS step for this is called Process RAD Tags

The Demultiplex

Output

- FASTQ files

The Demultiplex

Let's look at the output

- FASTQ files
- Reads, grouped by individual

The Demultiplex

Let's look at the output

- FASTQ files
- Reads, grouped by individual
- We haven't done any SNP calling. This is just the step that gets our data ready to do that

Initial Identification of SNPs

The STACKS step for this is called ustacks

Initial Identification of SNPs

Each RAD tag has usually been sequenced multiply per-individual

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This allows us to sort tags into "stacks" of identical and unique reads

Initial Identification of SNPs

Each RAD tag has usually been sequenced multiply per-individual
This allows us to sort tags into "stacks" of identical and unique reads
From these sets of identical and unique reads, we do a first pass at identifying SNPs.

Initial Identification of SNPs

Key Parameters

- -m: Minimum stack depth
- -M: Maximum mismatches allowed between reads in a stack

Initial Identification of SNPs

Other Parameters

- -i: ID for this sample

Exercise

One of the issues we discussed was biased missing data

Catalog Building

Once we have our within-individual stacks, we build a catalog of loci across individual catalogs (cstacks)

Catalog Building

Key Parameters

- -n: number of mismatches to allow between a putative tag, and a tag in the catalog

Outputting Data for Phylogenetics

The STACKS step for this is called populations

Outputting Data for Phylogenetics

A new file is needed, here: **the population map**

Outputting Data for Phylogenetics

Key Parameters

- -r: Percentage of individuals in a population that must have a locus to output it
- -m: Minimum stack depth at a locus

Exercise

Let's look at this output

Exercise

Let's look at this output

But we can also look in a more complex way: countPhyloMissing.sh and plotPhyloMissing.py

RAxML approximate likelihoods

RAxML

Dataset	L score
323320	-288336.664115
323340	-84377.460743
323360	-27407.770692
323380	-10281.525371
323390	-1699.210794

Lastly, let's build the tree

RAxML Approximate final L scores

Lastly, let's build the tree

Garli

Lastly, let's build the tree

Garli

Dataset	L score
323320	-287898.7874
323340	-84289.0263
323360	-27384.6012
323380	-10273.63021
323390	-1697.6284

Why

Tips	Number of unrooted (binary) trees	
4	3	
5	15	
6	105	
7	945	
8	10,395	
9	135,135	
10	2,027,025	
11	34,459,425	
12	654,729,075	
13	13,749,310,575	
14	316,234,143,225	
15	7,905,853,580,625	
16	213,458,046,676,875	
17	6,190,283,353,629,375	
18	191,898,783,962,510,625	
19	6,332,659,870,762,850,625	
20	22,164,309,5476,699,771,875	
21	8,200,794,532,637,891,559,375	
22	319,830,986,772,877,770,815,625	
23	13,113,070,457,687,988,603,440,625	> 21 moles of trees
24	563,862,029,680,583,509,947,946,875	

Why

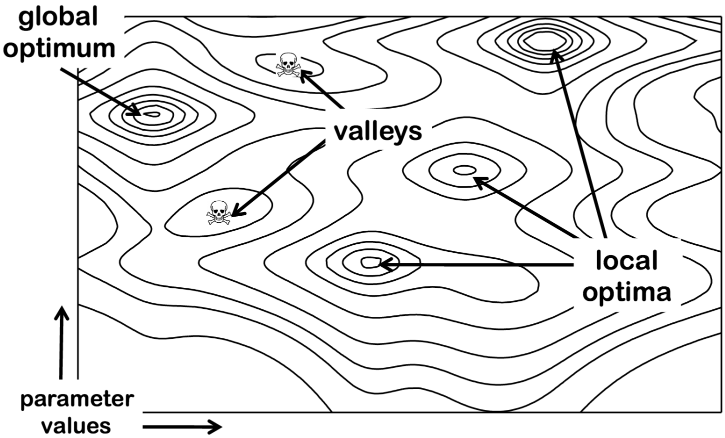

Image stolen, shamelessly, from Derrick Zwickl

Lastly, let's build the tree

Garli can take an input tree and optimize phylogenetic parameter estimates.

We actually had fantastic results doing this in a large scale phylogenetics paper (Wright et al. 2015)

Lastly, let's build the tree

Second-round optimization

Dataset	L score
323320	-287897.3609
323340	-84281.7290
323360	-27384.6012
323380	-10273.6302
323390	-1697.6283