Patterns of genetic variation in razorback sucker from Lakes Mohave and Mead





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Background

- Endemic to Colorado River system
- Live 40+ years
- Feed on algae, insect larvae, plankton, and detritus



- Grows up to 1 meter long and 5kg
- Abrupt, bony hump
- Iteroparous
- Highly fecund
 - ca. 200,000 eggs/large adult female



- Federally listed as endangered in 1991
 - 50+ yrs no natural recruitment in Lake Mohave
 - Water development
 - Non-native fish predation and competition







History of population declines

- Reservoirs fill
 - Populations expand
- Introduction of nonative species
 - Failure to recruit
 - Populations senesce and disappear
 - Demise hastened by large predators

Impact on genetic diversity

- Genetic diversity
 decreases with
 population size
 - Can have negative effects on health of population (e.g., inbreeding depression)
 - Can be used to monitor population size



Objective

 Use molecular markers to inform management of razorback sucker by monitoring patterns of genetic diversity



Mitochondrial DNA

- Small, maternally inherited
- Extremely variable
- SSCP
 - >33 haplotypes
- Parsimony analysis
 Differ by 1-2 bp





Nuclear DNA

- Microsatellites
- Tandem repeats (ex CACACACACACAC)



Microsatellites

- Biparental inheritance
- Also highly variable

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Individual 1: 72 76 bps 72 Individual 2: 74 76 bps 74 Individual 3: 76 76 bps 76

Statistics

- Allelic Richness: The number of alleles per locus corrected for sample size (not comparable across studies)
- Gene Diversity: A measure of genetic variation in a population (expected frequency of heterozygous individuals)
- F-statistics: measure distribution of genetic variation within and among groups
 - F_{IS} (within samples)
 - F_{ST} (among samples)
 - F_{sc} (among samples with regions/years)
 - F_{CT} (among regions or years)

Levels of focus/interest

- Geographic structure among extant populations (for context)
- Lake Mohave
- Lake Mead

Geographic samples

LOCATION	Ν
1. Lake Mohave	49
2. Lake Mead	16
3. Grand Canyon	NA
4. Lake Powell	23
5. Green River	29
6. Upper Colorado River	29

Samples collected in the mid to late 80's, early 90's



Levels of variation: microsatellites

	Allelic Richness							
				Green-	Upper			
Locus	Mohave	Mead	Powell	Yampa	Colorado	Total		
Xte1	2.00	2.00	2.00	2.00	2.00	2.00		
Xte2	2.40	2.94	2.00	1.60	2.90	2.73		
Xte4	6.04	3.87	5.31	5.24	1.00	5.13		
Xte7	8.62	6.87	4.79	3.80	2.59	7.16		
Xte8	12.18	10.69	8.78	8.08	7.45	11.20		
Xte10	8.42	7.99	7.93	7.11	4.00	8.07		
Xte11	10.16	8.87	8.74	9.29	3.90	9.96		
Xte12	7.96	7.93	6.81	7.26	4.00	7.80		
Xte14	6.23	4.81	5.31	5.11	1.00	5.13		
Xte16	15.44	13.00	11.28	7.50	5.56	12.54		
Xte17	10.22	9.00	5.67	7.93	3.00	8.72		
Xte18	6.74	4.94	4.00	4.52	3.68	5.84		
Xte19	10.34	8.75	7.62	4.63	2.90	8.80		
Xte20	17.95	18.42	11.38	8.68	2.68	15.00		
Xte22	17.33	14.55	10.64	10.62	4.97	14.57		
	9.47	8.31	6.82	6.22	3.44	8.31		

 Highest diversity downstream, in Lakes Mohave and Mead

Consistent with mtDNA

Population structure: microsatellites

$F_{ST} = 0.06$ Jackknife values mean = 0.06 <u>+</u> 0.01

From mtDNA $F_{ST} = 0.21$

STRUCTURE - Example

- Color = group identifier
- Columns = individuals
- Height = probability of assignment to specific groups

individual leg ara +191 ×251 ²robability of assignment loner eagle aravalioa cherry

k = 4 (50 replicates)

Geographic structure

- Consistency across replicates
- *K* = 2 or 3?
 - Upper Colorado River
 - Above and below Grand Canyon?
- Admixture
- Mohave and Mead not different from each other



Levels of focus/interest

- Geographic structure among extant populations
- Lake Mohave
- Lake Mead

How do we preserve the population in Lake Mohave?

- Need to increase population sizes while maintaining genetic diversity
 How?
 - Larvae have to be representative of wild adults
 - Repatriates have to reproduce



Lake Mohave Conservation management plan

- Initiated in mid-1990's
- Capture naturally produced larvae across regions throughout the spawning season to represent genetic diversity in the lake





- Reared in captivity
 - Hatcheries
 - Backwaters





- Release into the wild
 PIT tag
- Monitoring



- Native Fishes Work Group
- Genetics



mtDNA

- Previously published studies of mtDNA have shown that genetic variation is being maintained by this sampling strategy
- For example, variation among larval samples within areas, not among areas

Year	# samples	gene diversity	allelic richness
1997	13	0.703	4.79
1998	17	0.634	4.69
1999	11	0.639	4.37
2000	15	0.691	4.69
2001	9	0.688	4.54
2002	14	0.657	4.37
2003	14	0.693	4.70
2004	21	0.643	4.61
2005	17	0.695	5.11
2006	23	0.684	4.95
2007	12	0.721	5.81
ANOVA	4		
F		0.83	1.19
Р		0 60	0.30

Distribution of variation among larval samples

			among samples					
			among samples		within areas		among area	
Year	# samples	Ν	FST	Р	FSC	Р	FCT	Р
1997	13	339	0.083	<0.0001	0.101	<0.0001	-0.021	0.840
1998	19	485	0.043	<0.0001	0.046	<0.0001	-0.003	0.500
1999	13	294	0.041	<0.0001	0.053	<0.0001	-0.013	0.715
2000	16	367	0.049	<0.0001	0.058	<0.0001	-0.009	0.758
2001	10	230	0.100	<0.0001	0.101	<0.0001	-0.001	0.522
2002	14	348	0.020	<0.0001	0.024	<0.0001	-0.004	0.651
2003	14	370	0.06	<0.0001	0.037	<0.0001	0.023	0.069
2004	24	559	0.147	<0.0001	0.138	<0.0001	0.01	0.240
2005	17	437	0.059	<0.0001	0.058	<0.0001	0.001	0.380
2006	24	598	0.062	<0.0001	0.063	<0.0001	-0.0004	0.430
2007	13	308	0.043	<0.0001	0.054	<0.0001	-0.012	0.740
average			0.064		0.067		-0.003	

Variation among samples within areas, not among areas

Distribution of variation among larvae, adults, and repatriates

SOURCE

Among groups $F_{ST} = 0.003$ Among samples within groups $F_{SC} = 0.004$ Among larvae, repatriates, and adults $F_{CT} = -0.001$

No differences among larvae, repatriates, and adults!

What about nuclear genome?

- Genotyped 1560 Larvae (1997-2009) at 15 microsatellite loci
 - 7000+ larvae samples
 - Random sample of 120 from each year
 - Allows us to examine nuclear DNA across years



- Larval allelic richness

 Varied from 16.22 (2004) to 17.32 (1997)
 Average = 17.10
 - Allelic diversity has been maintained



Variation among larval samples

- Smaller than estimates
 from mtDNA
 - -Consistent with mode of inheritance
- Very little difference among samples within regions (F_{SC}) or among regions (F_{CT})
 - -Consistent across years

Year	F _{sc}	F _{CT}
1997	0.010	0.001
1998	0.009	0.002
1999	0.011	0.003
2000	0.013	-0.001
2001	0.016	0.001
2002	0.008	0.000
2003	0.005	0.008
2004	0.014	0.001
2005	0.007	0.001
2006	0.013	0.004
2007	0.003	-0.001
2008	0.012	0.002
2009	0.005	0.000
average	0.010	0.002

Sampling - adults

- Genotyped 979 adult (300 wild and 679 repatriate) fin clips at 15 microsatellite loci
- Repatriates stocked in years 1992-2008
- Allows us to examine nuclear DNA across stocking year classes



- Adult allelic richness
 - Varied from 7.07 (2008) to 8.04 (2003)
 - Average of 7.57 (allele numbers corrected for sample size)
 - Allelic diversity has been maintained across stocking years and relative to wild adults



Distribution of variation among stocking years

- F_{ST} (among year variation) = 0.004
- Similar genetic composition among repatriates across stocking years

Conclusions Lake Mohave

- All measures of genetic variation consistent among samples of larvae and repatriates
- What about transmission from larvae to adults?
- Requires direct comparison which has not been completed yet



Transmission of variation from larvae to repatriates?

- Cannot compare allelic richness directly from these analyses because of different sample sizes
- Gene diversities are comparable

 Repatriate diversity of 0.75 (SD = 0.01)
 Larval diversity of 0.74 (SD = 0.01)
- Therefore, program is maintaining genetic variation!

Levels of focus/interest

- Geographic structure among extant populations
- Lake Mohave
- Lake Mead

Samples

- Far fewer samples
- Larvae
 - Echo Bay (EBL) 1997 N = 25
 - Echo Bay (EBL) 2002 N = 30
 - Las Vegas Bay (LVL) 2002 N =27
- Adults
 - Mixed locations from late 80's N = 13
 - Echo Bay (EB) 2002 N = 11
 - Las Vegas Bay (LV) 2002 N =18

mtDNA

	Ν	gene diversity	allelic richness
larvae			
Echo Bay - 97	25	0.610	2.84
Echo Bay - 02	30	0.706	3.83
Las Vegas Bay - 02	27	0.641	3.22
adults			
mixed - late 80s	16	0.125	1.69
Echo Bay - 02	11	0.710	4.00
Las Vegas Bay - 02	18	0.660	4.07

- Generally lower diversity in larvae (excluding 80's adult sample)
- Increased diversity in newer samples, especially for the adults
- Lower gene diversity than Lake Mohave (ave. = 0.74)

Distribution of variation among Lake Mead samples

- F_{ST} (total among group variation) = 0.216
- F_{sc} (among samples within adults or larvae) = 0.221
- F_{CT} (adult vs. larvae) = 0.007
- Lots of variation, with differences due to individual samples (similar to Lake Mohave)
- Much larger than Lake Mohave
 - Largest estimate among larval samples within years was $F_{ST} = 0.147$ (ave = 0.064)
 - Among adults and larvae $F_{ST} = 0.003$
- Comparable to estimate for entire Colorado drainage $F_{ST} = 0.21$

What about nuclear genome?

	larvae			;			
	EBL - 97	EBL - 02	LVL - 02	mixed 80s	EB - 02	LV - 02	TOTAL
Xte1	1.99	2.00	1.94	2.00	2.00	2.00	1.99
Xte2	1.00	1.00	1.00	2.69	2.00	2.56	1.54
Xte7	4.14	5.36	7.05	7.48	6.00	6.54	6.88
Xte8	6.29	7.98	8.11	10.48	9.00	10.49	9.14
Xte11	8.06	8.90	6.96	9.36	10.00	8.91	9.21
Xte12	7.40	7.88	6.14	7.65	6.00	8.32	8.04
Xte16	9.14	9.20	8.59	11.00	9.00	10.81	11.44
Xte17	8.60	8.78	8.33	11.00	9.00	8.01	9.92
Xte18	5.33	5.72	5.31	5.85	4.00	5.74	5.59
Xte19	7.92	7.09	5.70	7.79	8.00	6.79	7.94
Xte20	9.99	9.86	9.76	13.83	13.00	12.35	12.54
Xte22	8.13	9.16	9.46	12.03	11.00	13.19	11.69
Xte24	9.07	9.47	10.45	9.36	8.00	14.12	11.62
Xte25	2.00	2.37	2.41	2.85	2.00	2.95	2.43
average	6.36	6.77	6.52	8.10	7.07	8.06	7.85

Allelic richness

- Less diversity in larvae than adults

Distribution of variation among samples

- F_{ST} (total among group variation) = 0.031
- F_{sc} (among samples within adults or larvae) = 0.033
- F_{CT} (adult vs. larvae) = -0.005
- Slightly higher than Lake Mohave
 - Average $F_{SC} = 0.010$
 - Average $F_{CT} = 0.002$
- Lower than estimate from entire drainage

 $-F_{ST} = 0.06$

Conclusions Lake Mead

- Less diversity in larvae than adults
- More variation among samples

 Sampling effect?
- Increased mtDNA diversity over time
 - Sampling effect?
 - Additional input source?
- Require additional sampling to understand dynamics of this system