Population Genetics and Breeding System of *Lophophora* spp.

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Population Genetics

‘Explain all that,’ said the Mock Turtle.

‘No, no! The adventures first,’ said the Gryphon in an impatient tone: ‘explanations take such a dreadful time.’

—Lewis Carroll,
The Adventures of Alice in Wonderland

Population Genetics: the Process

- Collect tissue samples (preferably *in situ*) from a number of individual plants in each population of interest.

- Extract DNA from all individual tissue samples.

- Select genetic markers – in this case, microsatellites, which are short repeating sequences of bases in DNA, e.g., GTGTGTGTGTGTGT (dinucleotide microsatellite) and CTTCTTCTTCTT (trinucleotide microsatellite).

  Microsatellites are inherited exactly like codominant genes, and they can give us genetic information about breeding systems and also about phylogenetic relationships among species, subspecies, varieties, hybrids, populations, and even among individuals *in situ* in wild populations.

Population Genetics: the Process

- Synthesize published primers or primers designed *de novo*. Primers are required for PCR.
- For each individual plant DNA, run PCR (polymerase chain reaction) with locus-specific primers and the enzyme DNA polymerase. PCR makes millions of copies of the microsatellite.
- Run the PCR product of each individual plant DNA on electrophoresis, which separates shorter DNA fragments from longer DNA fragments and tells their exact size.
- DNA fragments containing different forms of the same microsatellite are called alleles. Alleles are identified by their length in base pairs. E.g., if the allele containing the microsatellite $(AG)_{10}$ has a length of 80 base pairs, then the allele containing $(AG)_{11}$ will have a length of 80 + 2 = 82, the $(AG)_{12}$ allele will have length of 80 + 4 = 84, etc.

Population Genetics: the Process

- Every individual plant has two alleles of a given microsatellite: one allele on each homologous chromosome in a pair. Homologous chromosomes normally come in pairs.
- But there are usually more than two alleles in a population.
- If the two alleles are identical (same length), then the plant is a homozygote, and electrophoresis shows only a single peak.
- If the two alleles are different (not same length), then the plant is a heterozygote, and electrophoresis shows two peaks.

Population Genetics: the Process

- The length of the two microsatellite alleles in an individual plant's DNA, and their equal length (homozygosity) or unequal lengths (heterozygosity) constitute the genotype of the individual plant with regard to a specific microsatellite marker.

  Example: The six individuals sampled in a hypothetical population have the following genotypes:

  80 80 (homozygote)
  80 82 (heterozygote)
  80 84 (heterozygote)
  82 82 (homozygote)
  82 86 (heterozygote)
  86 86 (homozygote)

  The data show four known alleles in this population: 80, 82, 84 and 86.

  There are equal numbers of homozygotes and heterozygotes (three of each) among the six individuals sampled.

Population Genetic Data: Interpretation re Breeding System

- Inbreeding in a population leads to an increase in the fraction of homozygotes and a corresponding decrease in the fraction of heterozygotes in the population. Self-fertilization (autogamy) is the ultimate extreme of inbreeding.

- Many *Lophophora williamsii* populations are known to be comprised of individuals that are largely self-fertilizing (G. Rowley, 1980).

- When we find a population where all sampled individuals are uniformly homozygous for the same allele, that is evidence for a very high (up to 100%) rate of self-fertilization.

- When we find a population with some heterozygotes (but substantially less than 50% of the population) or all homozygotes but with more than one allele (e.g., genotypes like A_1A_1 and $A_2 A_2$), that is evidence for a lower, but non-zero, rate of self-fertilization.

Lophophora microsatellite data, Locus (Primers) Lw14

Montane population of *L. fricii*

Individual	Genotype
VIS-1	184 184
VIS-2	ND*
VIS-3	184 184
VIS-4	184 184
VIS-5	184 184
VIS-6	184 184
VIS-7	184 184

Laguna population of *L. fricii*

Individual	Genotype
AMP-1	184 184
AMP-2	184 184
AMP-3	184 184
AMP-4	184 184
AMP-5	184 184
AMP-6	184 184
AMP-7	184 184
AMP-8	184 184

*ND = no data.

Lophophora microsatellite data, Locus Lw14

Central population of *L. diffusa*

Individual	Genotype
QRM-1	***198*** ***198*** (?)
QRM-2	***198*** ***198*** (?)
QRM-3	178 178
QRM-4	178 178
QRM-5	178 178
QRM-6	178 178
QRM-7	178 178
QRM-8	178 178

Northern population of *L. diffusa*

Individual	Genotype
QRN-1	178 178
QRN-2	178 178
QRN-3	178 178
QRN-4	178 178
QRN-5	178 178
QRN-6	178 178
QRN-7	178 178

Lophophora microsatellite data, Locus Lw14

El Huizache pop of *L. williamsii*

Individual	Genotype
HUI-1	175 175
HUI-2	175 175
HUI-3	175 175
HUI-4	175 175
HUI-5	175 175
HUI-6	175 175
HUI-7	175 175
HUI-8	175 175

Dr. Arroyo pop of *L. williamsii*

Individual	Genotype
SOL-1	169 169
SOL-2	169 169
SOL-3	169 169
SOL-4	169 169
SOL-5	***166 166***
SOL-6	169 169
SOL-7	169 169
SOL-8	169 169

Lophophora microsatellite data, Locus Lw14*

South Texas pop of *L. williamsii*		West Texas pop of *L. williamsii*	
Individual	Genotype	Individual	Genotype
RES-1	159 159	STR-1	ND
RES-2	159 159	STR-2	165 165
RES-3	ND	STR-3	165 165
RES-4	159 159	STR-4	165 165
RES-5	159 159	STR-5	165 165
RES-6	159 159	STR-6	165 165
RES-7	159 159	STR-7	165 165
RES-8	159 159	STR-8	165 165

* The data for the two populations in this table were generated in 2005 with a different instrument than that used to generate the data reported here in other tables. The allele lengths in this table may not agree exactly with the allele lengths in the other tables due to procedural or calibration differences. It is nevertheless clear that the difference between the two alleles in this table is six bp, or two repeats of the Lw14 trinucleotide microsatellite.

Lophophora microsatellite data, Locus Lw14

Central pop of *L. diffusa*

Individual	Genotype
QRM-1	***198*** ***198*** (?)
QRM-2	***198*** ***198*** (?)
QRM-3	178 178
QRM-4	178 178
QRM-5	178 178
QRM-6	178 178
QRM-7	178 178
QRM-8	178 178

Northwestern pop of *L. koehresii*

Individual	Genotype
JNG-1	***198*** ***198*** (?)
JNG-2	178 178
JNG-3	178 178
JNG-4	178 178
JNG-5	178 178
JNG-6	178 178
JNG-7	178 178
JNG-8	178 178

Lophophora microsatellite data, Locus (Primers) Lw14

Central pop of *L. diffusa*

Individual	Genotype
QRM-1	***198 198*** (?)
QRM-2	***198 198*** (?)
QRM-3	178 178
QRM-4	178 178
QRM-5	178 178
QRM-6	178 178
QRM-7	178 178
QRM-8	178 178

Northwestern pop of *L. koehresii*

Individual	Genotype
JNG-1	***198 198*** (?)
JNG-2	178 178
JNG-3	178 178
JNG-4	178 178
JNG-5	178 178
JNG-6	178 178
JNG-7	178 178
JNG-8	178 178

Population of *L. alberto-vojtechii*

LAV-1	178 178
LAV-2	178 178
LAV-3	178 178
LAV-4	178 178
LAV-5	178 178

Lophophora microsatellite data, Locus Lw14

Central pop of *L. diffusa*

Individual	Genotype
QRM-1	***198 198*** (?)
QRM-2	***198 198*** (?)
QRM-3	178 178
QRM-4	178 178
QRM-5	178 178
QRM-6	178 178
QRM-7	178 178
QRM-8	178 178

Northwestern pop of *L. koehresii*

Individual	Genotype
JNG-1	***198 198*** (?)
JNG-2	178 178
JNG-3	178 178
JNG-4	178 178
JNG-5	178 178
JNG-6	178 178
JNG-7	178 178
JNG-8	178 178

Population of *L. alberto-vojtechii*

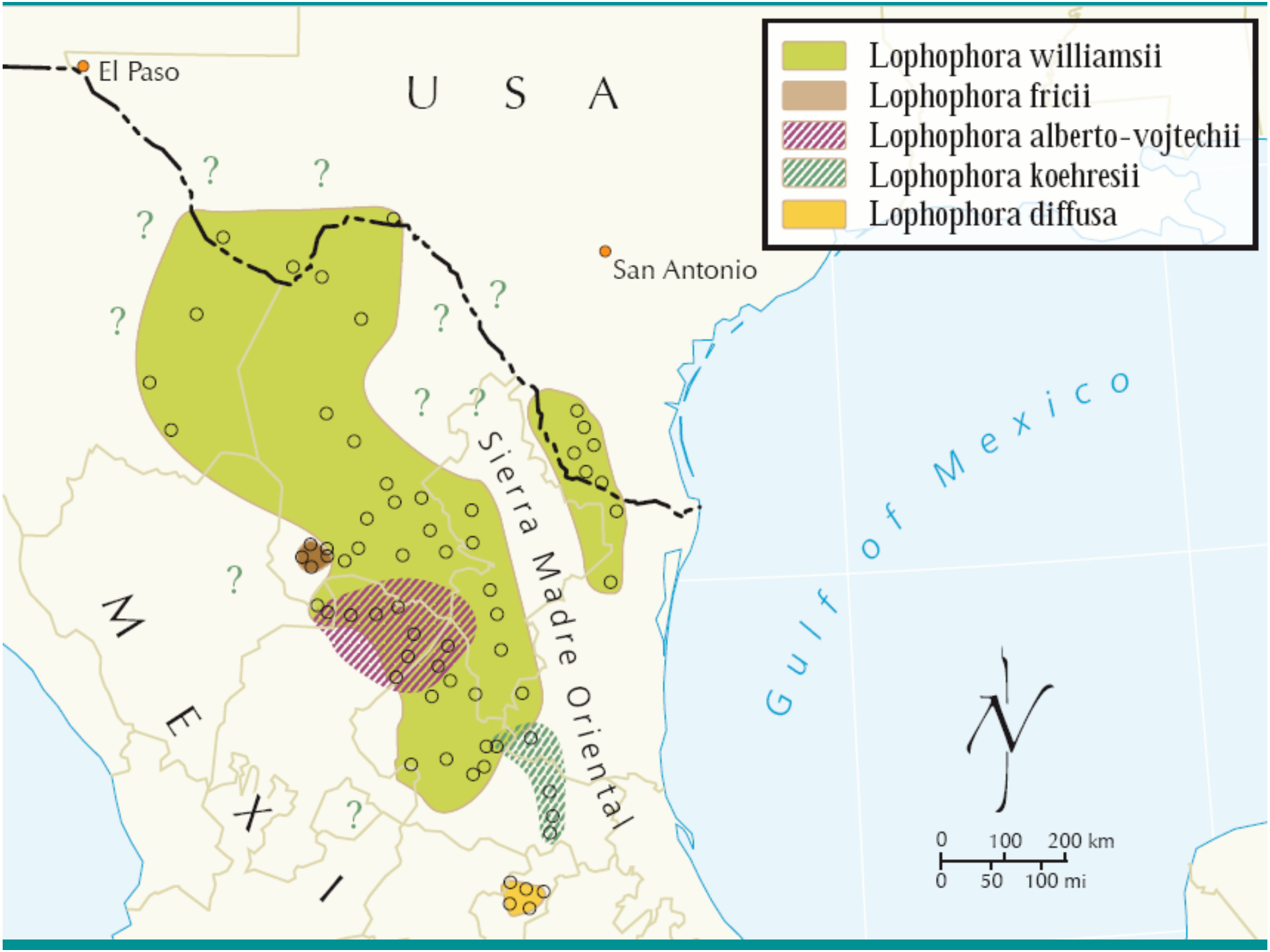
LAV-1	178 178
LAV-2	178 178
LAV-3	178 178
LAV-4	178 178
LAV-5	178 178

Three clones of *L. jourdaniana*

LJ-1	162 162(?)
LJ-2	162 162(?)
LJ-3	162 162(?)

Summary: genotype data for Lw14

Population (species)	Predominant Genotype
• HUI pop (*L. williamsii*)	175 175
• SOL pop (*L. williamsii*)	169 169
• W. TX pop (*L. williamsii*)	165 165
• S. TX pop (*L. williamsii*)	159 159
• *L. jourdaniana*	162 162
• QRM, QRN pops (*L. diffusa*)	178 178
• JNG pop (*L. koehresii*)	178 178
• N. SLP pop (*L. alberto-vojtechii*)	178 178
• VIS, AMP pops (*L. fricii*)	184 184


Lophophora williamsii
Lophophora fricii
Lophophora alberto-vojtechii
Lophophora koehresii
Lophophora diffusa
El Paso
San Antonio
U S A
M E X I
Sierra Madre Oriental
Gulf of Mexico
N
0 100 200 km
0 50 100 mi