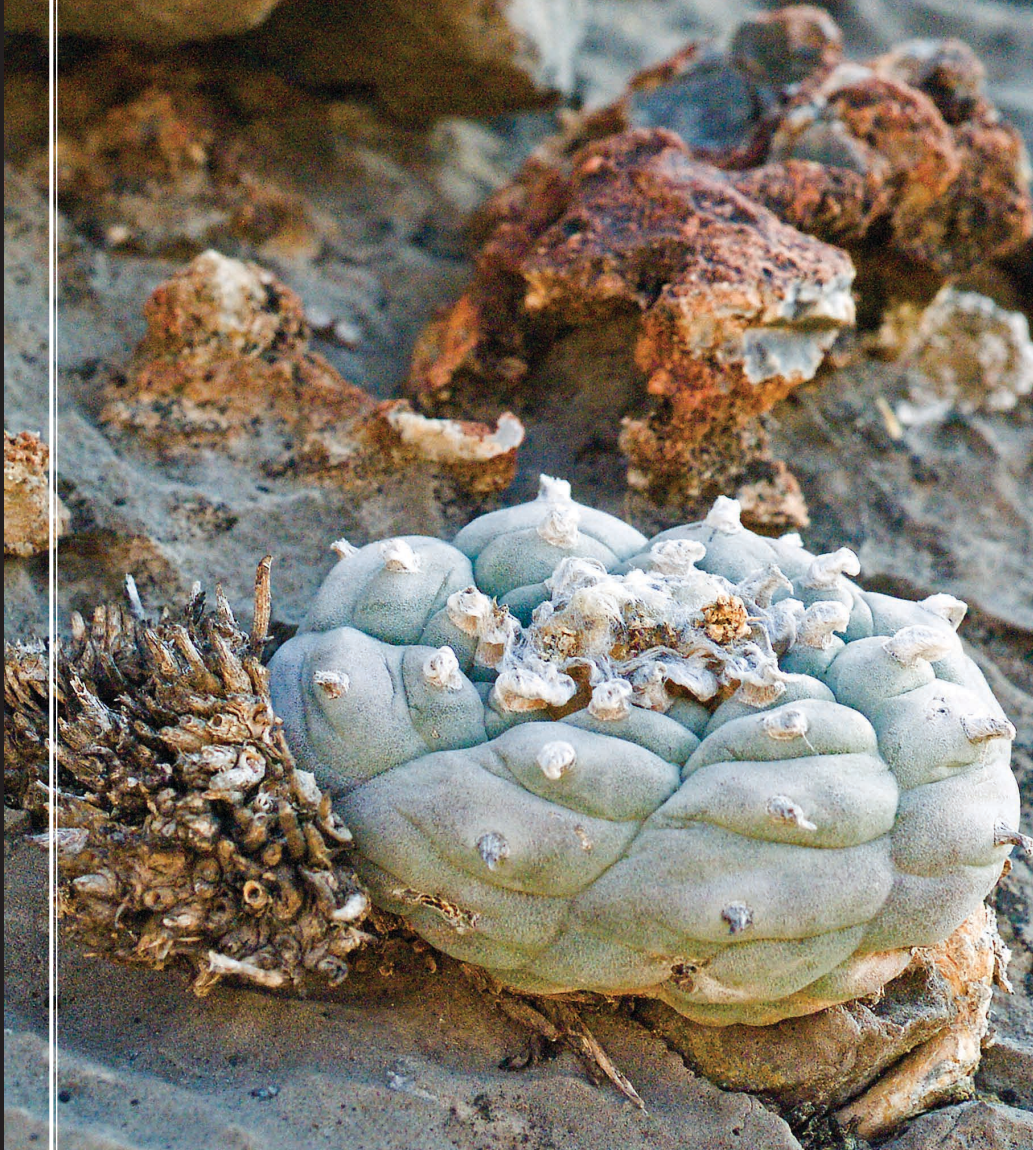


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Stalking the wild *Lophophora*

PART 2 *Zacatecas, San Luis Potosí, Nuevo León, and Tamaulipas*



Heading south from Saltillo, Federal Highway 54 leaves Coahuila and cuts across the eastern edge of the state of Zacatecas. Our first attempt to find a lophophora after crossing the border brought us to GPS coordinates now in the middle of a newly plowed field. The area was generally disturbed, and we found no *Lophophora williamsii* in the surrounding brush, although the habitat was superficially similar to the Tamaulipan thornscrub that makes up what members of the Native American Church call the “Peyote Gardens” of South Texas.

In the vicinity of San Tiburcio, Zacatecas, we found a typical population of *L. williamsii*, replete with all the usual companion plants of the Chihuahuan Desert, including candelilla (*Euphorbia antisiphilitica*), lechuguilla (*Agave lechuguilla*), leatherstem (*Jatropha dioica*), and tasajillo (*Opuntia leptocaulis*). We parked in a pulloff on the side of the highway and followed an old ranch road leading back into the desert scrub. Within 50 meters of walking we found our first small, mature cluster of *L. williamsii* right beside the road beneath a mesquite tree. It turned out that the road followed a contour of particularly good limestone soil, and the only plants we found for the first hour or so

were in that stratum. They were not abundant, and only when we were about to leave the site did Robert find a denser stand of plants on the northern slope of the next low limestone hill to the south.

San Luis Potosí

It seems as if everyone in the world who has any interest in *Lophophora* gravitates to the flats west and southwest of Real de Catorce in San Luis Potosí. We likewise succumbed to this attraction, partly to collect DNA samples from a well-known population, and partly to assess the impact of many years of “narcotourism” and other commercial enterprises that depend on the harvesting of peyote. On one side of the road where we stopped to investigate there was a newly plowed field. On the other side of the road was what appeared to be an old agricultural field with vestiges of plowed rows, now regrown with creosote bush and little else. There was some native brush in a strip running parallel to the road, and there we found a few small specimens of *L. williamsii*, but we were desperately trying to find just ten plants to complete our DNA sampling when a goatherd came walking along with about forty goats. We chatted about the marginal state of the goat business and local attitudes about peyote. He said that in spite of the suppos-



L. williamsii is sparse in the alluvial flats below Real de Catorce, San Luis Potosí, reportedly due to chronic over-harvesting concomitant to “narcotourism” and other commercial peyote harvesting enterprises, despite large signs warning: “The extraction and illegal trafficking of peyote is a federal offense.”

edly strict enforcement of laws to punish outsiders who might extract peyote from this area (which is protected as Wirikuta, the sacred land where the Huicholes come annually to gather peyote) there were people who had hauled out great quantities of peyote from local populations for sale in some unspecified distant market. I told him that we were searching for peyote for a scientific study, but that we were having difficulty finding enough plants. He looked down at the ground where we were talking and pointed with his herding stick: “There’s one.” And then there was another, and another, until we had our ten tissue samples. The sound of the goat bell disappeared into the deepening dusk as

we walked back to the truck. We found a small restaurant in the nearby town and enjoyed some local cuisine and a cold beer before heading for Matehuala.

My pickup began running hot as we approached the town of El Cedral (The Cedar Grove), so we bought some antifreeze and stayed at a convenient hotel near the Pemex station. I spent most of the next day getting a new water pump located, purchased, and installed in Matehuala while Robert and Lia processed a fraction of each of the tissue samples we had collected, grinding the tissue up into a solution designed to preserve DNA in the field until it can be extracted

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L. williamsii lays low at a site north of Doctor Arroyo, Nuevo León.



the Hitchhiker's Guide

to molecular systematics

The accompanying article can be read as a picaresque account of the quixotic adventures of botanists zig-zagging through northeastern Mexico from one set of GPS coordinates to the next, collecting DNA samples of various populations of cacti in the genus *Lophophora* and simultaneously taking stock of the conservation status of those populations. But what is the point of collecting those DNA samples? In Part 1 we described the process of extracting DNA from cactus tissue, but then what does one do with the extracted DNA?

The adjective “genetic” (from the noun “genesis”) refers to origins. DNA can be considered the point of origin of the processes that create and maintain the structure and function of living organisms. Traditional taxonomy (which concerns itself with naming and classifying organisms according to their similarities and differences) and systematics (which concerns itself with the relationships among organisms based on their evolutionary history) traditionally made use of morphological characters (the visible form of the plant) as the basis for evaluating relatedness among different organisms. In other words, up until the 20th century, if we wanted to compare different species and assess how closely related they were, we would look at their anatomical *structures* as the basis for comparison. (This was particularly convenient, because it also worked for fossils of long-extinct species.)

Then we became more adept in organic chemistry and found that closely related species of plants would produce identical or closely related chemicals (such as alkaloids), and in the 1960s there was a blossoming of plant systematics based on these *phytochemical* characters.

Meanwhile, the picture became more complicated as ethologists made systematists aware of *behavioral* differences that could be used to compare related species, while biochemistry was producing a generation of protein chemists who taught the systematists to do electrophoresis (a technique which separates molecules migrating through a gel by differences in

their electrical charge, shape, and/or size) to separate proteins such as enzymes, which could be used to detect distinctions between related genera or species, and sometimes even between subspecies/varieties.

All of those types of characters and techniques focused upon various aspects of the *phenotype* of the organism, which is the tangible or detectable expression of its underlying *genotype*. The genotype consists of the DNA sequence (sequence of nucleotides in the DNA) of a defined and specific part of the total genetic makeup (the genome) of the organism. While the structure of DNA had been elucidated by the middle of the 20th century, it wasn't until the last quarter of the century that DNA sequencing techniques became widely available. Once it was possible to analyze the exact sequence of nucleotides in DNA—and thus to know the genotype itself rather than its phenotypic manifestations—most of the older techniques became obsolete, at least in the minds of the emerging army of molecular biologists. Why, they reasoned, should one work with old, blunt instruments to obtain data that would at best yield an indirect, partial, and often ambiguous expression of the genotype, when one could now analyze the genotype itself, directly, totally and unambiguously, thereby cutting through to the ultimate genetic truth?

This view of the primacy of DNA research in US biological and medical science gained quick acceptance in circles that controlled the purse strings of government funding of academic research. (As Shakespeare might have put it, the DNA's the thing Wherein I'll catch the funding from the King.) And this in turn led to schisms in academic institutions, where the “Haves” (the well funded molecular biologists) were enviously derided as “gene jockeys” by the “Have-nots” (the organismal biologists) who continued to do biological research on a shoestring in the traditional ways. (Incidentally, as an organismal biologist who revels in the mysteries of the whole organism and simultaneously appreciates the analytical power that DNA research allows, I refuse to participate in this still-smoldering war.)

In practical terms, the first question to be answered is, what sort of DNA locus is most likely to be useful for this specific problem? The answer is largely determined by taxonomic hierarchy. For instance, if one is sorting out relationships among families within an order, it might be appropriate to use slowly evolving segments of DNA such as genes that code for functional proteins. The sites for viable mutations (the stuff of which evolution is made) are restricted in such a gene to nucleotides whose replacement does not cause severe or lethal dysfunction of the protein that is the product of the gene. That means that it

takes a relatively long time (evolution is measured in geologic time) for enough viable mutations to occur and become established in the gene pool, so that significant sequence differences can be seen between organisms representing different higher taxa.

If one is working at the species level, however, DNA segments of protein-coding genes are not likely to show sufficient change over the relatively short time it takes for species to evolve from a common ancestor. What is needed is rapidly mutating DNA. Such DNA is *selectively neutral*, meaning that a mutation in such a locus (segment of DNA) will have no effect on the survival or reproductive success of the organism. A neutral mutation has no effect on essential proteins, so the mutation has a reasonable chance of being preserved in the gene pool.

What kinds of DNA are presumed to be selectively neutral? (Here's where things get complicated.)

One type is known as *spacer DNA*, found between tandemly arranged genes. The function of spacer DNA is to separate "busy" genes that are subject to frequent transcription (reading). Spacer DNA is not itself transcribed to RNA as genes are.

Another type of selectively neutral DNA is transcribed. Coding segments of a gene (*exons*) are separated from each other by segments of DNA called *introns*, which are transcribed to RNA along with the exons of a gene, after which the RNA segments corresponding to the introns are edited out and the transcribed gene fragments spliced together to make a continuous, finished messenger RNA, which is then translated into a protein. Introns may not consist *entirely* of selectively neutral DNA, but major portions of many introns are thought to be mutationally neutral.

A third useful type of DNA locus is called a microsatellite. Such loci are likewise thought to be largely selectively neutral. Microsatellites consist of simple DNA-sequence repeats arranged in tandem; for instance, GAGAGAGA. These constitute the fastest-evolving type of DNA loci, because not only are they subject to "normal" mutations that occur at the rate of one mutation per 10,000 to 100,000 DNA base pairs per generation, but microsatellites also have their own accelerated type of mutation. When DNA is being replicated, the enzyme responsible for making a new DNA strand from the template of an old DNA strand is called DNA polymerase. What is postulated to happen with microsatellites is that when DNA polymerase is confronted with so many tandem repeats (like GAGAGAGA...), the enzyme undergoes "polymerase slippage," meaning that it either "drops a stitch" (that is, deletes one of the GA repeats in the example) or inserts one too many "stitches" (that is, inserts an extra GA repeat in the example). This process often results in a microsatellite locus with 10 or 15 different alleles (different DNA sequences

within the locus), all of which would differ from each other by the number of simple sequence repeats (the number of tandem GA repeats in the example given). Such a locus would be described as highly polymorphic and would likely be a sensitive genetic marker for analyzing either population structure or phylogenetic structure at the species or subspecies/variety level. (Are you still with me?)

So how do we generate usable data from our DNA samples using microsatellites? First, we go through a lengthy lab procedure to "capture" microsatellite loci from the DNA of our target species and grow bacterial clones containing the various microsatellites captured (the more loci used, the more sensitive and accurate the results will be). Then we sequence the microsatellite loci and use the sequences to design primers. If the primers work—that is, if they succeed in producing millions of copies of a given microsatellite in PCR (polymerase chain reaction)—then we send them to someone who puts fluorescent labels on the primers. At that point, after testing the fluorescent-labeled primers to confirm that they work, another PCR is run, using the fluorescent primers and DNA from our field-collected tissue samples. The products of that reaction are then put into an expensive electrophoresis instrument which detects the alleles of a given microsatellite by the fluorescence provided by the fluorescent primers. That process gives the exact length of each allele to the nearest single DNA base pair, so that you know how many different alleles you have detected in the population sampled, as well as the frequency of each allele in the population. It also tells you whether each individual plant sampled is a heterozygote (having different alleles on its two chromosomes that contain the locus) or a homozygote (having the same allele on both chromosomes). The latter information in turn tells you about the breeding system of the plant, with a high percentage of heterozygotes in the population indicating a high degree of outcrossing (individual plants fertilizing other individuals) and a high percentage of homozygotes indicating a high degree of inbreeding (self-fertilization being the extreme of inbreeding).

OK, then, skipping the rest of the population genetic analysis, how do we use the microsatellite dataset to determine how the various geographically defined groups of individuals sampled are related to each other phylogenetically? The short answer consists of one word: software. There are a number of programs that analyze microsatellite datasets and produce trees showing phylogenetic affinities among the various operational taxonomic units that we input. Would you like to know how many species of *Lophophora* there are? So would we, but we don't have the dataset completed yet, much less the analysis. Please stay tuned for the results of the analysis, coming soon at a *Haseltonia* near you.





- ▲ *L. koehresii* near Tula, Tamaulipas. Plants here grow in the mud beneath large nurse shrubs, are almost all solitary, and are relatively small compared to other species of *Lophophora*. Can you spot the African-native succulent, *Kalanchoe*?
- ◀ Near Miquihuana, Tamaulipas, the peyote plants are nearly all caespitose (clump forming).

► Wild *Lophophora* continued from page 223

in the lab. Matehuala was already hot at the end of May, and by four o'clock in the afternoon we were all quite ready to get on the road and stir up a breeze, when finally the truck was declared "listo."

Nuevo León

We headed for the fair city of Doctor Arroyo, and then north for a few miles, where we pulled into a small village nestled against some low mountains. A rain shower was just ebbing, and we had about an hour of daylight left. It didn't take long to find the first *L. williamsii*, but we were pleasantly amazed by the abundance and diversity of other cactus species. We gawked over all these species that none of us had ever seen before and took many pictures. Eventually one of the villag-

ers showed up to see what we were doing. When he found out we were interested in cacti he gave us a brief ethnobotanical tour of the common local species of edible and medicinal plants. This was delightful and informative, but used up precious daylight.

The GPS coordinates we had were misleading; following them, I ended up high on a mountain overlooking the village, where I got a fabulous view of the sun setting, but encountered no *Lophophora*. In the end, most of the *L. williamsii* plants we found were right along the road that skirted the edge of the village. The last two samples were collected in the dark. We had an unmemorable supper out of cans from the back of the truck, and slept soundly on the ground until dawn arrived in a mist.

After attaining a caffeinated semblance of

consciousness, we walked around the village to say our goodbyes to our lecturer/host of the previous evening, then fired up the truck and struck off to the east.

Tamaulipas

Miquihuana is a town on the western edge of Tamaulipas, at about the same latitude as Ciudad Victoria on the other side of the Sierra Madre Oriental. The population of *L. williamsii* near Miquihuana was one that Ted Anderson examined in his PhD thesis¹. It is a unique population that continues to puzzle and fascinate students of *Lophophora* to this day. It appears that virtually 100% of the plants are caespitose, and the plants begin to sprout lateral branches as seedlings², whereas the plants in other *Lophophora* populations generally start such branching only after reaching maturity, if at all. In terms of stem morphology and color, the Miquihuana plants bear a striking resemblance to the plants of the “Peyote Gardens” of South Texas, some 300 km to the north. In terms of their breeding system, however, they seem to have a closer affinity to the populations around El Huizache, about 120 km to the south. The northern plants are known to be autogamous³ (self-fertile), whereas greenhouse observations⁴ suggest that the Miquihuana plants, like those of El Huizache to the south, are obligate outcrossers (self-sterile). So how could we possibly neglect to include the Miquihuana population in our DNA-based phylogenetic study of the genus?

Locating the plants in Miquihuana was another matter. The only GPS coordinates we had led us to a roadside location where we were unable to find a single plant in an hour of careful searching, though the habitat looked reasonable for *Lophophora*. We put away the GPS data, and I fell back on my imprecise memory of the plants’ location from a visit some years before. After a chain of successive conversations with local people, we finally found someone who knew where the peyote grew. But then we had to get permission to collect our samples, which involved talking with a local official who, when I produced a blanket *permiso* that had been skillfully crafted by Héctor Hernández in anticipation of such situations, stared at it for a moment, then handed it back to me and asked me to read it to him. It only slowly dawned on me that this man could not read. The only condition he imposed on us was that we not divulge the location of the population to anyone, as such publicity could only result in problems, including increased risk of decimation of the rather small population, which the local people value greatly and harvest sustainably for therapeutic use (particularly as a

topical analgesic for sore muscles). We collected our tissue samples from the relatively dense population of multi-stemmed plants growing among small agaves and *Larrea*, said our thanks and goodbyes, and departed.

From Miquihuana, the gravel road took us eastward, up and over an arm of the Sierra Madre Oriental, through forests of arborescent yuccas in the high canyons, and down in a southeasterly direction until we hit Highway 101. We turned south, heading to the northernmost known population of *L. koehresii* in the town of Tula. We got to the spot after a hard thundershower, just as it was getting dark, and slept in the truck parked by the side of the road.

The next morning we awoke to a thoroughly soaked desert. The *L. koehresii* were there, all right—some of them within a few steps of the road, covered with wet mud. Others nearby had been washed to a pristine green by the rain. It was the first time I had ever seen *Lophophora* growing in mud flats, but it would not be the last. One of the hallmarks of *L. koehresii* is its ecological disposition to spurn the (often hilly) limestone habitat of *L. williamsii* in favor of low-lying mud flats. In spite of the fact that there was considerable human foot traffic through the area, probably associated with nearby agriculture, we saw no sign of harvesting. This may be a direct result of the fact that, like *L. fricii* and *L. diffusa*, *L. koehresii* is lacking in pharmacologically active concentrations of mescaline. These non-*williamsii* species of *Lophophora* have gained the reputation among Huicholes as “the peyote that makes you sleepy”⁵, which is perfectly compatible with data indicating that pellotine is the principal alkaloid in these species⁶. Pellotine was marketed as a sleeping aid in the early 20th century, before it was rendered economically obsolete by the advent of barbiturates, which proved much less expensive to manufacture⁷. By the time we finished exploring the Tula population and collecting the required tissue samples, we were all—like many of the *Lophophora koehresii*—well covered with mud.

Continued in Part 3: “*San Luis Potosí (again), Querétaro, and Mexico City.*” 🍷

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