Phylogeny of Sonoran Desert Milkweeds Resolved with Plastid Genome Sequences

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Abstract: The Sonoran Desert Clade milkweeds have very unusual traits, including species that are nearly leafless and shrubby. This clade includes 9 species for which we estimate a phylogenetic tree and use this information to better understand why species within this clade are diverging from their ancestral species. To do this we use plastid genomes to find genetic differences among the species. We assembled and aligned plastomes for multiple individuals of each species and found the maximum likelihood phylogenic tree. From the results we found that the Sonoran Desert Clade forms one clade rather than multiple. We found interesting relations between some of the species within the Sonoran Desert Clade. There was hybridization that we found within the results. This will help us further our study on the evolution of the *Asclepias* species.

Keywords: *Asclepias*; genome skimming; plastome; contigs; chloroplast content

Introduction

The milkweed genus *Asclepias* has an impact on the field of ecology and has provided a greater understanding of the evolution of plant reproduction strategies (Wyatt and Broyles 1994). The evolution in milkweed leaf surfaces provides data that shows the cause of adaptation to water efficiency (Agrawal et al. 2009a). Another major focus is the coevolution of *Asclepias* defenses and cardenolide-sequestering herbivores, for example the monarch butterfly (Malcomb and Brower 1986). Milkweed species produce toxic compounds for defense called cardenolides and a white latex. The monarch butterfly is one of several species that has been discovered use *Asclepias* species as a food plant despite these defenses.

The genus *Asclepias* has over 130 species that are located in North America, the northern parts of South America, and multiple parts of Mexico. The phylogeny of *Asclepias* consists of 15 well-supported clades that clashes strongly with classification formed on floral traits (Woodson 1954). This implies extreme lability of flower morphology and occurrences of convergence in floral forms. From the phylogenetic framework, Fishbein and others formulated and tested their hypotheses for the evolution of plant defenses against herbivores, including the existence of plant defense syndromes (Agrawal and Fishbein 2006), evolutionary increase and decline in plant defenses (Agrawal and Fishbein 2008; Agrawal et al. 2008, 2009b), and a connection between plant defenses and adaptive radiation (Agrawal et al. 2009a). Understanding the evolution of *Asclepias* has been based on plastid phylogenies (Fishbein et al. 2011, 2018). Determining the phylogeny of *Asclepias* is more tractable because of the probable absence of polyploidy and apomixis (Wyatt and Broyles 1994; Albers & Meve 2001). The most important discovery about the phylogeny of *Asclepias* so far is that all South American species share a common ancestor within the Incarnatae clade (Fishbein et al. 2018).

In my project, I am contributing to a study to better estimate the phylogeny of the genus *Asclepias* from plastome sequences (Fishbein et al. 2018). In particular, my part of the project is focused on the Sonoran Desert Clade. A plastome is a full DNA sequence of the plastid (a general term for chloroplasts and related organelles) chromosome. The main

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function of the chloroplast is to achieve the process of photosynthesis (Neuhaus et al. 2000). The plastid genome is present on a single chromosome that is separate from the chromosomes in the nucleus.

From past studies on the Sonoran Desert Clade there has been a discovery of interlocus incongruence (Straub et al. 2011). Fishbein et al (2011), found *A. subulata/A. masonii* and *A. albicans/A. subaphylla* to be sister-species, but these relationships weren't found by Straub et al. (2011) using nuclear and mitochondrial DNA sequences. Conflicting phylogenies of the plastid genome and other genomes in the core Sonoran Desert Clade may derive from the effect of additional within-species sampling, perhaps involving incomplete linkage sorting (Straub et al. 2011).

In my project, I am working with all 9 species of the Sonoran Desert Clade: *A. cutleri,* located in the Colorado Plateau; *A. coulteri*, located in the Sierra Madre Oriental; *A. leptopus,* found in the Sierra Madre Occidental*; A. macrotis,* located in the Chihuahuan Desert; and *A. sperryi,* also found in the Chihuahuan Desert. In addition, I am working with the core, four species of unusually large, leafless milkweed shrubs from the Sonoran Desert and adjacent regions: *A. albicans* and *A.subulata* are common throughout the Sonoran Desert; *A. subaphylla,* is located on a narrow strip of coastal dunes just south of the Sonoran Desert; and *A. masonii* is located on an island off the coast of Baja California Sur within the Sonoran Desert. What makes these species unique for milkweeds is that they are nearly leafless and occur, in very xeric vegetation types. To study the phylogeny of Sonoran Desert Clade species I am analyzing data from over 50 samples. Figure 1. shows where the species are located geographically.

There are two main objectives of my research. First, we are trying to answer what the differences are in plastome sequence within these species and between species. In particular how many Single Nucleotide Polymorphisms (SNPs) are there and
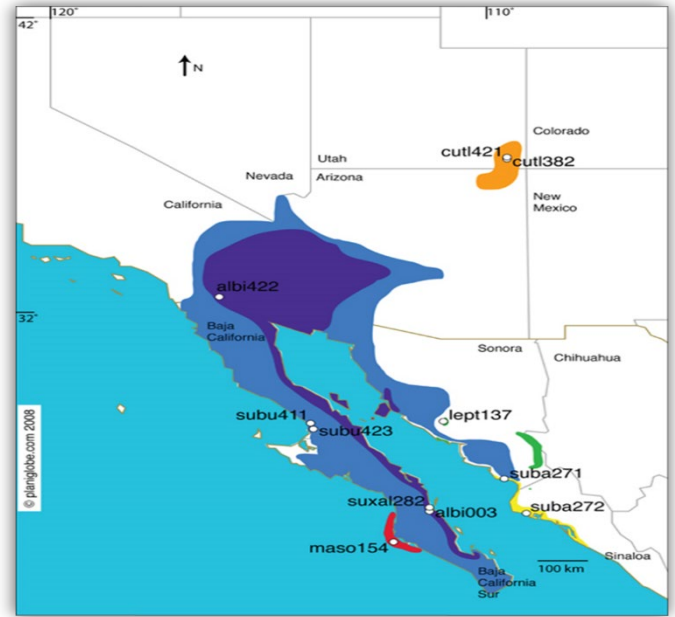

Figure 1: Location of Sonoran Desert Clade species: A. cutleri *(orange),* A. leptopus *(green),* A. masonii *(red),* A. subaphylla *(yellow),* A. subulata *(blue & purple). Three other species occur in the east and are not displayed (*A. coulteri*,* A. macrotis*,* A. sperryi*).*

where they are located in the plastomes. Second, the plastomes will be used to estimate a phylogenetic tree. The expected results from this research are to identify why species are diverging from their ancestral species. Researches assume that the cause of evolution is the need to adapt for survival. However, researchers do not always have evidence for how evolution takes place. For this report, I will only present results from the second objective of analyzing the phylogeny.

Methods

Prior to this project DNA of the samples was extracted from field collections and herbarium specimens. The genomic DNA was sequenced using "genome skimming", which is a method for obtaining plastome sequences (Straub et al. 2011). The data set I analyzed included previous published sequences for the Sonoran Desert Clade, new sequences obtained from Dr. Shannon Straub's lab (unpubl. data), and 17 new species I assembled (Table 1).

Table 1: Voucher information for the 17 newly assembled plastomes.

Species	Voucher and Library Database Number
Asclepias albicans	Fishbein 6465 DNA 00412
Asclepias coulteri	Steinmann 5133 DNA 01790
Asclepias coulteri	Zamudio 10782 DN 01797
Asclepias coulteri	Hernández 1497 DNA01847
Asclepias cutleri	Coburn 2549 DNA01606
Asclepias cutleri	Coburn 2533b DNA0164
Asclepias cutleri	Lynch 12122 DNA 00917
Asclepias cutleri	Coburn 2530 DNA01659
Asclepias leptopus	Van Devender 2016-33 DNA01788&2132
Asclepias leptopus	Reina 2016-47 DNA01821
Asclepias leptopus	Fishbein 2164 DNA02013
Asclepias macrotis	Reina 2006-695 DNA00589
Asclepias macrotis	McLaughlin 5976 DNA00825
Asclepias macrotis	McLaughlin 5976 DNA00825
Asclepias macrotis	Coburn 2643 DNA01751
Asclepias masonii	Fishbein 3099 DNA01787
Asclepias sperryi	McVaugh 10659 DNA01596

To assemble the plastomes of each sample, I used computer programs called Geneious (Fishbein et al. 2018) and IOGA (Bakker et al. 2016) following the steps of Fishbein et al. (2018).

First, sequence reads were filtered and trimmed to remove low quality data. Then, using Geneious or IOGA I made a *de novo* assembly of the sequence reads of each sample. In the *de novo* assembly reads are stacked together if they have long regions of overlapping similarity. The stacks of these sequencing reads are called contigs. Next, I found portions of contigs that had a high similarity to a reference plastome from (*A. nivea*_022431) using BLAST (Fishbein et al. 2018). These were aligned to the reference to produce a draft plastome of each sample. To finish the assembly of the plastome sequence the trimmed reads were mapped to the draft to fill in gaps between contigs.

After assembling the plastomes a multiple sequence alignment was formed using the online implementation of MAFFT (Castresana et al. 2000). To properly root the phylogenetic tree, representatives of American and African milkweeds were included in the alignment and used as outgroups.

Mesquite (Fishbein et al. 2018) was used to exclude poorly aligned parts of the alignment using GBLOCKS (Fishbein et al. 2018). To obtain the phylogenetic tree from the alignment IQTREE (Nguyen et al., 2015; Chernomor et al., 2016) was used. First, IQTREE was used to find the best fitting model of sequence evolution. This was then used to estimate the maximum likelihood (ML) phylogeny with clade support estimated by fast bootstrapping.

Table 2: These values show the amount of depth of coverage, number of reads, and length of assembled plastomes from the new 17 samples.

Individuals within the Species	Depth of Coverage	Reads Assembled	Length of Assembled Plastome (BP)
A. albicans 412	38.1	376,737	136,024
A. coulteri 1790	44	53,549	145,930
A. coulteri 1797	67.8	83,892	145,930
A. coulteri 1847	75.5	96,622	145,930
A. cutleri 1606	310.9	341,066	134,004
A. cutleri 1645	347	376,737	136,024
A. cutleri 0917	106.1	146,955	135,347
A. cutleri 1659	227.3	246,798	136,069
A. leptopus 2132	55.8	64,520	136,308
A. leptopus 1821	121.4	138,136	136,251
A. leptopus 2013	211.8	214,830	136,946
A. macrotis 0589	150.7	174,424	134,772
A. macrotis 0825	360.6	411,110	134,924
A. macrotis 1742	187.7	188,484	136,165
A. macrotis 1751	292.2	288,904	132,178
A. macrotis 1787	26.8	39,914	129,899
A. sperryi 1596	61.9	89,164	135,669

Results

I assembled new plastomes from 17 samples. The samples ranged from 39,914 to 411,100 reads assembled per sample, 26.8 to 360.6 depth of coverage of assembled plastomes, and 129,899 to 136,946 total length of the assembled plastomes (Table 2). I found that the species from the Sonoran Desert Clade formed a single clade (Figure 2). All of the samples for five of the nine species were found to be each other's closest relatives: *A. cutleri*, *A. macrotis*, *A. sperryi*, *A. leptopus*, and *A. masonii*. There was only one sample of *A. subaphylla* so there was not enough information to know whether all
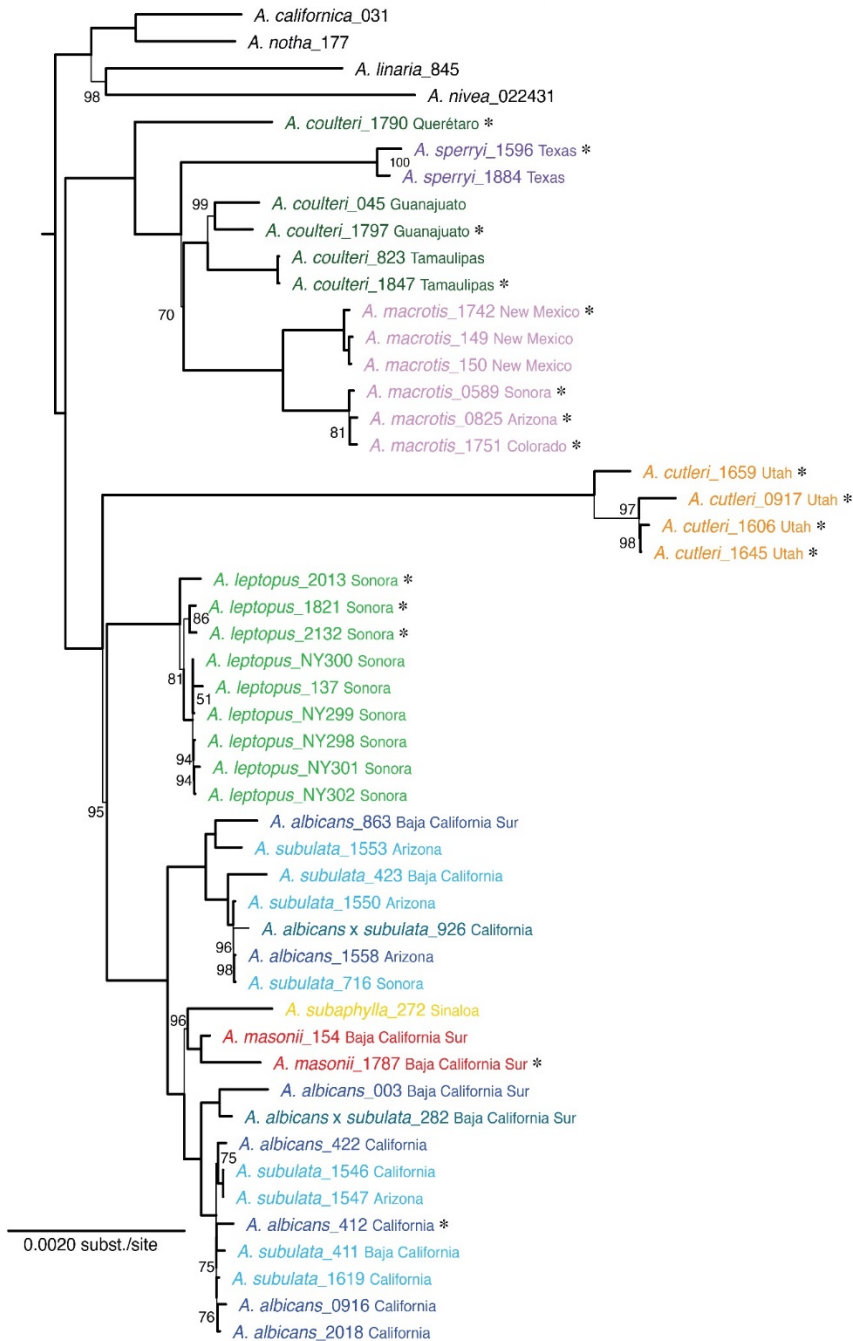
Figure 2: Maximum likelihood tree of the Sonoran Desert clade tree. Colors represent individuals of the same species. States are included from where the individual is collected. Branches in bold have 100% bootstrap support; numbers indicate support less than 100%.

samples would form a group of closest relatives.

For the remaining three species there was at least one individual that grouped with a different species (Figure 2.). For example, *Asclepias coulteri* #1790 was found to be as closely related to samples of *A. macrotis* and *A. sperryi* as to the remaining *A. coulteri* samples. More conspicuously, samples of *A. albicans* and *A. subulata* were intermixed in two separate clades (Figure 2).

Discussion

The phylogeny of the *Asclepias* plastome sequences is resolved with bootstrap values mostly between 90 and 100%. However, the resolution of the phylogeny is better supported here rather than past studies. The results demonstrate the original step in diversification of eastern and western clades (Figure 2). The eastern clade includes: *A. coulteri*, *A. sperryi*, and *A. macrotis* (Figure 2). The western clade includes: *A. cutleri*, *A. leptopus*, *A. subulata*, *A. subaphylla*, *A. masonii*, and *A. albicans*. From this we can see that individuals within a species that came from relatively close geographical locations seem to be more closely related on the phylogenetic tree (Figure 2).

The phylogeny shows that the large desert shrubs (*A. masonii*, *A. subaphylla*, *A. albicans*, *A. subulata*) evolved from a small ancestral species that would have been similar to *A. cutleri*, *A. leptopus*, *A. macrotis*, *A. sperryi*, and *A. coulteri*. Also shows *A. cutleri* on a very long branch compared to other species on the tree. This may be a result of the species undergoing more evolutionary change than other species, but it is unknown why and this will require further study.

An interesting relationship found was the relationship between *A. masonii* and *A.*

*subulata (*Figure 2)*.* We expected that *A. masonii* and *A. subaphylla* were separately evolved from the ancestral shrubby desert milkweed because they are geographically separated. These species are very rare and are found in separate geographical regions. Therefore, it was interesting to see how closely they are related from the phylogeny.

There is known hybridization between *A. albicans* and *A. subulata.* The sampling included two suspected hybrids, which were placed in two different clades, indicating that they had two separate origins (Figure 2).Increased sampling of individuals in this study showed that most species are genetically isolated from each other, but that *A. albicans* and *A. subulata* seem to be exchanging plastomes extensively. From this we can begin to understand how it is very common for these two species to experience hybridization.

These results are found useful in furthering the knowledge and understanding of relationships of the species within the Sonoran Desert Clade. This contributes to understanding the pattern of evolution of *Asclepias* species and how they have adapted to the dry environments of the Sonoran Desert Clade.

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