JT SCIENCE

A newsletter to promote a better understanding of the natural, physical, & cultural environment in Joshua Tree National Park

Managing Recreational Use in a Changing Landscape at Joshua Tree National Park 2

Joshua Tree's Native Legumes and their Microbial Partners: Effects of Host Species and Location 9

Insights Into the Clonal Tales of Chollas 15

JT SCIENCE

VOLUME 04

FALL 2024

OUR MISSION
Joshua Tree National Park Association (*www.joshuatree.org*) works in partnership with Joshua Tree National Park to help in its achievement of programming goals in education and interpretation, along with scientific and historical research and activities.

BEING A PART OF THE ADVENTURE
We operate four visitor centers and one park store that are often the first stop for visitors from around the world. We also offer a field institute, with classes taught by experts in natural sciences, cultural history, and the arts; and we raise funds via donations and our membership program. Visit a park store to learn about wildflower identification, birding, geology, stargazing, native plants and local history; pick up climbing and hiking guides; or sign up for a Desert Institute field class and make the park your classroom.

YOUR MEMBERSHIP MATTERS
As a member you support scientific research, the park's historical collections, youth programs, and assist in the preservation of our fragile desert. Email **membership@joshuatree.org** to find out more!

JT SCIENCE
Editor: Tasha La Doux
Design: Andrea Good
Oversight Committee:
Sheri Shiflett, Jacqueline Guevara, Michael Vamstad, Jay Goodwin, and Anna Tegarden

All papers are peer-reviewed anonymously for the authors prior to acceptance.

Questions?
Contact jtresearchgrant@joshuatree.org

Welcome to JT Science

The mission of JT Science is to promote a better understanding of the natural, physical, and cultural environment in Joshua Tree National Park (JTNP). Each issue highlights scientific endeavors being conducted in JTNP and provides an opportunity for scientists, land managers, and the general public to know and appreciate this desert region.

In memory of Marian Huff Tremblay
In May of 2023, we lost one of our long-standing and ardent supporters, Marian H. Tremblay. Marian was the Director of Business Operations at Joshua Tree National Park Association for 18 years. While she performed her job with fervor and proficiency, she did more than manage budgets and programs for the park. Marian truly cared about the future of JTNP and all things desert. To honor Marian's many years of hard work and dedication to the previous iterations of the Graduate Student Research Grant, we have renamed the grant: it is now called the Marian Huff Tremblay Science Scholarship.

Joshua Tree National Park Association is providing funding for the JTNP Science Research Grant Program, directly supporting this science-based newsletter for the public, as well as annual funding for the Marian Huff Tremblay Science Scholarship. The goal of the scholarship is to support graduate student researchers conducting independent field studies in JTNP and more specifically to support high-priority research needs that inform park management of socio-cultural, natural, and wilderness resources identified by the park.

Grant awardees will participate in the *Slice of Science* lecture series hosted by the Desert Institute. In addition, the students will provide manuscripts for JT Science, public outreach materials for JTNP Interpretation staff, as well as final reports summarizing their findings. By offering up to $5,000 to each student, this program provides opportunities for the student to gain experience with grant and report writing skills, budget management, and most importantly, to demonstrate how their research can apply to land management issues.

To date, we have supported over 40 students, through which we have gained insight into such things as visitor awareness of tortoises; conservation implications for fringe-toed lizards and Joshua trees; microbial diversity in soils; rare plant life history; insect inventories; and discoveries of new-to-science species of poppies and green algae. The impact of the JTNP Science Research Grant Program and the contributions made by these scientists have been richly rewarding.

Enjoy the newsletter.

Managing Recreational Use in a Changing Landscape at Joshua Tree National Park

By Jane Rodgers[1] and Sheri Shiflett[1], Ph.D.

[1]Affiliation: Joshua Tree National Park

INTRODUCTION

Joshua Tree National Park (JTNP) was first designated as a National Monument in 1939 and elevated to National Park status with the passing of the California Desert Protection Act in 1994. Currently the park encompasses nearly 800,000 acres, most of which is roadless Wilderness, providing expansive opportunities for visitors to experience solitude and personal challenge in an undeveloped desert landscape. The park also provides campgrounds, interpretive tours, signage at pull-outs, as well as many miles of short hiking trails. Until recently, these developed areas, which have expanded in footprint since the 1970s to accommodate gradually increasing use, provisioned ample opportunity and space for the various types of recreational use occurring in the park. However, annual visitation at JTNP exceeded two million for the first time ever in 2015 and nearly three million annual visits by 2018. This increase far outpaced the park's capacity to support such a rapid uptick in use, especially for brief stops to take social media photos and use restrooms (Fig. 1) (*1*).

A study published in 2024 showed that since 2010, United States National Parks with high social media exposure, such as JTNP, have experienced visitation increases of 16–22% relative to parks with less exposure (*2*). By 2013, smartphones were owned by over 50% of the U.S. population (*3*) and quickly became a common tool for trip planning.

> Access to information transformed the desert from a risky, inhospitable destination into one that is inviting, hip, and liberating.

Visitors can quickly dispel the myth of the desert being a treacherous place that is always hot, dry, and full of snakes by using smartphone applications that provide reliable weather forecasts, navigational tools, and connections to amenities (e.g. lodging, gas, supplies). Smartphones also created endless opportunities for real-time popularizing of photogenic destinations. Many reasons have been proposed for the doubling of park visitation between 2015 and 2018 in JTNP; however, in our opinion, a strong case can be made that the combination of access to information by smartphones and increased use of social media platforms (*2, 3, 4*) created a snowball effect firmly establishing the park not only as a popular destination for recreation, but also as a backdrop for advertising, films, weddings, and social media influencer posts.

The dramatic increase in visitation in a short period of time put park managers in a reactive mode. Managers were metaphorically "building the plane while flying it." In other words, the last decade has been a balancing act for JTNP employees to conduct day-to-day park

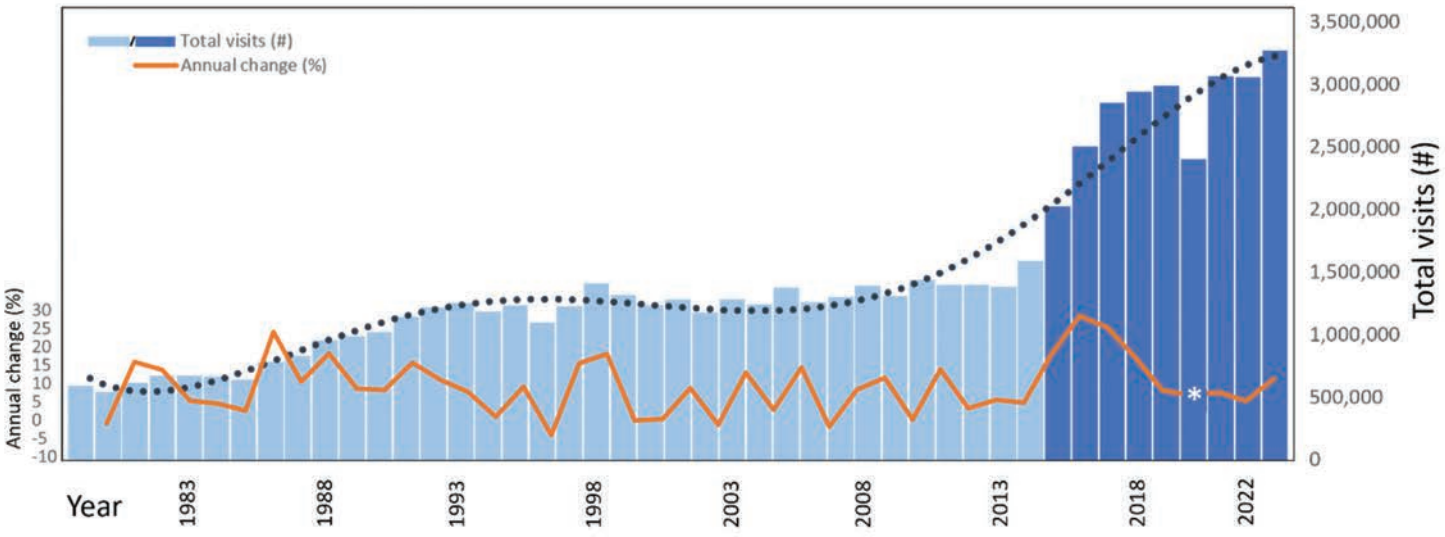


Figure 1. Visitation rates at JTNP from 1979–2023. Blue columns show the total number of visits each year, while the dashed line represents a polynomial best-fit line (R-squared = 0.94). Rate of change in visitation from year to year is represented by an orange line. Light blue bars (1979–2014) correspond to an average annual rate of change of 3%, whereas dark blue bars (2015–2023) correspond to an average annual rate of change of 10%. **Due to anomalous visitation associated with COVID-19, the year 2020 was excluded from calculations of annual change in visitation.* **Data from NPS Stats: NPS Visitor Use Statistics** (*1*).

operations while they perform triage actions (e.g., all hands-on-deck traffic management, rapid-response signage and messaging, painting curbing red) in the moment and build long-term visitor use management (VUM) strategies for the future.

Faced with unprecedented traffic congestion, user conflict, and damage to park resources, a tactic of immediate triage was employed in the short term, and with time, the triage has been coupled with data-informed strategic planning, which has proven effective in tempering the havoc created by the sheer numbers of people in the park. Park managers estimate it will take another ten years of facility improvements and stewardship actions to achieve sustainable visitation, assuming visitation remains near the current levels. However, if the visitation rate continues to grow, there is no telling if or where it might top out. The park surpassed 3 million visits in 2021, and by 2023, it witnessed nearly 3.3 million visits (Fig. 1).

As visitation numbers have risen, so has damage to park resources (e.g., vegetation trampling, soil compaction, roadkill, trail proliferation, graffiti, illegal off-road driving, etc.) at or near popular destinations. This resource damage is a direct result of increased vehicles and visitors concentrated in areas made popular through the park brochure and publicly generated online information (e.g., blogs and social media posts). Parking lots quickly reach full capacity, causing people to make destructive choices with their vehicles, such as parking outside official parking areas on fragile habitat and crushing native vegetation (Fig. 2). The sheer number of people gathering in areas that are not meant to hold large crowds has resulted in the proliferation of informal and unwanted "social" trails (Fig. 3).

> In desert environments it only takes a few individuals walking through an area to easily create what appears to be a trail, which in turn attracts more use, compaction, and widening of the unwanted trail.

To combat issues such as trail proliferation and trampling sensitive soil crusts, other desert parks (e.g., Arches NP, Canyonlands NP) and community organizations (e.g., Moab Trail Ambassadors) have launched remarkably successful social media campaigns and programs, demonstrated to influence visitor behavior towards enhanced park stewardship, which could serve as models for JTNP (*5*). Starting in 2017, JTNP social media platforms embraced the "Recreate Responsibly" campaign to expand messaging for both public safety and resource protection; the impact of these types of campaigns can be hard to measure.

To mitigate issues associated with increased visitation at JTNP, park managers and staff have been working in two parallel tracks: immediate triage and long-term strategic planning. Both tracks use the principles of Engineering, Education, and Enforcement (the "3Es"), a safety and management paradigm promoted by the Interagency Visitor Use Management Council (*6, 7*). The "3Es" provide strategies to increase recreational opportunities, reduce impacts of visitor use, increase durability of the resource, or limit use where appropriate (*6, 7*). At JTNP, solving visitor use challenges entails approaching each problem with due consideration to feasible solutions that consider visitor engineering of physical solutions (e.g. fencing, improved parking, clearer signage), education, and/or enforcement of park regulations. The 3E approach applies to immediate problem solving, as well as strategic long-term solutions. Triage efforts are nimble, include some risk of failure, and may not always address the root cause of a recreational use issue. In contrast, long-term strategies require enough time to gather information, obtain funding, meet planning requirements, implement solutions, and test their effectiveness. Both approaches require patience, public trust, and endurance. With this article, we hope to convey some of the challenges the park has encountered, as well as the outcomes of the park's efforts to mitigate the issues.

EMERGING ISSUES

Studying the change in visitation over time demonstrates how quickly the landscape changed and the issue of "too many people in the park" emerged. Starting in 1979 through 2014, JTNP experienced an average annual growth rate in visitation of 3% (representing 28,550 additional visits per year), after which visitation rose dramatically and average annual growth rate from 2015–2023 was 10% (representing 283,700 additional visits per year, an order of magnitude greater than that of the preceding 35 years) (Fig. 1). In 2015 and 2016, the growth rate was 27% and 24%, respectively, representing approximately 450,000 additional visits each year (Fig. 1). To put this into

Figure 2. More visitors than parking spaces results in illegal and unsafe parking along road edges. Visitors are opportunistic by parking illegally: A) within the roadbed; B) pulling off the road into the vegetation; or C) driving up and over the curb into the vegetation. Over time, these illegal pull-outs have increased the width of road shoulders and the number of unwanted "social trails" as vegetation is crushed and soils are disturbed by vehicles and foot traffic. **Photo credit: NPS**

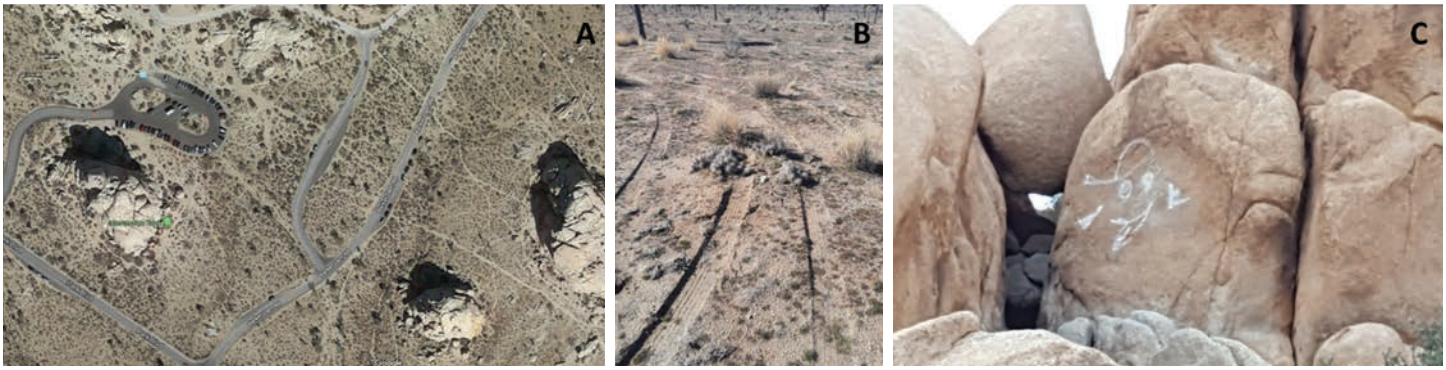


Figure 3. *A*) Proliferation of informal (i.e., social) trails and areas of trampled vegetation stemming from parking areas and roads. Social trails are often observed leading to features of interest, such as rocky outcroppings or climbing routes. *B*) Illegal off-highway vehicle intrusions crush sensitive vegetation and biological soil crusts. These illegal routes also spread undesired, invasive plants away from road shoulders and into interior park areas. *C*) Graffiti and vandalism increase with increased visitation. In the previous 80 years, park management had few records of significant graffiti. Today, park staff maintain an expansive graffiti removal program focused on rapid cleanup. Since 2016, approximately 1,100 incidences of graffiti have been recorded, and over half of these have been remediated by park staff. **Photo credit: NPS**

perspective, a 22% growth rate in 1986 equated to only 142,000 additional visits. Another way to visualize the incredible jump in visitation is to break the last 44 years into time periods of similar visitation numbers, for example: 1) from 1979–1989, visitation was on average 700,000 visits per year; 2) but for the next several decades, from 1990–2013, visitation had more or less doubled to an average of 1.3 million visits per year; and 3) then the numbers doubled again such that between 2014–2023, an average of 2.7 million visits were recorded per year (Figs. 1 and 4). Most visitation at JTNP occurs from Oct–May (i.e., the busy season), yet, from 2014–2023, the number of visits during the hottest months of the year (Jun–Sep) surpassed those during the busy season from 1990–2013 (Fig. 4) (*8*).

Thus, visitation rates are increasing seasonally and the park is experiencing more visitation in historically slower times of the year (i.e., in summer).

Not only does this create the challenge of managing for this volume of visitors when staffing is lower (e.g., seasonal rangers typically leave by May and return in November), but also there are more incidents of heat-related rescues as more visitors arrive at the park in hotter times of the year and are not prepared for the heat.

These unforeseen dramatic increases in visitation over the past decade forced the park into a state of triage and the following recreational use issues were identified as major problems:

MAJOR PROBLEMS DUE TO DRAMATIC INCREASES IN JTNP VISITATION

- Extensive entrance lines blocking residential driveways and access for emergency vehicles
- Visitor conflict in popular parking lots due to intense competition for parking spots
- Visitor conflict in first-come, first-served campgrounds with visitors arguing or taking campsites without regard for park policies or regulations
- Overflowing dumpsters and vault toilets required increased servicing
- Visitors illegally using backpacking trailheads as car campsites
- Increasing numbers of overnight backpackers and day hikers during hotter months that are unprepared for desert conditions (*8, 9*)
- Proliferation of unwanted social trails and trampling of vegetation
- Increase in illegal off-highway vehicle (OHV) routes (Fig. 3)
- Increase in graffiti and vandalism (Fig. 3)

IMMEDIATE TRIAGE

By 2016 the park was using existing staff and resources to improve problems that were impacting sensitive resources and visitor experience. Decisions were based on field observations, traffic counters, self-registration backpacking tags (2016–2017 data), satellite imagery, register receipts, and social media data (*10*). Use of existing, real-time data proved essential to understanding visitor use concerns and in minimizing the risks associated with implementing quick, novel actions. In response to immediate needs, park managers had to find ways to use existing staff and resources to mitigate negative impacts.

Triage-type actions included:

- **Used existing staff, regardless of rank or position, to assist with entrance station traffic flow and parking lot management.** An all-hands-on-deck approach provided the immediate NPS presence needed to mitigate chaos and confusion and had the added benefit of putting staff from different departments together into positions where they could experience, firsthand, the visitor use challenges.

- **Invited all staff to be a part of the conversation when developing problem-solving strategies.** This also maximized the use of interdisciplinary teams.

- **Reviewed proposed solutions weekly at management team meetings.** Proposals were based on firsthand observations provided by all staff. These observations were coupled with specialized expertise in public education, effective signage and messaging, and transportation rules and regulations to quickly develop on-the-ground solutions (Fig. 5).

- **New signage was quickly fabricated and posted to address the following problems:** 1) reduce vehicle conflicts in narrow roadways and parking lots; 2) deter unsuitable vehicles from driving 4x4-only roads; and 3) encourage visitors to arrive at the park early in the morning, as most parking lots filled up by 10 a.m. (Fig. 5).

• For the first time in the history of the park, curbing was painted red to reduce illegal and unsafe parking. Examples of unsafe parking included blocking janitorial staff from accessing restrooms and creating safety hazards on blind curves.

• Allowing cars to enter without payment when the line to enter the park exceeded one mile or if visitors were sitting in traffic for ≥30 minutes. These conditions caused a safety problem for emergency vehicle access and it blocked neighboring driveways and streets. Payment could still be collected upon their exiting.

STRATEGIC PLANNING & RESEARCH

One approach for mitigating future impacts of increased recreational use includes hosting think tank sessions and promoting research studies. These are actions that will be ongoing and continue to inform future management teams. These studies require funding and a substantial amount of time to implement, but garner long-term stability for various types of resources at the park. Examples of high priority topics to study include: NPS traffic congestion assessment, parking lot usage and turnover rates, inventory of formal and informal parking, visitor origin/destination, visitor use surveys, visitor impacts to cultural and natural resources, and soliciting public and stakeholder input. Some studies have provided novel information, while others simply confirm what staff have been observing in the field, all of which are important for managing recreational use. Examples of research conducted thus far include, but are not limited to:

• Data from one study demonstrated that backcountry wilderness use was highest at trailheads close to popular car camping areas, averaging one-night stays with two visitors per party; these visitors were primarily Californian and Saturdays were the most frequent arrival day (*10*). These data informed the establishment of a new backcountry permit system to increase user education with an online video and improve resource protection along the highly popular Boy Scout Trail by establishing designated campsites. The rest of the park remains at large for backpacking use, and permit data will continue to inform future needs for additional designated campsites in wilderness (Fig. 6).

• A 2019 visitor survey affirmed the need for increased visitor outreach by the park for

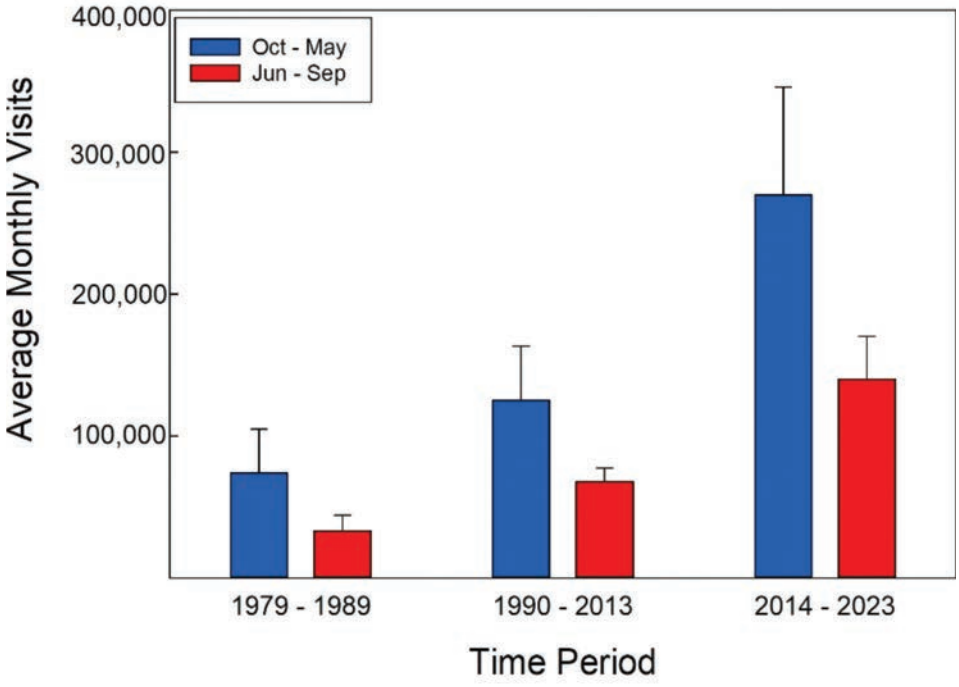


Figure 4. Average monthly visitation during 1979–1989, 1990–2013, and 2014–2023 for the busy season (Oct–May) and the less-busy season (Jun–Sep). Values are shown with ± 1 standard deviation. Notably, the Jun–Sep monthly average visits for 2014–2023 (140,107 ± 30,208) surpasses those of the busy season (Oct–May) for the preceding time period (125,243 ± 38,007). Time periods were selected based on periods of time with similar visitation numbers/rates. **Data from NPS Stats: NPS Visitor Use Statistics** (*8*)

Figure 5. *A*) Previous signage styles and sizes were modest and subtle on the desert landscape, as shown with the typical brown signs in this photo. Today managers are using color, size, and clear language to improve visitor orientation and education (e.g., bright yellow sign with very specific wording). *B*) Park managers can no longer assume what signage will instill compliance with park regulations. Testing signage with options and timed observations improves the likelihood that signs accomplish their intended purpose. *C & D*) Signage must be repeated and clearly visible to visitors who may be confused or lack trip planning. Some visitors ignore messages such as "full" or "reservations required" and will continue seeking recreational options despite adequate signage. *E*) Hike Smart informational signs have been placed at various trailheads throughout the park to improve visitor safety messaging. Increased staffing in key locations improves messaging and provides visitors with more options in times of need. **Photo credit: NPS**

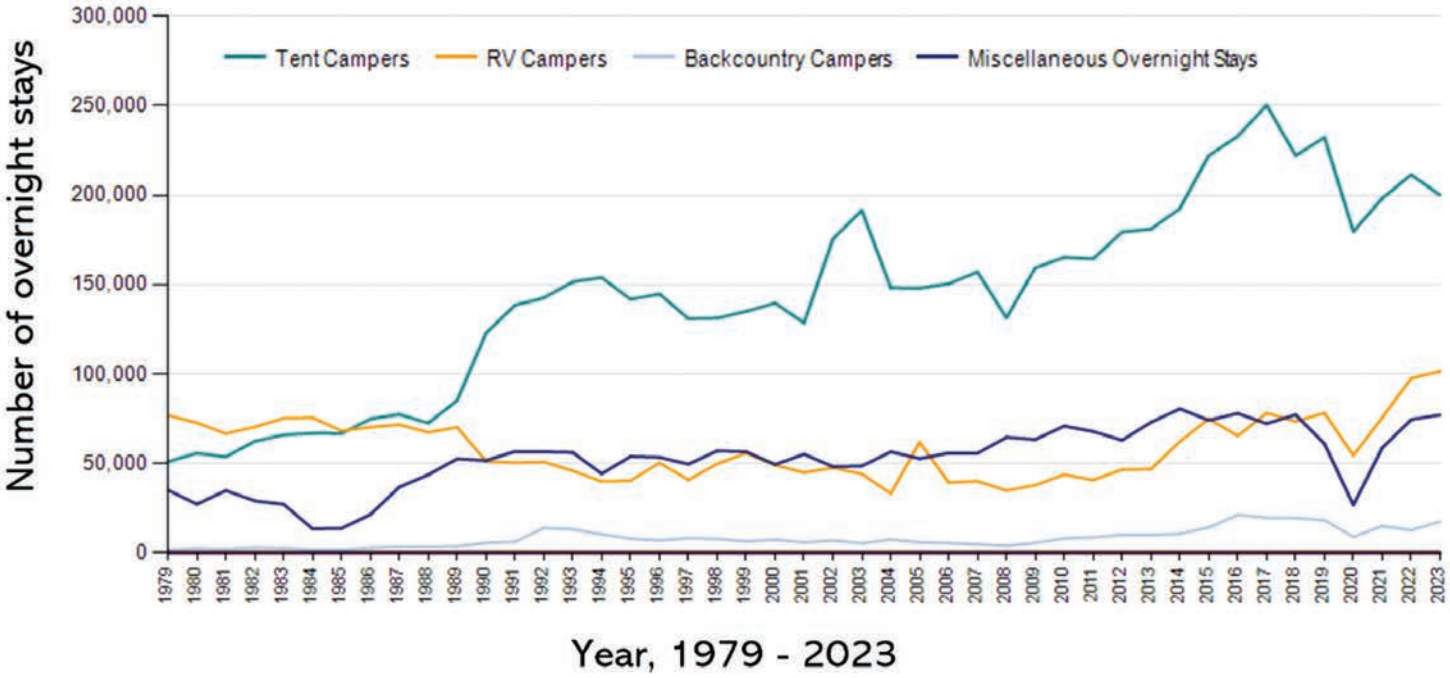


Figure 6. Number of overnight stays at JTNP between 1979 and 2023 comparing tent campers, RV campers, backcountry campers (i.e., backpackers), and miscellaneous campers (i.e., group campsites). **Data and figure adapted from NPS Stats: NPS Visitor Use Statistics** (*9*)

trip planning and provided insight on the willingness of visitors to seek out information digitally. Data from 2019 were compared to 2010 data (*11*), showing increases in public participation in several park activities. This proved helpful in reallocating outreach and management for activities with dramatically increased interest, such as astronomy programs (25% participation to 61% participation), bouldering (8% to 15%), and day hiking (46% to 94%) (*12*). Their data also showed that visitors relied on the park website for trip planning; at present, the park has doubled the investment in digital media, especially in the NPS App (Fig. 7).

• Another study used Bluetooth readers to document visitor origin/destination and answered questions about where visitors were going and how (*13*). Results showed 52% of visitors enter the park between 11 a.m. and 3 p.m., nearly 80% of visitors entering at Cottonwood continue northward and exit at Hwy 62, 28% of visitors go to Barker Dam, and 16% of visitors travel to Keys View. Data have been used to improve scheduling and staffing at fee stations to maximize visitor contact with park employees. These data were also used to develop a parking distribution and improvement plan.

• A 2021 pilot study testing effectiveness of "no pets on trails" signage indicated that nearly 75% of visitors with pets observed during an 8-hour period did not see existing signage prohibiting pets or chose not to comply with it (*14*). New signage was installed at Barker Dam during this study, in which adjustments to sign size, information provided, and image quality were tested for effectiveness. Compliance with the new signage was observed on two dates and yielded a 93% success rate (Fig. 5). Importantly, there were unintended consequences to increased compliance with these rules, such as visitors leaving pets in their vehicle while hiking. This provides motivation to continue testing and monitoring signage for effectiveness.

OUTCOMES & LESSONS LEARNED

Over the last five years the park has had time to develop and test long-term strategies that aim to alleviate the increased recreational use at JTNP. Examples include:

• **Moving the West Entrance Fee Station:** To reduce traffic build-up in residential neighborhoods in the community of Joshua Tree, the entrance station will be moved up the park road approximately 0.5 miles past the boundary. Construction begins in 2025.

• **Parking Lot Improvements:** To reduce out of bounds parking and crushing of vegetation, landscaping islands will be converted to additional parking spaces. The park recently expanded parking at Barker Dam and Wall Street Mill, and has plans to add parking to Keys View, Lost Horse Mine Trailhead, and Creosote Trailhead.

• **Public shuttle buses were tested in 2018-2020. Results were mixed.** Ridership was negatively impacted by the lack of external, centralized long-term parking lots. Ridership barriers also included the lack of cell phone coverage in the park and lack of food and water; these conditions made the shuttle feel somewhat unpredictable and there were concerns about being "left" in the park at the end of the day. Positive results indicated a willingness to use the shuttle after entering the park. Once visitors find a parking spot, there was increased interest and willingness to use the shuttle for internal transport. The park continues seeking parking options in local gateway communities.

• **Improve Visitor Services:** The park is saving fee monies to construct a new visitor center in Cottonwood. The southern end of the park has increased in popularity, particularly for star gazing. Improvements will provide visitors with a more secure location for trip planning, air-conditioned respite during hotter periods, and expanded exhibits and information specific to the Colorado Desert and regional paleontological resources.

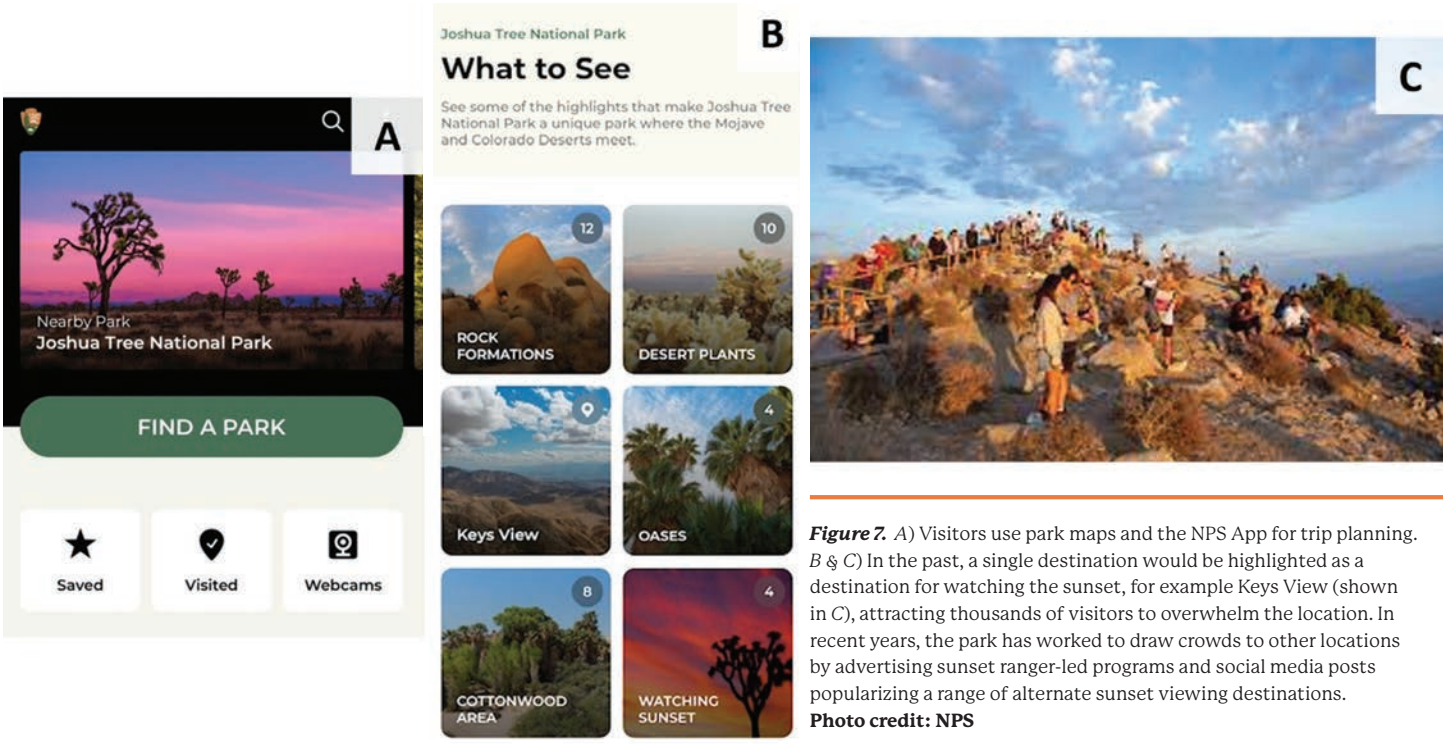


Figure 7. *A*) Visitors use park maps and the NPS App for trip planning. *B* & *C*) In the past, a single destination would be highlighted as a destination for watching the sunset, for example Keys View (shown in *C*), attracting thousands of visitors to overwhelm the location. In recent years, the park has worked to draw crowds to other locations by advertising sunset ranger-led programs and social media posts popularizing a range of alternate sunset viewing destinations. **Photo credit: NPS**

• Improve Visitor Preparedness: In partnership with the Joshua Tree National Park Association (JTNPA), the park launched the "Don't Die Today" campaign, which is based upon the "Hike Smart" program Grand Canyon NP initially developed (*15*). The "Don't Die Today" campaign includes informative signage at trailheads and pamphlets handed out at visitor centers (Fig. 5). The signs and pamphlets provide visitors with safety tips for desert hiking and survival, and a reminder of the Ten Essentials for hiking and camping (e.g., water, food, layers, first-aid kit, etc.) (*16*). Additionally, a preventative search and rescue (PSAR) program was launched in 2022 and employs a combination of JTNP staff and volunteers to rove park trails providing safety messages, heat illness prevention tips, trail information, etc. Since its implementation, the PSAR program has contacted > 255,821 visitors and documented > 31,996 preventative actions to alter unsafe visitor behavior. Moreover, the park has robust digital and social media programs which encourage visitors to do advance trip planning, provide visitor education, and park alerts regarding events and closures.

Figure 8. Image of vehicle lights exiting JTNP during periods of extremely high visitation. Social platforms and news outlets often amplify episodic events happening at the park, such as meteor showers and snowstorms, putting park staff in reaction mode to handle surges in traffic. This can be especially challenging at night or during the early morning hours when staffing may be low or conditions may be unsafe. **Photo credit: NPS**

• Tactics to Manage Surges in Visitation: Staff have developed Incident Action Plans (IAP) that can be used during surprise or planned surges in visitation. In addition to a steadily increasing rate of visitation, when popular astronomical events occur coincident with ample media coverage, JTNP often experiences unwieldy surges in visitation (Fig. 8). For instance, during the 2023 Perseids meteor shower, thousands of visitors arrived at the park on the same night. A similar surge in traffic and visitation was observed with the Aurora Borealis phenomenon in March 2024. Pre-planning using an IAP for the Perseids in 2024 demonstrated the usefulness of such planning, with crowding and chaos much reduced, and local communities relieved to see manageable levels of visitation.

LOOKING AHEAD

Since 2022, JTNP continues to be a destination supporting upwards of three million visits per year. With this amount of visitation comes major challenges, as the park is required to protect for future generations the heritage, natural, and wilderness resources within its boundaries.

What does the future hold? At present, increases in visitation appear to have leveled off and the park has a five-year plan to make critical infrastructure improvements to benefit the protection of environmental and heritage values and improve visitor experience. For now, the engineering, education, and enforcement approach is working; however, if annual visitation continues to increase and/or summer use levels increase, managers will face hard decisions involving the regulation of visitor use. Managers and local residents continue asking about timed reservations or other limits on access. However, because the park has numerous entry points and covers such a large area with multiple destinations/campgrounds, it is difficult to assess when any particular area is exceeding capacity. Restricting the number of visitors entering the park is not currently being considered, but managers are continuing to monitor the situation while investing in education and infrastructure improvements.

REFERENCES

1. National Park Service, "Annual Park Recreation Visits (1941 - Last Calendar Year)," Joshua Tree NP (JOTR) Reports, NPS Stats: NPS Visitor Use Statistics (accessed on 08/30/2024); https://irma.nps.gov/Stats/Reports/Park/JOTR.

2. C.J. Wichman, Social media influences National Park visitation. *PNAS* **121**, e2310417121 (2024); https://doi.org/10.1073/pnas.2310417121.

3. Pew Research Center, "Mobile Fact Sheet," (Washington, D.C., November 13, 2024); https://www.pewresearch.org/internet/fact-sheet/mobile.

4. Pew Research Center, "Social Media Fact Sheet," (Washington, D.C., November 13, 2024); https://www.pewresearch.org/internet/fact-sheet/social-media.

5. I. Wheeler, "Managing public land outdoor recreation for improved visitor experience and resource protection: comparing direct and indirect visitor use management tools," PhD Dissertation, Utah State University (2024); https://digitalcommons.usu.edu/etd2023/242.

6. Interagency Visitor Use Management Council (IVUMC), "Visitor Capacity Guidebook: Managing the Amounts and Types of Visitor Use to Achieve Desired Conditions, Edition One," (IVUMC, Lakewood, CO, 2019); https://visitorusemanagement.nps.gov.

7. R.E. Manning, L.E. Anderson, P.R. Pettengill, *Managing Outdoor Recreation: Case Studies in the National Parks* (CABI Publishing, 2nd edition, 2017).

8. National Park Service, "Recreation Visits by Month (1979 - Current Calendar Year)," Joshua Tree NP (JOTR) Reports, NPS Stats: NPS Visitor Use Statistics (accessed on 12/10/2024); https://irma.nps.gov/Stats/Reports/Park/JOTR.

9. National Park Service, "Overnight Stays (1979 - Last Calendar Year)," Joshua Tree NP (JOTR) Reports, NPS Stats: NPS Visitor Use Statistics (accessed on 08/30/2024); https://irma.nps.gov/Stats/SSRSReports/Park%20Specific%20Reports/Overnight%20Stays%20(1979%20-%20Last%20Calendar%20Year)?Park=JOTR.

10. T. Green, "Backcountry Overnight Use at Joshua Tree National Park 2016-2017," (National Park Service, Joshua Tree National Park, December 2019); https://irma.nps.gov/DataStore/Reference/Profile/2304878.

11. C. Jette, A. Blotkamp, Y. Le, S.J. Hollenhorst, "Joshua Tree National Park Visitor Study: Fall 2010," (National Park Service, Fort Collins, Colorado, October 2011); https://irma.nps.gov/DataStore/Reference/Profile/2236395.

12. E.E. Perry, M.T. Brownlee, R. Powell, R. Sharp, A. Sene-Harper, D.T. Dagan, S. Dopkins, C. Ehrett, B. Fowler, B. Lacy, S. Ogletree, W. Owen, K. Sikorsky, H. Smith, J. Smith, J. Thompson, "Visitor Characteristics Across and Within Seasons at Joshua Tree National Park," (National Park Service, Joshua Tree National Park, November 2020); https://irma.nps.gov/DataStore/Reference/Profile/2284830.

13. U.S. Department of Transportation - Federal Highway Administration, "Evaluation of New Traffic Data Collection Technology for Visitor Use Patterns: Methodology and Lessons Learned from Deployment at Four Federal Land Management Sites," (National Park Service, October 2021); https://irma.nps.gov/DataStore/Reference/Profile/2304945.

14. National Park Service, [Unpublished data testing effectiveness of "No Pets on Trails" signage] Joshua Tree National Park (2021).

15. National Park Service, "Hike Smart," National Park Service Article (last updated June 13, 2024); https://www.nps.gov/articles/hiking-safety.htm.

16. National Park Service, "The Ten Essentials," National Park Service Article (last updated July 19, 2023); https://www.nps.gov/articles/10essentials.htm.

AUTHOR BIOGRAPHIES

Graduating from UC Berkeley with a degree in Forest Management, **Jane Rodgers** began her career in conservation as a volunteer with the U.S. Peace Corps in Niger, West Africa. In 1994, Jane was offered a job at Joshua Tree National Park, managing the native plant nursery and eventually the entire vegetation program. In 2003, she moved to Point Reyes National Seashore as the park's vegetation ecologist, conserving rare plant species, implementing large-scale efforts to control coastal invasive plant species, and supporting range conservation and fire management and planning. In 2023 Jane took the helm as the first woman Superintendent at JTNP. She continues to be fully engaged in visitor use management and partnering with neighboring communities and organizations, and enjoying the desert with her wife Kate and their two rascally terriers, Birdie and Grit.

Dr. Sheri Shiflett is the Division Lead for Science and Resource Stewardship at Joshua Tree National Park. Since graduating from Virginia Commonwealth University in 2013 with a Ph.D. in plant ecology, she has spent her career bridging the gap between academic ecological research and management of natural resources. While an Assistant Professor at University of North Carolina Wilmington, she taught human dimensions of natural resources management and as a Science Coordinator at Yosemite National Park, she developed studies on recreational impacts on natural resources to inform visitor use planning. Sheri is an avid lover of nature and enjoys backpacking.

Joshua Tree's Native Legumes and their Microbial Partners: *Effects of Host Species and Location*

By Spencer Peterman[1]

[1]Affiliation: Sachs Lab; Department of Evolution, Ecology, and Organismal Biology; University of California, Riverside

Associations between plants and microbes, such as bacteria and fungi, are extremely common and widespread. While some of these interactions are negative, including parasitism and disease, many provide benefit to the host plant, for example by providing it with nutrients or protection from pathogens (*1*).

Figure 1. A-B) Habitat of (A) *Acmispon strigosus* and (B) *Acmispon americanus* in Joshua Tree National Park from which *Acmispon*-associated soils were sampled. Insets display photographs of specimens of the plant species (*note: not from JTNP*). C-D) Maps showing the locations of the (C) *A. strigosus* and (D) *A. americanus* populations that were sampled relative to Skull Rock Trail and Barker Dam, respectively. Colored rectangles show the approximate location of the populations that were sampled. **Photo credits: habitat photos by Spencer Peterman. *A. strigosus* photo by Jim Morefield (license: CC BY-SA). *A. americanus* photo by Stickpen (public domain).**

BACKGROUND

One of the most well studied and ecologically important plant-microbe interactions occurs between legume plants (family Fabaceae) and nitrogen-fixing, soil-dwelling bacteria known as rhizobia (*2*). This interaction is an example of symbiosis, in which two different types of organisms live in close association with each other. Rhizobia can live on their own in the soil or in association with a host legume, where they initiate the formation of organs called root nodules (*3*). In nodules, rhizobia convert ('fix') atmospheric nitrogen into usable forms such as ammonia, which the host can use to build important molecules such as proteins. In return for fixed nitrogen, rhizobia receive carbon from the host plant. However, there is often a high degree of specificity in the interaction: not all legumes are compatible with all rhizobia, and vice versa (*4*). A given strain of rhizobia (i.e., a set of genetically identical or near-identical rhizobia) can typically only form root nodules on ('nodulate') a small range of host plants, and even strains that can nodulate a host plant vary in how effectively they can provide that host with fixed nitrogen (*4*).

Legumes have a suite of mechanisms to ensure that their rhizobial partners are of high quality. For example, legumes can 'sanction', or punish, low-quality strains and provide greater rewards to high-quality strains, increasing the abundance of high-quality strains in root nodules (*5, 6*). Rhizobia are released into the soil once nodules decay, so higher-performing strains should be replenished into the soil at higher rates, potentially allowing legumes to locally enrich the soil with rhizobia that are beneficial to them. A substantial body of literature on plant-soil feedbacks shows that plants can change the composition of soil microbes, which in turn affects plant community composition (*7, 8*). With the sophisticated punishment/reward mechanisms that legumes have at their disposal, they might be expected to have a particularly strong ability to reshape the function of soil microbial communities in their favor. However, rhizobia also have a free-living phase of their lifecycle, where they live in the soil and are not associated with a host plant (*3*). Compared to host plants, the soil environment requires rhizobia to have a very different set of traits in order to successfully survive and reproduce, so natural selection imposed by the soil environment might override selection by hosts for mutualistic traits (*9, 10*). This study aimed to separate out the effects of host legume species and soil environment on the benefits provided by whole soil microbial communities.

Site	Latitude	Longitude	Host species	Date collected	Distance b/w samples (m)
JTNP	33.9965474	-116.0639309	*A. strigosus*	2/17/2023	8400
	34.0315366	-116.1446517	*A. americanus*	2/26/2023	
Stunt Ranch	34.0908942	-118.6589809	*A. strigosus*	3/4/2023	361
	34.0937954	-118.657218	*A. americanus*	2/20/2023	
UCR	33.9663544	-117.3220894	*A. strigosus*	2/22/2023	938
	33.9745286	-117.3195534	*A. americanus*	2/26/2023	
Motte Rimrock	33.8002489	-117.2580473	*A. strigosus*	2/21/2023	41
	33.8005172	-117.2583555	*A. americanus*	2/21/2023	

Table 1. Information about soil samples.

Joshua Tree National Park (JTNP) is home to a large diversity of legume species.

This study focused on legumes in the genus *Acmispon*, which includes annual and perennial plants that range across large portions of western North America, including several species that live in JTNP, such as *A. glaber*, *A. americanus*, and *A. strigosus*.

OBJECTIVES

This study had three primary objectives:

1. To determine whether legumes host soil microbial communities that provide substantial growth benefits to host plants;

2. To investigate whether the effects described in objective (1) are host-species-specific;

3. To investigate whether these effects are location- or soil-specific.

METHODS

PLANT SAMPLING:

General sampling design

Two legume species were selected for sampling, *Acmispon strigosus* and *A. americanus* (Fig. 1A-B). These are annual species that live across a wide range in the western United States, including highly overlapping ranges within California (*11, 12*). *A. strigosus* and *A. americanus* plants and soils were sampled from four sites spanning an ecological gradient in Southern California: JTNP (desert), the University of California Riverside (UCR) campus and the UCR Motte Rimrock Reserve (inland), and the UCLA Stunt Ranch Santa Monica Mountains Reserve (coastal) (Fig. 2). Ecologically diverse sampling sites were chosen to ensure that the results of the study could be generalized to multiple different ecological contexts.

Two plants (one of each focal species) and their root-associated soil were sampled from each site for eight samples total. This design ensured that samples were replicated across host species and sites. First, populations of both focal species were identified. Care was taken to ensure that the selected populations were as close to each other as possible to control for differences in the physical/chemical characteristics of different soils at the same site; however, some pairs of samples were relatively distant from each other due to limitations in where suitable populations could be found at each site (Table 1). Next, soil was sampled within a radius of 10cm and a depth of 15cm around a single plant that matched the following criteria: 1) the plant had true leaves, 2) there were no other plants within the 10cm radius, and 3) there were nodules on the roots of the plant. An ethanol-sterilized shovel was used to dig up the soil sample to prevent cross-contamination. The soil was

then bagged and taken back to the laboratory for processing and use in a greenhouse experiment. Standard soil analysis on the samples is planned to measure their physical and chemical properties, but results are still being awaited. Information about the soil samples is summarized in Table 1.

PLANT SAMPLING:

Gathering of JTNP samples

At JTNP, *A. strigosus* was sampled on February 17, 2023, near the Skull Rock Trail, from a population of about 15 individuals (Fig. 1A). The soil here was very dry and coarse-grained. *A. americanus* was sampled on February 26, 2023, along a stream bank near the Barker Dam Trail (Fig. 1B). The population here consisted of about 30 individuals, and it was the only population of *A. americanus* that could be found in the park. The relative scarcity of *A. americanus* in the park constrained choices for paired sampling locations, leading to the JTNP samples being about 8 km apart, much further apart than at the other sampling sites (Table 1).

INOCULUM PREPARATION

In the lab, microbes were isolated from each soil sample so that they could be introduced to *Acmispon* plants for a greenhouse experiment. First, soil samples were passed through a 2mm USA standard sieve to separate out fine-grained particles. Sieved soils were then mixed with sterilized, deionized water, and the mixtures were filtered through cheesecloth. The filtrates, containing soil microbes, were left to settle overnight. The top portion of each filtrate was then transferred to a new pair of bottles, one of which was used as a 'live' treatment containing microbes, and the other of which was autoclave-sterilized and used as a 'dead' (control) treatment containing no microbes but having the same chemical properties as the live treatment (*13*).

GREENHOUSE EXPERIMENT

Four species of *Acmispon* were grown in the laboratory, including the focal sample species (*A. strigosus* and *A. americanus*) as well as *A. glaber* and *A. parviflorus*, which are also widespread across California and have substantial range overlap with the other two species. All of these species except *A. parviflorus* occur in JTNP. The original experimental design included exposing plants to soil microbes from all four host species; however, despite broad range overlap between the species, few sites have 4 or even 3 of the species living in close proximity to each other. The 2 focal sampling species, *A. strigosus* and *A. americanus*, were chosen based on unpublished data suggesting that they host genetically divergent communities of rhizobia.

Once *Acmispon* seedlings developed true leaves (after about three weeks of growth), they were moved to a greenhouse and inoculated with soil filtrates by dripping 10 mL of filtrate onto the soil surface. Each species received filtrates from all 8 samples, with 10 replicates of the live filtrates and 5 replicates of the dead filtrates, for a total of 480 plants. Plants were fertilized weekly with 5 mL of nitrogen-free Jensen's nutrient solution (*14*).

Effect	*F* ratio	*p* value
Host inoc	5.2854	0.0229575*
Host isol	5.8757	0.0008224*
Soil site	0.4568	0.6342337
Interaction between host inoc and host isol	8.0621	5.34E-05*
Interaction between host isol and soil site	1.6923	0.1877698
Interaction between host inoc and soil site	1.5893	0.1543809

Table 2. Results of an ANOVA on the linear mixed model for host relative growth. Asterisks in the 'p-value' column display which variables had a statistically significant effect on plant growth. Abbreviations: 'host inoc' = host species that was inoculated in the experiment; 'host isol' = host species from which microbes were isolated during soil sampling.

Figure 2. Map of sampling sites in Southern California. Two soil samples, one each from *A. strigosus* and *A. americanus*, were taken from each site. **Map created by Audrey Burr.**

PLANT HARVEST AND TRAIT MEASUREMENT

Plants grew in the greenhouse for about 8 weeks, allowing enough time for substantial growth effects from microbes while ensuring that root nodules did not start to senesce (i.e., deteriorate with age, which leads to a decrease in the amount of rhizobia in the nodule). Plants were then harvested and soil was washed off roots. Plants were dissected into shoot, root, and nodule portions, which were stored in separate coin envelopes and dried at 60°C for at least four days. Two nodules were randomly sampled from each live-inoculated plant and stored at -20°C for future bacterial genotyping work. Dried plant samples were weighed to obtain measurements of plant growth

and investment into symbiosis. Host growth response was assessed as the ratio of the shoot biomass of live-inoculated plants to the shoot biomass of plants receiving the corresponding dead inoculum, giving a measure of how much benefit (if any) plants obtained from soil microbes alone (*15*).

STATISTICAL ANALYSIS

Plant growth response was modeled using a linear mixed model with host of soil filtrate origin, host inoculated in the greenhouse, soil filtrate sampling site, and their interactions as fixed effects; number of days post-inoculation that plants were harvested as a covariate; and greenhouse block as a random effect. This model allowed for host species-based effects on symbiosis traits to be separated out from the effects of location, while also testing whether these effects interact (i.e., whether hosts have specialized responses to microbes from particular species and/or locations). Once data are obtained on the chemical characteristics of the soil samples, they will also be included as a covariate in the model, to account for any differences in growth driven by differences in the chemistry of the filtrates (e.g., pH, nitrogen, or phosphorus concentrations).

RESULTS

HOST SPECIFICITY

Results for host growth response are summarized in Figure 3 and Table 2. *Acmispon* growth response depended much more strongly on host species than on location. Responses were specific to both the host species of soil origin and the host species that was inoculated in the greenhouse. For example, *A. strigosus* ($t = 2.6914$, $p = 0.01002$) and *A. americanus* ($t = 3.9759$, $p = 0.00034$) each received greater benefit from microbes sourced from their own species than from the other. *A. parviflorus* likewise benefited more from *A. americanus* than *A. strigosus* microbes ($t = 3.2197$, $p = 0.0026$), while *A. glaber* did not show any significant difference in response to microbes from either species ($t = 0.2709$, $p = 0.7878$; Fig. 4). These results suggest that different *Acmispon* hosts condition the traits of soil microbes in different ways, and that this conditioning feeds back to benefit host plant growth in a species-specific way. Patterns of specificity may reflect evolutionary relationships between *Acmispon* species: although phylogenetic data for the genus are currently scant, morphological comparisons suggest that *A. americanus* and *A. parviflorus* are closely related (*16*), congruent with the similar specificity they displayed here. In addition, that same morphological study (*16*), as well as a single-gene molecular phylogeny (*17*), both suggest that *A. glaber* and *A. strigosus* are not closely related within the genus, to each other, or to the other two species.

Despite this evidence of host specificity, *Acmispon* species were, in many cases, broadly cross-compatible with each others' microbes. In other words, *Acmispon* species usually received significantly greater benefit from live filtrates than from dead filtrates, regardless of host of soil origin, although host of soil origin affected the degree of benefit. Thus, while these closely-related species have evidently evolved differences in symbiotic specificity, they

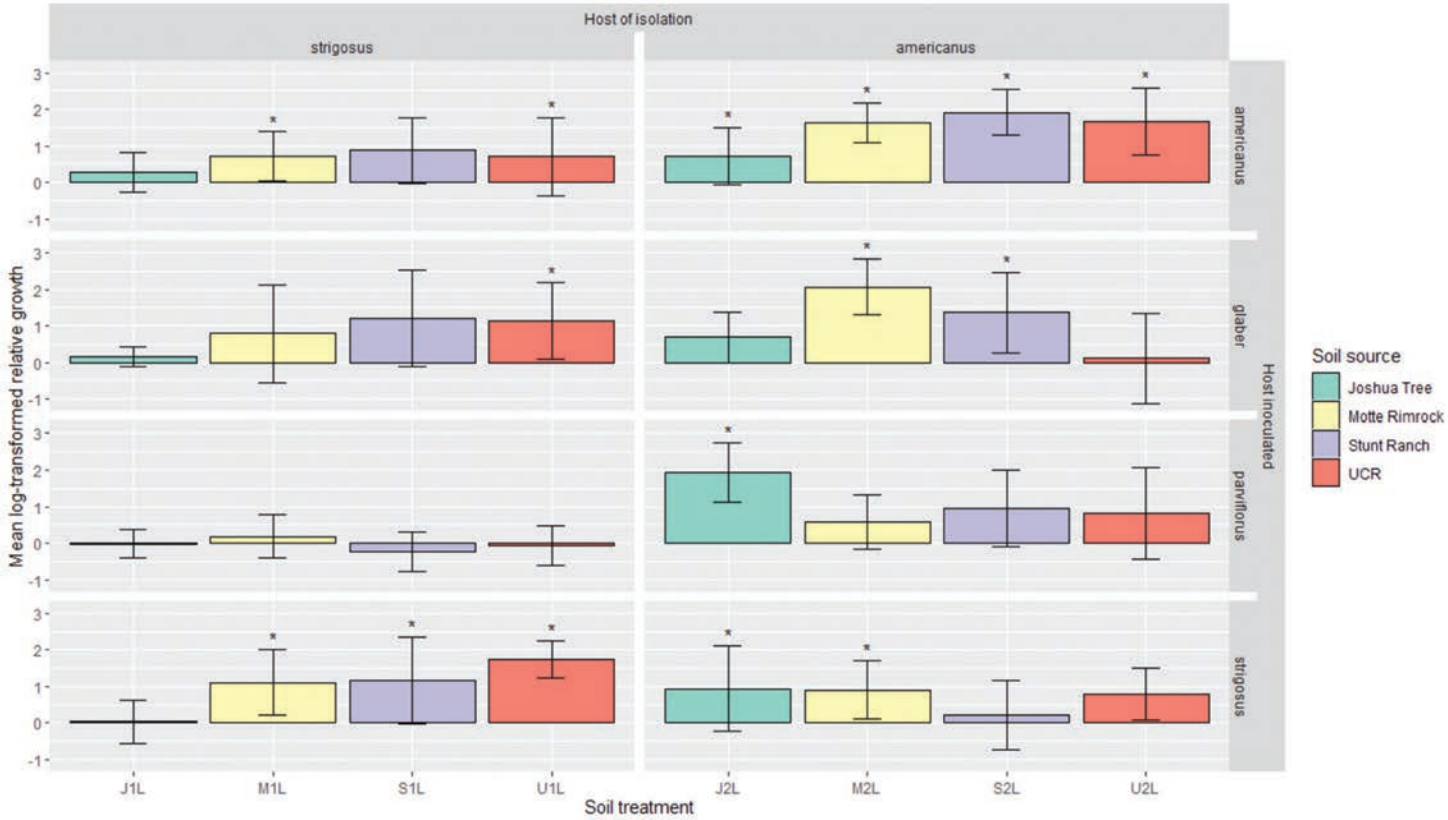


Figure 3. Bar plots showing the mean growth response of each inoculated species (horizontal facets) to filtrates from the two sampled host species (vertical facets). Growth response was measured as the ratio of the shoot mass of live-inoculated to dead-inoculated (control) plants. These values were log-transformed to meet model assumptions, so a value of 0 indicates a shoot mass equal to the controls, while higher values indicate growth benefit from microbes. Error bars indicate standard error. Asterisks (*) indicate a significant growth benefit relative to controls. Soil treatment codes: J = Joshua Tree, M = Motte Rimrock, S = Stunt Ranch, U = UCR; 1 = *A. strigosus*, 2 = *A. americanus*; L = live inoculum.

can still largely benefit from the same sets of microbes. The one exception to this pattern was *A. parviflorus*, which benefited from some *A. americanus*-derived filtrates but did not benefit from any *A. strigosus* filtrates (Fig. 3).

LOCATION SPECIFICITY

By contrast to the strong observed host effects, the location from which the soil was sampled had no apparent effect on host growth response, and none of the host species preferred soil microbes sourced from any particular location (Fig. 5). The lack of a location effect was particularly striking at the Motte Rimrock Reserve, where *A. strigosus* and *A. americanus* soils were sampled within ~50m of each other, within the same plant community and soil type, but had significantly different effects dependent on the species they were inoculated onto. This result, together with the observed host effects, suggests that hosts can find, and locally enrich the soil with, compatible microbes across very different locations and soil types.

NODULATION DATA

Because the soil filtrates likely contained diverse microbial communities, it is not possible to attribute the observed growth effects directly to rhizobia. However, data on root nodules from the experimental plants are consistent with the hypothesis that growth benefits to live-inoculated plants compared to controls were driven in part by rhizobia. All species formed root nodules in response to all soil filtrates, except *A. parviflorus* in response to *A. strigosus* filtrates (consistent with the lack of observed growth benefit to *A. parviflorus* from these samples).

Unfortunately, the JTNP soil sample associated with *A. strigosus* failed to produce root nodules on any of the tested species. This was probably not due to a complete absence of rhizobia in the sample, as root nodules were verified to be present on the sample plant. It is possible that the dryness of the soil sample led to a very low abundance of rhizobia in the filtrate. Unfortunately, subsets of the inoculum itself were not saved for testing, but in future work, DNA sequencing can be performed on the preserved soil sample to identify the bacteria present in the sample and perhaps shed some light on the lack of plant response to the inoculum.

MANAGEMENT IMPLICATIONS

The results of this study could have implications for efforts to conserve JTNP's native legumes. While the lack of response to the JTNP

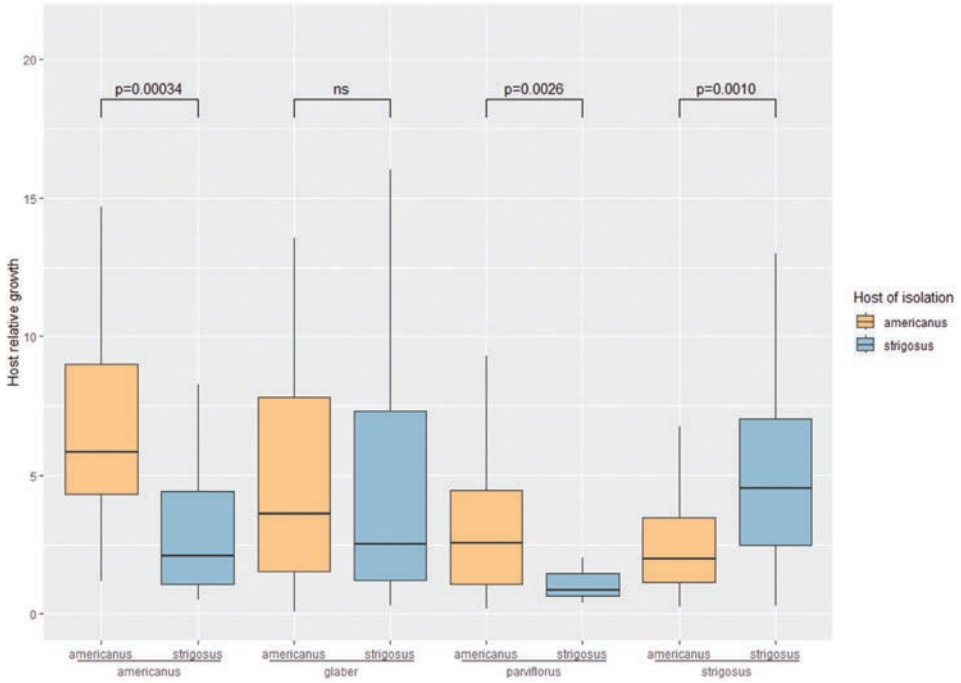


Figure 4. Boxplots comparing the growth response of the four host species in the greenhouse experiment to filtrates derived from *A. americanus* and *A. strigosus*. Names on the bottom of the x-axis indicate the host species receiving a filtrate, while names on the top of the x-axis indicate the host species from which those filtrates were derived. Note that all four filtrates that came from the same host species are pooled together in this figure. 'Host relative growth' is the ratio of the shoot mass of live-inoculated plants to dead-inoculated (control) plants, indicating how much benefit was obtained from microbes in the soil sample. 'ns' indicates no significant difference in response to *A. strigosus/americanus* microbes, while $p<0.05$ indicates a significant difference, as determined by a two-tailed t-test.

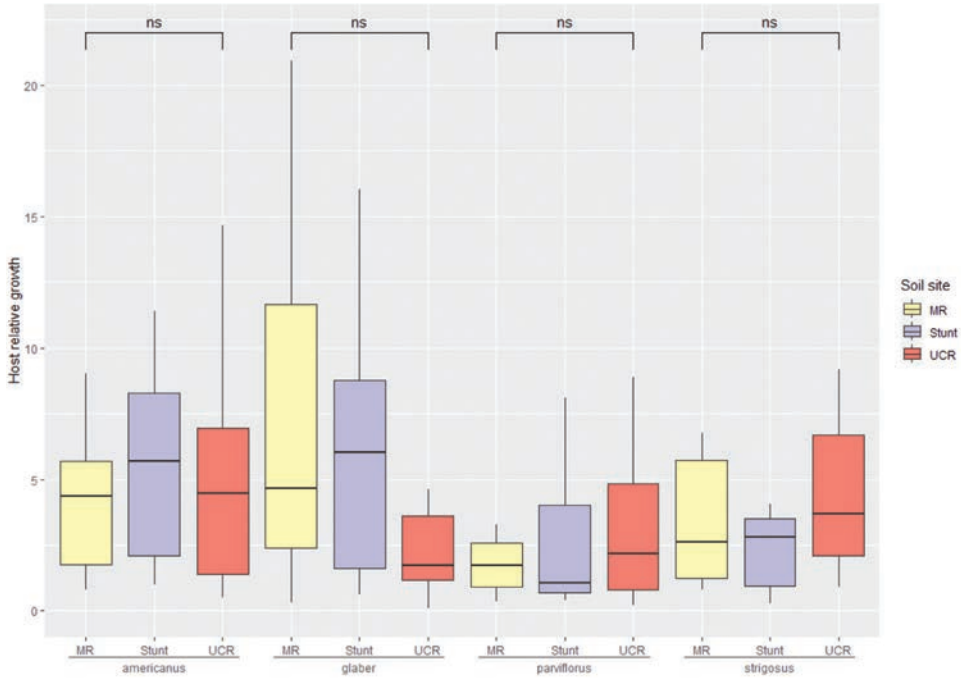


Figure 5. Boxplots comparing the growth response of the four host species in the greenhouse experiment to filtrates derived from different sites, including UCR, the UCR Motte Rimrock Reserve, and the UCLA Stunt Ranch Reserve. Results from JTNP soil filtrates were excluded from the model because the lack of nodulation response (and lack of a corresponding growth response in any of the tested species) would produce misleading results. All pairwise comparisons were performed; in each case, 'ns' indicates that there were no significant differences in response to samples from the three sampling sites, as determined by Tukey's HSD test.

A. strigosus-associated inoculum makes it difficult to compare differences in symbiosis between *Acmispon* species within the park itself, the broader results of this study suggest that *Acmispon* species do not have much specificity to microbes from particular locations or soil types. Conservation and restoration projects involving bioinoculants should focus more on the host species, rather than location, of origin of microbes; thus, bioinoculants containing microbes sourced from distant locations may still provide benefit to legume hosts. The fact that all of the species in this study were able to benefit from microbial communities that were originally associated with a different species suggests that high levels of host specificity will not be a problem for conserving legumes, at least not in the genus *Acmispon*.

CONCLUSIONS

This study provides evidence that JTNP's native legumes condition the microbial communities in the soil near their roots to provide greater benefits to themselves and that they do so in a host species-specific, but not location-specific, way. Although the lack of data on *A. strigosus*-associated rhizobia at JTNP is unfortunate, the results of this study suggest that highly specific host species-location interactions are not a conservation concern for JTNP's native legumes. Future work will focus on sequencing the DNA of bacteria in soil samples and root nodules from this experiment, which will clarify how legumes shape and respond to the composition and diversity of soil microbial communities.

LITERATURE CITED

1. M. L. Friesen, S. S. Porter, S. C. Stark, E. J. von Wettberg, J. L. Sachs, E. Martinez-Romero, Microbially Mediated Plant Functional Traits. *Annu. Rev. Ecol. Evol. Syst.* **42**, 23–46 (2011).

2. A. M. Hirsch, M. R. Lum, J. A. Downie, What Makes the Rhizobia-Legume Symbiosis So Special? *Plant Physiol.* **127**, 1484–1492 (2001).

3. J. L. Sachs, M. O. Ehinger, E. L. Simms, Origins of cheating and loss of symbiosis in wild Bradyrhizobium. *J. Evol. Biol.* **23**, 1075–1089 (2010).

4. D. Wang, S. Yang, F. Tang, H. Zhu, Symbiosis specificity in the legume – rhizobial mutualism. *Cell. Microbiol.* **14**, 334–342 (2012).

5. E. T. Kiers, R. A. Rousseau, S. A. West, R. F. Denison, Host sanctions and the legume–rhizobium mutualism. *Nature* **425**, 78–81 (2003).

6. J. U. Regus, K. W. Quides, M. R. O'Neill, R. Suzuki, E. A. Savory, J. H. Chang, J. L. Sachs, Cell autonomous sanctions in legumes target ineffective rhizobia in nodules with mixed infections. *Am. J. Bot.* **104**, 1299–1312 (2017).

7. J. A. Schweitzer, J. K. Bailey, D. G. Fischer, C. J. LeRoy, E. V. Lonsdorf, T. G. Whitham, S. C. Hart, Plant–Soil–Microorganism Interactions: Heritable Relationship Between Plant Genotype and Associated Soil Microorganisms. *Ecology* **89**, 773–781 (2008).

8. W. H. van der Putten, R. D. Bardgett, J. D. Bever, T. M. Bezemer, B. B. Casper, T. Fukami, P. Kardol, J. N. Klironomos, A. Kulmatiski, J. A. Schweitzer, K. N. Suding, T. F. J. Van de Voorde, D. A. Wardle, Plant–soil feedbacks: the past, the present and future challenges. *J. Ecol.* **101**, 265–276 (2013).

9. R. F. Denison, E. T. Kiers, Lifestyle alternatives for rhizobia: mutualism, parasitism, and forgoing symbiosis. *FEMS Microbiol. Lett.* **237**, 187–193 (2004).

10. L. T. Burghardt, B. Epstein, J. Guhlin, M. S. Nelson, M. R. Taylor, N. D. Young, M. J. Sadowsky, P. Tiffin, Select and resequence reveals relative fitness of bacteria in symbiotic and free-living environments. *Proc. Natl. Acad. Sci.* **115**, 2425–2430 (2018).

11. Acmispon americanus var. americanus. https://ucjeps.berkeley.edu/eflora/eflora_display.php?tid=91832.

12. Acmispon strigosus. https://ucjeps.berkeley.edu/eflora/eflora_display.php?tid=91722.

13. M. Manci, O. G. Mercado, R. X. Camantigue, T. Nguyen, J. Rothschild, F. Khairi, S. Neal, W. F. Farsamin, M. T. Lampe, I. A. Perez, T. H. Le, G. S. Ortiz-Barbosa, L. Torres-Martínez, J. L. Sachs, Live soil inocula, not host population or domestication status, is the predominant driver of growth benefits to cowpea. *Plant Soil* **482**, 585–600 (2023).

14. P. Somasegaran, H. J. Hoben, *Handbook for Rhizobia: Methods in Legume-Rhizobium Technology* (Springer Science & Business Media, 2012).

15. J. U. Regus, K. A. Gano, A. C. Hollowell, V. Sofish, J. L. Sachs, Lotus hosts delimit the mutualism–parasitism continuum of Bradyrhizobium. *J. Evol. Biol.* **28**, 447–456 (2015).

16. A. M. Arambarri, S. A. Stenglein, M. N. Colares, M. C. Novoa, Taxonomy of the New World species of Lotus (Leguminosae: Loteae). *Aust. J. Bot.* **53**, 797–812 (2005).

17. G. V. Degtjareva, T. E. Kramina, D. D. Sokoloff, T. H. Samigullin, C. M. Valiejo-Roman, A. S. Antonov, Phylogeny of the genus Lotus (Leguminosae, Loteae): evidence from nrITS sequences and morphology. *Can. J. Bot.* **84**, 813–830 (2006).

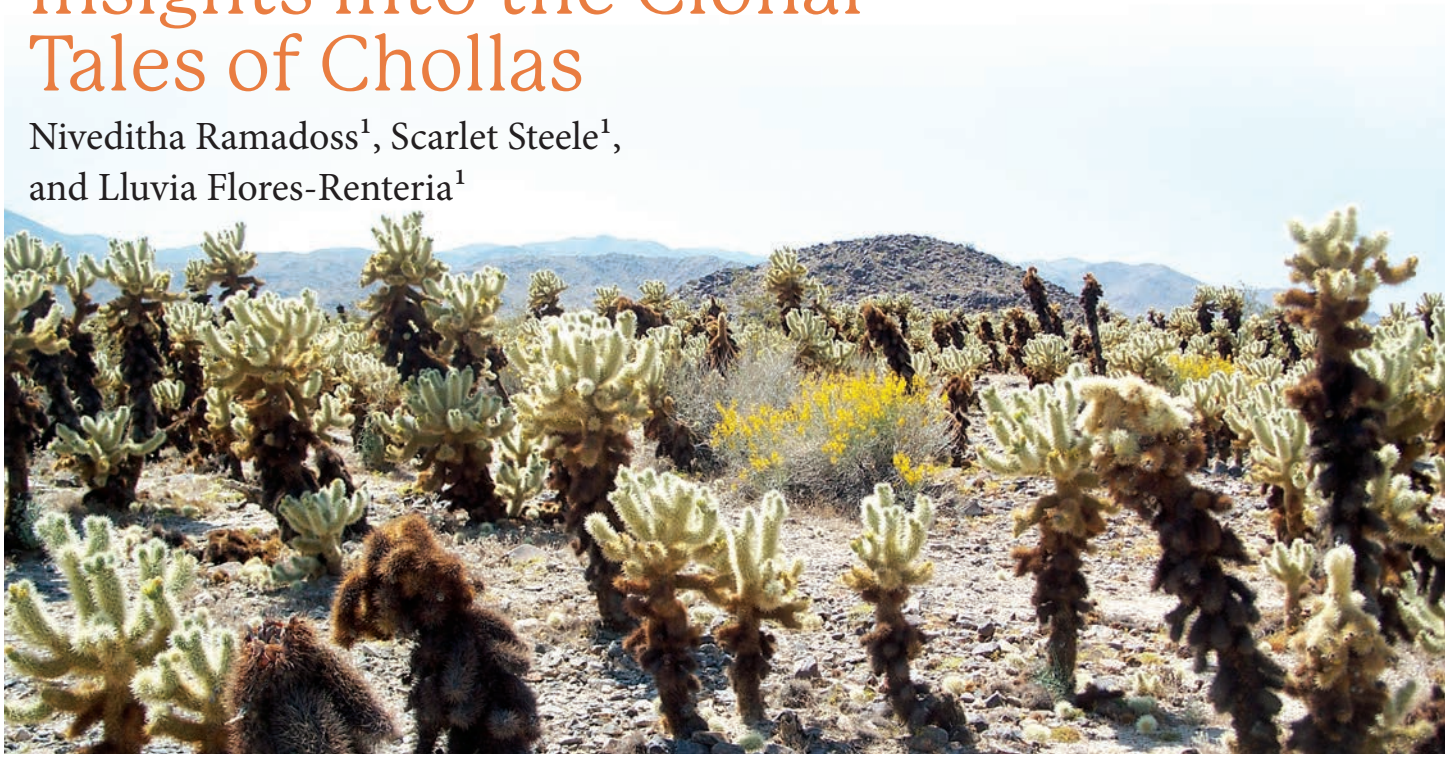
AUTHOR BIOGRAPHY

I am a fourth-year PhD student in the Evolution, Ecology, and Organismal Biology department at the University of California, Riverside. My research interests include symbiosis, coevolution, and the evolution of host specificity. Having grown up in California, I've always enjoyed the state's diverse natural environments and I'm glad to be able to continue doing that through my field work.

Author doing fieldwork at Granite Mountains Desert Research Center.
Photo credit: Audrey Burr

Insights into the Clonal Tales of Chollas

Niveditha Ramadoss[1], Scarlet Steele[1], and Lluvia Flores-Renteria[1]



A stand of *Cylindropuntia bigelovii* (teddybear cactus). **Photo credit: Mari Stewart**

ABSTRACT

Chollas belong to a prominent group of cacti thriving in the warm deserts of North America. *Cylindropuntia* — the largest genus among the chollas, comprises approximately 40 species that exhibit diverse sexual systems, including gynodioecy, dioecy, and hermaphroditism. This genus is of ecological importance, supporting a variety of native birds, animals, and pollinators, especially bees. Although studies on *Cylindropuntia* have recently seen increased activity, many aspects of their biology remain poorly understood, particularly the extent of their asexual reproduction. Most species of *Cylindropuntia* have the ability to clonally reproduce by dropping vegetative segments that can root and develop into new individuals. Determining the extent of clonal reproduction in these species is essential for understanding their genetic diversity and resilience to environmental changes which are critical factors for their effective management. This study aims to assess the degree of clonal reproduction in four species of *Cylindropuntia* (*C. echinocarpa, C. ramosissima, C. bigelovii, and C. ganderi*), the first three of which are distributed in Joshua Tree National Park (JTNP).

We hypothesized that all species would be associated with some level of clonal reproduction except the triploid populations of *C. bigelovii*, which must be purely clonal as it cannot successfully sexually reproduce.

To achieve our goal, we sampled the four *Cylindropuntia* species with different sexual systems from JTNP and Bureau of Land Management (BLM) lands. The plant tissues were dried, DNA was extracted and sequenced for SNPs (Single Nucleotide Polymorphisms), which are small differences in DNA using the Next Generation sequencing technology of DArTseq. The SNPs were assessed for clonality using the GenoDive software. All sampled species exhibited some degree of clonal reproduction with *C. bigelovii* having the highest level. Our findings indicate that *C. bigelovii* is predominantly clonal and requires careful management due to limited genetic variation, which may hinder its ability to adapt to environmental changes. Collectively, our results enhance the understanding of clonal dynamics in *Cylindropuntia* species and pave the way for future studies exploring broader ecological and evolutionary implications.

BACKGROUND

Species can reproduce either sexually or asexually, with the evolution of sexual reproduction from asexual reproduction marking one of the most significant transitions in the history of life (*1*). Despite this, asexual reproduction remains widespread throughout the tree of life (*2*). Asexual reproduction, where offspring are produced from a single parent without the need for gamete fertilization, is particularly common among flowering plants (*2, 3*). Angiosperms utilize two main methods for asexual reproduction: clonal propagation (vegetative propagation) and apomixis (*4*). The

[1]Affiliation: San Diego State University

most widespread form of asexual reproduction in plants is clonal propagation (vegetative propagation), wherein parental genotypes (genets) generate vegetative units (ramets) capable of independent growth, reproduction, and dispersal (*2*). Clonal propagation produces offspring that are genetically identical to the parent. There is a spectrum observed in clonality of plants with species exhibiting strictly clonal reproduction to facultative clonal reproduction (*5*). Research on plant clonality has largely been limited to obligately clonal plants and a few model species (*6*) highlighting the need to study a broader range of species.

The consequences of clonal reproduction differ among plant species and even among populations within a species (*7*). Certain species have shifted away from sexual reproduction in favor of clonal reproduction in certain habitats or regions of their geographic range (*8–10*). This is mainly because sexual reproduction demands greater energy input, time, and resources (*10–12*). Clonal reproduction becomes advantageous when there is a need for reproductive assurance in situations where sexual reproduction is difficult, such as low pollinator abundance or environmental stress (*10, 13, 14*). Conversely, clonal reproduction also has disadvantages as it restricts genetic variation and opportunities for recombination, hindering adaptive evolution. Over time, cloning can lead to the accumulation of harmful mutations, making the species more vulnerable to environmental changes (*10, 13, 14*). This particularly affects the rare species (because of their small population size) and complicates the conservation efforts as it is often difficult for biologists to distinguish between genets and ramets in the field, which can lead to overestimations of population size and viability (*15, 16*). Thus, understanding the extent of clonal reproduction in rare species is important for accurately estimating their population size and genetic diversity, which are key factors in conservation efforts.

Cacti, a critical species in arid and semiarid ecosystems across the Americas, rank as the fifth most threatened taxonomic group, with 31% of species facing extinction risks (*17*). Our paper focuses on the genus *Cylindropuntia* (commonly known as chollas), which includes ~39 species and has diverse sexual systems such as hermaphroditism (individual plants have flowers with both male and female reproductive organs), functional dioecy (individual plants carry flowers with both male and female parts, but only one sex is functional) and gynodioecy (a population includes both **hermaphroditic individuals** and **female flower-only individuals**) (*18, 19*). *Cylindropuntia spp*. are well known for their ability to reproduce clonally, with some species relying exclusively on this mode of reproduction (*20*). They produce genetically identical clones through their terminal joints that readily dislodge with minimal disturbance like wind or animal contact and efficiently root once they reach the soil (*21–23*). This enables them to rapidly expand and establish thick, localized monocultures in arid and semi-arid environments (*23–25*). Moreover, chollas can dominate resources such as water and soil nutrients, ultimately affecting biodiversity (*23, 26*). Certain species grow in dense clusters that form impenetrable fences for movement of wildlife and livestock, thereby complicating habitat restoration (*20, 24*). Most *Cylindropuntia* species lack genetic studies (*27, 28*), so investigating the clonality of these species through genetic data not only enhances our understanding of their reproductive strategies but also contributes valuable data to support conservation efforts. Our study includes three species from diploid populations of *Cylindropuntia*: *C. echinocarpa* (CE), *C. ganderi* (CG), and *C. ramosissima* (CR), as well as one triploid population of *C. bigelovii* (CB) (Fig. 1) (*29*). CB is reported to reproduce vegetatively (*20, 30*) and a previous study based on biomechanics of asexual reproduction (*30*) on these four species suggested that CB was

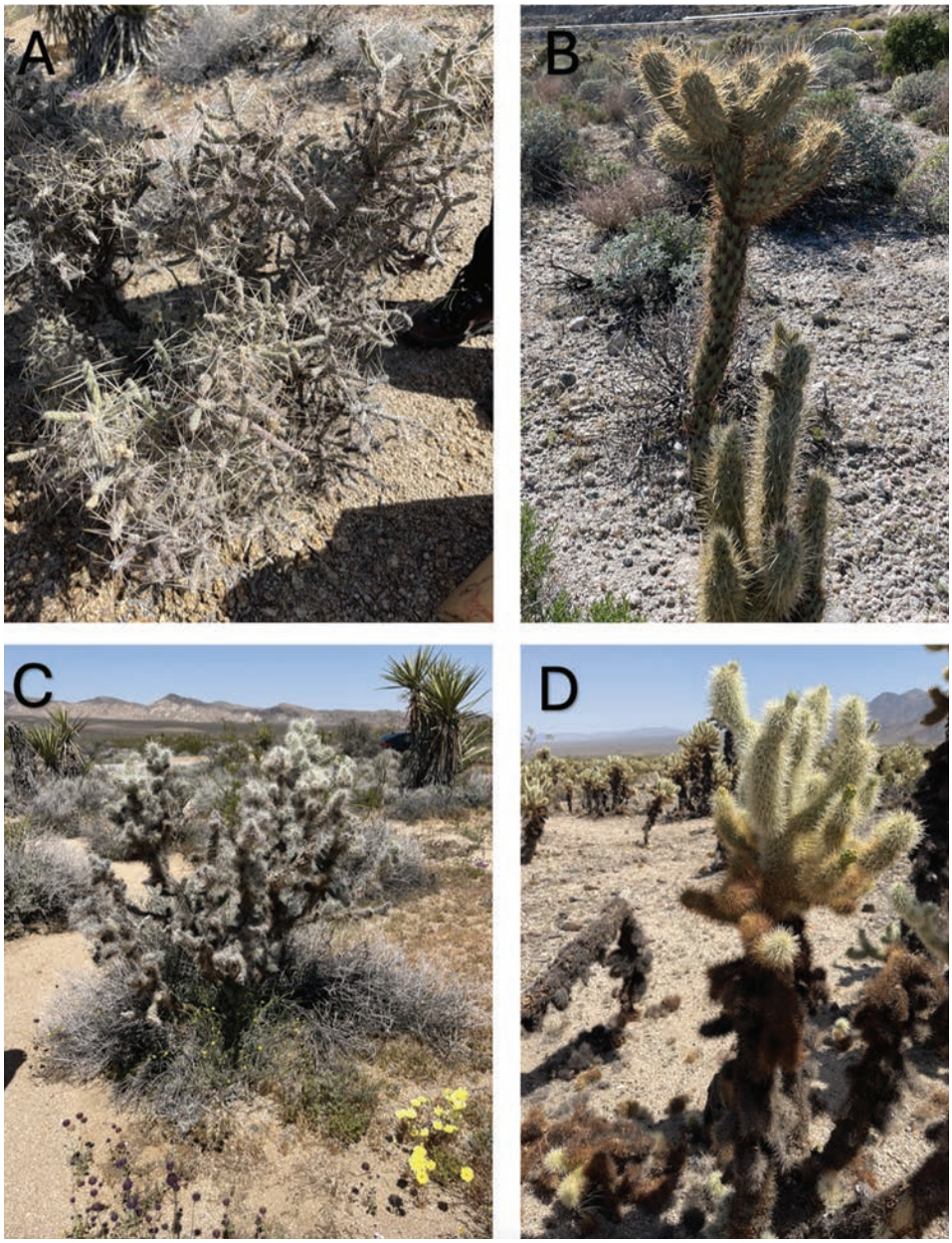


Figure 1. The *Cylindropuntia* species sampled in this study. Starting from top left A) *C. ramosissima*; B) *C. ganderi*; C) *C. echinocarpa*; D) *C. bigelovii*. A, C and D were collected at JTNP while B was from BLM lands in Imperial County. **Photo credit: N.Ramadoss**

the only species among the four to exhibit extensive vegetative propagation. Its terminal joints possess key traits that facilitate vegetative reproduction, including a high spine frequency, biomechanically stiff yet weak junctions that promote detachment, and a strong rooting ability. In contrast, none of the other species CE, CG or CR exhibited more than two of these traits. However, a genetic study would provide a more direct and accurate assessment of clonality by confirming the presence of genetically identical (clonal) individuals.

OBJECTIVE

The goal of our research is to assess the degree of clonal reproduction in *Cylindropuntia* species. Our study included three diploid populations of species (Fig. 1), *C. echinocarpa, C. ganderi, C. ramosissima* and one triploid population of *C. bigelovii* (*29*). We hypothesize that all species will have some levels of clonal reproduction except *C. bigelovii* which must be completely clonal as it is a triploid that cannot successfully sexually reproduce.

GENERAL METHODS

SAMPLING

To assess the degree of clonal reproduction, we collected fragments of four *Cylindropuntia* species. The populations we sampled have restricted distributions (Figs. 2–5). We collected one fragment from at least 15 individuals of four species — *C. echinocarpa* (CE), *C. ganderi* (CG), *C. bigelovii* (CB) and *C. ramosissima* (CR). The CG samples were collected from BLM lands (CAD07000-FY2023.003), while the other three species were obtained from JTNP (JOTR-2022-SCI-0018). The fragments were potted in small containers with a soil mix containing 70% potting mix, 15% sand and 15% perlite, and placed in the greenhouse. They were irrigated every three weeks with tap water. This allowed for fresh plant material collection to optimize DNA extraction.

DNA EXTRACTION AND SEQUENCING

Approximately 100 mg of tissue was cut from the cholla fragments and dried. To minimize excess moisture, only the epidermis and collenchyma were carefully excised for DNA extraction. Plant genomic DNA was then extracted using the DNeasy Plant Mini Kit (Qiagen). The presence of high molecular weight DNA bands were confirmed via gel electrophoresis and shipped to the Diversity Array Technology company (DArT) for sequencing SNPs (*31*). A SNP stands for single-nucleotide polymorphism which is a small difference in the DNA sequence between individuals, basically a single letter change in the genetic code that is common across the genome. The DArTseq technique has proven effective in genetic studies of polyploids and non-model organisms (*32, 33*). In polyploids, the SNPs are derived from a single homeolog using bioinformatic algorithms that filter SNPs

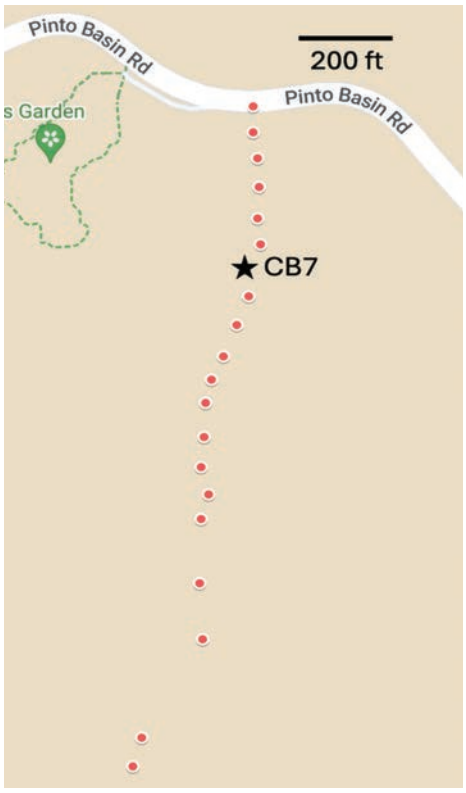


Figure 2. The distribution map of CB samples. The black star represents the individual forming distinct clonal lineage, while red circles indicate ramets belonging to the same genet. **Map generated using Google My Maps.**

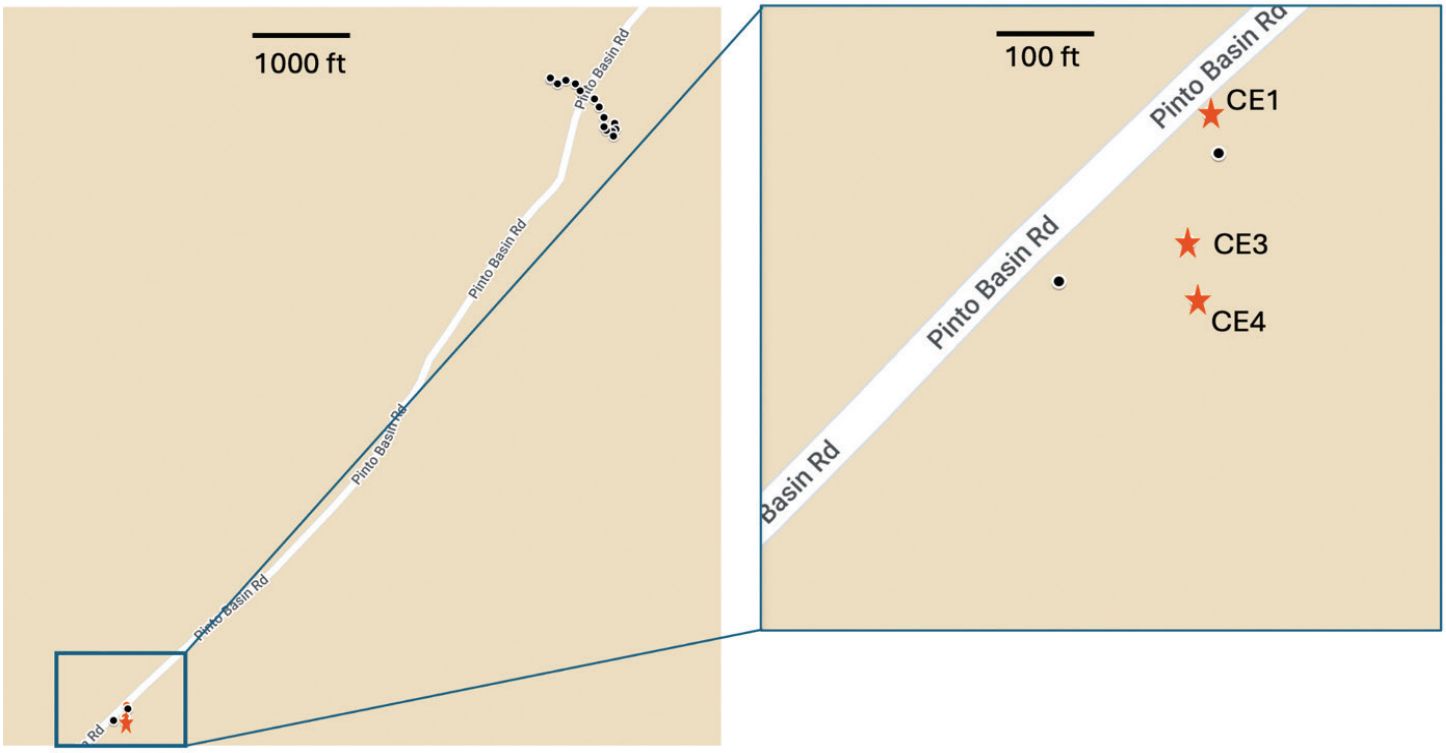


Figure 3. The distribution map of CB samples. The black star represents the individual forming distinct clonal lineage, while red circles indicate ramets belonging to the same genet. **Map generated using Google My Maps.**

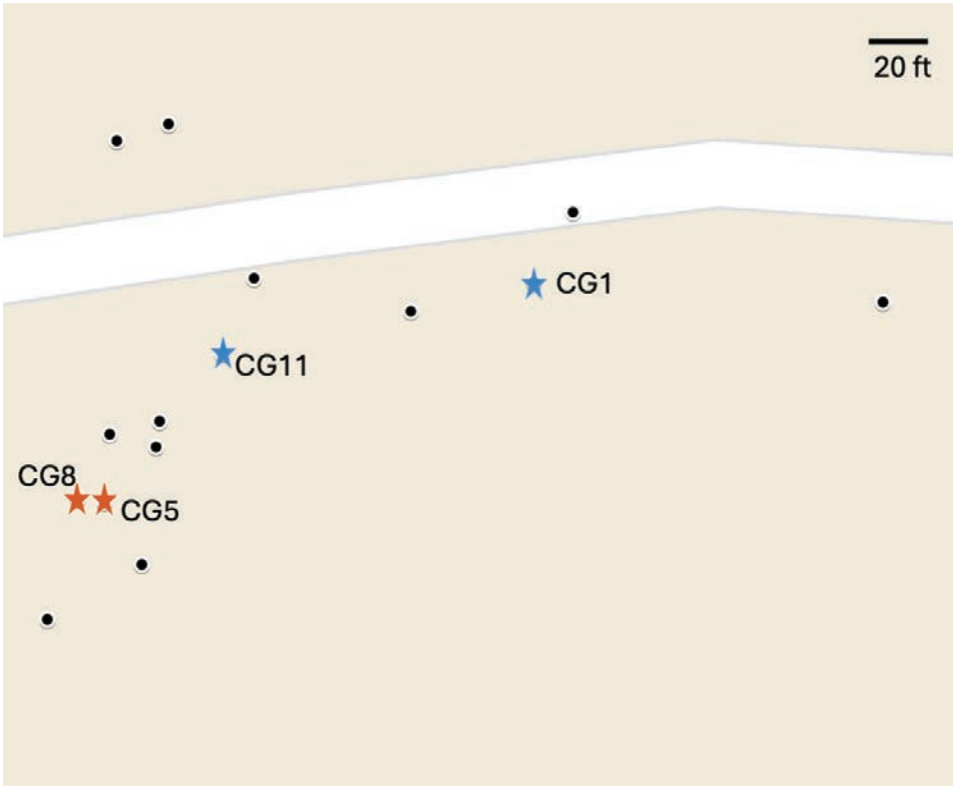


Figure 4. The distribution map of CG samples. Black circles represent individuals forming distinct clonal lineages, while colored stars indicate ramets belonging to the same genet, with each genet distinguished by a unique color. The sample IDs for ramets are given next to them. **Map generated using Google My Maps.**

based on sequence similarity and methylation status, that differs between homeologs (*34*).

CLONALITY ANALYSIS

To assess the degree of clonal reproduction, clonality analysis was conducted with the infinite allele model for each species separately, using GenoDive software (*35*). This program clusters individuals into clonal groups based on a genetic distance threshold. The threshold was identified by selecting a value between two peaks in the frequency distribution of pairwise genetic distances (*36*). The first peak, representing smaller genetic distances, is typically attributed to somatic mutations or genotyping errors, while the second peak corresponds to greater genetic variation between distinct genotypes (*37*). The probability of finding the observed clonal structure under sexual reproduction was tested using corrected Nei's diversity index (*38*) using 1000 permutations.

RESULTS

The DArTseq method identified a comprehensive set of SNP markers in all species, with a 100% call rate, producing a total of 10,839 SNPs. For the triploid CB, 2 genets were found, with 1 being a distinct clonal lineage while the other contained 19 ramets (Fig. 2). For the diploid CE, we found 17 distinct clonal lineages out of the 20 individuals, and

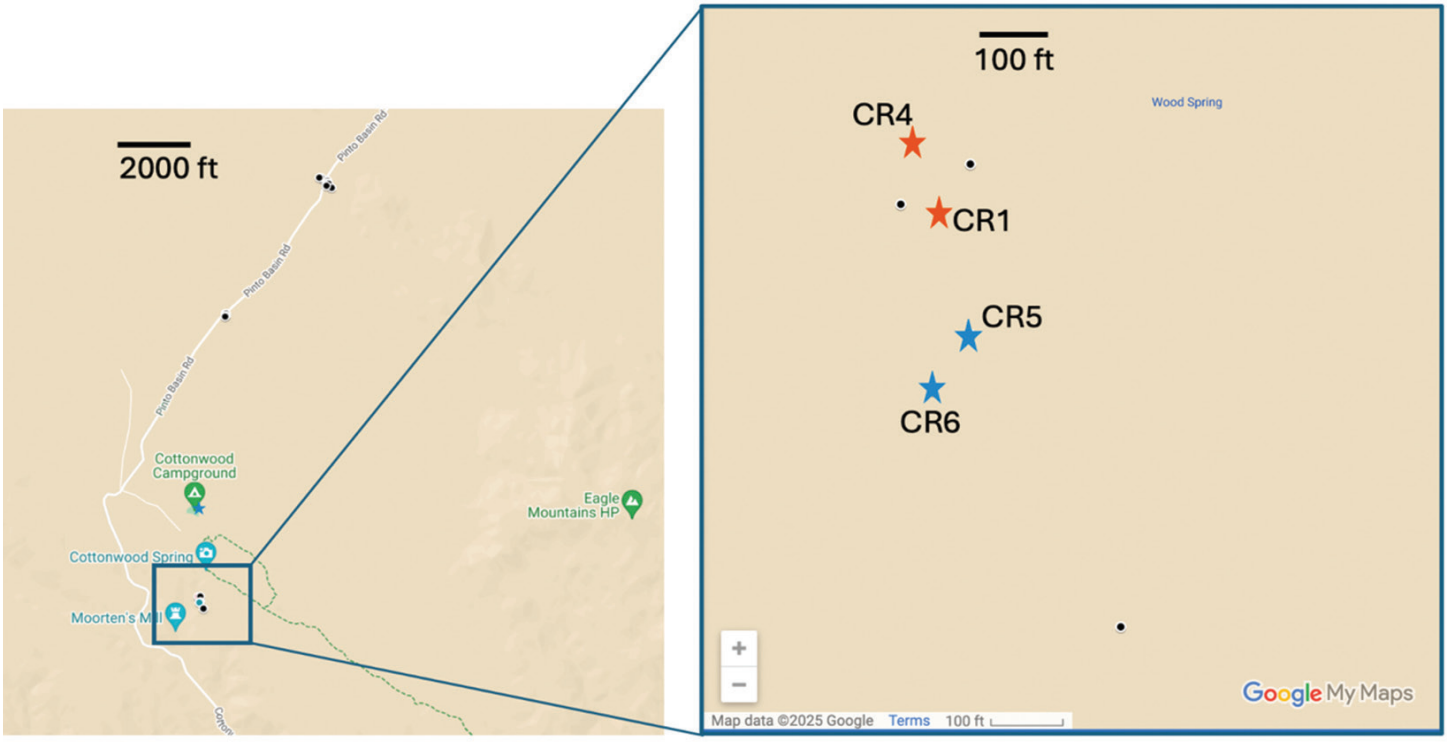


Figure 5. (Left) The full distribution map of CR samples. (Right) Zoomed-in map of CR distribution to clearly visualize individuals of the same clonal group. Black circles represent individuals forming distinct clonal lineages, while colored stars indicate ramets belonging to the same genet, with each genet distinguished by a unique color. The sample IDs for ramets are given next to them. **Map generated using Google My Maps.**

one clonal group including three individuals (Fig. 3). For CG, 11 distinct clonal lineages out of the 15 individuals and two clonal groups including two individuals each were identified (Fig. 4). For CR, 16 distinct clonal lineages out of the 20 individuals, and two clonal groups including two individuals each were identified (Fig. 5). The list of individuals belonging to the same clonal lineage or genet across all four species, along with the distances between them are presented below (*Table 1*). For all species, the null hypothesis that the probability of observing the recorded clonal diversity under random mating, was rejected.

DISCUSSION

The aim of our paper is to assess the degree of clonal reproduction in four *Cylindropuntia* species. The species we sampled have restricted distribution (*19*) and assessing their clonality has significant implications for their conservation (*37, 39, 40, 41*). Clonal reproduction can complicate population size estimation, as a population may comprise numerous ramets belonging to only a few genets (*41*). Consequently, censuses based solely on visible individuals may overestimate the number of genets or distinct individuals, potentially masking the genetic vulnerability of these plants (*37*). Moreover, without knowledge of the extent of clonality, general strategies for *in situ* conservation or *ex situ* collections may be irrelevant (*39, 40, 42*).

> Therefore, understanding clonality is essential for devising effective conservation management strategies for rare plants such as chollas.

Our results showed that all species exhibited some degree of clonality but it varied largely by ploidy level.

Previous research suggested that *Cylindropuntia ganderi* is unlikely to reproduce clonally due to the absence of detached terminal joints around the parent plants, which could facilitate the formation of new plants and large number of fibers and the resulting biomechanics (*30*). Additionally, CG exhibits strong terminal joint junctions that prevent detachment, limiting clonal propagation through this mechanism (*30, 43*). However, our findings reveal evidence of asexual reproduction in CG. It revealed 11 distinct clonal lineages out of 15 sampled individuals, along with two clonal groups containing two individuals each. In one clonal group, the two individuals were geographically close, consistent with the fact that asexual reproduction leads to clumped distributions (*43*). Interestingly, the second clonal group included individuals that were not in close proximity, suggesting that clonal fragments may be capable of dispersing over greater distances. This dispersal could be facilitated by wildlife or environmental factors such as wind. We have personally observed desert rats moving cholla fragments to fortify their burrows, which might play a role in clonal fragment dispersal.

For *C. echinocarpa*, we identified 17 distinct clonal lineages among the 20 individuals analyzed, along with one clonal group comprising three individuals. All members of this group were geographically close to each other. Similarly, for *C. ramosissima*, 16 distinct clonal lineages were identified among 20 individuals, with two clonal groups

Species (sample size)	No of genets found	Genets with more than one ramet	Ramet IDs that belong to the same genet	Approximate Distance between ramets (feet)
C. ganderi (15)	13	2	CG1 and CG11	81
			CG5 and CG8	11
C. ramosissima (20)	18	2	CR1 and CR3	102
			CR5 and CR6	88
C. echinocarpa (20)	19	1	CE1,CE3 and CE4	128 (CE1 and CE3)
				59 (CE3 and CE4)
				187 (CE1 and CE4)
C. bigelovii (20)	2	1	CB1-CB20 except CB7	70 (between each consecutive pair)

Table 1. List of individuals forming the same clonal lineage/genet in all four species and the distance between them.

of two individuals each, also found in close proximity. These findings suggest that vegetative reproduction through the rooting of detached fragments occurs in both species and complements the biomechanical findings of Bobich and Nobel (*30*). In that study, the authors noted that CE stems exhibited low rooting success. While this suggests that vegetative reproduction may be mechanically limited, the presence of small individuals near larger adults in the field indicated that clonal propagation does occur to some extent. Our genetic results support this interpretation, confirming the presence of clonal individuals in CE and suggesting that while biomechanical constraints may reduce the frequency of clonal reproduction, they do not eliminate it.

As hypothesized, *C. bigelovii* exhibited a high degree of clonality, with 19 out of 20 samples belonging to the same clonal group. However, individual 7 represented a distinct clonal lineage. The clonal distance between individual 7 and the rest of the individuals is in the thousands, whereas distances among the other individuals range between 50 and 100 (genetic distance units). Notably, individual 7 exhibited signs of admixture with CE in our genetic study (*44*), raising the possibility that it was translocated from a different population during restoration efforts or long distance dispersal by mammals. Signals of admixture between the two species may reflect historical hybridization, incomplete lineage sorting, or rare sexual reproduction events as CB occasionally produces viable gametes (*45*). However comprehensive phylogenomic approaches are necessary to confirm this. Another hypothesis for this genetic variation could be attributed to the Meselson effect (*46*), a mechanism that enhances heterozygosity in asexual species through the irreversible accumulation of mutations in homologous chromosomes over successive generations. The individual 7 might have resulted from a fragment dispersed from a different parent. The joints of CB are characterized by having mechanically weak terminal joints and the highest spine density per tubercle area, increasing the likelihood of spines attaching to passing vertebrates and facilitating greater dispersal (*30*). In fact, while commonly known as the teddy-bear cholla, CB has also been called a "jumping cholla," as their stems are easily detached (Fig. 6) (*47*). Previous studies have shown that side-propagules disperse more readily than top-propagules and that they attach best to medium-length fur, such as that of coyotes (*30,43*). Additionally, *Neotoma lepida* (desert woodrats) have been observed actively seeking CB propagules for use in their dens and as a food source (*48*). Predators, such as coyotes, that forage in woodrat nests are likely to encounter propagules placed around the dens and subsequently disperse them (*49, 43*). The high colonization rate of CB may represent its primary survival mechanism (*43*). However, a high degree of clonality reduces the potential for genetic variation, which is essential for adaptation (*50, 51*). Consequently, it is crucial to prioritize the conservation of CB in the face of impending climate change.

Figure 6. *Cylindropuntia bigelovii* is commonly known as "jumping cholla" because its spiny segments easily detach at the joints. Due to the high density of spines, the segments can easily attach to anything that brushes against them. **Photo credit: N. Ramadoss**

CONCLUSION AND MANAGEMENT IMPLICATIONS

Understanding the degree of clonality in cholla species provides critical insights for prioritizing conservation strategies to safeguard these unique desert plants. We found that all four sampled cholla species exhibit some degree of clonality, including those previously thought not to reproduce clonally. This finding highlights the potential for these species to create a misleading perception of abundance, as multiple individuals may be the same genetically. To address this, management plans should incorporate genetic tools to accurately differentiate between clonal ramets and distinct genets.

Our findings indicate that *C. bigelovii* in JTNP is predominantly clonal and exhibits limited genetic variation at the population level, which may hinder its capacity for short-term adaptation to environmental change. Although Baker et al. (2009) suggest that triploidy in *C. bigelovii* may represent a form of punctuated evolution involving rare haploid and diploid gametes, our results highlight the importance of monitoring and managing contemporary genetic diversity within JTNP populations. One potential management strategy is to propagate the rare genotype, like that of individual 7, to preserve its unique genetic composition and potential adaptive significance. Moreover, CB has the potential to spread aggressively in disturbed habitats, outcompeting other native species. Effective management strategies should focus on monitoring such expansions, especially in sensitive ecosystems.

ACKNOWLEDGEMENTS
We sincerely appreciate the invaluable assistance of Rosalinda Diaz, Zhuba Goldenlamb, and Tasha La Doux in the collection of samples.

REFERENCES

1. J. Maynard Smith, E. Szathmáry, *The Major Transitions in Evolution* (Oxford Univ. Press, Oxford, 1995), 346 pp.

2. S.C. Barrett, Influences of clonality on plant sexual reproduction. *Proceedings of the National Academy of Sciences*, **112**(29), 8859-8866 (2015).

3. L. Klimes, J. Klimešová, R. Hendriks, J. van Groenendael, Clonal plant architecture: a comparative analysis of form and function. In *The Ecology and Evolution of Clonal Plants*, H. de Kroon, J. van Groenendael, Eds. (Backhuys, Leiden, 1997), pp. 1–29.

4. A.J. Richards, *Plant Breeding Systems* (Chapman and Hall, London, UK, 1997).

5. V . Douhovnikoff, R. S. Dodd, Epigenetics: a potential mechanism for clonal plant success. *Plant Ecology* **216**, 227–233 (2015).

6. J. Klimesova, G. Ottaviani, T. Charles Dominique, G. Campetella, R. Canullo, S. Chelli, T. Herben, Incorporating clonality into the plant ecology research agenda. *Trends in Plant Science* **26**, 1236–1247 (2021).

7. C.G. Eckert, The loss of sex in clonal plants. In Ecology and Evolutionary Biology of Clonal Plants: Proceedings of Clone2000, an International Workshop held in Obergurgl, Austria, 20–25 August 2000 (Springer Netherlands, 2002), pp. 279–298.

8. G.E. Hutchinson, *A Treatise on Limnology. Volume III. Limnological Botany* (John Wiley & Sons, New York, NY, USA, 1975).

9. C.T. Philbrick, D.H. Les, Evolution of aquatic angiosperm reproductive systems. *BioScience* **46**, 813–826 (1996).

10. Z. Wang, L. Xie, C.M. Prather, H. Guo, G. Han, C. Ma, What drives the shift between sexual and clonal reproduction of *Caragana stenophylla* along a climatic aridity gradient? *BMC Plant Biology* **18**, 1–10 (2018).

11. H. Olofsson, P. Lundberg, The twofold cost of sex unfolded. *Evolutionary Ecology Research* **9**, 1119–1129 (2007).

12. E. Horandl, A combinational theory for maintenance of sex. *Heredity* **103**, 445–457 (2009).

13. C.H. Lin, M.N. Miriti, K. Goodell, Demographic consequences of greater clonal than sexual reproduction in *Dicentra canadensis. Ecology and Evolution* **6**, 3871–3883 (2016).

14. G.D. Duncan, A.G. Ellis, F. Forest, G.A. Verboom, Strong habitat and seasonal phenology effects on the evolution of self-compatibility, clonality, and pollinator shifts in *Lachenalia* (Asparagaceae: Scilloideae). *New Phytologist* (2024).

15. G.R. Hanko, M.T. Vogel, V. Negrón-Ortiz, R.C. Moore, High prevalence of clonal reproduction and low genetic diversity in *Scutellaria floridana*, a federally threatened Florida-endemic mint. *Plants* **12**, 919 (2023).

16. V.J. Tepedino, Overestimating population sizes of rare clonal plants. *Conservation Biology 26*, 945–947 (2012).

17. B. Goettsch, C. HiltonTaylor, G. Cruz-Piñón, J.P. Duffy, A. Frances, H.M. Hernández, et al., High proportion of cactus species threatened with extinction. *Nature Plants* **1**, 1–7 (2015).

18. L.C. Majure, M.A. Baker, M. Cloud-Hughes, A. Salywon, K.M. Neubig, Phylogenomics in Cactaceae: A case study using the chollas *sensu lato* (Cylindropuntieae, Opuntioideae) reveals a common pattern out of the Chihuahuan and Sonoran deserts. *American Journal of Botany* **106**, 1327–1345 (2019).

19. N. Ramadoss, A. Ordúño-Baez, C. Portillo, S. Steele, J. Rebman, L. Flores-Rentería, Unraveling the development behind unisexual flowers in *Cylindropuntia wolfii* (Cactaceae). *BMC Plant Biology* **22**, 94 (2022).

20. L.D. Benson, L.B. Hamilton, *The cacti of the United States and Canada* (Stanford University Press, Stanford, 1982), p. 1044.

21. A.C. Gibson, P.S. Nobel, *The Cactus Primer* (Harvard University Press, Cambridge, 1986).

22. L.J.S. Allen, E.J. Allen, C.R.G. Kunst, R.E. Sosebee, A diffusion model for dispersal of *Opuntia imbricata* (cholla) on rangeland. *Journal of Ecology* **79**, 1123–1135 (1991).

23. M. Chuk, Invasive cacti—a threat to the rangelands of Australia. In: D.J. Eldridge, C. Waters, eds. *Proceedings of the 16th Biennial Conference of the Australian Rangeland Society*, Perth, Bourke (Australian Rangeland Society, Perth, Australia, 2010) https://rangelandsgateway.org/sites/default/files/2021-01/arsbc-2010-chuk.pdf. Accessed Feb 1, 2025.

24. P. Soorae, Jumping cholla—the ornamental that became a wildlife and livestock barrier. *Selecting the Best Wild Fruits* **25** (2001).

25. P.K. Jones, J. Oakey, M.D. Day, J.S. Vitelli, Improving the effectiveness of the release strategy for the array of *Dactylopius tomentosus* biotypes for the biocontrol of *Cylindropuntia* species by using DNA analysis.

26. R.D. Cox, B.G. Johns, Dragged-rail treatments for reducing cholla infestation in southeast New Mexico, USA. *Weed Technology* **28**, 286–289 (2014).

27. P.C. Guerrero, L.C. Majure, A. Cornejo-Romero, T. Hernández-Hernández, Phylogenetic relationships and evolutionary trends in the Cactus family. *Journal of Heredity* **110**, 4–21 (2019).

28. N. Ramadoss, S. Steele, L. Flores-Rentería, Influence of sexual dimorphism and dichromatism on reproductive success in a rare native cactus. Oecologia 203, 383–394 (2023).

29. M.A. Baker, D.J. Pinkava, Chromosome numbers in some cacti of western North America—IX. *Haseltonia* **25**, 5–29 (2018).

30. E.G. Bobich, P.S. Nobel, Vegetative reproduction as related to biomechanics, morphology and anatomy of four cholla cactus species in the Sonoran Desert. *Annals of Botany* **87**, 485–493 (2001).

31. R. Buck, D. Ortega-Del Vecchyo, C. Gehring, R. Michelson, D. Flores-Rentería, B. Klein, L. Flores-Rentería, Sequential hybridization may have facilitated ecological transitions in the Southwestern pinyon pine syngameon. *New Phytologist* **237**, 2435–2449 (2023).

32. Q. Sohail, A. Manickavelu, T. Ban, Genetic diversity analysis of Afghan wheat landraces (*Triticum aestivum*) using DArT markers. *Genetic Resources and Crop Evolution* **62**, 1147–1157 (2015).

33. F. Shams, F. Dyer, R. Thompson, R. P. Duncan, J. D. Thiem, A. Kilian, T. Ezaz, Application of DArTseqderived SNP tags for comparative genome analysis in fishes; an alternative pipeline using sequence data from a nontraditional model species, *Macquaria ambigua*. *PLoS One* **14**, e0226365 (2019).

34. C. Sansaloni, J. Franco, B. Santos, et al., Diversity analysis of 80,000 wheat accessions reveals consequences and opportunities of selection footprints. *Nature Communications* **11**, 4572 (2020).

35. P.G. Meirmans, P.H. Van Tienderen, GENOTYPE and GENODIVE: Two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* **4**, 792–794 (2004).

36. M. Tsujimoto, K.S. Araki, M.N. Honjo, M. Yasugi, A.J. Nagano, S. Akama, H. Kudoh, Genet assignment and population structure analysis in a clonal forest floor herb, *Cardamine leucantha*, using RADseq. *AoB Plants* **12**, 080 (2020).

37. M.A. Sydes, R. Peakall, Extensive clonality in the endangered shrub *Haloragodendron lucasii* (Haloragaceae) revealed by allozymes and RAPDs. *Molecular Ecology* **7**, 87–93 (1998).

38. M. Nei, Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89(3)**, 583-590 (1978).

39. Z.W. Xie, Y.Q. Lu, S. Ge, D.Y. Hong, F.Z. Li, Clonality in wild rice (*Oryza rufipogon*, Poaceae) and its implications for conservation management. *American Journal of Botany* **88**, 1058–1064 (2001).

40. S. Fox, L. Eberth, J.B. Floyd, C. Pederson, L. Stafford, N. Tillmanns, A.B. Morris, The importance of understanding clonal structure for species listing and recovery: case studies from the rare Oconee bells (*Shortia brevistyla* and *Shortia galacifolia*; Diapensiaceae) and the federally endangered bunched arrowhead (*Sagittaria fasciculata*; Alismataceae). *Conservation Genetics* **1**, 1–18 (2024).

41. N.C. Ellstrand, M.L. Roose, Patterns of genotypic diversity in clonal plant species. *American Journal of Botany* **74**, 123–131 (1987).

42. D.J. Coates, Genetic diversity and population genetic structure in the rare Chittering grass wattle *Acacia anomola*Court. *Australian Journal of Botany* **36**, 22–27 (1988).

43. R. Fong, L. Johnson, J. Saucedo, Patterns of vegetative reproduction and distribution of *Cylindropuntia. California Ecology and Conservation Research* (2019).

44. N. Ramadoss, S. Steele, L. Flores-Renteria, Prickly Problems: Cylindropuntia's low genetic diversity despite inbreeding avoidance, *Ecology and Evolution, under review* (2025).

45. M.A. Baker, J.P. Rebman, B.D. Parfitt, D.J. Pinkava, A.D. Zimmerman, Chromosome numbers in some cacti of Western North America-VIII. *Haseltonia* **15**, 117–134 (2009).

46. D.B. Mark Welch, M. Meselson, Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. *Science* **288**, 1211–1215 (2000).

47. J.A. MacMahon, *Deserts*. The Audubon Society Nature Guides (Alfred A. Knopf, New York, NY, 1985).

48. J.H. Brown, G.A. Lieberman, W.F. Dengler, Woodrats and cholla: dependence of a small mammal population on the density of cacti. *Ecology* **53**, 310–313 (1972).

49. T.A. Vaughan, S.T. Schwartz, Behavioral ecology of an insular woodrat. *Journal of Mammalogy* **61**, 205–218 (1980).

50. J. Silvertown, The evolutionary maintenance of sexual reproduction: evidence from the ecological distribution of asexual reproduction in clonal plants. *International Journal of Plant Sciences* **169**, 157–168 (2008).

51. V.N. Suarez-Santiago, J. Provan, A.T. Romero-García, S. Ben-Menni Schuler, Genetic diversity and phylogeography of the relict tree fern *Culcita macrocarpa*: Influence of clonality and breeding system on genetic variation. *Plants* **13**, 1587 (2024).

AUTHOR BIOGRAPHY

Dr. Ramadoss is a postdoctoral researcher at San Diego State University working under Dr. Flores-Renteria, specializing in the evolutionary ecology of native plants. Her PhD research explored sexual systems and their ecological and genetic consequences in Cylindropuntia species, a key genus in North American deserts. Currently, her postdoctoral work spans projects on hybridization in Arbutus species and clonality in Agave. She is passionate about understanding plant reproductive strategies and their evolutionary implications.

Front cover: Congested roads in Joshua Tree National Park. **Photo credit: NPS/EmilyHassell**
Back cover: A stand of *Cylindropuntia bigelovii* (teddybear cactus). **Photo credit: Mari Stewart**

JOSHUA TREE NATIONAL PARK ASSOCIATION

74485 National Park Drive
Twentynine Palms, CA 92277

www.joshuatree.org

Email mail@joshuatree.org

Phone (760) 367-5525

JT Science
https://joshuatree.org/jt-science-newsletter/
jtresearchgrant@joshuatree.org

Grant Program
https://www.nps.gov/jotr/learn/nature/grantprogram.htm
jtresearchgrant@joshuatree.org

Research in JTNP
https://www.nps.gov/jotr/learn/nature/research_permits.htm

Desert Institute www.joshuatree.org/field-classes