



Science Newsletter

Grinnell resurveys document the colonization of the Zone-tailed Hawk (*Buteo albonotatus*) in Mojave National Preserve

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Beginning in 1908, Joseph Grinnell, pioneer ecologist and first director of the Museum of Vertebrate Zoology (MVZ) at the University of California, Berkeley, launched his mission to thoroughly document the fauna of western North America before it was forever transformed by human population growth and land-use changes. With the financial support of Annie Alexander, he and his colleagues amassed data from over 700 locations, primarily in California. When reflecting on the value of his work, he noted, “*This value will not, however, be realized until the lapse of many years, possibly a century, assuming that our material is safely preserved. And this is that the student of the future will have access to the original record of faunal conditions in California and the West, wherever we now work.*” (1)

These efforts first reached the Mojave Desert in 1914, with field work in and around the region of the Providence Mountains summarized by Johnson et al. (2) and entailing “367 man-days” extending to 1945, with the major part from 1938 to 1940. They collected a total of 2,582 specimens of vertebrates, and each biologist left



Figure 1. MVZ party in Cedar Canyon, 1 June 1938. From left to right: Elmer Aldrich, Dale Arvey, Dave Johnson, Tom Rodgers, and Joseph Grinnell. Photo used with the permission of the Museum of Vertebrate Zoology, University of California, Berkeley.

detailed notes of his or her daily observations. Beginning in 1945, the work extended into Joshua Tree National Park (then a national monument) (3).

Now, with the support of the National Science Foundation, biologists from the MVZ, San Diego Natural History Museum (SDNHM), University of New Mexico, and University of California, Santa Cruz, are retracing the steps of Grinnell and his students to document the changes in California’s desert fauna. We hope to tease apart the relative roles of climate, physiology, and habitat in shaping changes at the levels of both the species and the community.

Resurveys in Mojave National Preserve began in 2015 and will continue through 2019. The first trip our SDNHM ornithology team has made, to Cedar Canyon in the Mid Hills of the Providence Mountains from 28 April to 2 May 2016,

paralleled the visit of Joseph Grinnell, David H. Johnson, Elmer C. Aldrich, Dale Arvey, and Thomas L. Rodgers from 19 May to 4 June 1938 (Figure 1). Cedar Canyon was one of the sites more intensively covered by the MVZ, entailing not only the 17-day spring visit but also a winter visit, 6–8 January 1938.

Already this single trip has revealed some notable changes, stressing the importance of the careful documentation initiated by Grinnell. For example, the Gray Vireo (*Vireo vicinior*) has evidently disappeared, while new colonists are the Rufous-crowned Sparrow (*Aimophila ruficeps*), Anna’s Hummingbird (*Calypte anna*), Black-tailed Gnatcatcher (*Poliophtila melanura*), and Zone-tailed Hawk (*Buteo albonotatus*).

Colonization of the Zone-tailed Hawk is particularly notable because it represents a substantial expansion of the breeding range into

In this Issue:

- Page 1. Grinnell resurveys document the colonization of the Zone-tailed Hawk (*Buteo albonotatus*) in Mojave National Preserve
- Page 4. Desert salt flats as refugia for terrestrial arthropods
- Page 8. Phenology mediates reproductive success in the desert annual *Chylismia brevipes*

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California, beyond its former northern limits in Baja California and Arizona. In April 2016, Hargrove, Unitt, and Squires encountered the territorial male, and then located the nest and incubating female in a single-leaf pinyon (*Pinus monophylla*) near Cedar Canyon (Figure 2). Maikis followed up to confirm the pair's success, photographing a fledgling on 12 July (Figure 3). This is one of only a few nests documented in California.

Though discovery of this nest was exciting for us, it was not too surprising because the Zone-tailed Hawk has been observed in Mojave National Preserve as far back as 2004 (4). The species has occurred regularly since 2009, and a fledged juvenile was photographed at Mid Hills Campground in 2012. In 2014 David Huggins (University of Nevada, Reno) located a nest less than 200 m away from the nest we observed in 2016, possibly from the same pair, and Chelsea Hawk (MVZ) observed likely this same pair in Cedar Canyon during Grinnell resurvey work in April 2015. In 2016 alone, two Zone-tailed Hawks were reported via www.ebird.org in early April at the Mid Hills Campground, 5.8 km south of our nest, another pair was at Live Oak Spring about 2.3 km northwest of our nest in July and August, and there were multiple other reports of single individuals around the region.

Elsewhere in Mojave National Preserve, Todd Keeler-Wolf photographed a Zone-tailed Hawk nest near Cottonwood Spring in the Granite Mountains in April 2011. In June 2016, at least, this nest area was active again—Maikis photographed a pair and nest and then observed a fledgling on 7 July 2016. Considering all the observations in the Mid Hills, Cedar Canyon, and Granite Mountains over the last 12 years, it seems likely that there may now be several pairs of Zone-tailed Hawks nesting in Mojave National Preserve, although an inventory is needed to determine the total breeding population in the area.

The Zone-tailed Hawk was recorded in California as early as 1862 (5), but only five occurrences, mostly near the Mexican border, were known by 1944, when Grinnell and Miller (6) considered it a rare vagrant in fall and winter. Since then, fall and winter records have increased, to the point that



Figure 2. Adult female Zone-tailed Hawk incubating on nest in pinyon tree, Cedar Canyon (Photo taken by Lea Squires, 30 April 2016).



Figure 3. Fledgling Zone-tailed Hawk near nest, Cedar Canyon (Photo by Troy Maikis, 12 July 2016).

the California Bird Records Committee discontinued reviewing reports of the Zone-tailed Hawk in 1998. In recent years, several individuals are noted in southern California every winter. The first two spring records were near San Diego in 1945 and at Morongo Valley in 1970 (7). A pair nested unsuccessfully in the Santa Rosa

Mountains from 1979 to 1981 (8), and a pair at Hot Springs Mountain, San Diego County, fledged one young in 1986 (9). But neither of these previous nestings was followed by an apparent colonization as we now see in the Mojave Desert. Since 2003 the Zone-tailed Hawk has occurred at other scattered locations in

California during the breeding season annually, so there may be other new nesting sites yet to be discovered.

Because it occurs in such low density throughout its range, the Zone-tailed Hawk is one of those species typically disqualified from trend analyses based on the results of broad-scale monitoring programs. But it is a conspicuous hawk, highly defensive of its nesting site, screaming loudly at passing intruders, as we experienced (Figure 4). Its plumage is distinctive: almost black with the tail boldly banded with white. It is uncannily similar to a Turkey Vulture (*Cathartes aura*) in its general appearance and flight style—which is thought to be a type of mimicry enabling it to surprise prey accustomed to the innocuous vulture (10). Otherwise, confusion is likely only with the Common Black Hawk (*Buteogallus anthracinus*), another more tropical species beginning to reach California (11) but in far fewer numbers.

To the south and southeast of California, the Zone-tailed Hawk has a wide range that has apparently expanded but for unclear reasons (10). According to Corman (12), its range in Arizona “has steadily advanced northward ... in the past 50 years.” Although this generally northward spread is consistent with predictions of climate warming, research is needed to identify changes in other possible factors, such as habitat and prey availability. Monitoring would be needed to reveal differences in population growth and sustainability.

Often, it can be difficult to distinguish between a true expansion of a species’ geographic range from extensions discovered through increased exploration by ornithologists. However, Grinnell’s teams covered the sites of Zone-tailed Hawk nesting in the Santa Rosa Mountains in 1908 and the Granite Mountains in 1938 and 1940, as well as Cedar Canyon in 1938. And succeeding ornithologists, such as Steve Cardiff and Van Remsen (13), studied the mountains of Mojave National Preserve as well. This gives us confidence that the advent of the Zone-tailed Hawk and other species represents a real change, not an artifact. Further resurveys over these next three years will give us an opportunity to document these faunal changes carefully, and



Figure 4. Adult male Zone-tailed Hawk calling near the nest, Cedar Canyon (Photo taken by Lea Squires, 29 April 2016).

collaboration will provide further insights and interpretation in a broad ecological context.

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Desert salt flats as refugia for terrestrial arthropods

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At first glance, salt flats may not appear to be hospitable to animal life (Figure 1). Often located in hot, arid places, they can readily flood, and their salinity presents special challenges to water regulation. However, there is evidence that these seemingly inhospitable habitats may act as refugia (a location of an isolated population of a once more widespread species) for terrestrial arthropods (1). Soda Lake in Mojave National Preserve is one such salt flat that is a remnant of a much wetter period in the history of southwestern North America. The Desert Southwest, which includes some of the driest deserts on Earth, was largely composed of riparian woodland and scrub habitats before the Quaternary began (1.64 million years ago). During the Pleistocene (2.5 million to 11,700 years ago) there were several advances and retreats of glaciers, and during times of



Figure 1. Panamint Lake, Panamint Valley, California. A typical and seemingly inhospitable salt flat.

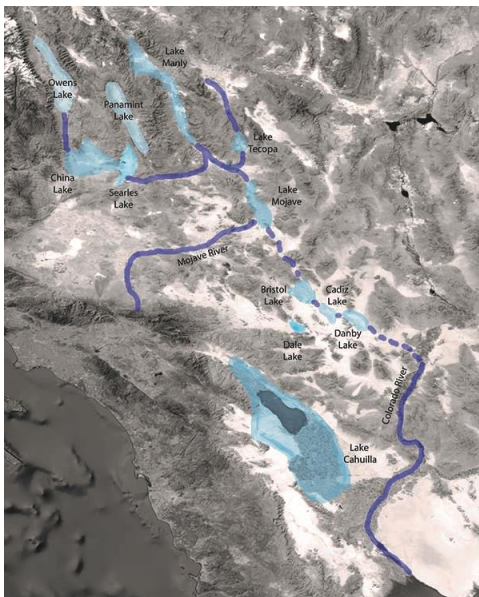


Figure 2. Ancient (2.5 million to 126,000 years BP) waterways in the Mojave and Colorado Deserts of southern California. Dashed lines indicate connections disputed by geologic evidence. This is not comprehensive or to scale, but meant to provide a general idea of what the Southwest was like prior to desertification.

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advancement when the overall climate was cooler and wetter, many large lakes occupied the Southwest. Some of these lakes were even connected to one another via river corridors (Figure 2); a much different past climatic regime is evidenced by the presence of arroyos and dry lake beds. Occasionally these features flood, revealing ancient corridors that aquatic organisms, such as springsnails and pupfish, once used to disperse (2, 3, 4, 5, 6), and today some of the dry lake beds are covered with a salt crust. These crusts form due to chemicals that leach out of the surrounding mountains or are left over from rapid evaporation of water during desertification, often occurring in an enclosed basin that precludes the salts from being washed away. These crusts differ among the lakes in both salinity and chemical composition (7).

Despite the harshness, a community of arthropods lives secret lives in the salt flats, making their homes in the layer between the salt crust and the soil and/or in cracks and holes in the salt or the substrate. Thus far, our research has primarily focused on the salt flat spider

Saltonia incerta (Banks, 1898) (Figure 3). When we began our study, only a handful of specimens (3) had been collected since its discovery, the species was presumed extinct (8), and its relationship to other spiders was elusive. *Saltonia incerta* was first collected in 1897 in Salton, California. This type locality (location from where a species is first recorded) had since been submerged when the Colorado River flooded the Salton Basin in 1905, creating the Salton Sea. A second specimen was collected in 1941 on the west side of the Salton Sea at Fish Springs; however, that locality was also lost, being transformed by human development. *Saltonia incerta* was also collected at an alleged third locality, but the exact location is unclear (9), making re-collection impossible. In the 1990s, much to everyone's surprise, the species was collected in pitfall traps from two localities some distance away from the Salton Basin – China Lake and Soda Lake (D. Ubick & W. Savary, unpubl. data). This result led the first author to explore other salt flats throughout the Desert Southwest in search of the spider. *Saltonia incerta* has now been found at 15 salt flats,

including sites as far east as New Mexico (1) (Figure 4).

Because *Saltonia incerta* is found under the salt crust of salt flats and not in the intervening desert, we aimed to determine the factors responsible for this striking distribution. We focused on three scenarios – one of complete isolation, one of dispersal during intermittent flooding, and a scenario involving aerial dispersal. The first scenario assumes that suitable habitats were more extensive in the wetter periods of the past and, as desertification occurred, the spiders retreated to areas where moisture persisted. The second scenario is governed by the premise that spiders would, assuming water is a limiting factor, rarely disperse except in times of flooding when there is sufficient moisture in the intervening areas for the spiders to survive the journey. Finally, a scenario of aerial dispersal relies on the ability of spiders to balloon (a form of dispersal in which immature spiders drift on air currents using silk threads) to suitable habitat. Each scenario produces distinct patterns in the genetic connectivity of populations. These patterns can be predicted both among individual salt flats, as well as among groups of salt flats located within ancient drainage basins. Analyzing the DNA of the spiders allowed us to determine which scenario was most likely based on these patterns.

We analyzed both a mitochondrial DNA gene (Cox1) and a nuclear DNA gene (H3) for 81 spiders from 13 salt flats in 6 drainage systems



Figure 3. The spider (*Saltonia incerta*) on its web made within the thin layer between salt crust and soil substrate on the surface of the dry lake bed.

(for detailed methodology, see (1)). Spiders were assigned to the following drainage basins – 1) Owens River Drainage, 2) Amargosa River Drainage, 3) Mojave River Drainage, 4) Bristol Basin Drainage, 5) Colorado River Drainage, and 6) Lake Otero (Figures 2, 4). We found a total of 41 haplotypes (unique DNA sequences). There is deep genetic divergence between the populations of New Mexico (Lake Otero) spiders and the populations of California spiders (Figure 5), and although no morphological differences were detected, they have likely been separated from each other for a long time/many generations. Within California, there were several salt flats with genetically unique populations that

corresponded to two ancient lake beds. These include specimens from ancient Lake Tecopa, and Badwater and Cottonball Basin specimens from ancient Lake Manly in Death Valley. Only the New Mexico specimens exhibit strong genetic isolation in relation to their drainage basin. This latter result is perhaps not surprising given that Lake Otero is a closed basin, without possible connections to other basins. An AMOVA (Analysis of Molecular Variance) indicated significant genetic variation at three levels: 1) among basins, 2) among individual salt flats within basins, and 3) among individuals within salt flats. Because of apparent admixture of genes between the Bristol and Colorado River Drainage Basins, we examined these groups for evidence of migration. The results of this analysis indicate that migration rates in either direction are very low, but slightly higher for migration from the Colorado River Drainage Basin to the Bristol Basin (south to north). Modeling migration rates also allowed us to determine that populations may have diverged 1.59 million to 89,300 years ago which is credible considering geological and climatological data. Finally, no pattern of isolation by distance was detected, indicating that the distance between salt flats or drainages is not important for predicting genetic divergence.



Figure 4. All known localities of *Saltonia incerta*, including three new ones that have yet to be included in analyses: Panamint Lake, California and Holloman Wildlife Refuge and Lazy Lagoon at Bottomless Lakes State Park, New Mexico.

Of the three proposed scenarios responsible for the distribution of *Saltonia incerta*, the data most strongly support complete isolation, the scenario

where suitable habitats were more extensive in the wetter periods of the past and, as desertification occurred, the spiders retreated to areas where moisture persisted. The data are not consistent with the dispersal via intermittent waterways scenario because we would expect less genetic change if this was the case. Additionally, many of the patterns present in the data are inconsistent with geological data. We also consider a scenario of aerial ballooning unlikely given the amount of genetic variation found in the data. Ballooning is also a very risky behavior for animals that live in a specialized habitat because there is no guarantee that they will land in a suitable area. Furthermore, there have been no observations of ballooning in *Saltonia incerta*. We also examined wind patterns and wind regime data to determine whether the directionality of migration mirrored wind direction. Prevailing winds in the Mojave Desert are usually from the west and the south (10), indicating migration could occur from the Colorado Drainage to the Bristol Basin. Our results indicate a very low probability of migration in this direction. Also, when examining more local wind patterns determined by topographical features, there is an overall trend of wind moving from west to east (11). This is opposite to the pattern indicated by the data, and is thus inconsistent with the species dispersing via ballooning. The scenario that populations are isolated with little to no gene flow is supported in all analyses and is consistent with geological data. There is high genetic structure between basins and between salt flats within basins, but low genetic structure within salt flats. Overall, the evidence thus far indicates that *S. incerta* was once more widespread than it is currently, and as drying has occurred, this species has retreated to salt flat habitats (7).

Although living in a salt flat may seem like a bizarre life history strategy, it does have its advantages. First, there is almost always some moisture between the underlying substrate and the salt crust. Second, the temperature beneath the salt is much cooler than the ambient air temperature. Finally, there are very few predators. Therefore, if animals can tolerate the osmotic challenges, this habitat may act as a type of oasis in an otherwise harsh environment. In addition to these advantages, once we

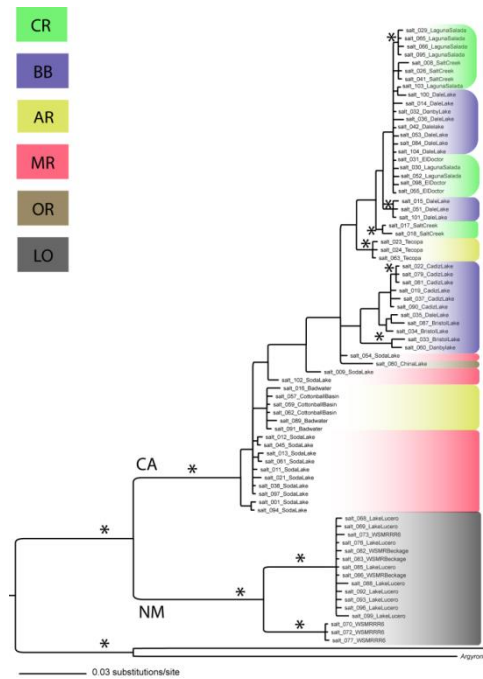


Figure 5. 50% majority rule consensus phylogram from 120,000 trees. Asterisks denote nodes supported by >95% Bayesian posterior probabilities. Colors represent drainages. CR = Colorado River, BB = Bristol Basin, AR = Amargosa River, MR = Mojave River, OR = Owens River, LO = Lake Otero, CA = California, NM = New Mexico.

consider where *Saltonia incerta* resides within the spider tree of life, salt flats may not seem like such a peculiar place to make a living.

Saltonia incerta was originally placed in Agelenidae (12), but was moved to Dictynidae by Lehtinen (13). In a study by Spagna et al. (14), *S. incerta* formed a clade (evolutionarily related group) with 2 other genera – *Paratheuma* and *Argyroneta* – neither of which were placed in Agelenidae or Dictynidae. The former was placed in Desidae based on morphological characters. Many desids are intertidal spiders that have enlarged tracheal trunks. *Paratheuma* is also an intertidal spider with enlarged tracheal trunks; however, this shared morphological character may represent an adaptation to a semi-aquatic lifestyle rather than a shared evolutionary history. First described by Banks (15) from Bermuda, Roth and Brown (16) later described a species from the shores of the Sea of Cortez, with many other species described from Pacific Islands, eastern and western Asia, including Iran (17, 18, 19, 20). In the latter paper, Zamani et al. (20) transferred *Paratheuma* to Dictynidae from Desidae based on genitalic characteristics, often

used for grouping and separating spider family, genera, and species.

The latter species that formed a clade with *Saltonia incerta*, *Argyroneta*, has been placed in several families, including its own – Argyronetidae – over the years owing to its unique morphology and lifestyle. According to the World Spider Catalog (21), this genus is currently placed in Cybaeidae. This spider is the only known representative of Araneae to live fully submerged in freshwater. Therefore, given this group of related “aquatic” spiders (14), *Saltonia*’s affinity for remnant aquatic habitats makes sense because this species may have been pre-adapted to living in such habitats. An ongoing study by the authors and colleagues is reconsidering the current placement of *Argyroneta* and other semi-aquatic spiders.

Saltonia incerta is not the only arthropod to exploit salt flat habitats. The salt flats are home to entire communities of arthropods, many of which are endemic. These include brine flies (*Ephedra*), salt flat ant-like flower beetles (*Tanarthrus*) (Figure 6), as well as other spiders of the families Lycosidae and Salticidae. Currently we are focusing on these groups to produce community-level data. The salt flats are excellent natural laboratories of nearly repeated ecosystems. Future work will be to obtain more molecular data that includes additional markers for *S. incerta* as well as additional samples of the spider to produce more detailed population history results. There are also areas where the spider may be that we have yet to find it, so we are still looking for additional distribution records. For instance, in early 2016, we found the species at three new localities: Panamint Lake in Panamint Valley, California and Bottomless Lakes State Park and Holloman AFB Wildlife Refuge in New Mexico. Notably, on any given salt flat at any given time, two distinct sizes of adults can be collected – small adults and large adults. We aren’t yet sure why this occurs, although one idea is that smaller adults reproduce sooner and therefore require fewer resources: i.e. a reproductive strategy ensuring a next generation is produced even when resources are scarce.

Next, we want to expand our studies to include a broad range of arthropod groups from which to

obtain genetic data. This will include arthropods that are sedentary (spiders) and those that fly (beetles, flies) to compare evolutionary histories and produce robust evolutionary hypotheses for salt flat organisms of the Desert Southwest. We also plan to focus more intensely on a small number of salt flats, including Soda Lake, to examine community level processes, such as food webs, using systematic collecting and new genetic methods like DNA 'barcoding'. Finally, we will examine the physiological mechanisms of heat and salt tolerance in some of the arthropod groups. It has been suggested that differences in the chemical make-up of the salt flats may have been responsible for speciation in some fly species (7).

The Mojave Desert is truly a special place that harbors many endemic organisms, often associated with unique microhabitats with very limited geographical range. Numerous habitats in the Desert Southwest, such as dune systems and salt flats, support their own suite of organisms, adding to the biological diversity found in these ecosystems. Unfortunately, this biodiversity is rarely accounted for; instead the desert is often considered a wasteland without much biodiversity, and it is constantly under threat from development and other human impacts. Currently, much of the desert, including land in the immediate vicinity of Soda Lake in Mojave National Preserve, is being considered for use as large solar farms (23, 24). This is occurring despite potentially negative environmental impacts caused by solar farms and despite the availability of alternative methods for meeting our energy demands like solar panels on rooftops in urban areas (25, 26). Although there are studies demonstrating the detrimental effects of habitat alteration to the arthropods that depend on these sensitive habitats (22), these organisms have rarely, if ever, been considered when assessing the environmental impacts for proposed solar projects. Long term assessment of salt flat arthropod communities can provide insight into the disturbance of these unique habitats; however, the pace of alternative energy development in the Desert Southwest may limit our ability to learn enough about these ecosystems before they are irrevocably altered.

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Figure 6. Salt flat ant-like flower beetles of the genus *Tanarthrus*.

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Phenology mediates reproductive success in the desert annual *Chylismia brevipes*

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Phenology, the timing of life history events such as germination or reproduction, strongly influences plant performance (1,2,3,4,5). Phenological events in plants are triggered by a variety of climatic cues, such as rainfall, temperature, photoperiod and soil moisture, all of which can vary between years. Long-term studies on spring phenology have demonstrated earlier flowering times as an effect of climate warming, documenting shifts of a few days up to a month over the past century (e.g., 6,7,8,9,10,11). However, these patterns are not as well documented or apparent in arid and semi-arid ecosystems (but see 12,13,14,15).

These results have generated interest in how a shift in flowering phenology will overlap with a shift in pollinator activity, since interacting species may respond to different climatic cues (16,17,18) or respond differently to the same climatic cue. Disrupted synchrony between flowering time and pollinator activity due to climate change will likely result in decreased reproductive success (19,20,21). Alternatively, interacting plants and pollinators may have similar responses to climate change, with limited or no disruption to their interaction (e.g. 22,23). In addition, herbivore phenology may be affected by shifting plant phenology, which could then indirectly modify interactions with pollinators and have consequences for plant performance (24,25,26,27,28,29).

Desert annual plants experience strong constraints on the timing of reproduction: they must complete their reproduction in a relatively short time period. The timing of flowering varies each year, depending on the arrival of winter



Figure 1. *Chylismia brevipes* flowers and fruit (Photo: Wikipedia Commons and K. Gerst).

rainfall and temperature patterns. This variable nature of rainfall and temperature in the desert during the winter months leads to large fluctuations in population dynamics and a short reproductive season for winter annual species (30, 31). The short window of time available to reproduce means that desert annual plants may be more vulnerable to mismatches with pollinators than perennials, which may flower longer due to greater storage of resources and are often able to reproduce over multiple years. Desert winter annuals germinate, grow, and complete their life cycle in the relatively cooler months when soil moisture is generally more available. They are limited by heat and drought, which constrain photosynthesis and ultimately determine the end of the winter annual growing season (32, 33).

This study examines how the timing of reproduction, pollination and herbivory affects reproductive success in the desert annual *Chylismia brevipes* (Onagraceae) over three flowering seasons. We explored the degree of plant-pollinator-herbivore synchrony and concomitant seed production through the flowering season. We predicted reduced fruit and seed set when plant and pollinator phenology are asynchronous. Additionally, we predicted greater damage to plants from insect herbivores with

increased plant-pollinator synchrony because insect herbivores and pollinators are expected to respond similarly to phenological cues.

Chylismia brevipes, a self-incompatible obligate outcrosser, germinates after winter rainfall and completes its life cycle by April or May (34). *Chylismia brevipes* has diurnal yellow flowers that are primarily visited by bees, and can stay open for more than four days when not fertilized (Figure 1). The study site used in this research is located at the south end of the Bristol Mountains in the Mojave Desert of San Bernardino County, California (34°37'13.66 N, 115°40'30.05 W) (Figure 2). Temperature and precipitation data were retrieved from the Mid Hills weather station in Mojave National Preserve (<http://www.wrcc.dri.edu/wraws/>) (Figure 3).

Data were collected on two occasions approximately one month apart during the flowering season (representing early season versus late season plants). These time periods differed across years based on observations of when the population was experiencing peak flowering; the proportion of individuals flowering in the population was monitored throughout winter and spring to make this assessment. Plants were included in the study if they had at least three open flowers. The number of individuals

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monitored per time period ranged from 22-70, depending on how many plants were flowering at the site. Measurements were made on separate cohorts of individuals early and late in the season rather than repeated measures of the same individuals; plants were selected only if they were in an early phase of flowering. The total number of flowers, fruits, and flower buds were recorded, as well as the fruit set (proportion of flowers that set fruit). We were able to calculate the cumulative number of flowers and fruit set because the corolla falls off 1 to 5 days after opening, but the inferior ovary remains on the plant whether the ovules have been fertilized (exhibiting swollen fruit) or not (exhibiting thin fruit). Plants were marked with a small label around the base of the stem and the GPS location was taken for relocation. Plants were re-visited three to four weeks after initial observation to collect fruits, which were then used to assess fruit length, ovule number, and seed number in the laboratory. "Seed set" was calculated as the number of fertilized seeds divided by the total number of ovules (fertilized and unfertilized). Seed set measures the within-fruit fertilization rate. To determine if flowers were pollen limited in their reproduction, we compared seed set of flowers that were naturally pollinated (control) to flowers that were hand-pollinated using pollen from neighboring plants (35). Pollen was applied by collecting and removing anthers that were shedding pollen from at least three plants in the vicinity that were not included in the study, and rubbing the anthers on the receptive stigmas of the focal flower. The majority of plants completed their reproduction within this three to four week period.

To quantify pollination intensity, styles from the oldest open flower on the primary flowering stalk were collected from each tagged individual and immediately placed in 70% ethanol. Styles were softened and stained with aniline blue dye (35). Each style was observed under a fluorescence microscope and was assigned a pollination intensity based on the number of germinated pollen grains with visible pollen tubes.

For each tagged individual, insect herbivore damage on leaf, stem, and fruit tissue was assessed at each visit. Damage was rated on a scale from 0-3 where 0 = no damage, 1= minimal



Figure 2. Population of flowering *Chylismia brevipes* in the Bristol Mountains (Photo: K.Gerst).

damage (< 25% of tissue), 2= substantial damage (25-75% of tissue) 3: intense damage (>75% of tissue). If caterpillar or beetle chewing was evident, herbivory was deemed to be the cause for damage.

Statistical analyses were performed in SAS 9.1.3 (SAS Institute, Cary, NC, USA). For all analyses, timing of reproduction and year were treated as fixed effects. Proportional fruit to flower ratio ("fruit set") and seed to ovule ratio ("seed set") data were analyzed using a generalized linear model assuming a binomial distribution and logit link function in PROC GLIMMIX to quantify differences in reproduction between early vs late season plants and between years. Pollen tube density, total number of seeds per fruit ("total seeds"), and herbivory score were each analyzed separately using PROC GLM to quantify reproduction and herbivore damage differences between early vs late season plants and between years. Differences in individual means were tested using Tukey-Kramer HSD comparisons within treatments. Pollen limitation was assessed using paired t-tests to test for a difference between seed number per fruit of control vs hand-pollinated flowers for each time period. Reproductive phenology began progressively later in each study year. In 2008, peak flowering

occurred in February; in 2009 peak flowering occurred in March; in 2010 peak flowering occurred in April. Temperature patterns did not differ strongly between years, however, given the shift in peak period, flowering occurred at increasingly warmer periods, with flowering occurring during the coolest period in 2008 and the warmest period in 2010 (Figure 3).

Generally, the bulk of the rainfall in the region was relatively early in 2008 when peak flowering occurred in February, and relatively late in 2010 when peak flowering occurred in April. In all three years, *Hyles lineata* (Lepidoptera: Sphingidae) larvae were the predominant herbivores and in 2010 an adult chrysomelid leaf beetle was also an abundant leaf consumer. *Hyles* larvae were observed consuming leaf, stem, flower, and fruit tissue (Figure 4). It was not uncommon to observe larvae consume an entire plant.

We examined how pollen limitation affects reproduction through the season by hand-pollinating flowers with outcross pollen and comparing the fruit set and total seeds to naturally pollinated flowers. Total seeds produced from hand-pollinated flowers were higher than naturally pollinated fruits in both early and late season 2008 plants, as well as late season plants from 2010 (Table 1); hand-pollination did not

influence seed production in late 2008 plants or any 2009 plants. Thus, *C. brevipes* was pollen limited in the year with early rainfall and at the end of a year with late-season flowering.

There were more germinated pollen grains within styles late in the season compared to early in the season in 2008 (Figure 5a). In addition, the number of germinated pollen grains in styles was higher in 2008 compared to later years. This indicates that pollen delivery was highest in the year with the earliest plant reproductive phenology. However, this pattern was driven by the high values during the late season time period in 2008. Greater differences in early versus late season pollen deposition are likely to reflect greater plant-pollinator asynchrony, as observed in 2008. On the other hand, greater similarity in early versus late season pollen deposition might indicate greater plant-pollinator synchrony, as in 2009 and 2010, which were years with intermediate and late plant reproductive phenology.

There were no differences in fruit set for early versus late season plants in any year or between years (Figure 5b). Early season seed set (seed to ovule ratio) was higher than late season seed set across all three years (Figure 5c), whereas total seeds (seeds per fruit) was only higher for early season plants in 2009, as compared to late season plants (Figure 5d). Seed set and total seeds were higher in 2008, compared to 2009 and 2010.

Herbivory was lower for early season plants in 2008, perhaps as a result of asynchronous herbivore emergence that early in the season (Figure 5e). For the two years in which reproduction occurred later in the season, the differences in herbivory for early versus late season plants were small and non-significant, indicating greater plant-herbivore synchrony on those years.

Over three spring flowering seasons in the Mojave Desert, we observed different plant phenological patterns. For plants that flowered in 2008, germination-triggering winter rainfall was early, leading to particularly early plant reproductive phenology, whereas in the following two years germination-triggering rainfall did not

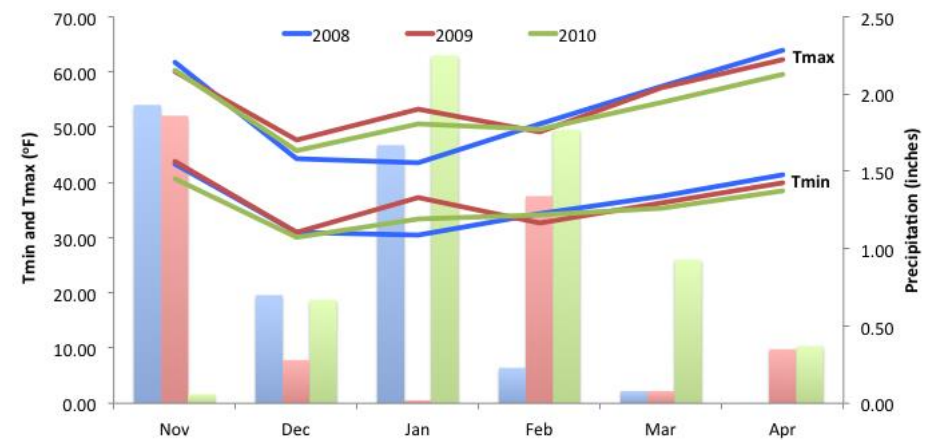


Figure 3. Temperature and precipitation patterns within key periods of the three-year study obtained from the Mid Hills weather station within Mojave National Preserve.



Figure 4. *Hyles lineata* larvae consuming *Chylismia brevipes* fruits and flowers (Photo: K. Gerst).

		Control	Hand-pollination	N	t-value	p value
2008	early	78.86 ± 5.03	90.54 ± 5.12	63	2.49	0.016
	late	61.77 ± 5.84	90.92 ± 4.76	51	4.24	< 0.0001
2009	early	52.73 ± 4.96	54.63 ± 5.86	38	0.38	0.709
	late	29.88 ± 8.95	33.75 ± 7.52	10	0.53	0.615
2010	early	41.38 ± 6.73	50.95 ± 6.61	39	1.40	0.169
	late	22.89 ± 3.99	40.16 ± 5.39	36	3.39	0.002

Table 1. Paired t-test results for total seed number in control vs hand-pollinated fruits from both early and late season plants. Significant differences (p < 0.05) between seed numbers in control vs hand-pollinated fruit are in bold.

occur until later in the season, resulting in progressively later growth and reproduction (Figure 3). In 2010, rainfall did not occur until mid-January, resulting in the latest peak flowering we observed (mid-April). We hypothesized that asynchronous phenology of pollinators and plants would result in reduced reproductive opportunities and that synchrony with herbivores would reduce plant reproductive success. We

found evidence that asynchrony between plants and pollinators occurred when plant reproductive phenology was early (February). Additionally, we found evidence that when plant reproductive phenology was late in the season, there was greater synchrony between plants and herbivores.

Pollinator activity, inferred from the number of

germinated pollen tubes in styles of open flowers, was lower early in the 2008 season compared to later. Previous research has shown that the timing of rainfall is a primary factor driving plant germination and growth and hence flowering time in desert annuals (36, 37). However, insect development, activity, and spring emergence is more strongly tied to temperature (38, 39). Thus, the depression in activity of the primary insect visitors to these plants in 2008 was likely due to the early germination and growth induced by the early rainfall that precipitation year, which led to flowering in February, a relatively cool period for insect activity relative to later spring months. Alternatively, if flowering synchrony was reduced in years with early flowering, it is possible that there is a reduced availability of pollen rather than pollinators.

The timing of herbivory can be a major factor affecting plant reproduction (40, 41). While greater plant-pollinator synchrony is beneficial, there may be an associated cost in terms of greater plant-herbivore synchrony. We found greater overall herbivore damage in the two years with later reproduction (specifically, late in 2009 and all of 2010). In fact, we observed plants being entirely consumed by herbivores before they had even begun flowering during 2010. This result emphasizes the need to better understand the mechanistic responses to environmental cues associated with both plant and insect phenology in order to predict how interactions will respond to climate variability and climate change.

Environmental factors, including high and frequent early rainfall, contributed to high population density in 2008. High plant and flower density often leads to greater pollinator visitation (42, 43) and this appears to have been the case in our study as greatest overall pollen tube densities occurred in 2008, mostly due to high numbers late in the season. While the number of germinated pollen grains in styles during the early period in 2008 was similar to levels found the other years, it was low relative to that found later in 2008 and represents a cost of early phenology in this otherwise favorable year.

In years and seasons in which asynchrony occurs, it is possible that species are buffered against reproductive failure by having longer

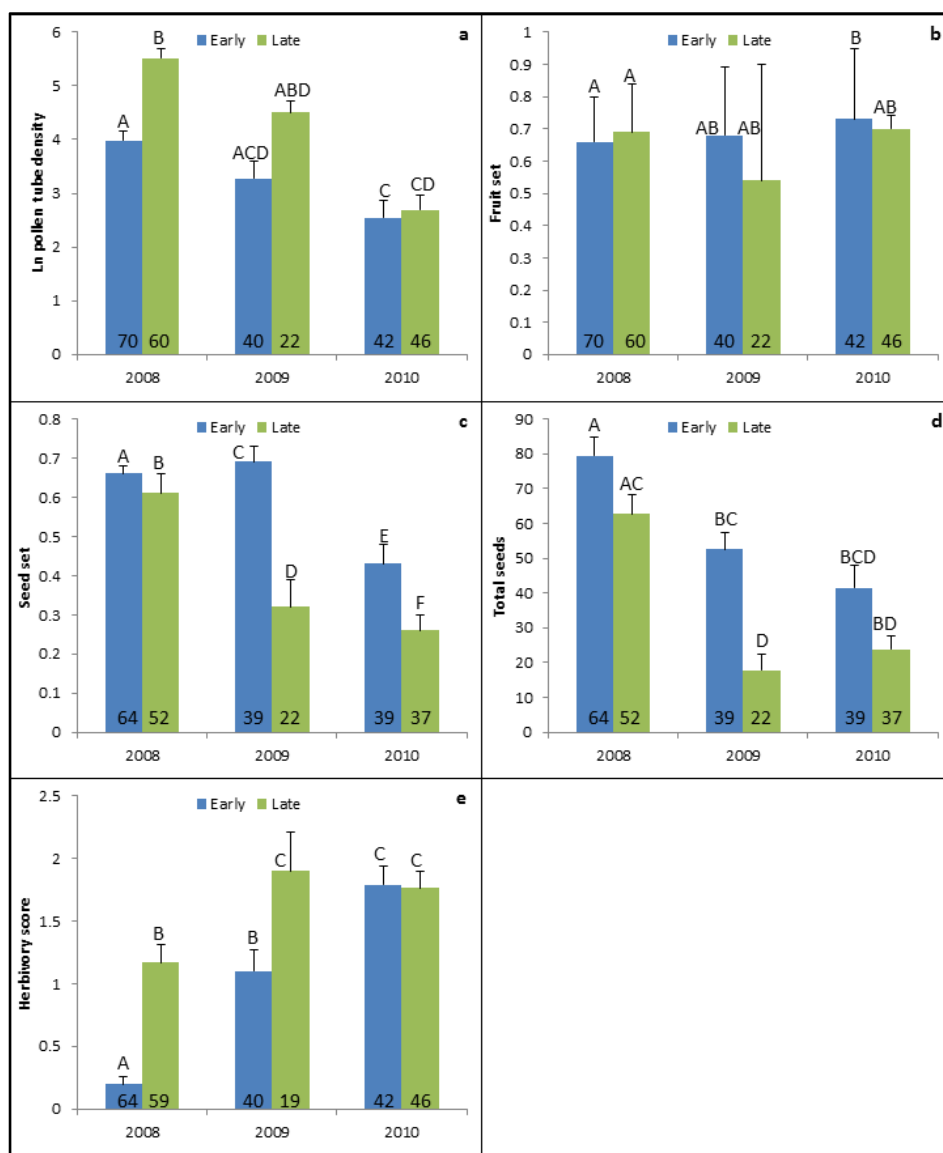


Figure 5a-e. Mean values for pollen tube density (a metric of pollination intensity), number of fertilized fruits per flower (“fruit set”), seeds per ovule (“seed set”), seeds per fruit (“total seeds”), and mean herbivory score for early and late season plants. Superscript letters represent significant Tukey-Kramer HSD differences ($P < 0.05$) within variables. Numbers within bars represent sample sizes.

reproductive periods or adaptations such as low ovule number and natural variability in pollinator communities (44). Additionally, desert annuals have the ability to buffer against the risk of asynchrony with pollinators by having long-lived seed banks and the potential for bet hedging via germination strategies (45). However, if the frequency of years in which asynchrony between plants and pollinators increases in the future, as has been demonstrated in other species (20), it is plausible to expect species that depend on pollinators to experience population decline. The increased risk of herbivory with late season reproductive phenology was also evident in this

study, demonstrating a potential conflict in determining an optimal phenological response to climate variability. Successful reproduction will depend on balancing the positive effects of optimal overlap with pollinators and avoiding the negative consequences of herbivory. This study is unique in its investigation of the role plant-pollinator-herbivore phenology plays in determining fitness in an annual species. We have shown that variable environmental conditions associated with desert annual phenology can contribute to the success of species based on the timing of their reproduction.

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