



Science Newsletter

Predator-prey interactions between rattlesnakes and kangaroo rats

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In any predator-prey interaction, the predator is attempting to subdue the prey and the prey is attempting to evade capture. Because both parties will have been shaped by natural selection to overcome the other, predator-prey interactions frequently involve extreme physical feats, including very rapid attack and evasion behaviors. As a result, it is difficult to study predator-prey interactions in the field under natural circumstances. However, we have pioneered techniques to record predator-prey interactions between two common denizens of the southwest deserts: rattlesnakes and kangaroo rats. We think the interaction between these two groups can be a model system for using high-speed videography to understand the factors that govern the outcome of high-speed attacks (i.e., what factors control whether a rattlesnake strike is successful or whether the kangaroo rat escapes).

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Figure 1. A sidewinder rattlesnake (*Crotalus cerastes*) in a stereotypical ambush coil.

Rattlesnakes (*Crotalus* and *Sistrurus*), which are common predators in the arid Southwest, are sit-and-wait ambush predators found throughout North and South America. When ambush hunting, rattlesnakes will 'sit' on the ground in a position known as an ambush coil (1) (Figure 1), then wait for potential prey to unknowingly approach the rattlesnake. If potential prey mistakenly wanders within the rattlesnake's strike range, the rattlesnake may then attempt to strike and inject venom. Whether the strike is successful depends on the ability of the prey to perceive the oncoming strike, rapidly react after recognizing the oncoming strike, and perform an evasive maneuver that removes the prey from the strike trajectory. In other words, the rattlesnake attempts to strike towards their prey to bite and inject venom prior to the prey being

able to perceive, react to, and avoid the oncoming strike. Rattlesnake strikes happen very quickly (average maximum velocity = 2.61 m/s, average maximum acceleration = 326 m/s²); typically contact with their prey occurs in <100 milliseconds (mean = 49 ms)(2), so rattlesnake prey have an amazingly short timeframe to escape. That said, kangaroo rats (*Dipodomys*), which are abundant bipedal rodents throughout the Southwest, have evolved an uncanny ability to avoid predation by rattlesnakes.

To understand fully why kangaroo rats are so effective at avoiding snake strikes, it is important to examine their unique morphology (Figure 2). First, kangaroo rats have evolved enlarged auditory bullae that effectively increase their ability to hear low frequency sounds, such as

those created by oncoming attacks from predators (both the strike of a rattlesnake and the swoop of an owl produce subtle, low frequency bursts of sound) (3). Second, kangaroo rats have large hindlimb muscles that can rapidly and powerfully contract, allowing kangaroo rats to perform amazing acrobatic leaps within milliseconds of perceiving a predator's attack (4, 5). When combined, the antipredator adaptations of kangaroo rats give them considerable ability to avoid strikes by rattlesnakes. Additionally, kangaroo rats are well known to perform a variety of antipredator signaling behaviors, including kicking sand in the direction of the predator (sand kicks, Figure 3; <https://youtu.be/cLfy-M9NWbl>), drumming on the ground with their hind feet (foot drumming), and repeatedly approaching then jumping away from ambush hunting snakes (jump backs; <https://youtu.be/Yc-qc6wfRKs>) (5, 6). Antipredator behaviors, such as the ones performed by kangaroo rats, are thought to mitigate the risk imposed by a predator by either informing the predator that it has been detected (perception advertisement) or of the physical ability of the kangaroo rat to escape (quality advertisement) (7). These behaviors are meant to both dissuade the predator from attacking and encourage the predator to cease pursuing the displaying individual by convincing the predator that it has little chance of a successful capture and that any attack would be futile.

In our initial studies of predator-prey interactions between rattlesnakes and kangaroo rats, we positioned video cameras filming at 30 frames per second (fps) to record ambush hunting sidewinder rattlesnakes (*Crotalus cerastes*) throughout the night, capturing any subsequent interactions that took place with desert kangaroo rats (*Dipodomys deserti*) (4). From the 36 recorded interactions, two aspects of the kangaroo rats' antipredator responses were found to drastically increase their ability to avoid predation by rattlesnakes. First, of the 23 strikes we recorded, only one strike resulted in the kangaroo rat getting bit and this kangaroo rat survived the encounter. The strikes were unsuccessful due almost entirely to the kangaroo rats being able to rapidly leap out of the path of the strike (<https://youtu.be/YeesFjACFJo>). As rattlesnake strikes occur rapidly and span short distances (~ 30 cm for sidewinder rattlesnakes),

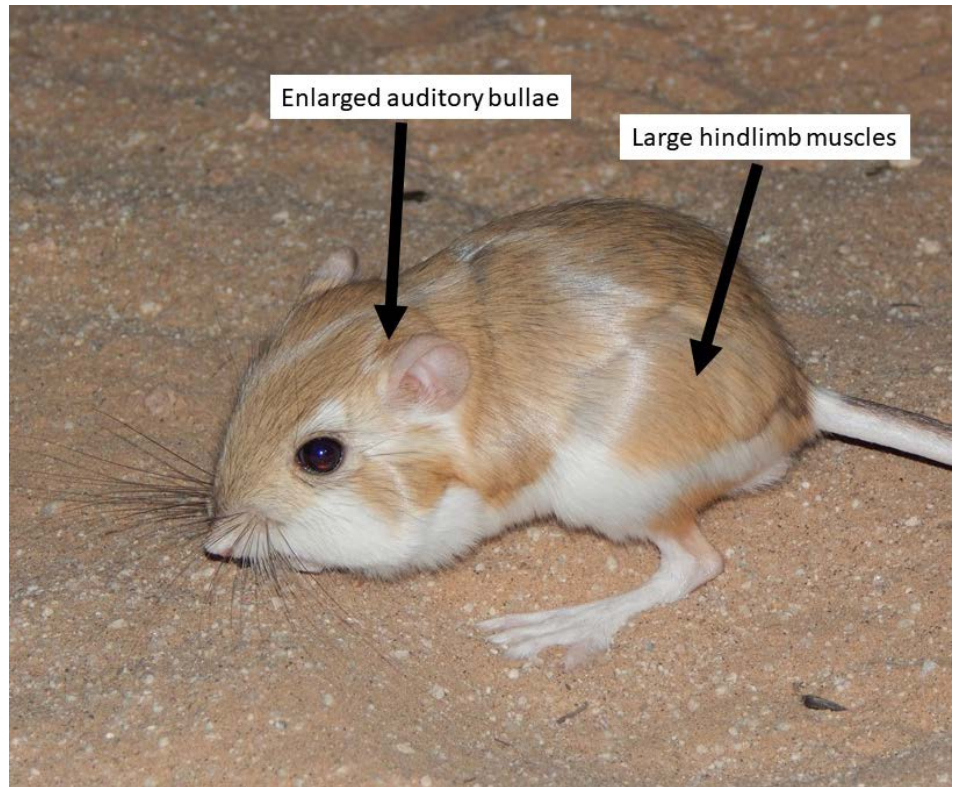


Figure 2. A foraging desert kangaroo rat (*Dipodomys deserti*) with key antipredator adaptations labeled.

rattlesnakes likely have little ability to change their strike trajectory mid-strike. Kangaroo rats appear to be able to take advantage of the ballistic nature of rattlesnake strikes by rapidly leaping and removing themselves from the strike trajectory, thereby causing the snake to miss its target. Second, when a kangaroo rat illustrated that it was aware of an ambush-hunting rattlesnake by performing any antipredator behavior (sand kick, foot drum, jump back), the rattlesnakes never attempted to strike the kangaroo rat following the antipredator behaviors. Thus, rattlesnakes only attempted to strike kangaroo rats that were unaware of the snake. Because the evasive leaping of kangaroo rats was apparently the key to their success, our next objective was to record this behavior in an experimental context and analyze it quantitatively.

To examine the evasive leaps more quantitatively, we motivated kangaroo rats to leap using a rattlesnake strike simulator (RSS) and filmed the subsequent leaps with cameras recording at 120 fps (8). The RSS is simply a spring-propelled cork launched out of PVC at nearly the same speed as a rattlesnake strike

(~2.8 m/s) (Figure 4), and it is not harmful to the kangaroo rat. Kangaroo rats were tested under two experimental treatments: control (not snake-exposed) and "primed" (recently exposed to a rattlesnake). To expose kangaroo rats to a rattlesnake, a rattlesnake was placed near the RSS and the kangaroo rats were allowed to interact with the snake. Once a kangaroo rat interacted with the snake, as evident by an expression of any of the three main antipredator behaviors directed towards the snake, we removed the snake and fired the RSS at the kangaroo rat when it returned later in the evening. The differences in the kinematics of the evasive leaps between the control and primed treatments were stark. Kangaroo rats that were recently exposed to rattlesnakes had quicker reaction times, faster take-off velocities, and jumped more vertically relative to the ground – indicating that primed kangaroo rats could more effectively avoid the strike than control kangaroo rats (<https://youtu.be/2lflsqyl63l>). Furthermore, for primed kangaroo rats, the reaction times were among the fastest recorded for any small mammal, with approximately one-third of individuals showing a reaction to the RSS between 8-17 milliseconds. Because it takes

between 100 and 400 milliseconds for a human to blink an eye, these kangaroo rats were responding in a mere fraction of the time it takes to blink! The increased performance of snake-exposed kangaroo rats is likely the reason why sidewinder rattlesnakes never attempted to strike kangaroo rats that were aware of the snake. That said, even kangaroo rats that were not exposed to snakes exhibited outstanding physical abilities, as they reacted in less than 40 milliseconds and displaced their body from the “strike” trajectory in less than 80 milliseconds, indicating that both control and primed kangaroo rats are elusive prey for snakes.

As a result of these studies, we know: 1) sidewinder rattlesnakes will only strike at kangaroo rats if they are unaware of the rattlesnake’s presence; 2) kangaroo rats can effectively dodge most rattlesnake strikes (5); and 3) the evasive leaps of kangaroo rats are extremely fast and well-adapted for moving the kangaroo rat out of the strike trajectory as rapidly as possible (8). These studies have been highly informative; however they still do not allow us to discern why some interactions result in the kangaroo rat getting captured and why some result in the kangaroo rat getting away. By closely examining the stereotypical series of events that occur in predator-prey interactions between these species, we can make several hypotheses as to why, in some instances, rattlesnakes may be able to capture kangaroo rats (Figure 5). Successful rattlesnakes must remain unnoticed until they are ready to strike, and then accurately and rapidly close the gap between their fangs and the kangaroo rat. Thus, we can hypothesize that rattlesnakes will be most successful at capturing kangaroo rats by: 1) striking when the kangaroo rat is closest and the time required to reach the kangaroo rat is minimal, 2) striking when muscle performance is not inhibited by low body temperature (although we need more experimental data to determine the extent to which rattlesnake strikes are limited by temperature), and 3) by striking rapidly (high strike acceleration and/or velocity). Conversely, we can hypothesize that the kangaroo rats can increase their likelihood of evading a strike by: 1) perceiving the strike shortly after the strike has been initiated, 2) reacting to the strike as early as possible, 3) leaping with high acceleration so that

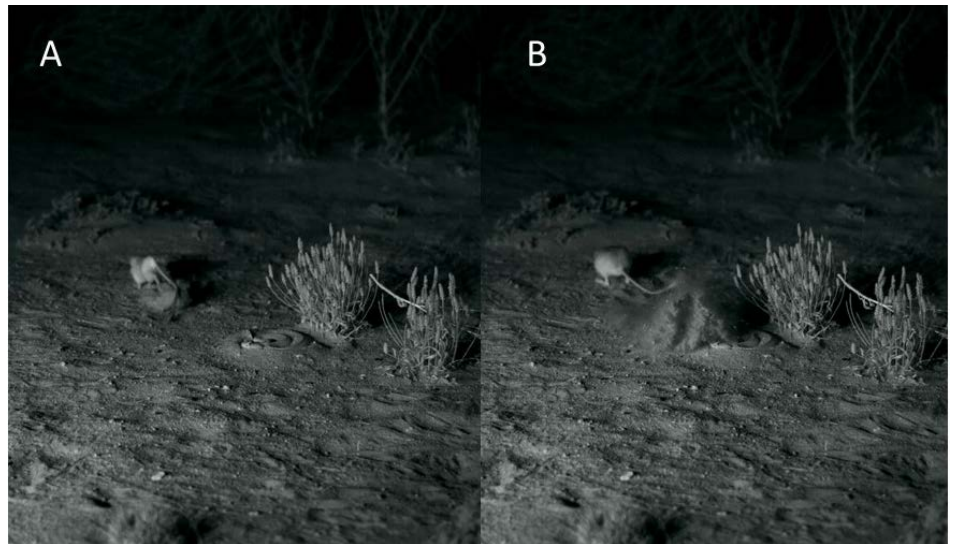


Figure 3. A desert kangaroo rat (*Dipodomys deserti*) kicking sand at a hunting sidewinder rattlesnake (*Crotalus cerastes*). A) The beginning of the sand kick. B) The sand as it contacts the snake.



Figure 4. A desert kangaroo rat leaping in response to the rattlesnake strike simulator (RSS). Note: the black marking on the hindquarters is from fur dye and is not a coloration typical of kangaroo rats.

it can quickly displace itself out of the strike trajectory, and 4) by leaping vertically to minimize the distance it has to travel to escape the strike trajectory. With this set of hypotheses in mind, our current study at Kelso Dunes in Mojave National Preserve is designed to provide insight into which, if any, of these hypotheses are supported by field data.

To evaluate these hypotheses, we are deploying paired and synchronized high-speed video cameras filming at a much higher resolution and frame rate (500 fps) to record natural interactions between rattlesnakes and kangaroo rats. By filming interactions with multiple cameras, we can calculate the three-dimensional movements of both species and extract the various kinematic measures (velocity, acceleration, reaction time,

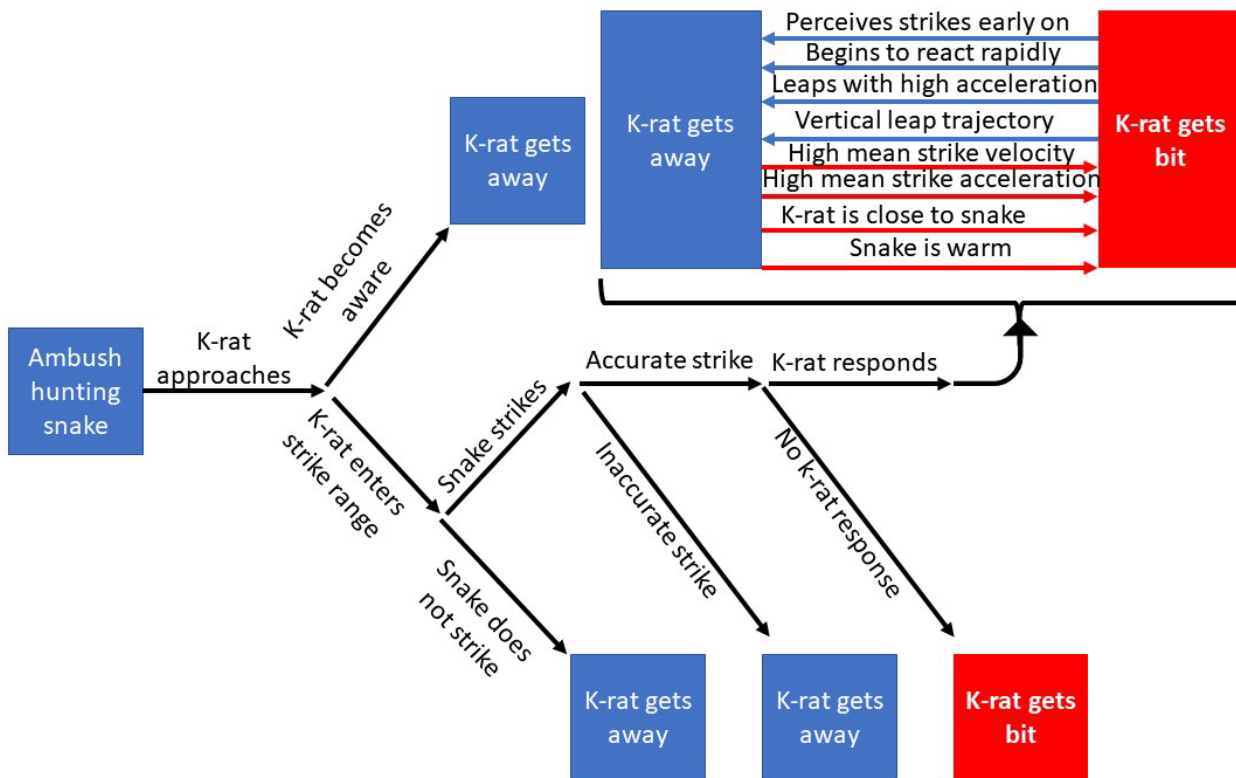


Figure 5. A diagram of the events during a predator-prey interaction between a rattlesnake and kangaroo rat (K-rat), and the potential factors that can influence the outcome of the interaction. Interactions can result in either the kangaroo rat getting away (blue squares) or the kangaroo rat getting bit (red squares). If a strike is accurate and the K-rat responds (upper right diagram), a variety of factors can influence the outcome of the strike; variables that increase the likelihood of the K-rat getting away are shown with blue arrows, while red arrows indicate variables that increase the likelihood of the K-rat getting bit.

etc.) needed to assess our hypotheses. Although simple in principle, this is logistically difficult to execute under field conditions. To record strikes, it is often necessary to carry in excess of 300 pounds of camera gear over a mile to a hunting snake. Despite the physical labor required to haul out our camera gear, we are not guaranteed to record a strike, as often times the snake will leave shortly after setting up the cameras without interacting with any kangaroo rats, forcing us to again walk the camera gear to another hunting snake. Consequently, we are still gathering the requisite data needed to make any definitive conclusions. However, some example footage from a recent and related publication (9) is viewable on our YouTube channel (https://youtu.be/jCxvIk8wS_8).

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California's glandular plants; where sticky situations are an advantage

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California has diverse plant life. This diversity takes many forms; for example, the state has towering 300-foot tall redwood trees as well as tiny 1-inch springtime wildflowers. A less noticed, but no less interesting, diversity exists on the surface of California's plants in the form of hairs, glands, and exudates (Figure 1). Nearly all the major families of western vascular plants have species with some combination of sticky, slimy, or oily substances on their surfaces. These plant species have a variety of common names that highlight these substances: tarweed, gumplant, stickystem, catchfly, viscid locoweed, tacky phacelia, and clammy chickweed. The scientific names are no less illustrative: *Geranium viscosissimum*, *Allophylum glutinosum*, *Phacelia glandulifera*, *Navarretia viscidula*, and *Chrysothamnus viscidiflorus*.

This variety of textures and the smells and intense tastes that so often accompany them are characteristic of California and the desert West's flora but are not universal. Sticky plants are characteristic of seasonally-dry environments worldwide (1), and you would be hard-pressed to find too many examples in tropical rainforests, boreal forests, or mixed deciduous Eastern United States forests. While little research has been directed towards these broad evolutionary and biogeographic trends, it is quite clear that a driving force is rainfall; when exposed to unseasonable rains (or the author's home sprinkler) the exudates causing stickiness or sliminess get washed off completely.

To an evolutionary biologist, this repeated occurrence of the same traits suggests a phenomenon called "convergent evolution," where unrelated plants have evolved a solution to a similar problem in a similar way. But what use is stickiness? Scientists, since long before the time of Darwin, and including Darwin, have considered this question carefully. For each

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Figure 1. California sand-entrapping plants. Clockwise from top left: *Alliciella leptomeria* (sand gilia); *Abronia alpina* (Ramshaw Meadow sand verbena); *Pholisma arenarium* (sand food); *Navarretia mellita* (honey-scented pincushion plant) with bee fly pollinator.

plant, the benefits may differ. One common use for stickiness is seed dispersal – brush against the fruit or seeds of *Boerhavia* (stickystems), *Bidens* (sticktight), *Arctostaphylos viscida* (sticky Manzanita) or *Galium* (sticky willy) and they will, more often than not, remain on your clothes, shoes or pets. They will, eventually, drop off – hopefully for them, in a place with a favorable habitat. The "stickiness" of these fruits or seeds can be physical or chemical. For example, *Galium* and *Bidens* use small hooked spines to grab on to fur or fabric, like Velcro, whereas *Boerhavia* and *Arctostaphylos* use sticky resins to the same end.

However, the majority of California sticky plants do not have sticky seeds, instead the stickiness occurs on the stems, leaves, buds, shoots or calyces. Therefore, evolutionary forces besides dispersal must have shaped these traits. A commonly cited, but rarely tested, hypothesis is that resins or mucus on plant surfaces can protect plants from harmful UV radiation. Whether this is true or not, in certain cases, the

presence of stickiness on stems or calyces, but not leaves, or on bottoms of leaves suggests that this explanation is insufficient to explain all cases.

If you look closely at a sticky plant, e.g. sand verbena (*Abronia* spp.) on a desert or coastal dune you will notice that the plant usually has a dense coating of sand on its surfaces. The sand verbenas are our best example of this trait – termed sand armor or "psammophory" ("sand-carrying" in Greek), but take a walk on the Kelso Dunes in Mojave National Preserve and you'll find "sand armor" not only on desert sand verbena (*Abronia villosa*) but also on sand gilia (*Alliciella leptomeria*), Ives' phacelia (*Phacelia ivesiana*), and many other species that have glandular-hairy surfaces (Figure 1). Look anywhere you have sand, and you will find psammophorous plants. For example, the sandy flats around Mono Lake harbor saltgrass (*Distichlis spicata*), another sand verbena (*Abronia turbinata*), and Mono gilia (*Alliciella monoensis*), all of which, upon close inspection,

will be wearing “sand armor.”

Having a coating of sand is probably both a blessing and a curse (2). The sand may block light from the plant and slow photosynthesis, but it may also protect the plant from abrasive sandstorms and/or reduce the drying impact of wind on plant surfaces by breaking up airflow. The sand particles may also catalyze condensation of moisture on the leaf surface or could discourage animals from eating the plant. Most of these potential functions of psammophory have not been tested yet, but in my own research I have looked at the latter function; more specifically, sand as a defense to reduce herbivory in the sand verbenas (*Abronia* spp.).

Sand caught on the plant surface could protect a plant in two ways. The first is that sand is unpalatable or detrimental to herbivores; this defensive function would probably work against most caterpillars, rabbits, deer, beetle larvae, and more. Secondly, since a plant covered in entrapped sand matches its background very closely, this camouflage could prevent herbivores from finding the plant. We would expect this to work more effectively against mammals than certain insects, which often use volatile plant chemicals to locate plants to eat or lay eggs on.

To determine whether the sand coating is defensive, and if so, how it is functioning, I performed a series of experiments on *Abronia latifolia* in Bodega Bay, California (3). For the first experiment, I located a population of 60 stems, and I removed sand from 30 stems every two weeks for 3 months with a soft wet sponge; the other 30 were left as sand-intact controls. At the end of the experiment, nearly 50% of leaves in the “washed” group had insect feeding damage on them, whereas in the sandy control group, only about 25% of the stems had damage. These data substantiate that sand does have a defensive function, but the exact mechanism was unclear. To elucidate the mechanism, I conducted a second experiment where I removed sand from 90 stems then added either tan sand (to match the color of the nearby substrate), green sand (to match the leaf), and left the remainder without sand. Those that had the sand removed, but no sand added, again suffered

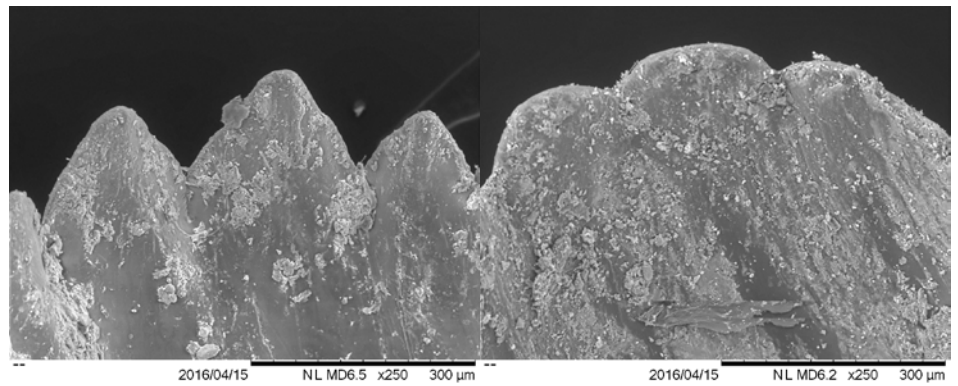


Figure 2. Left photo: A white-lined sphinx moth (*Hyles lineata*) mandible from a caterpillar fed on “clean” *Abronia latifolia* leaves. Right Photo: A worn-down mandible of a caterpillar fed on “sandy” *A. latifolia* leaves.

more herbivory (15%) than the plants with sand (~5%); however, it did not matter what color the sand was. From these experiments, I concluded that the sand coating functioned primarily as a physical defense, not as camouflage.

Despite the sand coating, herbivory is still common on many sandy plants. Whenever you find sand verbenas, you find caterpillars chewing on them, often those of the white-lined sphinx moth (*Hyles lineata*). These caterpillars, which can get to be the size of your finger and weigh up to seven grams (twice as heavy as a hummingbird!), relish *Abronia* and are often found eating it – even in really sandy areas. Given these observations, two obvious questions remained. Do they not care whether they eat sand? Does sand not affect them? To answer the first question, I created a choice test with 36 caterpillars, offering them “clean” versus “sandy” leaves from the sand plants. The caterpillars overwhelmingly chose (86%) to feed on the “clean” leaves, indicating that they prefer to eat sand-free leaves (4). They do care!

To find out if the presence of sand had a negative effect on white-lined sphinx moth (*Hyles lineata*) caterpillars (or if they just didn’t like it), I devised a second choice test. I fed 68 *Hyles lineata* caterpillars either “clean” or “sandy” leaves from *Abronia latifolia*. I created sandy leaves by collecting clean leaves and then dipping them in sand to emulate the sandy condition. As I observed in the field, the caterpillars provided sandy leaves ate just as fast as those given clean leaves; however, they grew far more slowly. This difference could be due to either reduced digestive efficiency, or that their

guts were filled with too much indigestible and nonnutritive material. While I could not rule out either explanation – and both likely occur to some degree – there is corroborating evidence for the former. I extracted the mandibles (the caterpillar equivalent of teeth) of caterpillars in both groups and examined them using a scanning electron microscope. Indeed, the mandibles of caterpillars in the “clean” group were normal, looking much like a spiky baseball mitt, whereas those in the “sandy” group were worn down to practically nothing (Figure 2), a result probably underlying the slower growth of these individuals. Sand led to both slower growth and a lower weight at pupation, leaving them vulnerable to predators for longer and resulting in adults who lay fewer eggs, meaning that they have lower reproductive fitness than larger caterpillars.

In places without windblown sand, sticky plants often get covered in other materials; bird feathers, windblown seeds, bits of falling leaves, or, after wildfires, ash. However, in my experience, the most commonly encountered material found sticking to a plant are the corpses of insects (Figure 3). Insects abound in the air, both night and day, and many land on plants to feed, rest, mate, or hunt. Other insects wander up plant stems from the ground. When insects end up on sticky plant surfaces, they usually remain there until rain washes them off or something consumes them. It is not hard to understand the obvious benefit to the plant of trapping insects; many insects, such as aphids, whiteflies, caterpillars, leaf beetles, and weevils survive by eating plants but stickiness and the difficulty of moving and feeding on sticky

materials largely prevents them from feeding on these plants.

This defense, however, is nowhere near perfect. Unsurprisingly, many insect herbivores have evolved ways to move around on sticky plant surfaces, as have their predators. These dueling arthropods – insect and mite herbivores versus the insect or spider predators that consume them – are the central cast of a commonly overlooked and completely fascinating set of defensive interactions occurring on sticky plants. The dead insects on sticky plant surfaces (usually small flies, wasps, and aphids) serve as a reliable food source for many predatory arthropods including assassin bugs, stilt bugs, and lynx spiders. In fact, some sticky plants are practically an all-you-can-eat bug buffet – a single sticky columbine (*Aquilegia eximia*) often has over 200 dead insects on its surface by the end of summer. Predatory arthropods will seek out plants with abundant dead insects on them and may never leave - living and reproducing on the plants. The presence of these predators is a good thing for the plant; when a female moth lays an egg on the surface of sticky columbine, wild tobacco (*Nicotiana attenuata*), common tarweed (*Madia elegans*), or Bolander's monkeyflower (*Diplacus bolanderi*), the waiting predators often devour the egg, or hatching caterpillar before it has time to damage the plant.

To examine the effect of these insect corpses on predators, I conducted an experiment on sticky columbine. Each week, for an entire summer, I surveyed all dead insects, predators, herbivores, and damage to the plant within a patch of columbines. At the same time, I removed all the dead insects from half of the plant during each survey. Those plants from which I removed the corpses had lower predator density (~50%) and had significantly more damage to them than the plants with a normal abundance of dead insects (5).

Next time you are wandering around and examining plants, take some time to touch them (carefully in some cases) and take notice of the variety of textures. And while looking more closely at them, you may find sand armor, dead insects, and predators standing guard, all due to surface qualities that you may not have noticed



Figure 3. Dead insects on the surface of some California plants. Clockwise from top left: *Mentzelia laevicaulis* (giant blazingstar); *Aquilegia eximia* (serpentine columbine); *Micranthes californica* (California saxifrage); *Mirabilis multiflora* (giant four o'clock).

previously. In fact, the study of the ecological interactions of sticky plants is quite new, and it is possible you may discover a previously unknown interaction occurring right under your nose.

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Intraspecific floral diversity in the California evening primrose, *Oenothera californica* subsp. *avita*

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Biologists since Darwin have been fascinated by the incredible diversity of flowers and flowering plants. Darwin referred to flowers as ‘beautiful contrivances’, intricate features shaped by natural selection that hold the key to angiosperm reproduction and diversification (1, 2). Many plants rely on flowers, and the pollinators they attract, for outcrossing and sexual reproduction. The intimate mutualism between flowering plants and insects has engendered an astounding diversity of floral shapes, color and fragrances through the process of coevolution (3). Flowers evolve in response to the morphology and ecology of the primary pollinating agent and pollinators in turn, evolve to more efficiently utilize floral resources like nectar and pollen. While we typically think of coevolution generating diversity over long timescales, the process of selection and evolution can occur at small spatial scales resulting in pervasive and often undetected variation within a single species (4, 5). These small-scale processes of intraspecific trait diversification are hypothesized to have given rise to the enormous diversity of angiosperms.

While pollinators may be the primary selective agents driving floral trait evolution, plants evolve in the context of abiotic factors and biotic interactions with co-occurring plant and animal communities. Edaphic factors, like soil composition, temperature and humidity can drive floral divergence; for example, species in high-UV environments have greater UV absorbing pigmentation (6). Furthermore, the plant community can facilitate trait divergence between co-flowering species to minimize competition for pollinators (7, 8). Recent studies also suggest that non-pollinator agents of selection such as

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Figure 1. Night-blooming *Oenothera californica* subsp. *avita* in Mojave National Preserve. Photo credit: K. Skogen.

herbivores, fungi and pathogens can alter plant-pollinator interactions and drive floral evolution (9). Together, geographic variation in abiotic and biotic factors can facilitate widespread intraspecific variation but few studies comprehensively examine the ecological processes that generate intraspecific diversification.

In this study, we examined floral trait variation and the ecological factors driving divergence in the California evening primrose, *Oenothera californica* subsp. *avita* (section Anogra, Onagraceae) (Figure 1). This taxon has a broad distribution (Figure 2) occurring across a wide range of biotic and abiotic communities, making it an ideal system in which to examine floral adaptation and divergence. Herbarium records of *O. californica* subsp. *avita* suggest that this taxon occurs in four distinct geographic areas 1) the western edge of the Mojave Desert, 2) the eastern Mojave Desert (including Mojave

National Preserve) within California, Nevada, and Arizona, 3) the Great Basin Desert in Nevada, and 4) at high elevations in the Eastern Sierra Nevada Mountains (Figure 2). The discontinuous distribution of *O. californica* subsp. *avita* suggests that gene flow is likely to be limited between populations. Thus, local adaptation to abiotic factors, pollinators and herbivores is expected to drive floral diversification.

Oenothera californica subsp. *avita* is a self-compatible, perennial evening primrose with large, long-tubed, scented, white flowers (Figure 1). Pollination occurs primarily by hawkmoths at dusk but bee visits are also common in the morning and evening. Typical of most moth-pollinated plants, the flowers open at dusk, remain open for 16-18 h, and fade by mid-morning the following day. Populations in dry desert climates usually flower in the spring (late March to May) whereas populations in the Sierras flower in the summer (early June).

One of the most common hawkmoth pollinators, the white-lined sphinx (*Hyles lineata*), is also a common herbivore on many evening primroses including *O. californica* subsp. *avita*. Female moths frequently oviposit while nectaring and their hornworm larvae feed on leaves, unopened buds and flowers. Because herbivory by caterpillars of *H. lineata* is so closely tied to hawkmoth pollination across the family Onagraceae, both pollinators and herbivores should drive floral trait divergence. Beetles in the genus *Altica* and small moths of the genus *Mompha* are also frequent herbivores on evening primroses.

To quantify floral trait divergence, we compared floral traits among five populations (Figure 2) across the distribution of *O. californica* subsp. *avita*. We focused our efforts on two components of floral biology: floral morphology and floral scent. Floral morphology, the shape and size of flowers, can determine pollen transfer efficiencies for different pollinators (3, 10). For example, the length of the floral tube and the floral flare (opening to the floral tube) mediates pollinator access to nectar and pollen placement. Hawkmoths with long tongues (proboscis) can efficiently access nectar in flowers with long tubes, whereas bees can be excluded from nectar access if the floral tube is too long or if the opening to the floral tube is too narrow to accommodate their body size. If bees are the predominant pollinators at a site, we would predict selection for shorter tubes and increased floral flare, as is seen in the related brown-eyed desert primrose (*Chylismia claviformis*), whereas if hawkmoths are dominant pollinators, flowers should have longer floral tubes.

At each of five sites, we measured four morphological traits: corolla diameter, tube length, floral flare and herkogamy (stigma-anther separation) on 15-30 individuals (Figure 3a). Corolla diameter was measured along two of the longest petal axes, perpendicular to the nectar tube. Floral tube length was measured from the top of the ovary to the point of sepal insertion at the distal end of the floral tube. Floral flare was measured as the diameter of the opening to the floral tube. Style and filament lengths were measured separately. Because anther filaments are attached to the floral tube (Figure 3a),

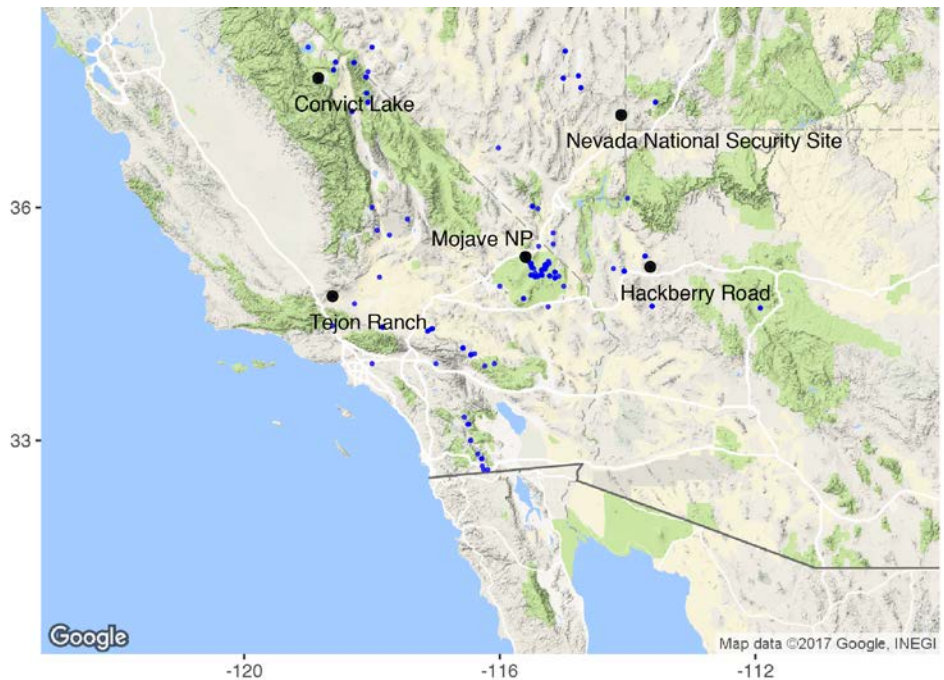


Figure 2. Distribution and collection sites of *Oenothera californica* subsp. *avita*. Georeferenced herbarium records, representative of the species distribution, are in blue and collection sites in this study are in black.

filament and floral tube lengths were summed and subtracted from the style to calculate herkogamy. Morphological measurements were made using digital calipers to the nearest 0.01 mm.

Whereas morphology mediates pollinator effectiveness, pollinator attraction to flowers is largely based on visual (11) and olfactory cues (12). *Oenothera californica* subsp. *avita* shows no variation in visual cues – both visible and UV reflectance are consistent geographically. To determine if olfactory cues have diversified between populations, we measured floral scent, the chemical odors released by flowers. We collected floral scent using standardized dynamic headspace collection methods (Figure 3b) for all populations following previously described protocols (13). We sampled one flower per plant and collected floral scent immediately after anthesis, between 18:00 and 20:00 hrs. Each flower was enclosed in a Reynolds (nylon resin) oven bag and affixed to the floral stem with plastic ties. Floral volatiles were collected in a cartridge containing an adsorbent material (10 mg of 80–100 mesh Super Q, Alltech Associates, Waukegan, Illinois, U.S.A.), packed into a Pasteur pipette. Air from the floral headspace, concentrated in the enclosing bag, was pulled at the flow rate of 250 ml/min through the cartridge

using a personal air sampler vacuum pump (Supelco, Berwick, Pennsylvania, U.S.A.). After 60 minutes of sampling, the cartridges were removed and volatiles eluted with 200 microliters of hexane into Teflon-capped borosilicate glass vials. Prior to analysis, we concentrated the samples to a uniform volume of 50 microliters and added 5 microliters of 0.03% toluene as an internal standard. The scent samples were run on a Shimadzu GC-17A equipped with a Shimadzu QP5000 quadrupole electron impact MS (Shimadzu Scientific Instruments, Columbia, Maryland, U.S.A.) detector. We collected morphology and floral scent from the same individual flowers.

To examine the ecological factors that might contribute to diversification, we evaluated the pollinator and herbivore communities at each of the five sites (Figure 4). We assessed herbivory by systematically inspecting leaves, fruits and flower buds for evidence of herbivory and herbivores. We measured the presence/absence and number of each herbivore on the plant and visually estimated the amount of leaf or flower damage as a percentage of the total. Herbivory and floral trait data were collected on the same plant. The primary herbivores on this taxon are *Hyles lineata* eggs and caterpillars, *Mompha* caterpillars and *Altica* beetles. To compare

differences in pollinator communities between sites, we conducted pollinator observations in the morning (~7:00) and in the evening (~18:00) for 30 to 60 minutes using a combination of 3-4 video cameras and 2-5 human observers. We observed an average of 22 flowers per site, per observation period. We identified most hawkmoths to species but bees were categorized based on their size (small, medium, large).

Two populations, the Nevada National Security Site, NV and Hackberry Road, AZ had flowers that differed morphologically from the other sites (Figure 5). Flowers from the Nevada National Security Site, NV were smaller in diameter, flare and herkogamy but had long floral tubes. In contrast, flowers from Hackberry Road, AZ were larger in diameter, floral flare, and herkogamy but had a much shorter floral tube. The smaller-tubed population at Hackberry Road had no hawkmoth visits and was the only population with bee visits (Figure 6a). These data fit the prediction that plants with pollinator communities dominated by bees should be under selection to reduce tube length. However, it is important to note that our pollination data were collected over the span of 2-4 days and provide a small window into the pollinator communities at each of these sites. While substantially more data are needed to fully understand the nature of pollinator-mediated selection at the Hackberry road site, these preliminary data show interesting patterns that merit further exploration.

Sites of *O. californica* subsp. *avita* showed striking differences in floral scent composition and emission rates (Figure 7). Each site emitted

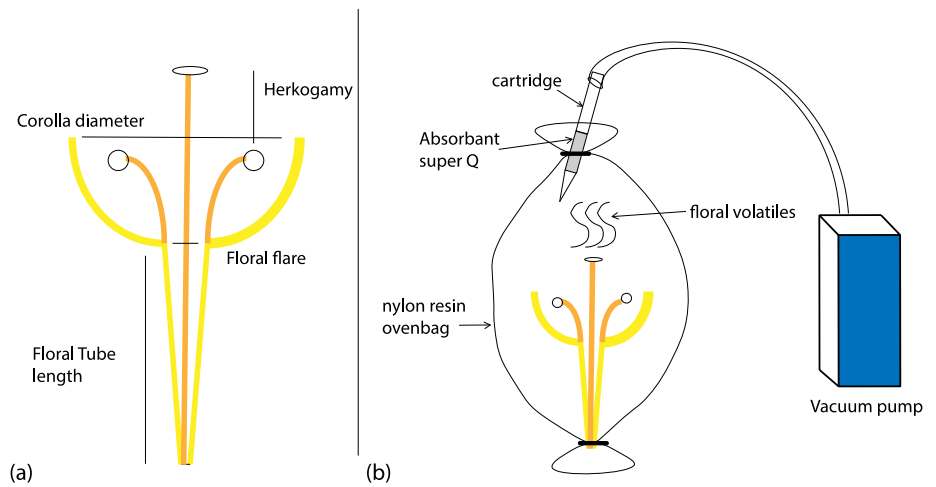


Figure 3. Floral trait measurements on *O. californica* subsp. *avita*. Figure 3a depicts the location of each morphological measurement on the flower. We measured all four morphological traits using digital calipers. Black lines indicate the location of each measurement on the flower. Figure 3b displays the setup for collecting floral scent compounds. A single flower was enclosed in a Reynolds (nylon resin) oven bag and affixed to the floral stem with plastic ties. Air from the floral headspace, concentrated in the enclosing bag, was pulled through a cartridge (Pasteur pipette) containing an adsorbent material (Super Q) using personal air sampler vacuum pump. To extract the chemicals, we washed the adsorbent material with 200 microliters of hexane.

a distinct floral blend with some compounds exclusively produced at a single site. *Oenothera californica* subsp. *avita* in the Western Mojave at Tejon Ranch, CA predominantly made (R) – linalool, a monoterpene compound that is a common component of floral scent in many taxonomically-diverse species. Tejon Ranch was the only linalool-emitting population in this study. Nitrogenous aldoxime compounds dominated the floral scent of plants in Hackberry Road, AZ but were absent from all other sites. The Sierra Nevada site at Convict Lake, CA was also the only site to produce alpha-farnesenes. Total emission rates also differed between the sites, such that linalool-producing flowers at Tejon Ranch, CA emitted the highest quantity of floral

scent and flowers at Convict Lake, CA emitted the lowest quantity.

Why has floral scent diversified so drastically between populations? While pollinator-mediated selection is the most commonly cited explanation for intraspecific floral scent variation (14), we see no obvious patterns that link pollination to floral scent in this study (Figure 6a). Three sites that had very distinct floral scent profiles were visited by the same hawkmoth species, *H. lineata*. *Hyles lineata* is the most widely distributed hawkmoth and has been documented to visit flowers from 13 different angiosperm families (15). Prior research has shown that *H. lineata* can physiologically respond to a broad range of



Figure 4. Left to right: *Hyles lineata* adult visiting an *O. californica* subsp. *avita* flower in Mojave National Preserve, *H. lineata* caterpillar and *Altica* beetle herbivory. Photo credits: K. Skogen, T. Jogesh, R. Raguso.

chemical compounds from many biosynthetic classes (15) and thus, it is not surprising that *H. lineata* visited flowers with distinctly different chemical compositions. Floral scent differentiation in this species is unlikely to have been driven by differences in pollinators between the sites.

Research in the last decade has shown herbivores can be an important selective force in the evolution of floral traits especially floral scent (16). Floral scents may attract both pollinators and herbivores and populations with high levels of herbivory might be under selection to reduce scent production or change the composition of floral scent. Sites in this study differed markedly in their herbivory rates (Figure 7b). Few plants (<25%) at Tejon Ranch, CA showed evidence of herbivory and herbivores were completely absent at Convict Lake, CA. Flower-feeding caterpillars of microlepidopterans in the genus *Mompha* were found at three sites. However, there is no discernable pattern between scent composition and the frequency of herbivory. Herbivores may not be an important selective force for a species that is self-compatible and perennial. It is interesting to note however, that populations with high emission rates of floral scent had higher pollinator visitation and lower herbivory.

Increased scent production has been associated with increased visitation rates and fitness in other plants (17) however, the relationship between emission rates and herbivory is counterintuitive if herbivores are also attracted to floral scent. Experimental assays are needed to fully understand how floral scent may mediate plant-herbivore interactions in this system.

Genetic drift may contribute to the extreme variation we observed in *O. californica* subsp. *avita* and a population genetic study is essential to fully understand how isolation and gene flow contribute to floral scent diversification among the five sites. The presence of site-specific scent profiles may also be indicative of hybridization events. *Oenothera californica* comprises three subspecies: *O. californica* subsp. *avita*, *O. californica* subsp. *californica* and *O. californica* subsp. *eurekaensis*, which are sister to *Oenothera arizonica* (18). *Oenothera californica* subsp. *eurekaensis* is federally threatened and endemic to the Eureka Dunes in Death Valley National

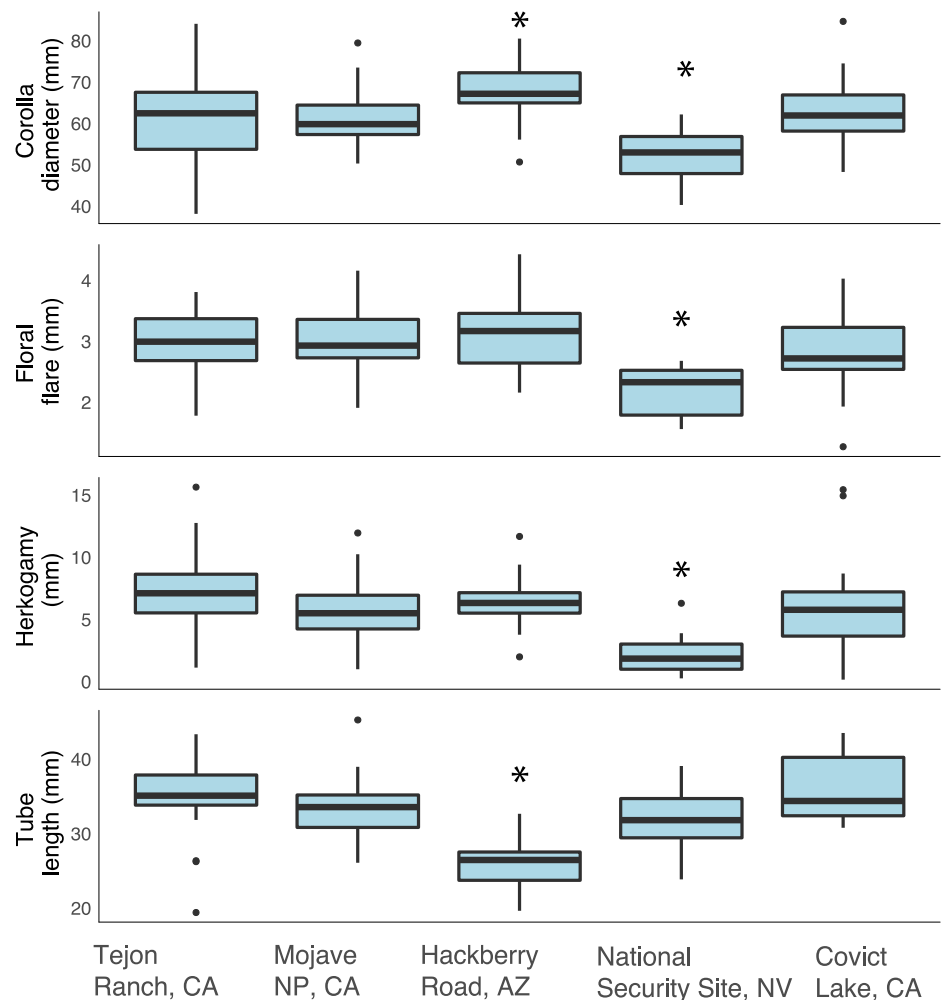


Figure 5. Floral morphology across five sites of *O. californica* subsp. *avita*. The boxes represent the full range of variation (the height of the box, top to bottom, represents the full variation) and the most common values (the median line is shown as a dark black line across the box) whereas the box itself represents the first and third quartiles within which most observations lie. Asterisks indicate populations that are significantly different from the others. Data were analyzed using a one-way ANOVA followed for a post-hoc Tukey's HSD test.

Park, CA and does not overlap in range with *O. californica* subsp. *avita*. However, at the western edge of its range, *O. californica* subsp. *avita* overlaps with *O. californica* subsp. *californica* and at the eastern edge of its range, *O. californica* subsp. *avita* overlaps with *O. arizonica*. It is possible that the western and eastern sites, Tejon Ranch, CA and Hackberry Road, AZ, respectively, have introgressed. Introgression might explain why plants at Tejon Ranch and plants at Hackberry Road have very different floral scent profiles (Figure 7).

Lastly, abiotic conditions like light, temperature and soil may also drive intraspecific variation in floral traits. Environmental variation can induce changes in the composition and emission rates of floral scent (17). Aside from the Convict Lake, CA

site in the Sierra Nevada Mountains, which experiences much lower annual temperatures and higher rainfall, the desert populations of *O. californica* subsp. *avita* do not differ in annual average temperatures and rainfall (Figure 8). Temperature and precipitation are unlikely to explain trait diversification between these sites. However, few studies have examined the importance of abiotic factors in shaping floral scent chemistry and factors like soil type and relative humidity might explain some of the variation in scent composition and emission rates.

Our study shows that *O. californica* subsp. *avita* harbors incredible intraspecific diversity, especially in floral scent. This pattern of intraspecific floral variation is pervasive across

many hawkmoth-pollinated evening primroses in North America. Of particular interest, the species of *Mompha* moth found on *O. californica* subsp. *avita* is currently undescribed (a new species to science). Specialist herbivores, like *Mompha*, have putatively co-diversified with their hosts but given their enigmatic biology, their diversity remains unexplored. Diversity is more than just the estimated 8.7 million species on earth. Measures to conserve biological diversity should take into account the ecological and evolutionary mechanisms that engender and maintain intraspecific variation as these microevolutionary processes have shaped the diversity of life.

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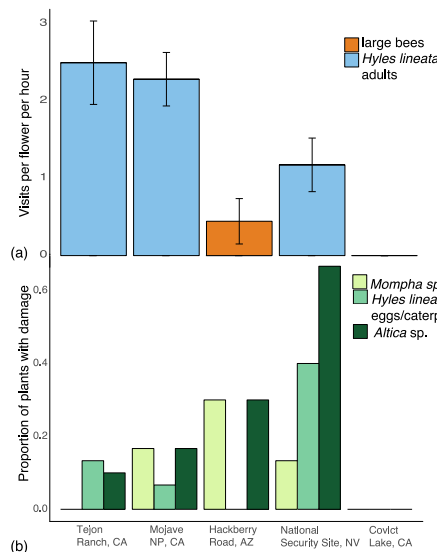


Figure 6. Variation in pollinator visitation rates (a) and herbivory (b) across five sites of *O. californica* subsp. *avita*.

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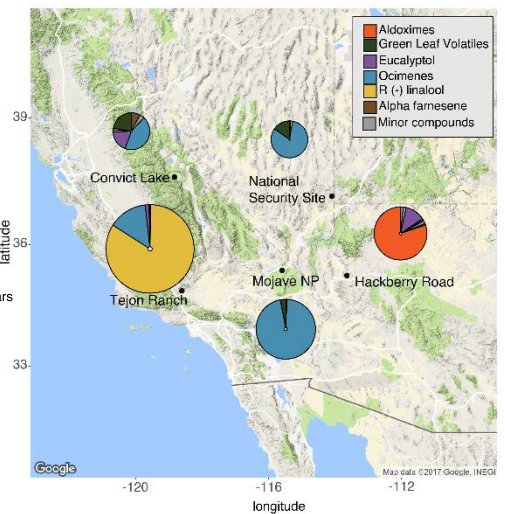


Figure 7. Floral scent composition across five sites of *O. californica* subsp. *avita*. Sections of the pie represent the average proportion of a compound in the total scent bouquet. The size of the pies indicates the total average emission rates per population (Tejon Ranch 0.17 mg/ml, Mojave NP 0.07 mg/ml, Hackberry Rd 0.04 mg/ml, NV National Security Site 0.01 mg/ml, Convict Lake 0.01 mg/ml)

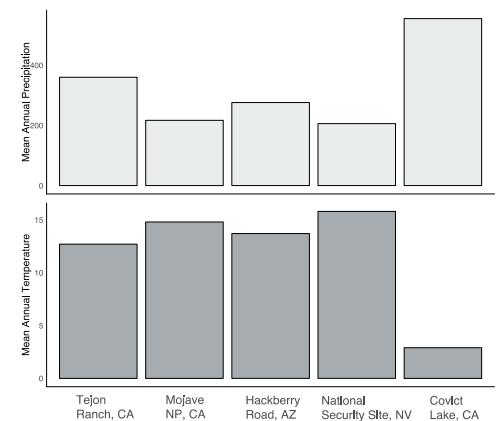


Figure 8. Mean annual temperature (°C) and precipitation (mm) obtained from BIOCLIM (<http://www.worldclim.org/bioclim>) across five sites of *Oenothera californica* subsp. *avita*.

Revisiting an old question in California botany: Why do many plant species have five-petaled flowers?

James Mickley¹ and Carl Schlichting¹

If one takes the time to observe many different species of flowering plants, one may notice that a large proportion of them have five-petaled flowers. Not only are five-petaled flowers widespread, but petal number is remarkably constant within plant families and is often used to differentiate among families (1, 2). Consistently five-petaled flowers, with few shifts to other petal numbers, is one of the defining characteristics of Pentapetalae: a massive clade of ~175 plant families comprising much of flowering plant diversity (3, 4).

These patterns in Pentapetalae raise some questions: why is petal number so invariant in Pentapetalae, and why is five petals the predominant number? Early botanists noticed these patterns in petal number, and the popular explanation was that pollinators preferred a certain number of petals, thereby selecting for that number as well as a reduction in variation (e.g., 5-7). In particular, during the 1950s, Elmar Leppik demonstrated that various pollinators were able to “count” by manipulating the number of petals on flowers to force pollinators to differentiate among them to gain rewards. He found that bees and butterflies could differentiate among petal numbers, but that flies, beetles and weevils could not (6, 8). From his observations, he noted anecdotally that bees preferred five-petaled flowers to other petal numbers (6).

Leppik’s work was well known at the time, even making the New York Times (9) and it attracted the attention of an eminent California botanist and evolutionary biologist, George Ledyard Stebbins. Stebbins was in the process of writing his second treatise on plant evolution (10), and his graduate student, Carl Huether, had been studying petal number variation in normally five-petaled *Leptosiphon androsaceus*

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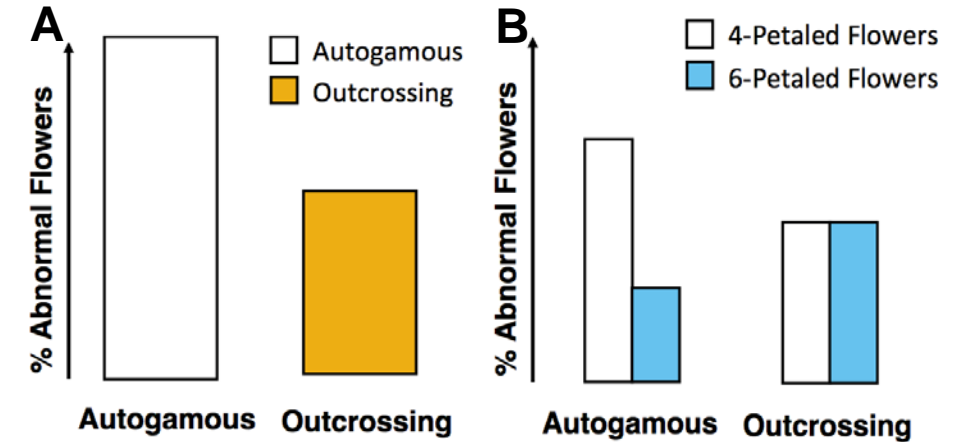


Figure 1. Hypothetical differences in the percentages of abnormally-petaled flowers between self-pollinated (autogamous) and outcrossing species under our hypotheses. In A), if pollinators select for invariant petal number, autogamous species would be expected to have a higher percentage of abnormal flowers because pollinator selection would be relaxed. In B), if higher petal numbers are costly, autogamous species would be expected to have more four-petaled flowers and perhaps also fewer six-petaled flowers than outcrossers.

(Polemoniaceae) in the mountains around San Francisco. Huether found that 1-4% of *L. androsaceus* flowers deviated from five petals in natural populations, with an increasingly higher proportion of four-petaled flowers, relative to six-petaled, as environmental conditions became drier (11). However, field observations suggested that the pollinators (bombyliid and syrphid flies) did not discriminate against four- or six-petaled flowers (10). Despite this apparent lack of pollinator preference, Stebbins concluded that a fixed petal number was adaptive and maintained by natural selection (10). He also called for more research on pollinator preferences for petal number (12), since this had not been thoroughly explored. Alas, in the ensuing 45 years, the field turned elsewhere.

We decided to revisit this old conundrum and assess the evidence for pollinator selection on petal number, or traits correlated with it, (13) using a different method: by comparing the amount of petal number variation between species that were pollinated by insects (outcrossing), and those that could self-pollinate without any insect assistance (autogamous). If selection by pollinators were responsible for the

predominance of five-petaled flowers, then species freed from such selection, such as autogamous species, might be expected to exhibit more variation in petal number (Figure 1A). In addition, if making petals is costly to the plant – they can lose a lot of water (14) and can also attract herbivores (15) – we might expect that autogamous plants would produce flowers with fewer than five petals (Figure 1B).

We have focused on species in the Polemoniaceae because they have natural variation for petal number, and there is evidence for a genetic basis for this variation (up to 16% petal number variants: 11, 16-18; Figure 2). In addition, the pollination biology of the Polemoniaceae is unusually well characterized, thanks to the work of Verne and Karen Grant, who were also California botanists and contemporaries of Stebbins. In a monumental effort, even by current standards, the Grants collected detailed data on 122 species in the Polemoniaceae, identifying the primary pollinators, and testing whether species could self-pollinate (19). This combination of data on petal number variation, primary pollinators, and mating systems provides a useful system to test

our two main hypotheses: 1) Autogamous species should have higher levels of petal number variation, compared to that of outcrossers, if pollinators are directly or indirectly selecting for five petals; and 2) Autogamous species should show a shift toward more four-petaled flowers, if having more petals is costly.

We selected two genera from the Polemoniaceae to work with: *Gilia* and *Saltugilia*. Both genera are desert annuals occurring in similar geographic regions and dry habitats, and each contains closely-related species that are autogamous or outcrossing. *Gilia cana* and *S. splendens* ssp. *splendens* are pollinated by bombyliid flies, and *G. sinuata*, *S. latimeri*, and *S. australis* are autogamous self-pollinated species (19, 20). For each species, petal number for a minimum of 500 flowers (# plants: 140-435) was recorded for three separate populations in April 2015 (Table 1). These populations were located across Southern California within Mojave National Preserve, Sweeney Granite Mountains Desert Research Center, Joshua Tree National Park, San Bernardino National Forest, the Pioneertown Mountains Preserve (Wildlands Conservancy), and natural areas managed by the Bureau of Land Management. Voucher specimens were collected for all the species sampled at each site and deposited in the CONN herbarium at the University of Connecticut (CONN00200743–CONN00200833). Data were summarized as per-individual proportions of each petal number.

All species we studied exhibited natural variation in petal number, ranging from < 1% of flowers that were not five-petaled in *S. splendens*, *S. australis*, and *G. sinuata*, to 4% in *S. latimeri*. In most cases, variants were four- or six-petaled, though more extreme variation was occasionally found (0.1% of flowers), particularly in *S. latimeri* (e.g., Figure 2). Since this extreme variation was rare, it was grouped with four- and six-petaled flowers for analysis. Species with more petal number variation mostly produced more six-petaled flowers; proportions of four-petaled flowers were similar across species. Despite these differences among species, autogamous species did not have greater variation in petal number compared to outcrossing species (Figure 3).

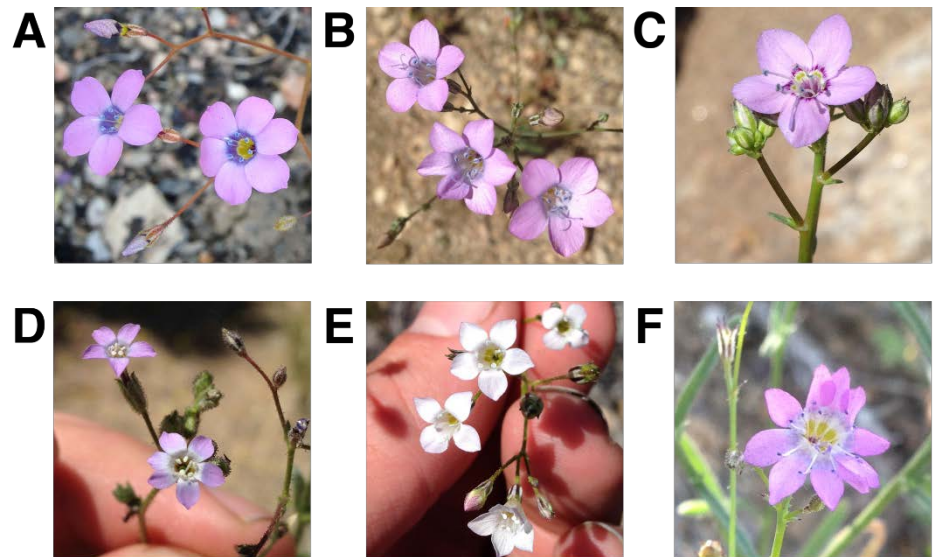


Figure 2. An assortment of variation in natural populations of petal number across species in *Gilia* and *Saltugilia* in the Polemoniaceae, which normally have five-petaled flowers. A) *Gilia cana* ssp. *speciformis*; B) *Saltugilia splendens* ssp. *splendens*; C) *S. caruifolia*; D) *G. sinuata*; E) *S. australis*; F) *S. latimeri*. Most abnormal flowers are either four-petaled or six-petaled, though more extreme variations exist (e.g., nine petals in F).

Table 1. A list of the field site locations where species were sampled across Southern California. Note that multiple species were sampled at several sites.

Site	Species	Latitude	Longitude
Granite Cove	<i>G. sinuata</i>	34.78238	-115.65548
Black Rock	<i>G. sinuata</i> , <i>S. latimeri</i> , <i>S. splendens</i> ssp. <i>splendens</i>	34.06982	-116.39351
Burns Crossroad	<i>G. sinuata</i>	34.22112	-116.62119
Kelbaker ¹	<i>G. cana</i> ssp. <i>speciformis</i>	35.20488	-115.87035
Aiken Mine	<i>G. cana</i> ssp. <i>speciformis</i>	35.18528	-115.76691
Rattlesnake Canyon	<i>G. cana</i> ssp. <i>bernardina</i>	34.23017	-116.65197
Smarts Quarry	<i>G. cana</i> ssp. <i>bernardina</i>	34.30404	-116.79989
Elata Ave.	<i>S. latimeri</i> , <i>S. splendens</i> ssp. <i>splendens</i>	34.07416	-116.41512
Elk Trail	<i>S. latimeri</i>	34.07486	-116.43531
Burns Spring	<i>S. splendens</i> ssp. <i>splendens</i>	34.20462	-116.57495
HWY 243 ²	<i>S. australis</i>	33.89241	-116.85896
S22 PCT 0.4S	<i>S. australis</i>	33.21182	-116.58227
S22 PCT 1.5S	<i>S. australis</i>	33.20837	-116.57798
S22 PCT 2.4S	<i>S. australis</i>	33.20389	-116.56817

¹ Small population with 12 plants and 53 flowers

² Small population with 75 plants and 124 flowers

Within genera, autogamous species exhibited more petal number variation in *Saltugilia*, but less in *Gilia* (Figure 3). Similarly, there was no overall pattern of autogamous species having more four-petaled or fewer six-petaled flowers, though autogamous species also showed divergent patterns for six-petaled flowers (Figure 3). In each case, the trends in *Saltugilia* were driven by *S. latimeri* – it had an unusually large proportion of six-petaled flowers.

Based on these results, there is no evidence that the absence of pollinators results in differences in selection on petal number in autogamous species, or that there are selective advantages to reducing petal number. Though these species were not pollinated by bees, as in Leppik's work (6), this calls into question the old assertion that pollinator preferences drove trait fixation on five petals. We do not find this result surprising, as it is difficult to conceive of a reason why a particular pollinator would prefer flowers with five petals over those with some other number, if other advertisement traits remained unchanged.

Differences among species are present in the proportions of four- and six-petaled flowers, both among those species we sampled, and among species in other studies (11, 17, 18). As noted above, *S. latimeri* showed substantially more petal number variation than other species we studied, particularly for flowers with six or more petals. *Saltugilia latimeri* is a recently described and poorly-studied species with small populations and a restricted geographic range, and perhaps random genetic drift has played a role in influencing the control of petal number. More species need to be assessed to determine what drives these differences in petal number among species and whether species such as *S. latimeri* are unusual outliers, or fit within the range of normal variation among species. We were unable to include two additional *Saltugilia* species (*S. caruifolia* and *S. splendens* ssp. *grantii*) because they bloom later in the season. These two species are pollinated by different pollinators: bees and hummingbirds, respectively.

Although most research on natural variation in petal number has occurred with species in the Polemoniaceae, it is less clear what patterns of natural petal number variation (if any) are present

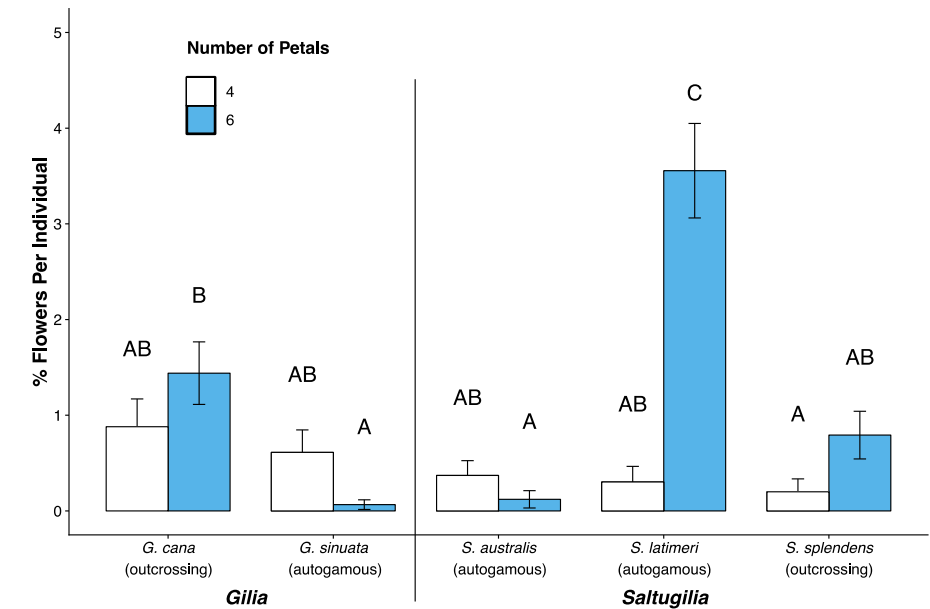


Figure 3. The difference in petal number variation for autogamous species relative to that of outcrossers within each genus. Error bars represent standard errors of the mean per individual percentages. Letters represent significant groupings from Tukey post-hoc tests with bonferroni corrections. In *Gilia*, there is less overall petal number variation and significantly fewer six-petaled flowers in the autogamous species (*G. sinuata*). In *Saltugilia*, the trends are reversed in *S. latimeri* (more overall variation, significantly more six-petaled flowers), while *S. australis* has less overall variation and fewer six-petaled flowers than the outcrosser. No genera show differences in the percentage of four-petaled flowers between autogamous and outcrossing species.

in other plant families. While pollinators may not be the primary factor constraining plants to five-petaled flowers, having a certain petal number may still be adaptive, as Stebbins theorized (10), but for other reasons. One possibility is that the genetic framework that produces a certain petal number may also control other traits. In this case, changes in petal number could result in changes to other floral or vegetative traits that might be maladaptive. Petal number is correlated with the numbers of sepals, stamens, and carpels (17, 18, 21), and changing petal number may adversely affect traits such as pollen production or seed set (18). Unfortunately, little is known about specific genetic and developmental processes that predictably lead to specific petal numbers. In our future work, we hope to explore aspects of these processes to ask further questions about whether having consistently five-petaled flowers is adaptive and why this is the predominant pattern in Pentapetalae.

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In Memoriam – Robert Fulton

With great sadness we report that Robert “Rob” Fulton, outstanding manager of the California State University Desert Studies Center for 32 years, passed away at the age of 63. Rob was a true naturalist and an exceptional desert ecologist. Over the years, Rob touched the lives of thousands of students and researchers by enthusiastically sharing his keen knowledge and passion for the desert. Rob served as mentor, friend, and colleague to many in the science and land management community of the Mojave Desert, including the editors of this newsletter. He will be dearly missed.

Cards and messages to Fulton's family may be sent in care of the CSU Desert Studies Consortium, College of Natural Sciences and Mathematics, Cal State Fullerton, 800 N. State College Blvd., Fullerton, CA 92831.

Links to other Memoriams:

<https://www.gofundme.com/rob-fulton-memorial-fund>

<http://news.fullerton.edu/2018su/robert-fulton.aspx>

<https://desertstudies.org/2018/07/02/passing-of-rob-fulton/>