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The population biology of dispersal and gene flow in the desert shrub *Acacia (Senegalia) greggii* A. Gray in the Mojave National Preserve

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Biology

by

Keith Donald Gaddis

2014

ABSTRACT OF THE DISSERTATION

The population biology of dispersal and gene flow

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Doctor of Philosophy in Biology

University of California, Los Angeles, 2014

Dean Victoria L. Sork, Committee Chair

Desert ecosystems are increasingly affected by the human-driven environmental pressures currently changing global landscapes and climates. Plant species are especially susceptible to these changes due to their inability to move except through propagules and a frequent reproductive dependence on dispersing animal species that could themselves be displaced. To identify the limitations climate and landscape already exert on the movement and reproduction of plant species in desert zones, we examined the desert shrub *Acacia (Senegalia) greggii* A. Gray in the Mojave National Preserve. In chapter one, we give an overview of the ecosystem and study species. In chapter two, we present determinants of reproductive success in *A. greggii* shrubs. Limited water availability in deserts leads to annual and spatial variation in floral production. The impact of this variation on the reproductive success of a plant depends on the response of dispersal agents and seed predators to fluctuating food resources. We conducted observational surveys and experimental manipulations, and found that greater floral abundance attracted a greater number of pollinators, increased fruit-set, decreased the seed predation rate,

and thus increased the pre-dispersal reproductive success of the shrub. In chapter three we present the historic and contemporary dispersal in *A. greggii*. We compared the genetic patterns of adults and pollen in a 4 km^2 area, and found widespread gene flow indicating pollen flow may be more extensive than seed dispersal. Despite this extensive movement, both adult and pollen genetic patterns were explained by separation between dry-washes, suggesting a potential dispersal corridor for pollen and seeds. In chapter four, we present our comparison of alternative landscape pathways derived from both climatic and topographic variables to explain regional genetic structure. We examined movement across 2,700 km^2 to determine if the fine-scale patterns observed in chapter three translate into regional connectivity. We again found widespread dispersal was best explained by gene flow along dry-washes. Our work documents a species with a well-adapted reproductive strategy and historically widespread dispersal. In spite of these results, the movement pattern of *A. greggii* is shaped by landscape features suggesting a potential impact of landscape change and development on future movement.

The Dissertation of Keith Donald Gaddis is approved.

Thomas W. Gillespie

Stephen P. Hubbell

Gregory S. Okin

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2014

To my mother for teaching me work ethic,

my father for giving me an appreciation for the natural world,

my Aunt Pat for pushing me into higher education,

and to my wife for believing in me when I didn't believe in myself.

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CHAPTER 1:

Introduction to a desert study system

Desert ecosystems are increasingly affected by the human-driven environmental pressures currently changing global landscapes and climates. Rising temperatures, aridity and development have already led to decreasing vegetation cover and increasing isolation of desert plant populations (Anyamba and Tucker 2005). For instance, in the desert Southwest of the United States, residential and industrial development is at an all-time high (Leu et al. 2008), and has led to a rise in air pollution (Brooks 2003), and the introduction of invasive species (Reible et al. 1982). Plant species are especially susceptible due to their inability to move except through propagules and a frequent reproductive dependence on dispersing animal species that could themselves be displaced. Moving allows a species to escape an unfavorable environment, find mates, and share genetic information that may help adapt to new environments. If changing climate and habitat destruction inhibits the mobility of a species, there is a greater likelihood of local or global extinction. It is essential that we identify the limitations climate and landscape already exert on the movement and reproduction of species in desert zones, like the American Southwest. Using this information we can inform future efforts to protect these fragile ecosystems as damage continues. The overall goal of this dissertation is to understand the patterns of genetic diversity and connectivity in a common desert shrub species. In this chapter, I will introduce the study system and provide background natural history information that enhances an understanding of the central chapters.

The Mojave Desert

The Mojave Desert is the most climatically and topographically diverse desert in North America. Situated between four states (California, Nevada, Utah, Arizona), the Mojave lies as a transition between the colder Great Basin Desert to the north, and hotter Sonoran Desert to the

south (Jaeger 1957, Rundel and Gibson 1996). Although much of the Mojave is high desert (lying between 900 and 1500 m in elevation) it contains the lowest and hottest point in the western hemisphere, Death Valley (86 m below sea level). Annually, the Mojave receives less than 300 mm of rainfall, and ranges in average temperature from 40 °F to 115 °F, with highs above 120 °F in the summer months, and lows well below freezing in winter (NOAA 2014). The majority of rainfall occurs in the colder months, with more sporadic high intensity storms occurring in summer. Because of the basin-range topography, elevation differs drastically over short distances leading to powerful variation in air density, temperature, and precipitation (Jaeger 1957, Rundel and Gibson 1996, Hereford et al. 2006). Although, the Mojave is the smallest US desert (65,000 km^2), it is home to four national parks, the most central of which is the Mojave National Preserve (6,224 km^2).

The Mojave Desert contains a large diversity of plant and animal life. The majority of Mojave fauna extend into the deserts to the north and south. Common animal species include the desert bighorn sheep (*Ovis canadensis nelsoni* Merriam), black-tailed jackrabbit (*Lepus californicus* Gray), and kangaroo rat (*Dipodomys sp.*), and include endemics such as the Mojave rattlesnake (*Crotalus scutulatus* Kennicott Merriam), western banded gecko (*Coleonyx variegatus* Baird), Mojave ground squirrel (*Spermophilus mohavensis* Merriam), and California leaf-nosed bat (*Macrotus californicus* Baird). One-quarter of Mojave plant species are endemic, for instance parry's saltbush (*Atriplex parryi* S. Watson), Mojave sage (*Salvia mohavensis* Greene), and the Joshua tree (*Yucca brevifolia* Engelm.). Shrub species dominate the landscape (i.e. blackbrush (Coleogyne ramosissima Torr.), brittlebush (*Encelia farinose* Torr. & A. Gray), creosote bush (*Larrea tridentata* (DC.) Coville), and Mojave yucca (*Yucca schidigera* Roezl ex Ortgies)), occurring in an often semi-uniform distribution. Though it has other unique characters,

it is the plant life that is often used to distinguish the Mojave from the other three North American deserts (Jaeger 1957).

Pollinators of the Mojave Desert

The Mojave contains a diverse assembly of pollinator species. In a 7 year survey of pollinating bees within the Nevada Test Site in southern Nye county, Nevada, Allred (1969) found 71 species in 35 Genera visiting 40 different plant species. Mojave pollinators are not restricted to bee species. For instance, Costa's hummingbirds (*Calypte costae* Bourcier) pollinate a suite of Southwest plant species including the Mojave plants barestem larkspur (*Delphinium scaposum* Greene), desert lavender flower (*Hyptis emoryi* Torr.), and firecracker penstemon (*Penstemon eatonii* A. Gray). In an multi-year survey of the arthropod community in the Nevada Test Site, Rundel & Gibson (1996) report a large diversity of insect pollinator species including some 61 species of bee flies (Bombilliydae), 26 families of butterflies and moths (Lepidoptera), in addition to numerous other potentially pollinating flies (Diptera) and beetles (Coleoptera). One of the best studied pollinator guilds of any Southwest plant species is that of the creosote bush (*Larrea tridentata*). Over 120 bee species have been documented visiting flowers of this plant, 25 of which are specialist pollinators (only forage pollen from single plant) (Hurd and Linsley 1975, Simpson et al. 1977, Minckley et al. 1999). In a survey of creosote pollinators in 47 sites throughout the Mojave, Sonoran, and Chihuahuan Deserts, Minckley et al. (1999) showed that spatial turnover was high for both specialist and generalist bees. This spatial variation occurred at distances 1 - 5 km apart; indicating environmental and ecological influences, in addition to resource availability, likely drive local pollinator assemblages (i.e. nesting site needs, water availability, or inter-annual variation in floral display).

Pollinator availability in the Mojave National Preserve is variable and, may be determined by local climatic forces. To estimate the baseline pollinator diversity we conducted a two-year pan trap survey (2009 and 2010) of insect pollinators at 23 sites in the Mojave National Preserve. Over five sampling periods, we collected 2277 bee and 159 wasp individuals in 37 and 57 species or morphospecies (Table 1-S1). Wasp diversity may be more composed of rare species than bees. We found 68% of wasp species collected were singletons compared to 34% of bees. Additionally, there was a stronger asymptote in the species accumulation curve of bees compared to wasps (Figure 1-1), indicating a greater decrease in likelihood of collecting a new species with each sample. We found a significant relationship between elevation at a sample site with both pollinator abundance ($R^2 = 0.09$, $P < 0.01$) and pollinator species richness ($R^2 = 0.20$, $P < 0.001$). Collecting times at these sites overlapped with the flowering times of multiple species that occur within the study range (i.e. *Acacia (Senegalia) greggii* A. Gray, *Psorothamnus spinosus* (A. Gray) Barneby, *Peritoma arborea* Greene, *Chilopsis linearis (Cav.) Sweet).* Thus, it is not clear if the relationship between elevation and pollinator availability is driven by the climatic limitations of the pollinator species, or is indirectly driven by the climatic influence on flowering time of the local plant species (Rathcke and Lacey 1985).

Seed Dispersers of the Mojave Desert

The animal seed disperser community of the Mojave is diverse, but may present a greater threat to reproduction through pre- and post-dispersal seed predation compared to other ecosystems. Mojave Desert animal seed dispersers include ungulates (Bighorn sheep, Mule deer (*Odocoileus Hemionus* Rafinesque)), birds (silky fly catcher (*Phainopepla nitens* Swainson), Northern mockingbird (*Mimus polyglottus* L.)), rodents (desert wood rat (*Neotoma lepida*

Thomas), white-tailed antelope squirrel (*Ammospermophilus leucurus* Merriam)), and insects (harvester ants (*Pogonomyrmex californicus* Cole)). Several studies have reported higher seed consumption rates in north American deserts relative to other world deserts, citing higher rodent activity as a primary cause (Brown et al. 1975, Mares and Rosenzweig 1978, Predavec 1997). For instance, in a study of kangaroo rat seed predation in the Mojave Desert, Soholt (1973) showed that over 95% of estimated seed product of *Erodium cicutarium* (L.) L'Hér. ex Aiton was consumed. In another study, Reichman (1979) showed that Southwest desert rodents were able to find and consume 100% of seeds artificially dispersed aboveground, and 76% of seeds artificially placed belowground. This seed predation intensity contributes to the minimal seed bank maintained by numerous Southwest plant species (Price and Joyner 1997), and may be a severe limitation to dispersal.

Acacia (Senegalia) greggii A. Gray

The species *Acacia (Senegalia) greggii* A. Gray is representative of numerous patchy shrub species throughout the Southwestern US. Like *Psorothamnus spinosus*, *Olneya tesota* A. Gray, and *Parkinsonia aculeata* L., *A. greggii* occurs primarily in dry-washes that occasionally flood during rainstorms. Flowers in this species occur in fragrant yellow cylindrical spikes. Like other Legumes, this species forms bean like pods that contain a variable numbers of thick-coated seeds. Although flowering typically occurs between June and July, and fruiting between July and August, the phenology of this species is affected by local climatic variation. In a phenological survey of 1000 shrubs across 23 sites over three years (2009, 2010, and 2011), we saw a significant relationship between the elevation of a sampling site and the average peak flowering time of the shrubs at that site ($R^2 = 0.64$, $P < 0.001$) (Figure 1-2). This relationship was likely

driven by temperature cues, whereby lower elevation sites reached higher temperatures at earlier dates than those at higher elevations. It is not clear how this phenological variation would affect the pollinator species or seed predators that rely on this species for food.

For the last 30,000 years, this species has likely had a semi-stable distribution. The occurrence of packrat middens has assisted in the ability to infer past distribution ranges of numerous plant species. Packrats create debris piles for their dens (middens). The burial of material in this process sometimes results in long term preservation of plant matter, which can then be carbon dated to estimate the period in which a species formerly occupied an area (Wells 1976). The five earliest records for this species, all greater than 20,000 years old, together occupy a large portion of the western range of this species (Figure 1-3). The youngest samples do occur at many sites on the edge of the current distribution; however the difference between these and younger samples suggest only a modest expansion within the last 10,000 years. A previous analysis of midden and current distribution data suggested that *A. greggii* distribution has been limited by mean winter temperatures (Holmgren 2005), suggesting the distribution of this species may be influenced by increasing temperature and aridity projected for the Southwest (Seager et al. 2007).

Preview of Dissertation

We address the limitations of landscape and climatic effects on the movement and reproduction of *A. greggii* in the Mojave National Preserve. Over three years we conducted a series of experimental and observational studies using ecological, remote sensing, and genetic tools. In chapter two, we examine the effect of increased floral abundance on reproductive success in *A. greggii*. Limited water resources in deserts lead to year-to-year variation in floral effort by many plant species. This variation may confer a benefit in wet years through increased

seed production and seed predator satiation. Conversely, the variation in floral abundance could lead to decreased pollinator efficiency, reducing fruit-set in flowering shrubs, and jeopardizing reproductive investment. In the third and fourth chapters, we examine dispersal patterns of *A. greggii* and the limitations of landscape features at two different spatial scales. In the third chapter, we examine fine scale genetic spatial structure within a 4 km2 area examining the distribution of genetic diversity in adults and dispersed pollen. In the fourth chapter, we examine regional movement patterns, testing for both landscape and climate effects on describing connectivity between 23 populations in 2,700 km^2 area. These studies expand our understanding of the ecology of this species, and give a framework in which to predict future responses of this and similar species to ongoing habitat and climate change.

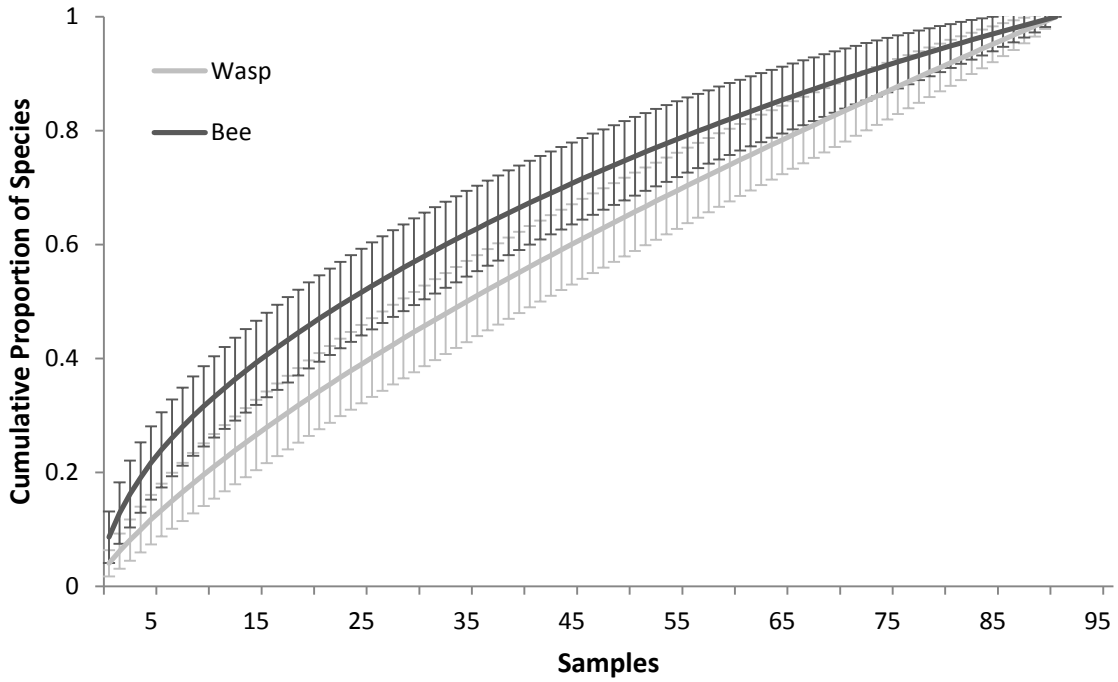

Figure 1-1: Species accumulation curve of 42 bee and 57 wasp species collected across 23 sites in the Mojave National Preserve. The curves indicate the mean cumulative proportion of species collected when adding a given sample based on 100 random permutations of the data, with error bars indicating the standard deviation around the mean (Ugland et al. 2003, Colwell et al. 2004, Kindt et al. 2006).

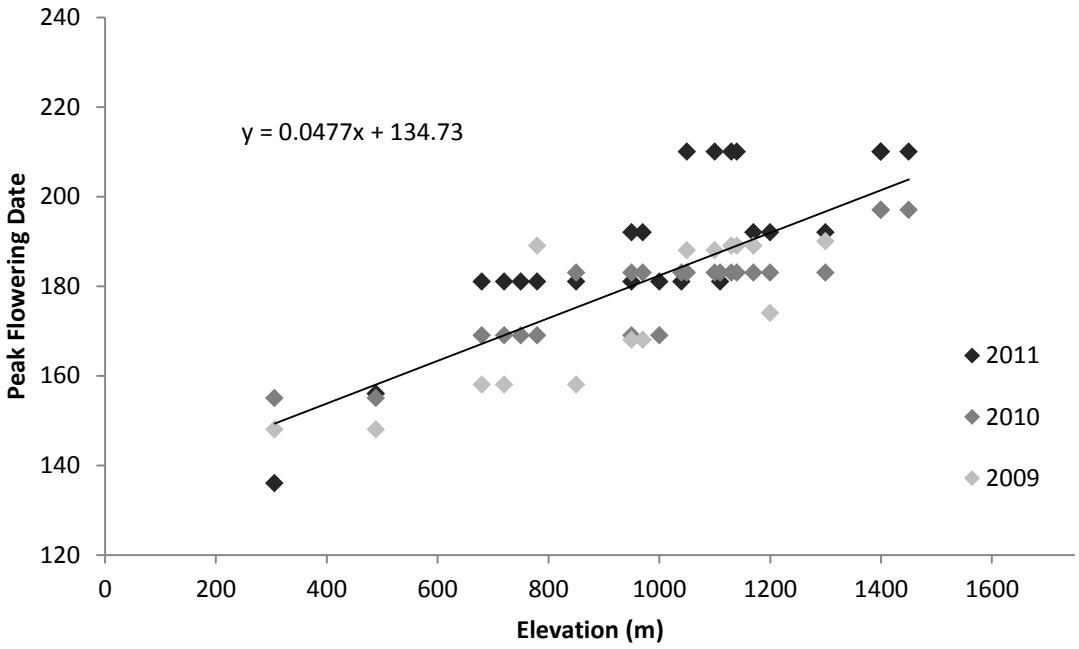

Figure 1-2: Relationship between peak flowering time and site elevation at 23 one-hectare sites in the Mojave National Preserve across three years. Peak flowering time is correlated with elevation using a linear regression model when examining the three years together ($R^2 = 0.64$, $P < 0.001$), 2011 alone (2011 $R^2 = 0.71$, $P < 0.001$), 2010 alone ($R^2 = 0.84$, $P < 0.001$), and 2009 alone ($R^2 = 0.66$, $P < 0.001$). The slope when examining all years is 0.048, indicating that a 100 m difference in elevation between sites translates to nearly five days difference in peak flowering. We estimated peak flowering date by identifying the period in which the number of flowers (or flowering shrubs) was higher than the number of buds or seed pods (or budding/seeding shrubs). If there were fewer flowers than buds in one period, and fewer flowers than seed pods in the subsequent period, peak flowering date was estimated to have occurred at the mid-point between the two periods.

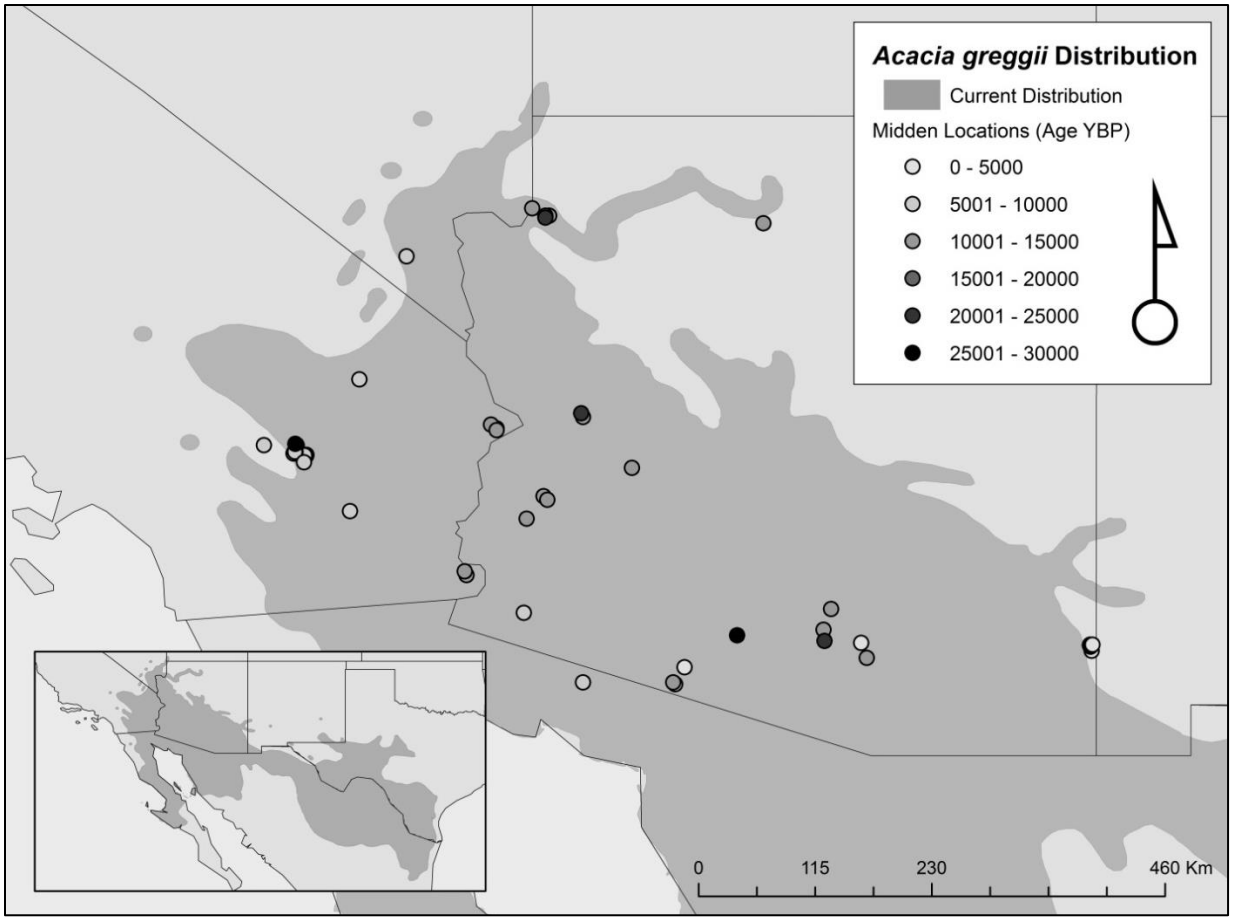

Figure 1-3: Map showing current and past distribution of *Acacia (Senegalia) greggii* in the Southwestern US. The darker shaded area in the full and inset map indicate the current distribution of *A. greggii* (Little Jr 1971). The point features on the map indicate locations of wood rat middens that have contained *A. greggii* plant material, and have been carbon dated to estimate the time period in which they were collected. Midden data was obtained from USGS/NOAA North American Packrat Midden Database online (http://geochange.er.usgs.gov/midden/).

Table 1-S1: Abundance of bee and wasp species collected during pan trap pollinator surveys of 23 sites within the Mojave National Preserve. The survey was conducted over two years (summers of 2009 (June and July) and 2010 (May, June, July)). Traps were laid in 100 m transects with alternating yellow, white, and blue color, and left for 8 hours during each collection period (Leong and Thorp 1999). Insects were identified to species when possible, or divided into morphospecies.

	Species	Abundance	Sites
Bee	*Anthidiini s.str*	1	1
Bee	*Anthidium palidiclypeum*	1	1
Bee	*Anthidium sp.*	10	4
Bee	*Apis mellifera*	7	2
Bee	*Ashmeadiella sp.*	2	2
Bee	*Ashmeadiella sp.*	12	2
Bee	*Augochlorella sp.*	5	2
Bee	*Ceratina morpho 1*	2	2
Bee	*Ceratina morpho 2*	2	2
Bee	*Chelostoma sp.*	1	1
Bee	*Colletes sp.*	2	1
Bee	*Diadasia sp.*	62	16
Bee	*Exomalopsis sp.*	55	10
Bee	*Halictus sp.*	16	3
Bee	*Heriades morpho 1*	71	19
Bee	*Heriades morpho 2*	54	18
Bee	*Hesperapis elegantula*	7	4
Bee	*Hoplitis sp.*	1	1
Bee	*Hylaeus sp.*	2	1
Bee	*Lasioglossum morpho 1*	1799	23
Bee	*Lasioglossum morpho 2*	121	9
Bee	*Megandrena enceliae*	2	2
Bee	*Melissodes sp.*	9	6
Bee	Morpho 1	11	6
Bee	*Nomia sp.*	1	1
Bee	*Perdita morpho 1*	1	1
Bee	*Perdita morpho 2*	1	1
Bee	*Perdita morpho 3*	1	1
Bee	*Perdita morpho 4*	2	2
Bee	*Perdita morpho 5*	1	1
Bee	*Perdita morpho 6*	3	1

	Species	Abundance	Sites
Bee	*Perdita morpho 7*	3	1
Bee	*Perdita morpho 8*	3	2
Bee	*Perdita morpho 9*	1	1
Bee	*Perdita morpho 10*	2	2
Bee	*Perdita morpho 11*	1	1
Bee	*Perdita morpho 12*	1	1
Bee	*Trachusa Heteranthidium*	1	1
Wasp	*Ammophila sp.*	3	2
Wasp	Morpho 3	2	2
Wasp	Morpho 4	1	1
Wasp	Morpho 7	2	1
Wasp	Morpho 8	2	1
Wasp	Morpho 9	2	2
Wasp	Morpho 11	1	1
Wasp	Morpho 16	1	1
Wasp	Morpho 17	1	1
Wasp	Morpho 18	1	1
Wasp	Morpho 19	1	1
Wasp	Morpho 20	1	1
Wasp	Morpho 21	1	1
Wasp	Morpho 22	1	1
Wasp	Morpho 23	1	1
Wasp	Morpho 25	1	1
Wasp	Morpho 26	1	1
Wasp	Morpho 30	1	1
Wasp	Morpho 34	1	1
Wasp	Morpho 40	1	1
Wasp	Morpho 41	1	1
Wasp	Morpho 42	49	19
Wasp	Morpho 43	4	4
Wasp	Morpho 44	2	2
Wasp	Morpho 45	1	1
Wasp	Morpho 46	6	5
Wasp	Morpho 47	7	7
Wasp	Morpho 48	10	6
Wasp	Morpho 49	7	6
Wasp	Morpho 50	4	2
Wasp	Morpho 52	7	3
Wasp	Morpho 54	2	1
Wasp	Morpho 56	1	1
Wasp	Morpho 57	1	1
Wasp	Morpho 60	1	1

	Species	Abundance	Sites
Wasp	Morpho 61	1	1
Wasp	Morpho 62	1	1
Wasp	Morpho 63	1	1
Wasp	Morpho 64	1	1
Wasp	Morpho 65	1	1
Wasp	Morpho 66	1	1
Wasp	Morpho 67	1	1
Wasp	Morpho 68	1	1
Wasp	Morpho 69	1	1
Wasp	Morpho 70	1	1
Wasp	Morpho 71	1	1
Wasp	Morpho 72	1	1
Wasp	Morpho 73	1	1
Wasp	Morpho 74	1	1
Wasp	Morpho 75	1	1
Wasp	Morpho 76	1	1
Wasp	Morpho 77	1	1
Wasp	Morpho 78	1	1
Wasp	Morpho 79	1	1
Wasp	Morpho 80	5	4
Wasp	Morpho 81	4	3
Wasp	Morpho 82	2	2

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CHAPTER 2:

Impact of floral display on fertilization success and seed predation in a common desert shrub *Acacia (Senegalia) greggii* A. Gray

ABSTRACT

Water availability in deserts is often spatially and temporally unpredictable, which leads to variation in the reproductive output of numerous plant species, producing more flowers in wet years or locations. The success of this behavior is dependent on the availability of pollinators to transport the increased pollen load, and ability of the increased seed abundance to either satiate seed predators or withstand their damage. To determine if increased floral display increases reproductive success, we conducted a series of observational and experimental studies in the desert shrub, *Acacia (Senegalia) greggii*, in the Mojave National Preserve. In this species, we test the assumptions that: (1) increased floral abundance will increase fertilization success, (2) increased fruit production will lead to seed predator satiation, and (3) seeds are capable of germination following predator damage. We surveyed bud, flower, and fruit abundance and pollinator occurrence for two years across 23 one-hectare sites in the Mojave National Preserve. In one of those years, we concurrently conducted an exclosure study to measure the level of fertilization success and seed predation. Additionally, we compared germination and survival in seeds exposed to low, medium, and extreme physical damage relative to an undamaged control. Pollinator abundance and fertilization success at the level of individual shrubs were both positively related to floral abundance, but the ratio of pollinators to flowers was unaffected. Although seed predation reduced fruit-set by 72% on average, increased floral production reduced the proportion of fruit lost to seed predation. Germination and survival was equal between the control and the two lower damage treatments, but actually increased in the extreme damage treatment. This study indicates a positive outcome of increased floral production, likely conferring a reproductive advantage on shrubs.

INTRODUCTION

Seed production and survival are two essential variables that define plant reproduction success. Variation in these two variables is dependent on likelihood of fertilization and risk of seed predation. In deserts, reproductive outputs of numerous plant species often match water availability, such that plants produce more flowers in wet years or locations (Noy-Meir 1973, Regal 1982, Kelly and Sork 2002). To the extent that increased flower production results in more plants, this variation in floral production can generate patchy plant distributions, as well as create an unpredictable food resource for the animal populations that forage on pollen or feed on seeds. A greater number of flowers could draw more pollinators (Levin and Kerster 1969, Augspurger 1980, Eckhart 1991, Thompson 2001, Mitchell et al. 2004), but also lower pollination efficiency (Andersson 1988, Grindeland et al. 2005). Similarly, increased seed production could attract more seed predators (Brody and Mitchell 1997), but decrease the proportion of seeds eaten (Silvertown 1980, Sork 1993, Kelly 1994, Herrera et al. 1998, Kelly et al. 2000, Kelly and Sork 2002), or the fraction of damage to seeds partially eaten (Bonal and Muñoz 2007). Together, these interactions may generate a series of trade-offs, wherein the costs and benefits of increased floral display depend on the plant's relationship with its animal community.

A rise in floral density in desert plants could lead to alternative reproductive consequences, depending on pollinator availability and behavior. Numerous studies have documented extreme forms of resource matching, where an overabundance of flowers and seeds are produced in resource rich years (Silvertown 1980, Norton and Kelly 1988, Kelly 1994, Herrera et al. 1998, Kelly and Sork 2002). The advantage of this behavior is dependent on the availability of a reliable pollen dispersal agent that will not be saturated by a rise in pollen abundance during peak years (Janzen 1971, Nilsson and Wastljung 1987, Brody and Mitchell

1997, Crawley 2000, Kelly and Sork 2002). Thus, the majority of species that employ similar mass flowering in other ecosystems are wind pollinated (Smith et al. 1990, Sork 1993, Herrera et al. 1998, Kelly et al. 2001, Kelly and Sork 2002). The consequences of a similar floral production spike in desert plants are unclear for several reasons. First, desert species often have patchy distributions that spatially separate potential pollen sources, decreasing the chance of pollen arrival, and limiting pollen availability (Cunningham 2000a, Groom 2001, Wilcock and Neiland 2002, Johnson et al. 2004, Amarasekare 2004, Knight et al. 2005). Second, desert plants are predominantly animal pollinated (Regal 1982). If pollinators are not sufficiently available during locally resource rich conditions, an increased floral output would be wasted. Last, though not uniformly observed (Augspurger 1980, 1981, Ghazoul 2005, Brys et al. 2008), pollinator efficiency in animal pollinated species may be reduced when presented with increased floral abundance, due to decreased pressure to travel between plants (less outcrossing) (Andersson 1988, Totland and Matthews 1998, Grindeland et al. 2005, Karron and Mitchell 2012), subsequently lowering fertilization success. In spite of this risk, there is an indication that increased floral display would lead to higher fertilization success in desert plants. Several studies show that pollinators increase their dispersal with increased distance between resources, and track variation in floral availability both spatially and temporally (Levin and Kerster 1969, Augspurger 1980, Eckhart 1991, Thompson 2001, Mitchell et al. 2004). Additionally, open landscapes such as those found in desert ecosystems may actually increase pollinator movement (Dick et al. 2003, Sork et al. 2005, Slavov et al. 2009, Kamm et al. 2010, Shohami and Nathan 2014), helping to maintain local populations, and allow for quick response to variation in floral production. Thus, to demonstrate a reproductive benefit of large floral displays, one would need to observe a positive relationship between floral and pollinator abundance and no decline in the

average number of pollinators per flower with rising floral density. More importantly, a lack of decline in fertilization success with increased floral abundance would indicate no decrease in pollinator efficiency with size of floral display.

A larger production of seeds or fruits following a larger floral display could assist in reducing seed predator effects. Predator satiation occurs when seed abundance for a given plant species exceeds predator demand, lowering the proportion of seeds eaten (Silvertown 1980, Sork 1993, Kelly 1994, Herrera et al. 1998, Kelly et al. 2000, Kelly and Sork 2002). In desert plants, seed predation represents one of the largest barriers to reproductive success. For resource poor deserts, seed crop from plants provides one of the most nutrient-rich food sources amenable either to immediate consumption or to long-term storage (Mares and Rosenzweig 1978, Crawley 2000). The value of seeds fuels competition among seed predators (Janzen 1971, Brown et al. 1975, 1979, Brown 1979, Davidson et al. 1984), driving the evolution of highly specialized skills adapted to find and consume seeds. As evidence of this pressure, numerous studies of seed predation in deserts have reported removal rates between 70 - 100% (Chew and Chew 1970, Brown et al. 1975, Whitford 1978, Reichman 1979, Abramsky 1983, McAuliffe 1990). Despite this pressure, there is some indication that predator satiation in this environment can occur. First, as with pollen, the frequently patchy distribution of plants in this ecosystem spatially separates seeds, making it harder for potential seed predators to find seeds and track year-to-year variation in production. Second, as desert plants are predominantly consumed by scatter-hoarding rodents (especially in North America (Brown et al. 1975, Mares and Rosenzweig 1978, Predavec 1997)), and not frugivores, there is a greater likelihood predator satiation will confer a net benefit to the plant due to decreased seed destruction, but not result in a large decrease in seed dispersal (Janzen 1971, Silvertown 1980, Herrera et al. 1998, Kelly and Sork 2002). Therefore, if seed

production is sufficient to satiate predators, the proportion of seeds or fruits consumed on a plant should decline with the size of the initial floral investment and/or fertilization success.

The effect of seed predation may also be mitigated by a seed's ability to tolerate damage and maintain viability following partial consumption by seed predators. Seed predator attacks may occasionally result in only partial destruction of seeds. For instance, rodents may not completely consume seeds (Abbott and Quink 1970, Forget et al. 1994, Loayza and Carvajal 2014), or may store them for later consumption (Brown et al. 1979, Thompson and Thompson 1980, Nikolai and Bramble 1983, Reichman 1983). Numerous insect predators use seeds as hosts for offspring that may only partially destroy the seed structure (Steele et al. 1993, Ollerton and Lack 1996, Dalling et al. 1997, Bonal and Muñoz 2007). If the seed embryo is not harmed in this process, this damage does not always preclude seed survival if the species is tolerant to partial loss of its cotyledon food stores (Halevy 1974, Janzen 1976, Dalling et al. 1997, Bonal and Muñoz 2007, Fox et al. 2012, Loayza and Carvajal 2014). In some cases this damage may result in an increase in germination success (Steele et al. 1993, Hauser 1994, Mucunguzi 1995, Mack 1998, Takakura 2002, Branco et al. 2002, Sousa et al. 2003) by allowing water to penetrate thick seed coats that normally require abrasion for germination (Went 1948, Coe and Coe 1987, Fox et al. 2012). If seeds do not show reduced germination or survival following damage, then partial damage presents a means of predator escape.

The common Southwestern United States shrub *Acacia (Senegalia) greggii* A. Gray (Fabaceae) provides an appropriate study system to test the effect of large floral displays on fertilization success and increased predator satiation. This species produces flowers in yellow cylindrical spikes that can number into the thousands on a given shrub. It is insect pollinated, and produces bean-like pods that are consumed by both insects and vertebrates (Monson and Kessler

1940, Lopez-Trujillo and Garcia-Elizondo 1995, Fox et al. 2012). Like many other desert species (Noy-Meir 1973, Chambers and MacMahon 1994, Meney et al. 1994, Price and Joyner 1997, Marone et al. 1998, Guo et al. 1998), *A. greggii* maintains little seed bank (Vanzant et al. 1997), suggesting a greater impact of pre-dispersal predation. A previous study of *A. greggii* showed increased germination when exposed to scarification, however, germination decreased under increasing bruchid beetle infestation (Fox et al. 2012). Like similar bruchid studies (Miller 1994) it was not clear if decreased germination was a general response to reduction of stores or a specific result of embryo destruction. Though not uniform across the genus (Peake 1952, Lamprey et al. 1974, Miller 1994, Mucunguzi 1995, Or and Ward 2003), bruchid infestation has been shown to increase germination in several African *Acacia* species (Halevy 1974, Hauser 1994, Miller 1994, Mucunguzi 1995).

In this study we investigate the consequences of increased floral production and seed predation in *A. greggii* within the Mojave National Preserve in California by testing the assumptions that (1) increased floral abundance will increase fertilization success, (2) increased fruit production will lead to seed predator satiation, and (3) seeds are capable of germination following predator damage. We evaluate assumptions 1 and 2 (Figure 2-1) using data from a two-year survey of floral phenology and pollinator abundance at 23 one-hectare sites, with an assessment of fertilization success and seed predation from an exclosure analysis of 85 shrubs in 18 of those sites. To address assumption 3, we compared germination and survival of 119 seeds exposed to simulated low, medium, and extreme predator damage with a control treatment over 34 days. If increased floral abundance positively affects reproductive success, there should be a tight relationship between floral and pollinator abundance, with no decline in the number of pollinators per flower or fertilization success with increased floral abundance. If seed predators

are satiated by increased production, then the proportion of fruits consumed should decline with initial floral abundance and/or fertilization success. If seeds are tolerant to predation damage, germination and survival success should be equal in the four simulated damage treatments.

METHODS

Study Species

Acacia greggii is a desert shrub occurring throughout the Southwestern United States into Northern Mexico. The shrub flowers in yellow cylindrical spikes (here called inflorescences) that produce bean-like pods (here called fruit), which can hold one to more than ten seeds. We refer to the number of fruits produced per inflorescence as 'fruit-set' (Kenrick and Knox 1989, Willmer and Stone 1997, Cunningham 2000b). Inflorescences begin bud formation in mid-May, flower in June and July, and fruit in July and August. Like many other dry-climate legumes, *A. greggii* fruits have a thick seed coat that often requires scarification to allow water penetration for germination (Fox et al. 2012). Each seed weighs between 0.1 to 0.5 g on average. Shrubs primarily occur along dry-washes, and have a patchy, but locally gregarious distribution.

Study Area

The study was conducted in the Mojave National Preserve in Eastern California (Figure 2-2), which is an arid region receiving less 200 mm of rainfall annually. The landscape is topographically diverse, with several mountain ranges and low land sand dunes separating study sites. We selected 23 one-hectare plots, located in sites from approximately 300 - 1500 m, and spanning an area of 3,000 km^2. Density of *A. greggii* at a site ranged from 10 to 143 individuals per hectare.

Sampling

In the summers of 2010 and 2011, we monitored budding, flowering, and fruit production in 1000 adult *A. greggii* shrubs across the 23 one-hectare plots, while concurrently surveying bee, butterfly, and wasp visitors to each shrub. During each period, we counted the total number of buds, flowers, and fruits for each shrub. Full seed development may take additional time after initial fruit formation. To avoid adding spurious relationships due to the timing of collection, we only counted fruit and not seed number. This measure provides a better method to estimate seed predators that likely respond to the number of visible seed pods, rather than to the number of seeds within pods (Crawley 2000). We took censuses every four weeks in 2010 (three periods), and every two weeks in 2011 (six periods). On the same day, we conducted one 30-minute AM and one 30-minuite PM pollinator survey, recording visitors visually or collecting them by sweep net. We provisionally divided pollinator species into morphospecies, and later identified to genus or species when dichotomous keys were available.

To determine fertilization success and seed predation rate, in the summer of 2010 we conducted an exclosure experiment at 18 sites with 1 - 5 shrubs per site and 85 shrubs overall. We identified nine branches on each shrub with equal numbers of budding inflorescences. To measure fertilization success, we allowed pollinator access to three of these branches, which we covered following flowering with mesh 'no-see-em' netting to exclude seed predators. To measure the amount of self-fertilization mediated without pollinator assistance, we covered three branches prior to flowering. We left the three remaining branches as unaltered controls to allow both pollinator and seed predator access. We measured seed predation as the difference in number of fruits between predator exposed and predator excluded/pollinator allowed branches,

divided by the number of fruits in the latter treatment. Difference in fruit-set between the three treatments was determined using a Kruskal-Wallis test.

To identify the effect of seed damage by seed predators, we collected 119 seeds from 34 mothers at 15 of the sites. Fox et al. (2012) showed that one bruchid destroys 6% of *A. greggii* seed mass on average, whereas five larvae destroyed 22.3% on average. Using this observation as a gauge, we weighed seeds and divided them among four simulated seed predation treatments, with no significant difference in initial weight of seeds divided among the four treatments ($P > 0.05$). In the first treatment, we simulated damage similar to a single bruchid infestation or major scarification event, by making slight cut on the seed edge of the seed coat, called 'clip' ($n = 22$). In the second treatment, we simulated damage seen in seeds infested by five bruchids by cutting one-quarter of the seed away ($n = 22$). In the third treatment, we simulated extreme predator damage, above what has been observed in previous *A. greggii* studies (Fox et al. 2012), by removing one-half of the seed ($n = 22$). The final treatment was a control with no damage to the seed ($n = 53$). Cutting was done to avoid damage to the embryo. We placed the seeds in separate petri dishes on moist paper towel, and monitored them for 34 days. Over that time we tracked germination and mortality.

Analysis

To determine if *A. greggii* is pollinator limited, we first analyzed the relationship of local floral abundance with pollinator abundance, pollinator richness, and the number of pollinators per flower. We compared the site total values for each measurement during the 9 sampling periods between 2010 and 2011 in the 23 sites using linear regression ($n = 61$). Sites in which no shrubs were flowering were excluded. Abundance values were log transformed for normality.

To examine the cause of variation in fertilization success, seed predation, and final fruit abundance across our study area, we first standardized datasets for comparison. Variables were measured as shrub means (fertilization success and seed predation), annual totals for a shrub (floral abundance and final fruit abundance), or annual site total (pollinator abundance) for the year 2010. Floral abundance was measured as the maximum total number of inflorescences counted on a shrub during any one survey (including unflowered buds). Using this measure of inflorescence number reduces the power to explain pollinator abundance, but improves the ability to detect other response variables. Final fruit abundance was measured as the total number of fruits on a shrub at the time exclosure branches were collected. Both floral and fruit abundance were log transformed for normality.

We used stepwise Akaike information criterion (AIC) model selection to compare alternative combinations of causal variables to explain fertilization success, seed predation, and final fruit abundance (Figure 2-1). All combinations of causal variables were compared, using the lowest AIC score as an indication of best model fit (Johnson and Omland 2004). Model selection for fertilization success was run a second time using site total floral abundance and site mean fertilization success to confirm that patterns observed using shrub totals and means were not influenced by pollinator abundance being measured at the level of the site.

Combining the three best-fit AIC models we created a Structural Equation Model (SEM) to quantify the relationships between variables and evaluate model performance. Structural equation modeling (Ullman and Bentler 2003) is an extension of Path Analysis (Wright 1921, 1934), that employs linear modeling techniques. Like in Path Analysis, we used SEM to succinctly present the causal relationships among variables using lines and arrows in a path diagram (Figure 2-1). In place of the simple regression approach of Path Analysis, we use SEM

to test the significance of each model connection, calculate the standardized regression coefficients (change in one parameter given a standard deviation change in another), and evaluate the coefficient of determination (R^2) (Mitchell 1992, Lei and Wu 2007). The goodness of SEM fit was determined using a X^2-test and a Tucker-Lewis Index (TLI). Non significance of the X^2-test indicates that there is no statistical difference between the explanatory ability of the user's model and a saturated model, with all variables connected. The TLI is less susceptible to sample size and non-normality compared to a X^2-test. TLI-values greater than 0.95 are accepted as a confirmation of good model fit (Hu and Bentler 1999, Hooper 2008). All SEM values and tests were run in the R package Lavaan (Rosseel 2012).

To determine if damage treatments affected the likelihood of a seed germinating and then surviving the trial, or of a seed dying, we compared the three damage treatments with the control using a factorial logistic regression. To determine if damage treatments affected germination timing, we compared the days until germination using an ANOVA.

RESULTS

Linear regression analysis showed that floral abundance significantly explained 60% of the variation in pollinator abundance ($R^2 = 0.60$, $P < 0.001$), and also significantly predicted pollinator species richness ($R^2 = 0.29$, $P < 0.001$) (Figure 2-3), but did not explain the number of pollinators per flower ($P > 0.10$). During the two-year survey, we recorded over 12,775 bee, wasp, and butterfly pollinators across 44 species (Tables 2-S1). Honey bees (*Apis mellifera* L.) made up 85% of the observations, with butterflies and wasps composing 2 and 6% respectively. Eighteen of the pollinator species observed were singletons for the study.

Pollinator and seed predator exposure affected the number of fruits per shrub. The three exclosure treatments differed significantly in fruit-set ($X^2 = 141$, $df = 2$, $P < 0.001$). Average pollination success (fruits per inflorescence in seed predator excluded bags) was 0.58, with a maximum of 2.1 and minimum of 0.03. Shrub branches excluding pollinators had an average fruit-set of 0.04, which was significantly higher than 0 (Wilcox signed rank test, $V = 1770$, $P < 0.001$), indicating some low level of self-fertilization without the assistance of pollinators. On average 72% of fruits were consumed. Seed predation was intense in many sites, with eight exclosure bags ripped open by obvious rodent action. Bags damaged in such a way were not included in the analysis.

The best-fit AIC model indicated floral abundance alone increased fertilization success and decreased seed predation. Additionally, we found that floral abundance, seed predation, and fertilization success together best predicted final fruit abundance on a shrub (Table 2-1). The fertilization success model did not change when run at the site level (Table 2-1), indicating no effect of pollinator sampling on model results. The fit measures of the SEM model indicate that including missing connections would not significantly improve the model performance ($X^2 =$

4.116, *df* = 3, *P* = 0.249, TLI = 0.961). All connections within the model were significant (Figure 2-4, Table 2-S2).

The factorial logistic regression showed that the treatment in which seeds were cut in half increased the likelihood of a seed germinating and then surviving the trial (68%) relative to the control (32%) ($z = 2.779$, $P < 0.01$, Figure 2-5). The other two damage treatments did not differ significantly from the control ($P > 0.05$, Figure 2-5). The three damage treatments germinated 6 days faster than the control on average (ANOVA, $F = 9.066$, $P < 0.001$), bit did not differ between each other in timing ($P > 0.05$). All ungerminated seeds in the three damage treatments died; however, only 25% of ungerminated control seeds died. The second factorial logistic regression showed total likelihood of mortality (examining ungerminated seeds and seedlings together) was higher in the clip (68%, $z = 3.076$, $P < 0.001$) and the one-quarter treatments (64%, $z = 2.768$, $P < 0.001$) relative to the control (28%).

DISCUSSION

This study demonstrates that plant reproductive success in *A. greggii* is predominantly determined by initial floral display and not by variation in fertilization success or seed predation rate. Pollinator abundance in the study area was tightly related to the flowering effort of shrubs (Figure 2-3). Within the spatial range of the study sites, pollinators were widespread and variation in pollinator abundance explained very little of the variation in fertilization success (Figure 2-4), indicating a reproductive advantage for large floral displays. We also found evidence in support of predator satiation: more fruits per plant resulted in a lower proportion of fruits consumed per shrub (Figure 2-4). Moreover, our seed predation simulation test showed that seed damage either enhanced or had no negative impact on seed survival and germination success. Thus, enhanced fruit production has a positive impact on plant reproductive success.

Pollination

Despite the patchy desert environment and harsh matrix, pollinator abundance was strongly correlated with floral abundance at a site during both years. Other studies of desert plant-pollinator interactions have shown high variability in pollinator abundance during plant flowering, driven by desynchronized flowering phenology and pollinator emergence (Waser 1979, Clark-Tapia and Molina-Freaner 2004), or local climate or environment deterring pollinator attraction (Lange and Scott 1999, González and Pérez 2010). There was no indication of pollinator limitation driven by variation in floral abundance (Figure 2-3). These results indicate pollinators respond to local floral availability, and are likely not limiting to *A. greggii* reproductive success within the study area.

Greater floral abundance increased the fertilization success of *A. greggii*. As the density of pollinators to flowers was unrelated to floral density (Figure 2-3), this result suggests potentially increased pollinator efficiency with higher floral density. Large floral displays often decrease pollinator movement between plants, lowering fertilization success in obligate outcrossing species (Augspurger 1981, Ghazoul 2005). We observed low levels of seed production on inflorescences in pollinator excluded bags, indicating some selfing ability in *A. greggii*. Thus, it is possible that a portion of the rise in fertilization success could be driven by increased within-shrub pollen transfer. It is clear pollinators improve fertilization success, as pollinator excluded bags decreased fruit-set by 93%. Regardless, fertilization success is best explained by floral abundance alone (Figure 2-4), likely due to the tight relationship between flower and pollinator abundance throughout the study area (Figure 2-3).

The major pollinator of *A. greggii* is a recently introduced species. Over 80% of observed pollinators were honeybees (*Apis mellifera*), which appear to be the 'Africanized' variety based on size characteristics (Daly and Balling 1978). These pollinators began arriving in the Southwestern US in the 1990s (Pinto et al. 2005). This finding introduces several questions about how the relationships we studied here may have been different in the recent past. Honeybees can displace competing pollinators to less profitable forage (Goulson 2003), suggesting other species may have pollinated *A. greggii* in greater abundance less than 30 years ago. The second most common pollinator observed on the shrub is also an *Apidae* species in the genus *Centris*. Like honeybees, this species is a medium size (~2 cm) hairy bee, with a bushy scopa efficient for pollen collection. In spite of this similarity, there was no evidence for competition between these species, as there was positive but weak correlation between abundance of *A. mellifera* and of *Centris sp.* (Pearson correlation, $r = 0.21$, $P > 0.05$). Additionally, although introduced

pollinators are predicted to increase pollen limitation (Knight et al. 2005), the results of model selection for fertilization success was the same when using log honeybee abundance and log total pollinator abundance. Regardless, reliance on a single major pollinator increases the likelihood of pollinator failure (Wilcock and Neiland 2002, Kremen et al. 2002), and raises concern for long-term stability given observed declines in domesticated honeybee populations (Kremen et al. 2002, Vanengelsdorp et al. 2009).

Predation

Increased floral abundance in *A. greggii* leads to predator satiation. We found that a decreased proportion of fruits were consumed in those shrubs that produced a larger number of initial inflorescences (Figure 2-4). The best model did not contain a link between pollination success and seed predation, probably because inflorescence number better explained the variance in pollination success and best predicted the total number of fruits produced before predation. Regardless, seed predation is still a large tax on shrub resources. On average 72% of fruits were consumed per shrub, which is similar to other studies of seed predation in desert plant populations (Chew and Chew 1970, Brown et al. 1975, Whitford 1978, Reichman 1979, Abramsky 1983, McAuliffe 1990).

We found evidence that rodents will consume seeds on the shrubs, even before they fall to the ground. Of the 504 branches in which we excluded seed predators using mesh bagging, eight were severely rodent damaged. Pre-dispersal predators are generally assumed to be primarily small insect specialists, with rodents acting on seeds post-dispersal (Crawley 2000). Previous work in the area has documented two kangaroo rat species, *Dipodomys merriami* Mearns and *D. panamintinus* Merriam, pocket mice, *Perognathus longimembris* Coues, and

several other omnivorous *Peromyscus* species (Price and Joyner 1997). Although these rodents do not frequently climb (Lemen and Freeman 1986, Price and Joyner 1997), the common wood rat (*Neotoma lepida* Thomas) does (Holbrook 1979), and occurs throughout the area (Smith 1995). This observation is a departure from the common separation of pre- and post-dispersal predator communities, and demonstrates the increased seed predation pressure on plants in this area.

Initial floral investment, seed predation, and pollination success determine the pre-dispersal fruit crop of a shrub. Together, these three variables explained 54% of the variance in final number of fruits per shrub (Figure 2-4). Though floral abundance has a strong influence on explaining the variation in the other two variables, we do not discount the effect seed predation and pollination success have on determining fruit production. Less than 58% of inflorescences produced a single pod, and 72% of fruits were consumed by pre-dispersal seed predators. In spite of this observation, the largest portion of variance in final fruit crop of a shrub was explained by floral abundance.

Seed Damage

We found damaged *A. greggii* seeds are capable of germination, and can actually outperform undamaged seeds. Two of the three damage treatments showed no difference in probability of germinating and surviving the trial; however, seeds cut in half had over twice the success rate as seeds in the control treatment (Figure 2-5). This enhanced performance following damage has also been reported in several larger seeded plants (Ernst et al. 1990, Steele et al. 1993, Vallejo-Marín et al. 2006, Loayza and Carvajal 2014). Nevertheless, other studies in smaller seeded species (many congeners of *A. greggii*) show reduced germination following

damage (Lamprey et al. 1974, Miller 1994, Mucunguzi 1995, Ollerton and Lack 1996, Koptur 1998, Or and Ward 2003). The increased germination rate we observe is probably due to greater water imbibing, as well as fewer resources required to break the tough outer seed coat. Numerous desert plant species require abrasion for germination to occur (Went 1948, Coe and Coe 1987, Fox et al. 2012), and physical damage is a means to this end. As further evidence, all three damage treatments in this study showed an average six-day difference in time to germination compared to the control (Figure 2-5). Previous work has shown a reduction in the proportion of seed damaged from partial seed predation with increased seed density (Bonal and Muñoz 2007). Thus increased floral production and subsequent predator satiation could present a potentially negative impact on germination. Further work examining density effects on partial seed consumption is needed to draw a conclusion about possible links between these processes.

Comparing our results with previous work, we find that damage does not universally improve seed performance. Fox et al. (2012) showed that although a single bruchid larva would not alter *A. greggii* germination probability, as the number of larvae in a seed increased, the probability of germination decreased. In this study, we observed increased germination and survival success in the 50% removal/extreme damage treatment (Figure 2-5). Even under the highest bruchid beetle infestation rate, Fox et al. (2012) found only 22% of a seed was removed on average. The difference in these two studies could be explained by the fact we avoided the embryo when administering the three damage treatments, whereas bruchids would not discriminate embryo from non-embryo. Thus, decreased germination related to increased bruchid infestation observed by Fox et al. (2012) was probably due to embryo damage, and not due to loss of cotyledon food stores. Additionally, this difference might be explained by the timing of the two experiments. In a study of bruchid effects on seed germination of *Acacia (Faidherbia)*

albia (Delile) A.Chev, Hauser (1994) showed that seeds with bruchid infestation had a higher germination when collected early in the season, while seeds collected late in the season were all dead. Although scarification by predators opens seeds up for water uptake, it also removes the thick coat that previously protected the seed inside from desiccation, infection and weathering (Chang et al. 2010, Fox et al. 2012). In this experiment we began germination trials immediately following physical damage. Fox et al. (2012) allowed beetles to emerge increasing the time after initial seed coat penetration in which additional damage could occur. Even so, the probability of mortality of a seed in this study was significantly greater for the clip and one-quarter treatments relative to the control ($P < 0.011$). This information reinforces that potential cost and benefits of seed predator action that may be dependent on timing and predator behavior.

Conclusion

This work demonstrates reproductive advantages of increased floral abundance in a desert shrub. A larger floral display led to a greater abundance of pollinators, and more importantly, higher fertilization success. There is a clear indication that increased fruit production leads to predator satiation (Figure 2-4), and therefore a greater return on the initial plant investment. Seeds partially damaged performed as well or better than undamaged seeds. Overall, the initial floral production explained the majority of the variation in final fruit density per shrub. These results indicate a relatively predictable outcome when varying flower production, likely conferring a reproductive advantage on those shrubs able to produce more flowers.

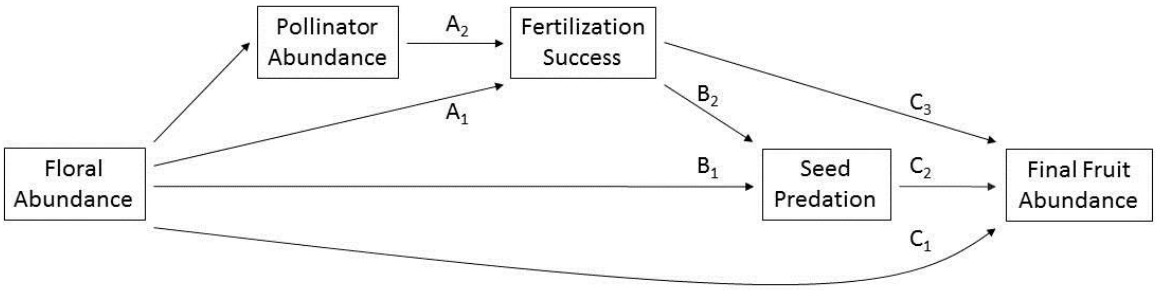

Figure 2-1: Hypothetical path diagram, with letters indicating the alternative direct and indirect connections to explain fertilization success (A), seed predation (B), and final fruit abundance (C) in *A. greggii*. The best combination of connections was evaluated using an AIC model selection. Final model fit was evaluated using X^2 and Tucker-Lewis Index (TLI).

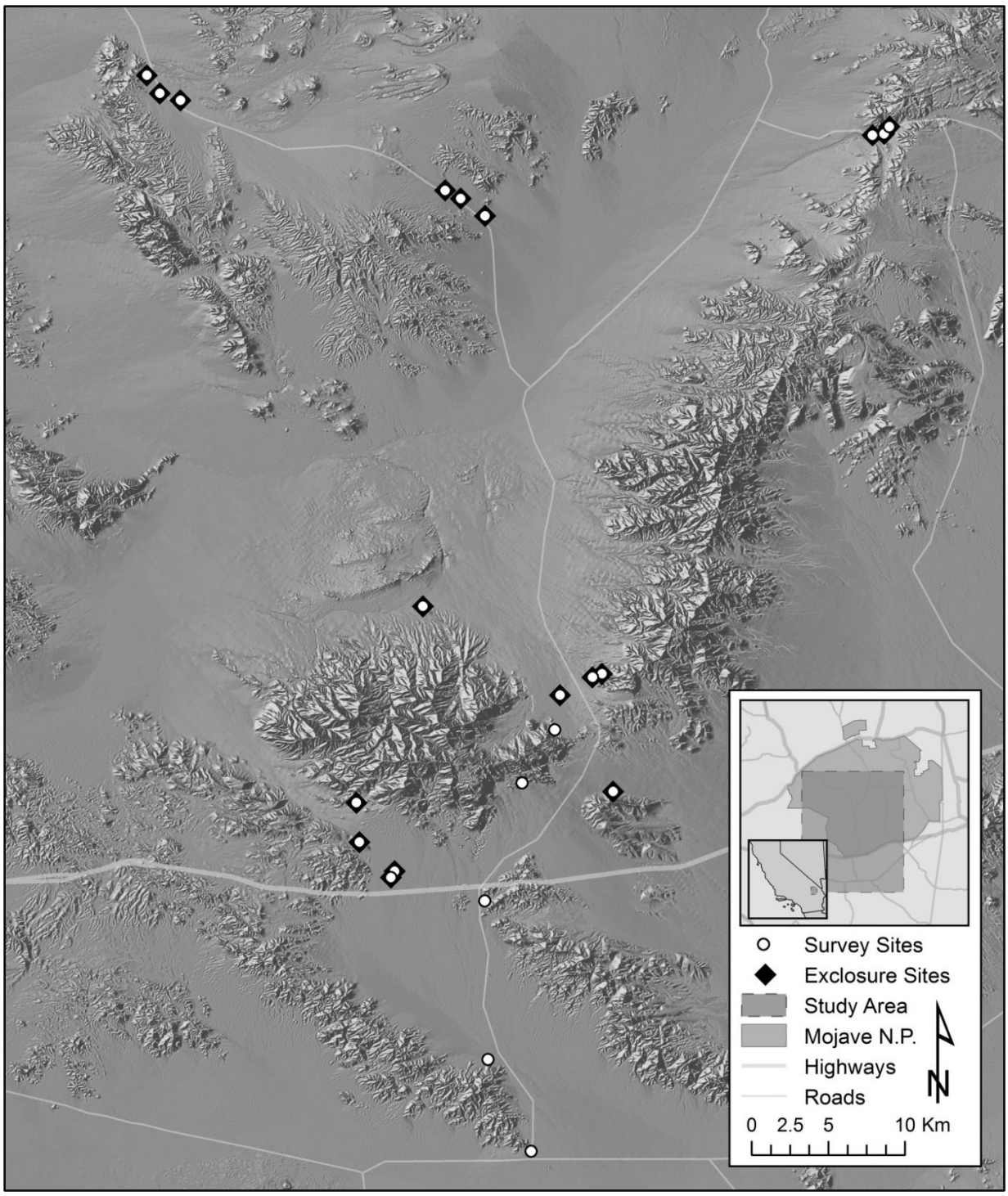

Figure 2-2: Map of 23 one-hectare sites in which phenology and pollinator abundance were monitored in 2010 and 2011. Also indicated are the 18 of these sites in which fertilization success and seed predation were estimated in 85 shrubs using exclosures in 2010.

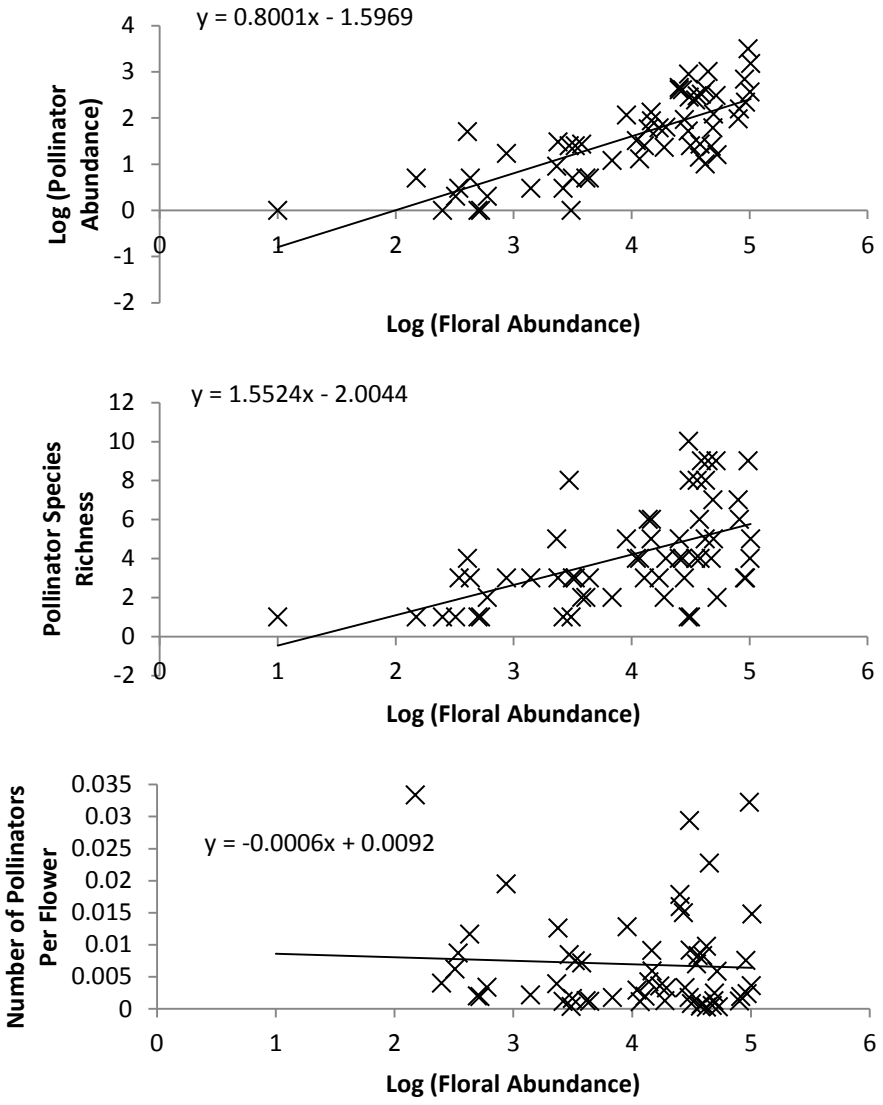

Figure 2-3: These linear regressions show a positive relationship between log floral abundance of *A. greggii* at a site with log pollinator abundance ($R^2 = 0.60$, $P < 0.001$), and pollinator species richness ($R^2 = 0.29$, $P < 0.001$), but no relationship with the number of pollinators per flower ($P > 0.10$). Values are based on site totals recorded during 9 survey periods in the summers of 2010 and 2011 at 23 one-hectare sample sites ($n = 61$). Two data points in the pollinator abundance per floral abundance variable were identified as y-outliers and significantly influential based on cook's distance ($P < 0.05$), and were dropped from the analysis ($n = 59$). Sampling periods in which no shrubs were flowering were removed from all analyses.

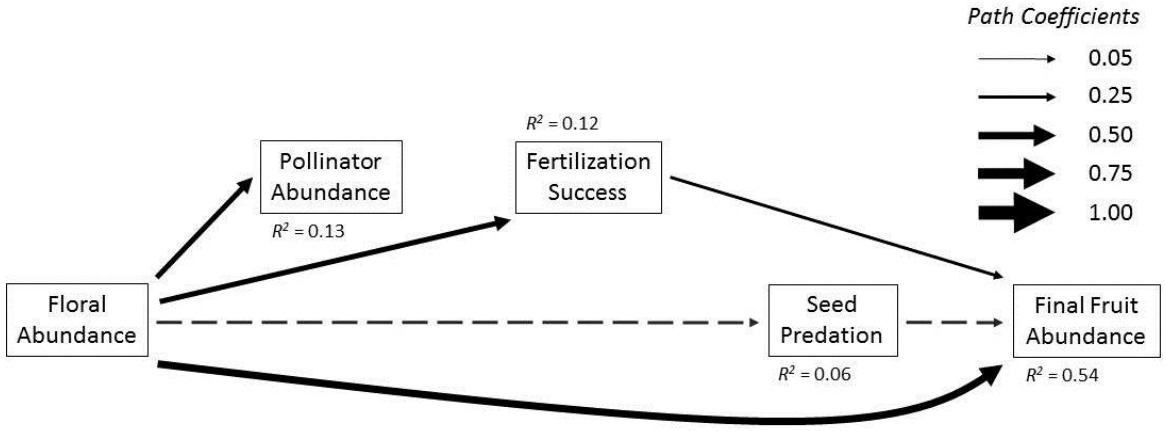

Figure 2-4: This figure shows the interaction of variables determining final fruit abundance for a shrub (n = 85) in the species *A. greggii*. The width of the arrows are proportional to the strength of the standardized path coefficient (see legend), with positive effects indicated by a solid line, and negative effects by a dashed line. The model contains no insignificant connections (P < 0.05). Abundance measures were log converted for normality. The model fit the data (X^2 = 4.116, df = 3, P = 0.249, TLI = 0.961).

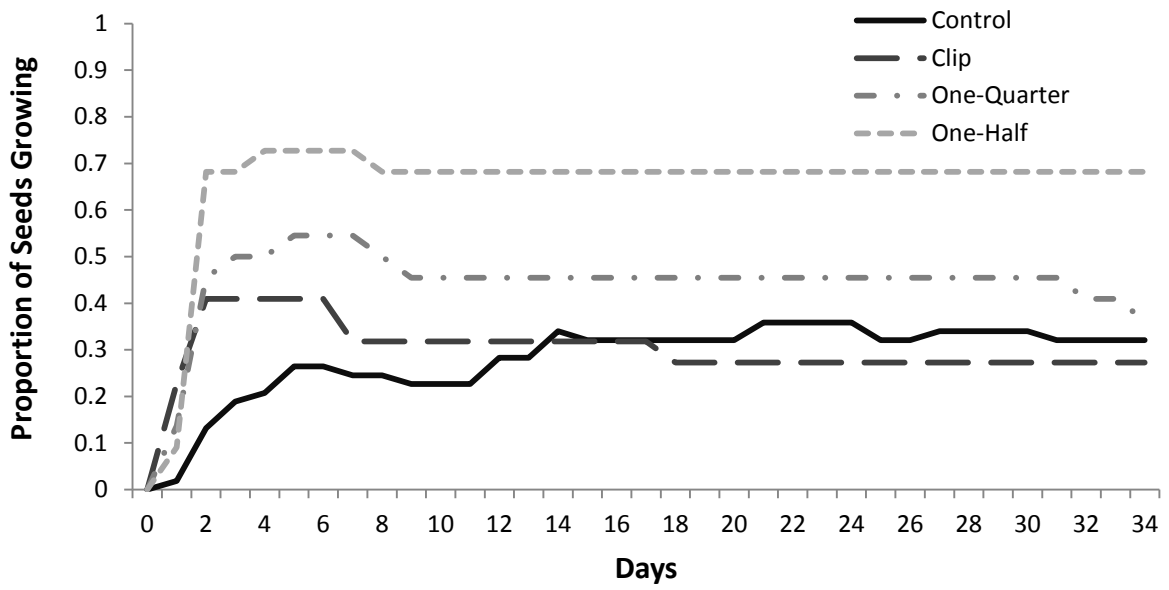

Figure 2-5: Life history chart showing germination success and survival of *A. greggii* seeds across the three damage treatments and control. The seeds cut by half had a significantly higher probability of developing into seedlings and surviving the trial than the control ($z = 2.779$, $P < 0.01$), while the other two treatments did not differ significantly ($P > 0.05$). All three damage treatments germinated significantly faster than the control (ANOVA, $F = 9.066$, $P < 0.001$), but showed no significant deviation between each other in timing ($P > 0.05$).

Table 2-1: Model selection by AIC scores to determine the best-fit explanatory variables for the three shrub measures: fertilization success, seed predation, and final fruit abundance. Lower values indicate better model fit. Model selection for fertilization success was run a second time using site mean values, AIC (Site), to confirm patterns were not influenced by pollinator abundance being measured at the level of the site. These values were used to inform the SEM model creation.

Candidate Model	AIC	Delta	AIC (Site)
Fertilization success			
Floral abundance	76.041	0.31	-4.2376
Floral abundance + Pollinator abundance	76.351	5.403	-2.8186
Pollinator abundance	81.754		6.7902
Seed Predation			
Floral Abundance	27.381	1.925	
Floral abundance + Fertilization success	29.306	3.164	
Fertilization success	32.47		
Final Fruit Abundance			
Floral Abundance + Fertilization success + Seed Predation	103.15	2.67	
Floral Abundance + Fertilization success	105.82	2.02	
Floral Abundance + Seed Predation	107.84	2.03	
Floral Abundance	109.87	29.64	
Fertilization success + Seed Predation	139.51	7.62	
Fertilization success	147.13	7.06	
Seed Predation	154.19		

Table 2-S1: Abundance of insect visitors to *Acacia greggii* recorded during 9 sampling periods in the summers of 2010 and 2011. The 'Sites' column indicates how many of the 23 study sites the species was observed in.

	Species	Abundance	Sites
Bee	*Apis mellifera*	10819	22
Bee	*Bombus sp.*	10	5
Bee	*Centris sp.*	939	18
Bee	*Exomalopsis sp.*	1	1
Bee	*Halictus sp.*	10	3
Bee	*Heriades sp.*	1	1
Bee	*Lasioglossum Dialictus sp.*	3	3
Bee	*Megachilli sp.*	9	6
Bee	*Melissodes sp.*	1	1
Bee	*Nomia sp.*	2	2
Wasp	*Polistes morpho 1*	363	17
Wasp	*Polistes morpho 2*	55	18
Wasp	*Ammophila sp.*	1	1
Wasp	*Steniolia nigripes*	151	21
Wasp	Morpho 3	1	1
Wasp	Morpho 28	1	1
Wasp	Morpho 29	1	1
Wasp	Morpho 31	1	1
Wasp	Morpho 32	1	1
Wasp	Morpho 33	1	1
Wasp	Morpho 35	1	1
Wasp	Morpho 37	1	1
Wasp	Morpho 38	1	1
Wasp	Morpho 39	1	1
Wasp	Morpho 51	2	2
Wasp	Morpho 53	2	2
Wasp	Morpho 55	10	6
Wasp	Morpho 58	1	1
Wasp	Morpho 59	1	1
Wasp	Morpho 60	70	14
Wasp	Morpho 61	9	7
Wasp	Morpho 62	23	9
Wasp	Morpho 63	3	2
Wasp	Morpho 65	12	8
Wasp	Morpho 66	19	11
Wasp	Morpho 67	11	4
Wasp	Morpho 68	20	1
Wasp	Morpho 69	1	1
Wasp	Morpho 70	8	2
Butterfly	*Colias eurytheme*	28	10
Butterfly	*Colias sp.*	1	1
Butterfly	*Danaus plexippus*	9	4
Butterfly	*Pontia beckerii*	3	3
Butterfly	*Satyrium sp.*	167	18

Table 2-S2: Standardized SEM path coefficients for (a) fully saturated theoretical model and (b) best fit final model (Figure 3). Significance of connections are indicated by asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

a. Saturated model	Pollinator Abundance	Fertilization Success	Seed Predation	Final Fruit Abundance
Floral Abundance	0.356***	0.299**	-0.206	0.632***
Pollinator Abundance		0.141	-0.173	-0.237**
Fertilization Success			0.055	0.244***
Seed Predation				-0.205**
R^2	0.127	0.139	0.088	0.577

b. Best fit model	Pollinator Abundance	Fertilization Success	Seed Predation	Final Fruit Abundance
Floral Abundance	0.356***	0.35**	-0.248*	0.54***
Pollinator Abundance				
Fertilization Success				0.24***
Seed Predation				-0.202**
R^2	0.127	0.122	0.061	0.543

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CHAPTER 3:

Pollen movement facilitates extensive gene flow in the desert shrub,

Acacia (Senegalia) greggii A. Gray

ABSTRACT

For desert plant species, there are two opposing interpretations of how the landscape influences movement. Desert plants tend to have discontinuous populations that are often small and separated by harsh intervening environments that could impede seed or pollen dispersal. Conversely, these factors can also increase gene flow by forcing animal dispersal vectors to travel greater distances for their food resources. We assessed the relative strength of these factors in the desert shrub *Acacia (Senegalia) greggii* A. Gray, a common Southwest species that has a patchy distribution restricted to dry-washes. Using ten microsatellite molecular markers within a 4 km^2 study area in the Mojave National Preserve, we address three specific goals: 1) to infer historical connectivity patterns and the distances over which gene movement has occurred, 2) to examine contemporary gene flow through pollen, and 3) to test whether features of the terrain (dry-washes and elevation) affect relatedness among adults and pollen movement over the landscape. We found that both historic and contemporary gene flow are very high in this species. Genetic structure was low between the dry-washes sampled in both adults (F_{ST} = 0.005) and pollen (ϕ_{GT} = 0.004). The low values of spatial autocorrelation among pollen pools relative to adults suggest that pollen movement is extensive and post-establishment processes create subsequent structure. Patterns of pollen genetic variation were significantly shaped by the spatial arrangement of dry-washes, whereas adult patterns of genetic variation were shaped by both dry-washes and elevation. This study helps resolve conflicting expectations for gene flow and genetic structure of desert plant species, and throws light on the potential threats to rare or endangered plants that have limited connectivity among discontinuous populations.

INTRODUCTION

Genetic connectivity is critically important to long term maintenance of genetic variation in many species, ultimately determining the ability of local populations to respond to their environment (Travis, 2003; Kremer *et al.*, 2012). This importance is especially apparent in deserts ecosystems where resource availability, particularly water, is restricted; and thus species tend to occur in spatially discontinuous, small, or low density populations separated by harsh intervening environments. In addition, a large proportion of desert plant species rely on animals for seed and pollen dispersal (Regal, 1982), which could further increase the uncertainty of connectivity between patches (Loveless and Hamrick, 1984; Hamrick and Godt, 1996). This combination of factors might cause a high level of intraspecific genetic divergence between clusters of individuals due to reduced gene flow (Loveless and Hamrick, 1984; Klinkhamer *et al.*, 1989; Kunin, 1997; Sork *et al.*, 1999; Nielsen and Ims, 2000; Ehlers *et al.*, 2002; Manel *et al.*, 2003; Ghazoul, 2005; Byrne *et al.*, 2007). Conversely, the same factors could increase movement by incentivizing dispersers to travel a greater distances for resources (Levin and Kerster, 1969; Ellstrand, 1992; Nattero *et al.*, 2011), or increasing the number of pollinator visits to a single plant (Stout *et al.*, 1998; Mustajärvi *et al.*, 2001; Ghazoul, 2005). In addition, open landscapes with spatially separated populations can increase connectivity relative to their continuous counterparts (Dick *et al.*, 2003; Sork *et al.*, 2005; Slavov *et al.*, 2009; Shohami and Nathan, 2014). Additionally, the predominance of perennial shrubs and seed dormancy in desert plants (Noy-Meir, 1973) allows for overlap of generations, which maintains a large effective population size, high genetic diversity, and reduces genetic differentiation among populations (Loveless and Hamrick, 1984; Hamrick and Godt, 1996). Thus, it is not clear if spatial

arrangement and environment in desert ecosystems will promote or hinder connectivity among plant populations.

Population genetic approaches provide a means of gauging the empirical reality of movement in desert plants. Genetic structure measures of adult populations infer historic gene flow among patches through pollen and seed movement (Loveless and Hamrick, 1984; Slatkin, 1985; Hamrick and Godt, 1996; Sork *et al.*, 1999). Spatial autocorrelation (Smouse and Peakall, 1999) estimates dispersal distance by examining genetic relatedness among individuals (or gametes) across space (Wright, 1943, 1978; Loiselle *et al.*, 1995; Hazlitt *et al.*, 2004; Epperson, 2005; Hardesty *et al.*, 2005). Previous studies of desert plants have often identified significant genetic differentiation ($F_{ST} > 0.1$) between populations for rare, threatened, or endangered species (Neel and Ellstrand, 2003; Neel and Cummings, 2003; Chen, Huang, *et al.*, 2009; Chen, Crawford, *et al.*, 2009; Haque *et al.*, 2010; Albrecht *et al.*, 2010; Migliore *et al.*, 2013; Clarke *et al.*, 2013), as well as common (Wesche *et al.*, 2011; Richardson and Meyer, 2012; Laport *et al.*, 2012; Lowry *et al.*, 2013). However, the majority of these studies have been conducted at regional spatial scales (> 10,000 km^2), and thus lack compelling evidence to determine dominant dispersal patterns for desert plants.

Genetic estimates of pollen flow provide a means to separate out the two gametic contributions to gene flow and compare contemporary and historic movement patterns. In general, pollen flow is more effective than seed dispersal at maintaining genetic connectivity because typically pollen moves farther than seed (Ennos, 1994), however, mating patterns can alternatively increase genetic structure if there is biparental inbreeding, selfing, or a small number of effective pollen donors (N_{ep}) (Griffin and Eckert, 2003; Vekemans and Hardy, 2004; Degen *et al.*, 2004). One can evaluate movement and the mating system through either indirect

estimation of genetic structure (i.e. MLTR (Ritland, 2002), TWOGENER (Smouse *et al.*, 2001)) or direct parentage analysis (Kalinowski *et al.*, 2007). The indirect structure approaches are appropriate when sampling large populations where it is not feasible to locate all pollen parents, while parentage approaches provide a more precise documentation of pollen flow events (Sork and Smouse, 2006). Genetic studies of desert pollen flow are few. Both Sampson (1998) and Ahmed *et al.* (2009) showed evidence for extensive pollen flow in desert trees with the former study documenting greater than 10 effective pollen donors per tree, and the later showing strong evidence for long distance pollen dispersal. Conversely, Slavov *et al.* (2009) found desert pollen pools of the tree species *Populus trichocarpa* Torr. & A. Gray were highly structured (ϕ_{FT} = 0.253) compared to their mesic counterparts (ϕ_{FT} = 0.052), indicating more restricted pollen flow in deserts.

The emerging field of landscape genetics provides a toolset with which to assess the influence of ecological, geomorphological, and environmental variables on gene flow (Manel *et al.*, 2003; Storfer *et al.*, 2007; Sork and Waits, 2010; Holderegger *et al.*, 2010). For instance, spatial autocorrelation analysis can be modified to determine the influence of a single variable on the relationship between distance and genetic relatedness (Hazlitt *et al.*, 2004; Dutech and Sork, 2005; Andrew *et al.*, 2007; Van Heerwaarden *et al.*, 2010). However, when examining multiple potential influences, differentiating the effect of each variable becomes more difficult. To address this issue, several studies have incorporated an AIC model selection approach to determine the best combination of variables to describe genetic differences (Garroway *et al.*, 2008; Pluess *et al.*, 2009; Murphy *et al.*, 2010; Jha and Kremen, 2013), and assess significance by comparing the best landscape model to a simple distance only model (Pluess *et al.*, 2009; Murphy *et al.*, 2010). Recent studies have shown that physical features of the landscape shape

movement in species with both restricted (Wang *et al.*, 2009; Vandergast *et al.*, 2009; Munshi-South, 2012), and widespread dispersal (Van Heerwaarden *et al.*, 2010; Jha and Kremen, 2013; Rico *et al.*, 2014), demonstrating the need to account for the effects of landscape variables especially when interpreting spatial genetic patterns in physically harsh environments like desert ecosystems.

The focal species in this study, the shrub *Acacia (Senegalia) greggii* A. Gray (Fabaceae) in the Mojave Desert, provides an appropriate system in which to study genetic connectivity in a desert plant. *Acacia greggii* is a common insect pollinated species that is widespread throughout the Southwestern United States and into Northern Mexico. This species is often restricted in distribution to dry-washes which may channel seed and pollen dispersal. Pollinators tend to concentrate foraging within or following the spatial distribution of a resource (Townsend and Levey, 2005; Almeida Vieira and Carvalho, 2008; Van Geert *et al.*, 2010), therefore, dry-washes, with a greater local abundance of flowering *A. greggii* shrubs, could create resource corridors for movement and restrict inter-wash pollen transfer. Seeds of *A. greggii* dispersed by rainfall run-off in dry-washes would likely experience seed coat abrasion, allowing the embryo inside water access, conferring seeds a greater likelihood of germinating and establishing. In addition to dry-washes, it is also possible dispersal in this species is restricted by elevation. In areas like the American Southwest the combination of topographic variation and aridity leads to steep climatic variability over a short distance (MacDonald, 2004; Kunkel *et al.*, 2013). Seeds dispersed from a higher or lower relative elevation may have lower survival compared to individuals dispersed at the same elevation with locally adapted genotypes (Rathcke and Lacey, 1985; Woodward, 1987; McKay and Latta, 2002; Kawecki and Ebert, 2004). Additionally, like many plant species (Rathcke and Lacey, 1985), flowering time in *A. greggii* is correlated with elevation, so that

individuals at lower elevations flower earlier than those at higher elevation (see Chapter 1), suggesting a possible barrier to pollen-mediated gene flow.

This chapter seeks to determine the scale of dispersal within a representative desert shrub, *A. greggii*, by examining the influence of distance and landscape on both adult and pollen genetic variation. We examine a 4 km^2 study area, using ten microsatellite genetic markers, to address three specific objectives. (1) We quantify historic connectivity patterns and the spatial scale of gene movement by analyzing the genetic structure and spatial autocorrelation of adult genotypes. (2) We estimate present gene flow through pollen using four methods: (i) Ritland's (1996, 2002) 'method of moments estimators' to analyze the degree of selfing, biparental inbreeding, and effective number of pollen donors; (ii) TWOGENER (Austerlitz and Smouse, 2001; Smouse *et al.*, 2001) to estimate the genetic structure among maternal pollen pools, and effective number of pollen donors; (iii) spatial autocorrelation analysis to assess the spatial scale of contemporary pollen movement; and (iv) paternity analysis to estimate dispersal distances directly. (3) We test for landscape effects on gene flow by examining the ability of dry-washes and elevation to describe relatedness among adults and pollen using a modified spatial autocorrelation analysis, and AIC model selection.

METHODS

Study Species

Acacia (Senegalia) greggii A. Gray (Fabaceae) is a common North American desert shrub species, occurring from the western Mojave Desert in California, to the Sonoran and Chihuahua Deserts in Northern Mexico. Across its range, *A. greggii* is often habitat restricted to dry-washes, where it can form dense local patches. Commonly called "catclaw acacia," this species is known for its distinct recurved spines. It produces yellow flowers in a cylindrical spike in the late spring to mid-summer, sometimes with a second flowering in early fall in wet years. In the Mojave, the shrub is pollinated by a community of bee, wasp, and butterfly species that is dominated by *Apis mellifera* L. (see Chapter 2), a non-native species introduced into this area. It sets seeds in bean-like pods in late summer.

Study Area

We conducted this study between the Granite and Providence Mountains in the Mojave National Preserve (centered around 34°50'30'' N, 115°37'50'' W). This arid zone receives less than 200 mm of rainfall annually. Habitat in the surrounding area ranges from pine-forest to barren sand dunes, and the shrub cover in the study area is dominated by *Larrea tridentata* (DC.) Coville (Zygophyllaceae). Density of *A. greggii* within dry-washes in the study area is as high as 46 individuals per hectare, compared with less than 1 individual per hectare in intervening areas. Elevation within 10 km of the study area ranges from 750 to 2050 m. The study area itself ranges from 1070 to 1177 m in elevation with a north facing slope.

Sampling

We examined two roughly parallel washes, each with an independent upstream watershed running out of the Providence and Granite Mountains (Figure 3-1). We established 1.5 km transects along each of these washes, and collected leaf tissue from 188 *A. greggii* shrubs spread out along them. We also collected 10 to 14 seeds from each of 10 mothers along each transect (Figure 3-1). Because *A. greggii* produces polyads (composite pollen grains (Kenrick and Knox, 1982)), we collected only one seed per pod to decrease the probability of genotyping seeds likely fertilized by the same dispersal event. Inclusion of multiple seeds per pod decreases the estimate of effective pollen donors and increases the pollen structure between sampled mothers (Muona *et al.*, 1991). We measured map distance and elevation difference between all shrub-pairs. Additionally all shrub-pairs were defined as having occurred within the same (within), or separate wash (between). Map distance ranged from 16.5 to 2111 m between mothers, and 5 to 2764 m between all sampled adult shrubs. Elevational offset ranged from 0 to 62 m between maternal shrubs, and up to 102 m between all adults. The total study area was 4 km^2.

Marker Development

We developed microsatellite molecular markers for this species. We used a Roche 454 Genome Sequencer FLX instrument to generate a suite of potential microsatellites. We extracted DNA using a modified Cetyltrimethylammonium Bromide (CTAB) protocol (Cullings 1992, Doyle & Doyle 1987), with an added pre-wash step to remove secondary compounds (Li et al. 2007). We prepared our library using 500 ng of DNA according to the GS FLX+ Series – XL+ protocol (Roche Applied Sciences, Indianapolis, USA). Library fragment size distribution, quality, and quantity were assessed on an Agilent 2100 Bioanalyzer. We then processed the

library through the GS FLX emPCR protocol for small and then large volumes. We performed sequencing on a GS FLX+ picotiterplate, which yielded 43,895 sequences.

We identified and scanned potential microsatellite loci. Of the 43,895 sequences scanned, we found multiple motifs with appropriate flanking primers using MsatCOMMANDER (Faircloth, 2008), including 43 tetramer repeats, 43 trimer repeats, and 240 dimer repeats. We tested 66 markers (42 di and 24 tri) for PCR suitability and variation in 14 individuals collected across the Mojave National Preserve. We used Qiagen multiplex kits to amplify all loci (in five different primer mixes) using 2.5 µl DNA, 5 µl multiplex mix, 1.1 µl water, 0.4µl bovine serum albumin (BSA), and 1µl primer mix: mix 1 (Agreg04, Agreg18, Agreg24); mix 2 (Agreg20, Agreg28); mix 3 (Agreg48 Agreg49); mix 4 (Agreg52, Agreg54); and mix 5 (Agreg45). All forward primers included an M13F(-20) sequence (GTAAAACGACGGCCAG) on the 5' end to attach a fluorescent label and allow genotyping by size (Boutin-Ganache *et al.*, 2001). The reaction cycle consisted of an initial 15 minute denaturing at 95 °C, followed by 26 cycles at 94 °C for 30 seconds, then annealing at 53 °C for 90 second, and extension at 72 °C for 60 seconds. We ran the following 20 cycles at 94 °C for 30 seconds, 53 °C for 90 seconds, and 72 °C for 60 seconds (Schweizer *et al.*, 2009). We ran the final extension step at 60°C for 30 minutes. We did fragment analysis at the UCLA Genotyping and Sequencing Core and we made genotype calls in the lab using GeneMapper® (Applied Biosystems). We eliminated monomorphic markers. We used Microchecker (Van Oosterhout *et al.*, 2004) to check for effect of stutter, allelic dropout, and null alleles within 30 individuals sampled within the middle transect. Additionally, we tested for linkage disequilibrium using GenePop (Raymond and Rousset, 1995) to ensure independence of loci. We also identified the rate of mistyping by comparing the genotypes of seeds to those of their respective mothers.

Historic Gene Flow

To determine historic connectivity and scale of dispersal within the study area, we calculated genetic structure and spatial autocorrelation in adults using GenAlEx (Peakall and Smouse, 2006). We calculated genetic structure among the 188 adults using an AMOVA (Excoffier *et al.*, 1992) to determine the genetic difference between the two washes (F_{ST}), and calculated the inbreeding coefficient (F_{IS}). We performed spatial autocorrelation analysis by measuring the genetic similarity between all pairs of individuals, grouping the pairs into geographic distance classes, and reducing the average kinship to a correlation coefficient (r). We chose the sizes of distance classes for this analysis to maintain evenness in sample sizes between groups, while still displaying informative patterns. For each distance class, GenAlEx provides two measures of significant departure from relatedness being no greater or less than expected at random. The first method creates confidence intervals around the calculated r values using bootstrapping. The second measure uses a permutation method to create confidence intervals around the null hypothesis of $r = 0$, based on the assumption of no spatial structure.

Contemporary Mating System and Pollen Flow

To evaluate the mating system in *A. greggii*, we used the program MLTR v. 3.2 (Ritland, 2002), which employs a maximum likelihood method to estimate single (t_s) and multi-locus (t_m) outcrossing rates, selfing ($1 - t_m$), mating between relatives (biparental inbreeding, $t_m - t_s$), and the fraction of full-sib offspring of a give maternal parent, i.e. offspring that share the same father (correlated paternity rate, r_p). One can then use correlated paternity to estimate the average effective number of pollen donors to a shrub ($N_{ep} = r_p^{-1}$). The 95% confidence intervals for these

estimates were calculated with 1000 bootstrap values (Ritland, 2002). Iterations for the program began with the suggested initial outcrossing rate $t = 0.90$, parental inbreeding $F = 0.1$, and correlated paternity $r_p = 0.1$.

To determine the level of contemporary pollen connectivity and estimate the scale of dispersal, we performed a TWOGENER analysis of pollen pool structure (Smouse *et al.*, 2001), and a spatial autocorrelation analysis of pollen haplotypes using GenAlEx (Peakall and Smouse, 2006). The pollen pool analysis used a hierarchical AMOVA to determine the global value of structure among all mothers (ϕ_{FT}), among mothers within washes (ϕ_{FG}), and among washes (ϕ_{GT}). We used the final ϕ_{FT} value to derive the average number of pollen donors (N_{ep}). To determine spatial autocorrelation in pollen, we used the same procedure and distance classes as with adults. We identified pollen haplotypes using an adapted Smouse *et al.* (2001) approach where maternal alleles are removed from each seed leaving behind paternal contribution. To resolve ambiguous loci (where maternal and paternal alleles cannot be determined due to equal probability of assignment to either parent) we treated pollen haplotypes as genotypes. Both alleles of ambiguous loci were kept as heterozygotes, and assigned loci were converted to homozygotes. We compared pollen and adult spatial autocorrelation through the significance within each distance class, bootstrapped confidence intervals for *r* estimates (Cabin *et al.*, 1998; Kalisz *et al.*, 2001; Dutech and Sork, 2005; Gaddis *et al.*, 2014), and the distance at which spatial genetic autocorrelation is significantly negative, called the 'genetic neighborhood' (Clark and Richardson, 2002; Hazlitt *et al.*, 2004; Epperson, 2005), for each dataset.

To supplement the indirect estimates, we estimated pollen dispersal directly using a maximum-likelihood approach in CERVUS 3.0 (Kalinowski *et al.*, 2007), based on ten locus genotypes for seeds, mothers, and potential fathers. All adults genotyped within the two transects

(including mothers) were included as potential fathers (n = 188). Maternal genotypes were included as a known variable. To determine the confidence level of paternity analysis, we ran simulations with 500,000 repetitions, 1% of loci mistyped, and 188 potential fathers for each seed. We set 95% as the level of strict confidence, and 80% as a relaxed level of confidence. We estimated that we had genotyped 10% of the potential pollen donors based on floral survey data identifying the average proportion *of A. greggii* shrubs that flowered within the Mojave National Preserve (see Chapter 2). To determine if the detected paternity events are a function of the position of the seeds and potential fathers sampled, indicating randomly assigned fathers, we compared the expected frequency of events in each distance class (using only ascribed seeds) with the observed frequency of events in each distance class using a Kolmogorov-Smirnov test (Sokal and Rohlf, 1995; Bittencourt and Sebbenn, 2007; Bacles and Ennos, 2008; Albaladejo *et al.*, 2012).

Landscape Effect

To determine the influence of landscape on movement in *A. greggii*, we examined the influences of elevation and dry-wash on genetic relatedness in adults and pollen. First, we adapted the spatial autocorrelation analyses for both data sets to determine *r* values in elevational separation classes, and compare pairs from the same wash with those occurring in different washes. Second, we used AIC model selection to compare the effect of elevation, wash, and log-distance on genetic relatedness using the Lynch and Ritland-estimator (LRM) (Lynch and Ritland, 1999) calculated in GenAlEx (Peakall and Smouse, 2006)). Like other relatedness measures, LRM coefficients vary from 1 to -1. Full-siblings would have a coefficient of 0.5, half-siblings 0.25, first cousins 0.125, and unrelated individuals should have a coefficient of 0 on

average. Negative values indicate increasing dissimilarity. We were concerned that multicollinearity of these three independent variables (elevation, distance, wash) might lead to spurious identification of landscape effects, as some variables had variance inflation factors greater than 5 (Marquardt, 1970; Neter *et al.*, 1989; Kennedy, 1992; Hair Jr. *et al.*, 1995). To reduce the likelihood of a Type I error, we used a residual approach to detecting the effects of the two landscape variables (York, 2012). To do this we independently regressed wash and elevation variables with log-distance, to determine the residuals of each relationship. These residuals, which capture the variation in wash and elevation not related to log-distance, were then used in subsequent AIC model selection as potential independent variables. We ran all possible combinations of variables as generalized linear models and we identified the best combination using a step-wise AIC model selection. We then evaluated the improvement of the best model to a simple log-distance model using a Cox approach for comparing non-nested models (Cox, 1961, 1962).

RESULTS

Marker Development

Of the 66 loci tested in 30 individuals, four were indiscernible or did not amplify, 52 were monomorphic or had excessive genotyping issues, and ten were found to be variable and reliable markers for *A. greggii*, with consistent parent to offspring fidelity (Table 3-S1). None of our ten variable markers showed evidence for stuttering, allelic dropout, or null alleles. After Bonferroni correction, we found no indication for linkage between loci. We observed less than 1% overall mistyping rate. We found no incidence of duplicate genotypes among the 188 adults we surveyed.

Historic Gene Flow

Though significant, genetic structure between adults in the two washes was low (F_{ST} = 0.005, $P < 0.05$). There was significant positive spatial genetic autocorrelation within adults up to 200 m, becoming significantly negative at 1000 m (Figure 3-2). The *r* coefficient lowered in each distance class (r_{20} = 0.154 ($P < 0.001$), r_{100} = 0.068 ($P < 0.001$), r_{200} = 0.024 ($P < 0.001$), r_{500} = 0.007 ($P > 0.05$), r_{1000} = -0.008 ($P < 0.01$), r_{2000} = -0.011 ($P < 0.001$)).

Contemporary Mating System and Pollen Flow

Biparental inbreeding ($t_m - t_s$ = 0.045) and selfing ($1 - t_m$ = 0.023) were both significantly greater than zero (Table 3-1). There was significant, but very low, genetic structure between pollen pools in the two washes (ϕ_{GT} = 0.004, $P < 0.05$). Pollen structure among mothers was higher (ϕ_{FT} = 0.033, $P < 0.001$), but still indicative of widespread gene flow. Because the inbreeding estimate was low and insignificant in adults (F_{IS} = 0.016, $P > 0.20$), there was no

need to correct for its effect on pollen pool structure. The estimated number of pollen donors based on ϕ_{FT} (N_{ep} = 15) was comparable to MLTR (N_{ep} = 20.4). Spatial autocorrelation was weaker in pollen pools compared to adults (Figure 3-2). Pollen haplotypes were significantly autocorrelated up to 100 m, however, *r* values were lower in pollen than for adults in all significant distance classes (r_{20} = 0.029 ($P < 0.001$), r_{100} = 0.016 ($P < 0.01$), r_{200} = 0.002 ($P > 0.1$), r_{500} = -0.004 ($P > 0.05$), r_{1000} = 0.001 ($P > 0.10$), r_{2000} = -0.003 ($P < 0.01$)). The bootstrapped confidence intervals of adults and pollen in the first two distance classes did not overlap, and the 'genetic neighborhood' of pollen was larger (2000 m) than adults (1000 m).

The paternity analysis provides comparable results to the indirect approaches, showing a large proportion of both near neighbor pollination and long distance dispersal events. We were able to identify the father for 15 seeds at 95% confidence, and 47 seeds at 80% confidence (Figure 3-3). Between 0 and 5 seeds per maternal shrub were ascribed a father. In three maternal shrubs we saw multiple paternity by the same father, with six paternal parents siring more than one seed. The majority of identified fathers (88%) only sired a single seed within our maternal sample. Of the 220 seeds sampled, 5 (0.023%) were determined to be sired by self-fertilization events. Of the 47 seeds ascribed a father, the average and median dispersal distance was 750 and 433 m respectively (Figure 3-4), with a greater proportion pollinated by fathers occurring within the same wash (70%) than occurring in separate washes. Frequency of observed dispersal events differed significantly from that expected based on the sampling frequency (Kolmogorov-Smirnov: D = 0.5192, $P < 0.001$), with the largest differences in the lower distance classes (Figure 3-4).

Landscape Effect

Relatedness (LRM) between adults ranged from 0.5 to -0.254, with an average value of -0.001. Pollen relatedness ranged from 0.478 to -0.212, with an average of -0.002. Average LRM of pollen within a mother was 0.013, and -0.003 between mothers. Relatedness among adults was best explained by distance, separation by wash, and difference in elevation (Table 3-2). In contrast, pollen relatedness was explained by distance and wash. Both best-fit landscape models for pollen ($P < 0.001$) and adults ($P < 0.001$) significantly improved a log-distance only model.

DISCUSSION

Collectively, our findings indicate that both historic and contemporary gene flow are very high in the desert shrub *Acacia greggii*, ensuring excellent connectivity among individuals throughout the study area. Though significant, we found low genetic structure between the dry-washes sampled in both adults and pollen. Our results suggest pollen flow is more widespread than seed dispersal. Compared to adults, spatial autocorrelation values were lower in pollen with a greater genetic neighborhood. This pattern is also evident in the results of the direct parentage analysis, which show that most documented pollen dispersal events occur at distances less than 100 m, but nevertheless longer-distance pollen dispersal events at low frequency occur at all distances up to the maximum distance studied. Despite this movement, we found that landscape variables significantly explained a portion of the genetic variance in both adults and pollen. Both wash and elevation were correlated with relatedness among adults, indicating some directing influence of washes to dispersal, but with more restricted movement over an elevational gradient. Pollen relatedness was only marginally correlated within washes, indicating pollinators may restrict movement along dry-washes, but show no restriction along elevation.

Adult genetic pattern indicates a history of widespread dispersal. Though significant, there was almost no genetic structure between the two washes ($F_{ST} = 0.005$, $P < 0.05$). There was significant spatial autocorrelation in adults up to 200 m, with a genetic neighborhood size approximately 1 km. These results show extensive gene flow, and is comparable to other studies examining less dense species with wind pollination (Dutech and Sork, 2005). This dispersal pattern appears similar to that of the shrub *Haloxylon ammodendron* (C.A.Meyer) Bunge in the deserts of northern China, which exhibits similarly low differentiation between populations separated by small distances (Sheng *et al.*, 2005). This finding raises concern for those studies

that have observed much higher genetic structure in more rare or endangered species in a comparable range (Nickrent and Wiens, 1989; Neel and Ellstrand, 2003; Neel and Cummings, 2003; Qian *et al.*, 2008), suggesting a potential danger should habitat damage or climate change occur, and dispersal is required to maintain regional populations.

Pollen flow in *A. greggii* was extensive when examined under both indirect (Table 3-1, Figure 3-2) and direct (Figure 3-3 & 3-4) analysis. We are aware of only one previous study (Klein *et al.*, 2008) that has reported lower pollen pool structure among maternal shrubs in an animal pollinated species. Both spatial autocorrelation and paternity analysis indicate a large proportion of near neighbor pollen movement events, with significant spatial autocorrelation at 20 and 100 m (Figure 3-2), and the greatest frequency of dispersal events within the 100 m paternity analysis distance class (Figure 3-4). However, results suggest long distance dispersal events are frequent in this species. In addition to the low level of structure among pollen pools, the genetic neighborhood estimate from spatial autocorrelation was 2 km, and half of detected paternity analysis events were greater than 430 m. Even within the 15 paternity events described with 95% confidence, there are still 3 events above 1 km, demonstrating the long tailed dispersal in this species. Only a few studies (Biscaia de Lacerda *et al.*, 2008; Kamm *et al.*, 2009; Ahmed *et al.*, 2009) have reported a larger average dispersal (750 m) using paternity analysis.

The analysis of mating pattern indicates a large number of effective pollen donors, yet a relatively small rate of ongoing self-pollination. Both TWOGENER and MLTR analyses indicated a large number of effective pollinators per mother ($15 < N_{ep} < 20$), and few studies of animal pollinated shrubs have reported higher N_{ep} values (Muona *et al.*, 1991; Klein *et al.*, 2008), though similar values have been more common among wind-pollinated species (Finkeldy, 1995; El-Kassaby and Jaquish, 1996; Mitton *et al.*, 1997; Robledo-Arnuncio *et al.*, 2004; De-Lucas *et*

al., 2008; Pakkad *et al.*, 2008). Pollen donor diversity in *A. greggii* is likely driven by the high local density, providing a large pool of potential fathers. Despite this competition among fathers, selfing rate was significant (s = 0.023). Although selfing can increase reproductive assurance, it can also lead to inbreeding depression in long lived species (Morgan *et al.*, 1997). There may be a reproductive advantage conferred to *A. greggii* individuals that move into unpopulated areas and exploit ephemeral or unused resources. Self-fertilization would allow a single individual to colonize a new area without the need of a second arrival to enable reproduction. Additionally, a large number of effective pollen donors, if common in this species, would relax pressure on self-incompatibility and allow for only partial incompatibility (Ferrer *et al.*, 2009). Regardless, selfing and the incidence of biparental inbreeding in pollen (Table 3-1) was not apparent in the level of inbreeding in adults, which was found to be insignificant ($F_{IS} = 0.016$, $P > 0.20$).

In spite of long distance dispersal and selfing, there does appear to be an influence of landscape shaping adult and pollen dispersal patterns. For pollen, both the AIC (Table 3-2) and the spatial autocorrelation analysis (Figure 3-2) show significant effects of both distance and wash in describing genetic variance. Dry-washes may channel pollen dispersal, acting as corridors for pollinator movement, meanwhile creating a partial inter-wash barrier to gene flow, and explaining the between wash pollen pool structure (Table 3-1). Elevation did not improve the AIC model explaining pair-wise pollen relatedness. Conversely, all three landscape variables influenced genetic patterns in adults. It is likely washes act as dispersal pathways for seeds during rainfall events, which accounts for the stronger influence of wash seen in the AIC coefficient and *r* values in spatial autocorrelation (Figure 3-2, Table 3-2). We present two possible explanations for the significant elevation effect found in adults and not pollen. First, seeds that cross an elevation gradient, which has both temperature and hydrological implications,

may be at a disadvantage compared to locally dispersed seeds whose parents have passed on genes advantageous to a particular elevation. Second, our pollen results display contemporary patterns which may not have been present during the time of adult shrub establishment. We have found that *A. greggii* is dominantly pollinated by *Apis mellifera* (see Chapter 2), which was introduced in the region in the mid-90s. It may be that pollination was previously more influenced by elevation, and has only recently become more widely dispersed.

These results indicate pollen is likely driving the widespread gene flow we observe in *A. greggii* adults. Spatial autocorrelation values were lower in pollen than adults, indicating less restriction (Loiselle *et al.*, 1995; Hardesty *et al.*, 2005), with a larger 'genetic neighborhood' (Figure 3-2). In addition, pollen was less affected by landscape with lower autocorrelation coefficients for all variables (Figure 3-2), and only a tenth of the variance being explained by the best-fit AIC model (Table 3-2). Although inevitably reflecting difference in contemporary versus historic context, these data do suggest pollen is less restricted by distance and landscape than is seed dispersal (Kalisz *et al.*, 2001).

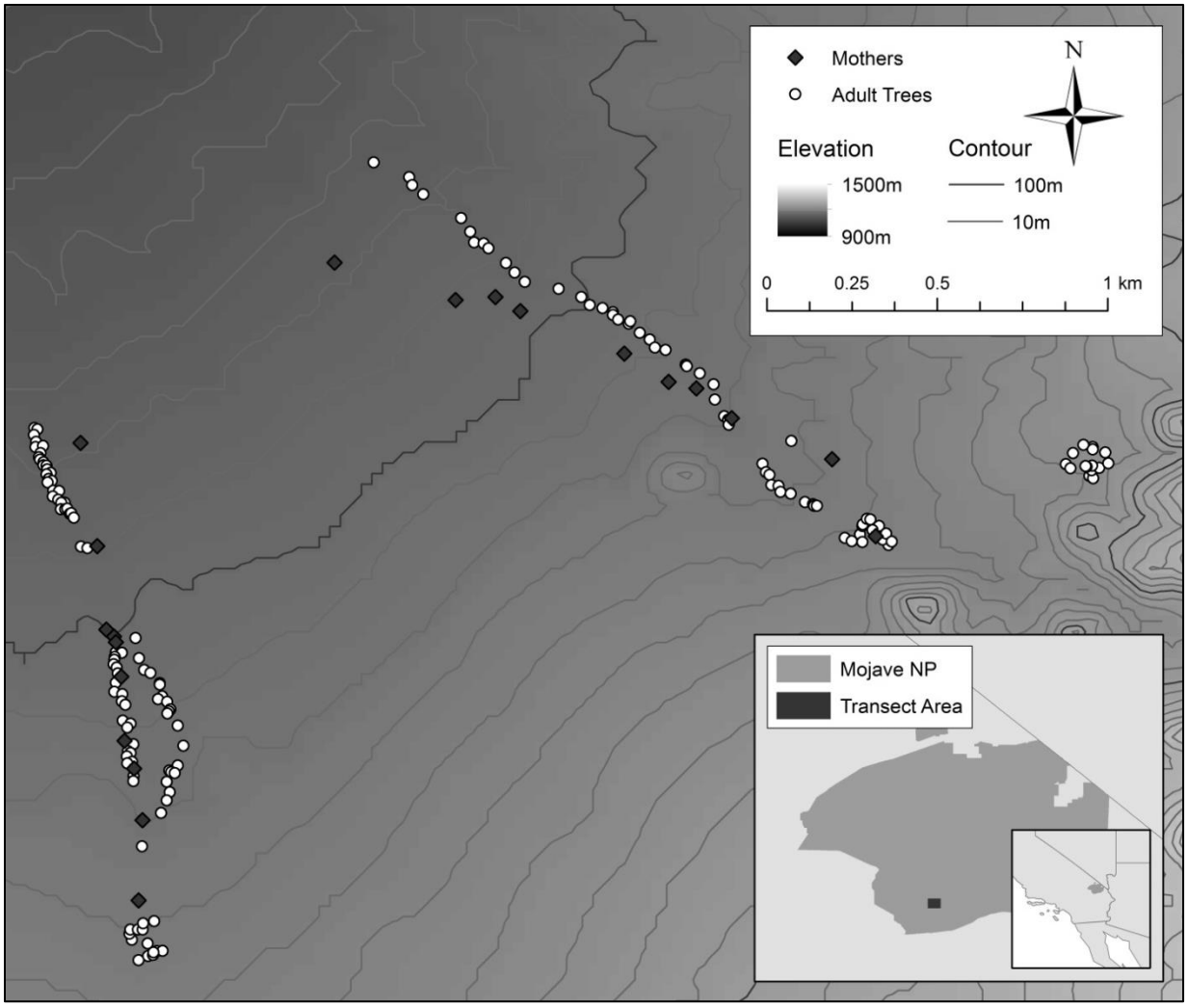

Figure 3-1: This map indicates the distribution of *A. greggii* shrubs (n = 188) sampled and mothers (n = 20) from which we collected seeds (n = 240).

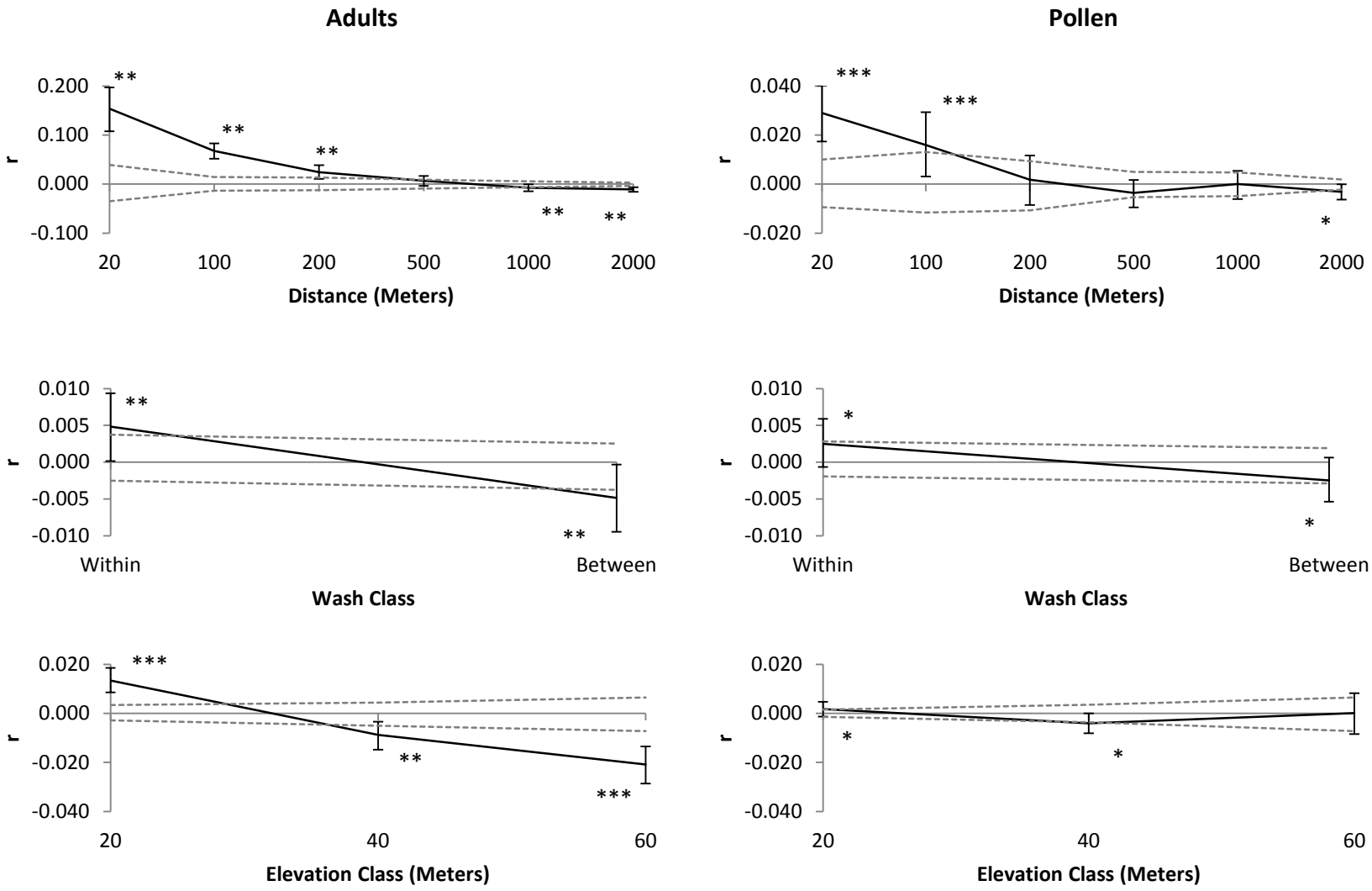

Figure 3-2: Spatial autocorrelation for adults (188 individuals) and pollen (240 seeds). The black line shows the autocorrelation coefficient value (*r*) at each class with the upper and lower 95% confidence intervals. The dashed lines represent the permuted 95% confidence interval from 999 simulations of *r* with no spatial structure. The asterisks (*) identify classes significantly different from 0.

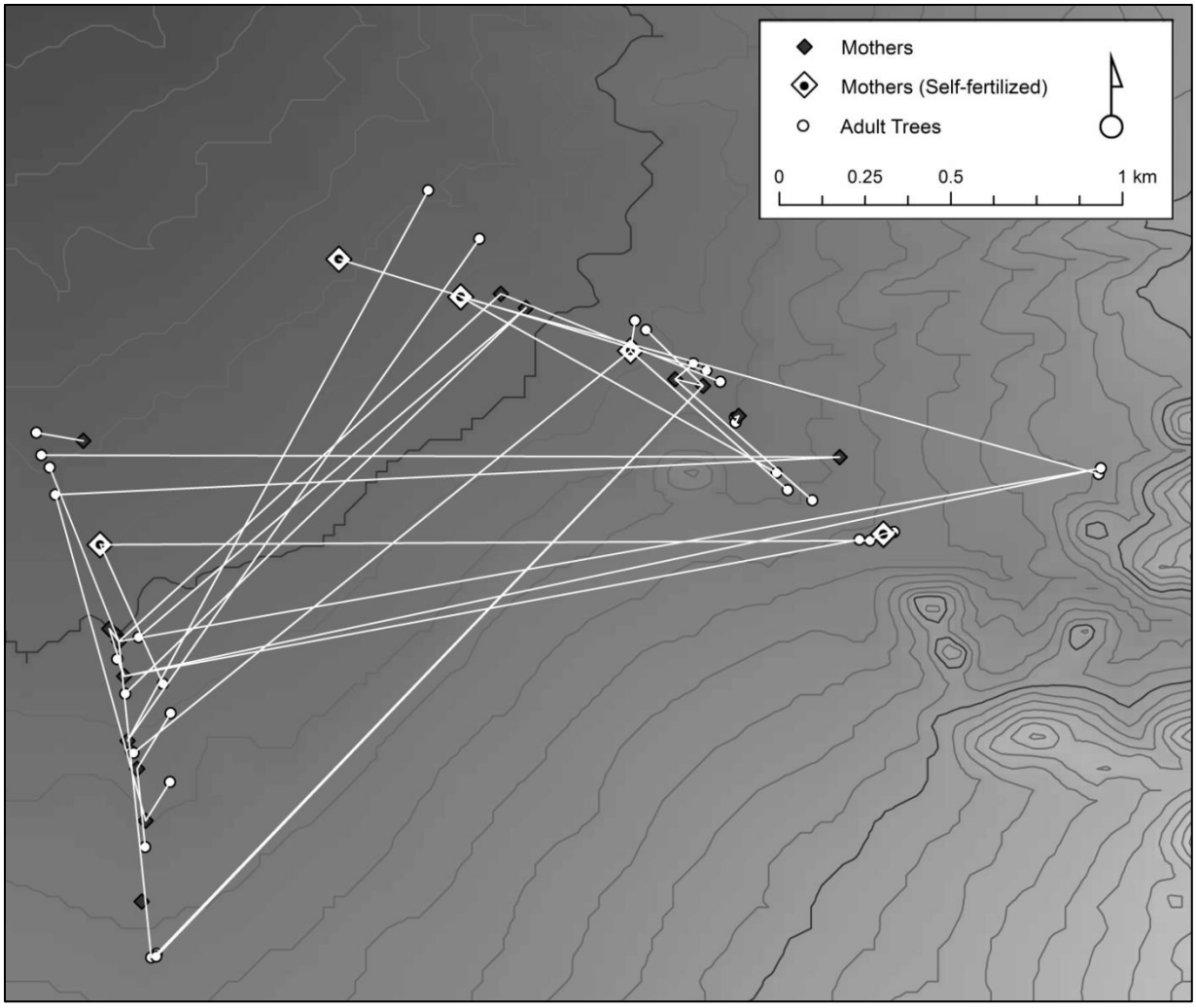

Figure 3-3: Map showing the 47 dispersal events (white lines) identified using paternity analysis. Five of these were self-fertilization events with 33 of the detected events occurring within a wash.

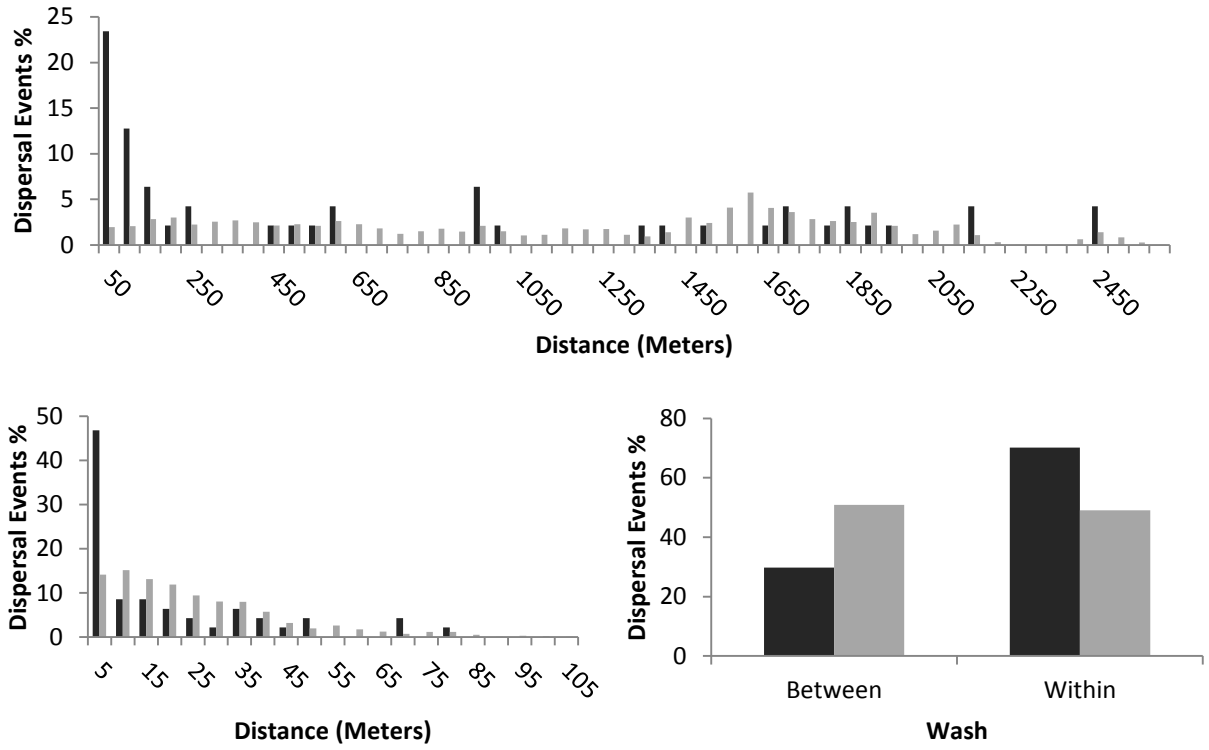

Figure 3-4: These graphs show the frequency of observed (black) and expected (gray) dispersal events based on paternity analysis within each of the independent landscape variable groups. Frequency of observed dispersal events differed significantly from that expected based on the sampling frequency (Kolmogorov-Smirnov: D = 0.5192, $P < 0.001$).

Table 3-1: Indirect dispersal estimates using TWOGENER and MLTR. Standard errors are within parentheses, and asterisks indicate significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Parameter	Estimate
TWOGENER	
Pollen pool structure:	
Among mothers ($\phi_{FT)}$	0.033***
Among mothers within washes (ϕ_{FG})	0.028***
Among washes	0.004*
Effective number of pollen donors (N_{ep})	15.2
MLTR	
Multi-locus outcrossing rate (r)	0.977 (0.008)
Multi-locus selfing rate (1 - t_m)	0.023 (0.008)
Average Single locus outcrossing rate (t_s)	0.932 (0.017)
Biparental inbreeding ($t_m - t_s$)	0.045 (0.017)
Effective number of pollen donors ($N_{ep} = r_p^{-1}$)	20.4 (4.78)

Table 3-2: These are the best-fit AIC model selection results indicating the relationship between pairwise relatedness (Lynch and Ritland-estimator) with landscape variables. Values below each variable represent the coefficient within the relationship. Asterisks indicate significance (˙P < 0.1, *P < 0.05, **P < 0.01, ***P < 0.001). The R^2 values are for the entire model, with accompanying asterisk indicating model significance. Each model was compared to a simple log-distance model using a Cox-test to verify significant improvement.

	Intercept	Log(Distance)	Wash	Elevation	R^2	AIC	Cox-test
Adults	0.014**	-0.003*	-0.009***	-0.002**	0.021***	-38679	Yes***
Pollen	0.009**	-0.001**	-0.001˙		0.002***	-63567	Yes***

Table 3-S1: These are the *Acacia greggii* marker characteristics across two washes. All samples (n = 188) were treated as the same population.

Marker	Forward primer 5'–3'	Reverse primer 5'–3'	Motif	A^1	$H_O{}^2$	$H_E{}^3$	$F_{IS}{}^4$	Size	Mistyping[5]
Agreg04	GTTGGGTACCAGGCATTAGC	ACGCTAAATATGTGCCTCACAAG	(GT)n	4	0.41	0.42	0.01	356-362	0%
Agreg18	GGATGCCCTCACCCAGAAG	CCCTTCTTCGTCCCATCAAG	(GT)n	2	0.51	0.46	-0.10	282-286	0%
Agreg20	TGGTTTAGGCATGGAACAGG	CGGTCCGATCGTCATACTAGC	(TA)n	10	0.73	0.74	0.01	172-204	1%
Agreg24	TGCCTGCAGAAACACCTTG	GCTTCTTCTAATGGCGACCAC	(GT)n	10	0.79	0.84	0.06	350-368	0%
Agreg28	ACTCCCAATATCGAGCAGGG	GGATCACTTGTGGAGTAGGC	(GT)n	10	0.79	0.81	0.02	222-241	1%
Agreg45	TGGCTTCCACATGGCTTTC	AGAGATGTTGGCCACCGAG	(TTC)n	6	0.69	0.71	0.03	239-254	1%
Agreg48	TCAGGTTTGAACATGCGGTG	AGCTGATCAACACCGGGAC	(TTC)n	6	0.62	0.56	-0.12	321-336	0%
Agreg49	TGCTTCCTCTTCCAAGCCC	TCTCCTGCACAGAAGTCGC	(CTT)n	3	0.40	0.41	0.01	204-216	0%
Agreg52	GGTTTCAGTGTCTGGGATCG	GGCAAACATTGGACCACTTC	(CTT)n	4	0.33	0.32	-0.03	278-293	0%
Agreg54	TCATCGGCGACGTTAACCC	TTGCACGCAAACTGCCTTG	(TTC)n	3	0.37	0.43	0.14	207-216	0%

1. Number of alleles (n = 188).
2. Observed heterozygosity (n = 188).
3. Expected heterozygosity (n = 188).
4. Inbreeding coefficient (n = 188).
5. Based on parent to offspring match in 20 mothers and 240 offspring.

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CHAPTER 4:

Impact of dry-wash pathways on gene flow and genetic diversity in the desert species *Acacia (Senegalia) greggii* A. Gray

ABSTRACT

Both the intrinsic dispersal ability and the extrinsic impact of landscape shape the movement of desert plant species. Many species exhibit patchy spatial distributions within a harsh intervening habitat matrix, but long distance dispersal may allow gene flow among patches. We propose here that desert dry-washes serve as dispersal pathways for the desert shrub, *Acacia (Senegalia) greggii*, capable of moving seeds long distances during heavy seasonal rainfall, promoting regional connectivity, and reducing genetic structure. To test the influence of dry-washes on landscape genetic patterns, we genotyped 345 individuals evenly across 23 one-hectare study sites in the Mojave National Preserve using ten microsatellite molecular markers. To determine if watersheds structure genetic patterns at the regional scale, we performed a hierarchical AMOVA, but found no significant differentiation between watersheds regionally ($F_{RT} = 0.00$, $P > 0.10$), and very low genetic structure among all populations ($F_{ST} = 0.03$, $P < 0.001$). To inspect inter-population patterns, we examined the relationship between genetic distance and four geographic distance measures using a Mantel test, and found that distance along dry-washes best explained genetic variance ($r = 0.47$, $P < 0.05$) when compared to Euclidean distance ($P > 0.05$), and two alternative least-cost path distances calculated by slope surface ($P > 0.05$) and habitat suitability ($P > 0.05$). Last, we examined genetic diversity (H_E and H_O) and relatedness among individuals within a site (LRM), and found they were best explained by upstream watershed area and slope at a site, and not by local population density or habitat suitability. This finding indicates that areas with greater water-flow due to higher slope, and a larger source of upstream seed donors have a greater amount of immigration. This study demonstrates that dry-washes are a key landscape feature that impacts the dispersal ability of a patchy desert plant species, and serves as a mechanism for regional connectivity.

INTRODUCTION

Demographic and genetic connectivity are important for the health and long-term viability of many populations of plant species. In plants, dispersal allows a species to escape unfavorable environments and colonize new habitats (Howe & Smallwood 1982; Travis 2003; Aitken *et al.* 2008; Kremer *et al.* 2012), homogenize genetic variation across populations (Slatkin 1985), increase genetic diversity locally (Loveless & Hamrick 1984), and lower the probability of inbreeding depression (Charlesworth & Charlesworth 1987). Dispersal is shaped both by the intrinsic traits of the organism, and features of the local landscape. Recent advances in the field of landscape genetics have increased our ability to identify explicit ecological, geomorphic, and climate effects on gene movement and genetic diversity (Manel *et al.* 2003; Storfer *et al.* 2007; Sork & Waits 2010; Holderegger *et al.* 2010). This emerging field has highlighted the importance of identifying which specific landscape factors are responsible in understanding plant dispersal patterns.

Desert environments present contrasting expectations for dispersal. Limited resources in desert environments, particularly water shortage, cause local, patchy distributions in many plant species which, along with a harsh intervening matrix, might lead one to expect limited connectivity among populations (Loveless & Hamrick 1984; Sork *et al.* 1999; Manel *et al.* 2003; Ghazoul 2005). On the other hand, this spatial arrangement could force animal dispersal agents to travel a greater distance for resources (Levin & Kerster 1969; Ellstrand 1992; Nattero *et al.* 2011), or open up the landscape, leading to greater frequency of long-distance dispersal events (Dick *et al.* 2003; Sork *et al.* 2005; Slavov *et al.* 2009; Shohami & Nathan 2014). Most measurements of gene flow in desert plant species appear to support the former assumption, namely high genetic divergence among populations that increases with the size of the study area

(Figure 4-1). Desert shrubs and trees show lower genetic structure relative to forbs or grasses, likely due to longer life span and overlap in generations, which reduce genetic divergence (Loveless & Hamrick 1984; Hamrick & Godt 1996). Regardless, even in long-lived species, measures of the amount of spatial genetic structure are generally greater than 0.1 at all sampling scales. Two key exceptions in this review are the studies of *Haloxylon ammodendron* (C.A.Meyer) Bunge (Amaranthaceae), a wind-pollinated shrub in China (Sheng *et al.* 2005), and *Washingtonia filifera* (Lindl.) H.Wendl. (Arecaceae), an insect pollinated shrub in the Sonoran desert (McClenaghan Jr & Beauchamp 1986). Both species have seeds that are putatively animal-dispersed and occur in patchy populations, but exhibit little genetic structure among sample sites. These patterns have been explained as evidence of selection, fragmentation of previously continuous habitat, but also long distance dispersal. It is not clear if these two studies represent exceptions to a rule or suggest a broader potential for connectivity in desert plants.

Dispersal pathways (or corridors) in desert landscapes may provide a mechanism for long distance dispersal. Using new tools in landscape genetics and geographic information systems (GIS), it is possible to assess the extent to which these pathways shape gene flow. Classically, dispersal has been examined as a simple relationship between genetic and Euclidean distance, known as isolation by distance or IBD (Wright 1943; Slatkin 1993; Rousset 1997). A landscape genetic approach would test whether ecological, geomorphic, and climate variables affect gene movement by replacing Euclidean distance with 'least-cost path' distance between populations (Adriaensen *et al.* 2003). A least-cost pathway between locations is determined by creating a cost surface, where the landscape is divided into pixels, each with a number value higher if an area is expected to be more permeable and lower if an area is expected to be more obstructive. Alternative pathways are then compared by summing the value of all pixels in the cost surface

that are crossed from one point to another. The least-cost path is the route that results in the lowest accumulated cost (e.g. Coulon et al. 2004; Broquet et al. 2006; Epps et al. 2007; Wang et al. 2009). Examples of surfaces used for plant and animal species include slope gradients (Spear *et al.* 2005; Cushman *et al.* 2006) or habitat suitability derived from ecological niche modeling (Trénel *et al.* 2008; Wang & Summers 2010). Another alternative is to measures distance along an explicitly defined landscape feature hypothesized to direct gene flow. For instance, several studies of animal species have used elevation models to determine pathways of major streams in a study region, and then compare the relationship between genetic and geographic distance along those streams (Vignieri 2005; Spear *et al.* 2005; Lowe *et al.* 2006; Richards-Zawacki 2009). To test the influence of landscape on gene flow, one can then compare the fit of genetic divergence among alternative distance measures to IBD (Arnaud 2003; Vignieri 2005; Spear *et al.* 2005; Richards-Zawacki 2009). Though effective in animal dispersal studies, this method has been little applied to plant species (Holderegger *et al.* 2010).

Variation in gene flow can also be inferred through the distribution of genetic diversity across the landscape. Local genetic diversity is shaped by population density (Wright 1969; Lande 1988), and habitat suitability (Collevatti *et al.* 2011; Ortego *et al.* 2012; Dubey *et al.* 2012; Wan *et al.* 2014). The greatest influence, however, is driven by the influx of new genetic variants through gene flow (Loveless & Hamrick 1984). It is therefore possible to examine whether specific local environmental variables are associated with higher immigration rates and local genetic diversity, to test explicit hypotheses of landscape influence on gene flow (Vellend 2004; Johansson *et al.* 2005; Gaddis *et al.* 2014; Thompson *et al.* 2014).

The Southwest shrub species *Acacia (Senegalia) greggii* A. Gray is an appropriate study system in which to examine landscape influence on gene flow in desert habitats. Like many other

desert shrub species, *A. greggii* has a patchy distribution that is predominantly restricted to dry-wash habitats. It produces seeds with thick seed coats that require abrasion or damage for germination when water is available (Went 1948; Coe & Coe 1987; Gutterman 1994; Fox *et al.* 2012). Increased rainfall during seasonal wet periods may provide a means of long distance seed dispersal in this species, while simultaneously causing scarification of the seed through sand abrasion, increasing the likelihood of germination. Therefore, we hypothesize that dry-washes serve as a dispersal corridor for this species. Although this means of dispersal has been suggested in the literature for numerous species (Went 1948; Bullock 1976; Fllner & Shmida 1981; Zedler 1981; McArthur 1989; Gutterman 1994), no studies have yet tested if such a pattern is observed in natural populations. Alternatively, animal dispersal agents might be deterred by travelling along the slope of a dry-wash pathway, suggesting gene flow along other pathways that avoid steep elevational change. In addition, the distribution of this species is restricted to mid-elevation habitat (Munz 1974; Weber 1981), suggesting a well-defined climatic niche that may restrict gene flow.

We examined gene flow along dry-washes in the desert shrub *A. greggii* in the Mojave National Preserve. We used ten microsatellite loci to study the genetic variation and structure of 345 adults collected evenly across 23 one-hectare sites (Figure 4-2) within four watersheds. We had three specific objectives: (1) To examine regional effects, we determined the genetic structure among watersheds and study sites using a hierarchical AMOVA. If dispersal along dry-washes affects regional patterns we would expect significant genetic structure among the four watersheds in the study area. (2) Next, we examined inter-population patterns by comparing the ability of four distance measures to explain pair-wise population genetic distance within watersheds. We measured (i) distance between populations along dry-washes, and compared its

explanatory ability to (ii) Euclidean distance and two other least-cost path distances based on likely constraining forces: (iii) slope and (iv) habitat suitability. (3) Last, we tested the contribution of slope, upstream watershed area, local adult density, and habitat suitability to genetic diversity and relatedness within the 23 study sites. If seeds are moved downstream during increased water-flow, then we would expect both slope and upstream watershed area to be correlated with increased local diversity and lower relatedness amongst individuals. Sites with steeper slopes should have stronger currents running through them, and sites with larger upstream watersheds would have both stronger water flow and a larger seed source, and thus both would have a greater diversity of locally deposited seeds. Gene flow by pollen may produce a similar association between genetic diversity and upstream watershed area, as downstream sites would lie at the midpoint between numerous populations, however, we would not expect a similar relationship due to pollen flow patterns. Because flowering time in this species is associated with elevation, we might expect sites with higher slope to have decreased genetic diversity due to lower gene flow driven by phenological separation. Regardless of movement driven by pollen or seed flow, we would expect sites in lower habitat suitability areas to have lower diversity, due to a decreased number of migrants capable of establishing in the area. Additionally, we would expect sites with greater density to harbor greater genetic diversity due to lower influence of genetic drift and founder effects (Wright 1969; Lande 1988).

METHODS

Study Species

Acacia (Senegalia) greggii A. Gray (Fabaceae) is a common desert shrub species, occurring from the western Mojave Desert in California to the Sonoran and Chihuahua Deserts in Northern Mexico. It forms yellow flowers in a cylindrical spike in the late spring to mid-summer, sometimes with a second flowering in early fall of wet years. In the Mojave, the shrub is pollinated by a community of bee, wasp, and butterfly species that is dominated by *Apis mellifera* L., an introduced bee species into the area (see Chapter 2). It sets seeds in bean-like pods in late summer. Putative animal dispersers include rodents, birds, and ungulate grazers. Zedler (1981) showed a surge in *A. greggii* seedlings in a previously unpopulated area following a rainstorm. Upon later inspection, it was noted that this newly established population lay downstream from a site with high adult density, suggesting that this species disperses seeds by water transport.

Study Area

The study was conducted in the Mojave National Preserve in Eastern California (Figure 4-1), which is an arid region that receives less than 200 mm of rainfall annually. The landscape is topographically diverse, with several mountain ranges and lowland sand dunes separating study sites. Vegetation throughout the area is dominated by creosote (*Larrea tridentata* (DC.) Coville (Zygophyllaceae)), but also contains higher elevation pine forests and Joshua tree (*Yucca brevifolia* Engelm. (Asparagaceae)) woodlands.

Sampling

We sampled leaf tissue from 15 shrubs at each of 23-one hectare sampling plots across the Mojave National Preserve (n = 345) (Figure 4-2). We preserved tissue in silica gel upon collection for later DNA extraction. Distances between sites varied from 0.4 to 73 km, and sites ranged in elevation from 300 to 1500 m. The total study area spanned 2,700 km^2. Density of *A. greggii* at a site ranged from 15 to 143 individuals per hectare.

Laboratory Methods

We extracted DNA using a modified Cetyltrimethylammonium Bromide (CTAB) protocol (Cullings 1992, Doyle & Doyle 1987), with an added pre-wash step to remove secondary compounds (Li et al. 2007). We used the Qiagen multiplex kit to amplify all loci using 2.5 µl DNA, 5 µl multiplex mix, 1.1 µl water, 0.4µl bovine serum albumin (BSA), and 1µl primer mix (see Chapter 3). All forward primers included an M13F(-20) sequence (GTAAAACGACGGCCAG) on the 5' end to attach a fluorescent label and allow genotyping by size (Boutin-Ganache *et al.* 2001). We performed fragment analysis at the UCLA Genotyping and Sequencing Core and we made genotype calls in the lab using GeneMapper® (Applied Biosystems). We tested genotypes for departure from Hardy–Weinberg equilibrium using a X^2 test in the program GenAlEx 6.5 (Peakall & Smouse 2006). Additionally, we tested for linkage disequilibrium using GenePop (Raymond & Rousset 1995) to ensure independence of loci. Sequential Bonferroni corrections were applied to account for multiple comparisons (Rice 1989).

Landscape Variables

We determined the shortest distance between all sites within dry-washes. We generated dry-wash watercourse pathways for the study area using the 'Hydrology' tool in the Spatial Analyst Toolbox in ArcGIS 10.1, referencing a Digital Elevation Model (DEM) for the area (100 m^2 resolution). This tool uses the topography within the DEM to determine the direction and accumulation of water flow within each pixel. From this surface, we defined all pixels with greater than 100 flow accumulation value (greater than 100 pixels draining into it), as part of the dry-wash pathway. We converted the dry-wash pathway into a network, and measured the shortest distance between all sites following the dry-wash within the same watershed using the Network Analyst Toolbox in ArcGIS 10.1. The final distance between each site along the dry-wash is refereed to here as 'dry-wash distance.'

We used ecological niche modeling to generate a habitat suitability layer for *A. greggii* throughout its range. To model suitability based on climate, we used a maximum entropy algorithm, MAXENT 3.3.3 (Phillips *et al.* 2004, 2006; Elith *et al.* 2011). This method examines the correspondence between species occurrence records and randomly sampled pixels (called background points) with climate variables. We then derive habitat suitability of a given pixel by comparing the likelihood of presence based on species occurrence records relative to the likelihood of occurrence based on background points. For this study we used 10,000 randomly selected pixels from the study area as background points. Species occurrence records were gathered from herbarium databases (Consortium of California Herbaria, http://ucjeps.berkeley.edu/consortium/; Southwest Environmental Information Network, http://swbiodiversity.org/seinet/; and the Global Biodiversity Information Facility, http://www.gbif.org/). We assessed the quality of occurrence records, removing those gathered

prior to 1950, with poor spatial resolution, or obvious geo-referencing issues, leaving 1332 final records. We mapped climate using 19 bioclimatic layers at 30-arc-second spatial resolution (~ 1 km^2) (Hijmans *et al.* 2005). The program randomly selected thirty percent of species occurrence records as a data validation sample to evaluate model performance under 10 replicate runs. We evaluated the performance of the species distribution model using the area under the receiving operator characteristics curve (AUC), which is the likelihood of the final model correctly assigning randomly sampled points as actual presence records or background points. AUC ranges from 0.5 (model performs as well as a randomly generated prediction) to 1 (maximum prediction). The final MAXENT output creates a grid raster layer indicating habitat suitability ranging from 0 (low) to 1 (high).

We calculated least-cost distances between sites based on two cost surfaces using the 'Distance' tools in the Spatial Analyst Toolbox in ArcGIS 10.1. The first cost surface we used was the MAXENT habitat suitability output, deriving a pathway that avoided areas of low habitat suitability. We refer to the least-cost distance between sites we derived from this surface as 'habitat suitability distance.' The second cost surface we created using a digital elevation model for the study area, and defined each pixel as the degree of slope in that area. The pathway we produced from this surface avoided areas of steep incline. We refer to the least-cost distance derived from this surface as 'slope distance.'

For all study sites we determined average slope and habitat suitability values for the one-hectare area. We extracted slope and habitat suitability from our raster cost surfaces. Additionally, we measured the area of upstream watershed, which is defined as the total area that drains into the dry-wash from a given study site upwards. We calculated upstream watershed

area from the stream pathway layer using the 'Hydrology' tool in the Spatial Analyst Toolbox in ArcGIS 10.1.

Analysis

To determine connectivity among sites and watersheds, we examined the partitioning of genetic variance among groups using an AMOVA (Excoffier *et al.* 1992). We first performed a hierarchical AMOVA using the software GenAlEx 6.5 (Peakall & Smouse 2006), to estimate the genetic structure among the four watersheds (F_{RT}) and among sites within the watersheds (F_{SR}). Additionally, we performed a standard AMOVA to determine global and pair-wise genetic structure among all sites (F_{ST}).

We compared the ability of 1) Euclidean, 2) dry-wash, 3) slope, and 4) habitat suitability distances to explain the variance in genetic distance, measured as F_{ST}. We used a Mantel test (Mantel 1967), to independently examine the correlation between the four geographic distance variables with genetic distance We report the correlation coefficient, equivalent to a Pearson product-moment correlation (r), and a P-value (P) calculated through a permutation test using 100,000 randomizations in the R package 'ncf.'

We examined the ability of four landscape variables to determine genetic diversity and relatedness at a site. We measured the observed heterozygosity (H_O), expected heterozygosity (H_E), and average relatedness at each site as indicators of genetic diversity using GenAlEx 6.5 (Peakall & Smouse 2006). We measured average relatedness using the Lynch and Ritland's measure (LRM), which has the greatest power relative to other relatedness measures when examining multiple polymorphic markers (Vekemans & Hardy 2004). The four landscape variables considered were density, watershed area, slope, and habitat suitability. We log transformed slope and watershed area for normality. We examined all combinations of these four

variables to explain genetic diversity and relatedness, comparing model-fit using AIC scores. We report the *P*-value and R^2 of each model when run as a simple linear regression.

RESULTS

None of the ten loci showed evidence of linkage disequilibrium after Bonferroni correction. Additionally, all loci and sites showed no evidence of significant departure from Hardy-Weinberg equilibrium (HWE), with the exception of Agreg52, which showed deviation from HWE at two sites where all individuals were homozygous for a single genotype, except for a single individual homozygous for an alternative genotype. We identified seven shrubs, distributed among six sites, as clones. We excluded these plants from subsequent analysis, leaving us with an *n* of 338.

The habitat suitability surface was consistent with contemporary range estimates for *A. greggii* (Little Jr 1971). The analysis showed a good fit of the modeled surface, with an AUC score for the test data of 0.971 (SD = 0.005, *n*= 10 replicated runs) on average. Model AUC was significantly greater than that of a random (AUC = 0.5) prediction (one-tailed Wilcoxon signed rank test; $P < 0.01$). Seven climatic variables had an importance greater than 5%: (measured as the percent drop in training AUC when the variable was excluded) bio5 (28.7%), bio12 (12.1%), bio9 (11.0%), bio6 (9.2%), bio11 (7.3%), bio19 (6.6%), and bio4 (6.4%).

We calculated all pairwise distances between sites. Site 15 occurred at a location where confidently ascribing a watershed and calculating dry-wash distance to other sites was difficult. To remove ambiguity, we removed site 15 from the hierarchical AMOVA and Mantel test, but included the site in the calculation of total F_{ST} among all sites, and within site analysis. Least-cost pathways differed greatly from direct Euclidean lines between sites (Figure 4-3). The largest Euclidean distance between sites was 73 km, but the largest distances using the habitat suitability distance, slope distance, and dry-wash distance were 112 km, 328 km, and 77 km, respectively.

We did not find significant regional genetic structure among watersheds (Table 4-1). However, there was both significant genetic structure among sites within regions (F_{SR} = 0.031, P < 0.001), and globally among all sites (F_{ST} = 0.031, P < 0.001).

We could best explain pairwise genetic distance by distance along a dry-wash (Table 4-2, Figure 4-4). Of the four distance measures examined, distance along dry-wash was the only one to have a significant relationship with genetic distance (r = 0.47, P < 0.05), although both slope and habitat suitability distances had higher r values than Euclidean distance. None of the three non-Euclidean distance values measured across all populations were significantly related to pairwise genetic distance (Table 4-2).

The best-fit models explaining the variation in three diversity measures all included slope as an explanatory variable, and additionally included the upstream watershed area for models explaining heterozygosity and relatedness (Table 4-3). None of the best-fit models included either local density or habitat suitability. All best-fit models were significant, with the greatest amount of variation explained in the relatedness (LRM) model (R^2 = 0.37, P < 0.01).

DISCUSSION

Our findings support the hypothesis that dry-wash pathways promote movement in *Acacia greggii.* Pairwise genetic distance between populations was best explained by distance along dry-washes rather than Euclidean distance or least-cost path distance calculated by slope or habitat suitability (Table 4-2, Figure 4-4). The slope at a site and the upstream watershed area best explained variation in genetic diversity and relatedness at a site (Table 4-3). Overall, there was little regional genetic structure indicating a history of widespread dispersal in this species.

Regional Analysis

The regional analysis showed a surprising lack of genetic structure among the four watersheds within the 2,600 km^2 study region (Table 4-1). These finding suggests that adults within the four watersheds are derived from the same ancestral population. Many of the watersheds have origins within the same mountain chains, which raises the possibility that gene flow emanated out of watershed headways. If the subsequent subpopulations maintain a large effective population sizes due to high dispersal rates, then the signature of the ancestral population would not disappear regardless of whether or not the separate watersheds are experiencing ongoing gene flow from the headway.

Acacia greggii has very little genetic structure compared to other desert plant species (Figure 4-1). Previous studies of desert shrubs or trees have found F_{ST} values near 0.22 on average. Here we report a global F_{ST} value of 0.03, which is lower than all but two of 34 species in our review, one of a desert forb in a 3 km^2 area (Archibald *et al.* 2001), and the other of *Haloxylon ammodendron,* mentioned previously (Sheng *et al.* 2005). Although *Haloxylon ammodendron* is also a long-lived shrub species, it is dissimilar to *A. greggii*, producing very

small seeds and relying on wind for pollination. Thus, prior to examining landscape influence on movement patterns, *A. greggii* appears to be uniquely genetically homogenous as a desert shrub among those studied.

Inter-population

Inferred gene movement along dry-washes best explained the variance in genetic distance between sites. Of the four measures of geographic distance examined, only dry-wash distance was significantly related to pairwise F_{ST} ($P < 0.05$, Table 4-3, Figure 4-4). Although this result supports the hypothesis that water flow along dry-washes assists in the dispersal of seeds, it does not allow us to comment on the relative contribution of pollen or seed flow in the observed genetic pattern. Regardless the pattern does indicate that either abiotic or biotic dispersal agents of this species are channeled along dry-washes. This result is similar to several animal studies demonstrating that riparian habitats act as dispersal corridors in complex landscapes (Spear *et al.* 2005; Lowe *et al.* 2006; Richards-Zawacki 2009).

This study illustrates the utility of landscape distance measures to describe gene flow in plants (McRae 2006; Trénel *et al.* 2008; Holderegger *et al.* 2010). Similar methods may be commonly avoided in other plant studies due to the complexity of plant gene flow in which two separate dispersal agents (seeds and pollen) contribute to the final regional pattern (Holderegger *et al.* 2010). We have shown here that regardless of this complexity, dominant movement patterns exist, and can be detected. In addition, the correlation coefficients for both habitat suitability and slope models were higher than the Euclidean model, with lower *P*-values (Table 4-3), indicating an improvement in explanatory ability. These results demonstrate the inability of simple Euclidean distance to explain variation in genetic patterns in a complex landscape.

Advances in landscape modeling have opened the door for explicit hypothesis testing that is amenable to both plant and animal species.

Within Population

Patterns of genetic diversity and relatedness within local populations provide strong evidence in support of the hypothesis that dry-washes promote gene flow. Relatedness was lower and diversity was higher in sites with greater upstream area and higher slope (although upstream area was not included in the best-fit observed heterozygosity model) (Table 4-4). The larger upstream watershed area creates a greater source from which downstream areas draw seeds, or serves as a mid-point for pollen flow. Areas with a smaller upstream area have a smaller population from which to draw, and thus lower local diversity. Sites with a higher slope will have faster water flow during wet periods, capable of carrying a greater load, and thus depositing a greater abundance and assortment of seeds. This association appears to be driven by seed dispersal, as we would have expected no relationship or for diversity at a site to be lower in high sloped sites due to decreased gene flow from phenological separation.

Conclusions

This study suggests a dispersal pattern and mechanism to explain a pattern of regional connectivity in a patchy desert plant species. *Acacia greggii* has a generally restricted distribution that would predict relatively high genetic divergence between populations, but instead we found unexpected low genetic structure. The observed relationship between dry-wash pathway distance with genetic distance, and water flow variables with genetic diversity, support the hypothesis that water flow disperses seeds downstream during infrequent rainfall events.

Pollen flow may also contribute to this pattern, but the influence of slope on genetic diversity and relatedness support a significant seed dispersal effect. Thus, our findings support the idea proposed by numerous ecological studies suggesting water flow as a mechanism of widespread dispersal in some desert plants, enhancing regional connectivity (Went 1948; Bullock 1976; Fllner & Shmida 1981; Zedler 1981; McArthur 1989; Gutterman 1994).

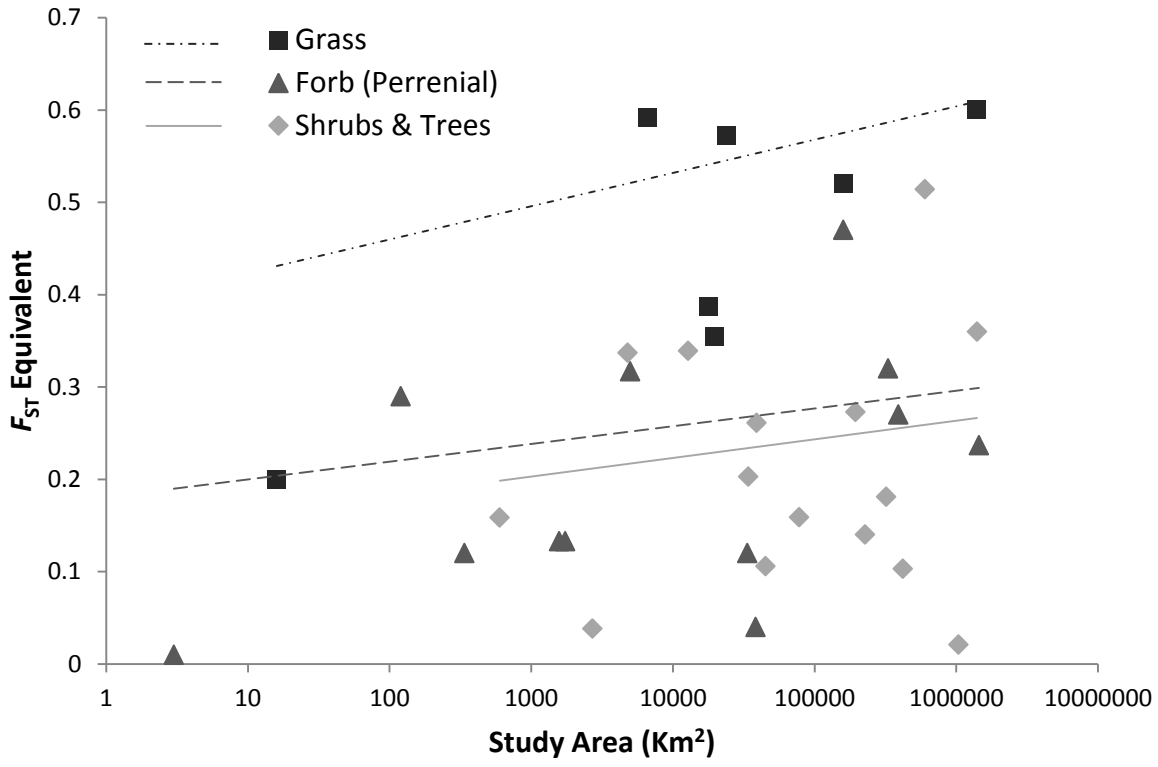

Figure 4-1: Relationship of global genetic structure among 34 plant species populations in desert ecosystems with total study area based on a review of previous literature. Genetic structure is measured as global F_{ST} or an equivalent measure (i.e. R_{ST}, Θ_{ST}, G_{ST}). Relationships are separated into basic plant groups: grasses, forbs, and shrubs and trees. (McClenaghan Jr & Beauchamp 1986; Nickrent & Wiens 1989; Wolff *et al.* 1997; Hickerson & Wolf 1998; Comes & Abbott 1999; Archibald *et al.* 2001; Neel & Ellstrand 2003; Navarro-Quezada *et al.* 2003; Knaus *et al.* 2005; Clark-Tapia *et al.* 2005; Sheng *et al.* 2005; Walker & Metcalf 2008; Qian *et al.* 2008; Chen *et al.* 2009b; a; Garrick *et al.* 2009; Rebernig *et al.* 2010; Haque *et al.* 2010; Albrecht *et al.* 2010; Volis *et al.* 2010; Wesche *et al.* 2011; Richardson & Meyer 2012; Laport *et al.* 2012; Migliore *et al.* 2013; Lowry *et al.* 2013; Milla-Tapia *et al.* 2013; Clarke *et al.* 2013; Hamasha *et al.* 2013)

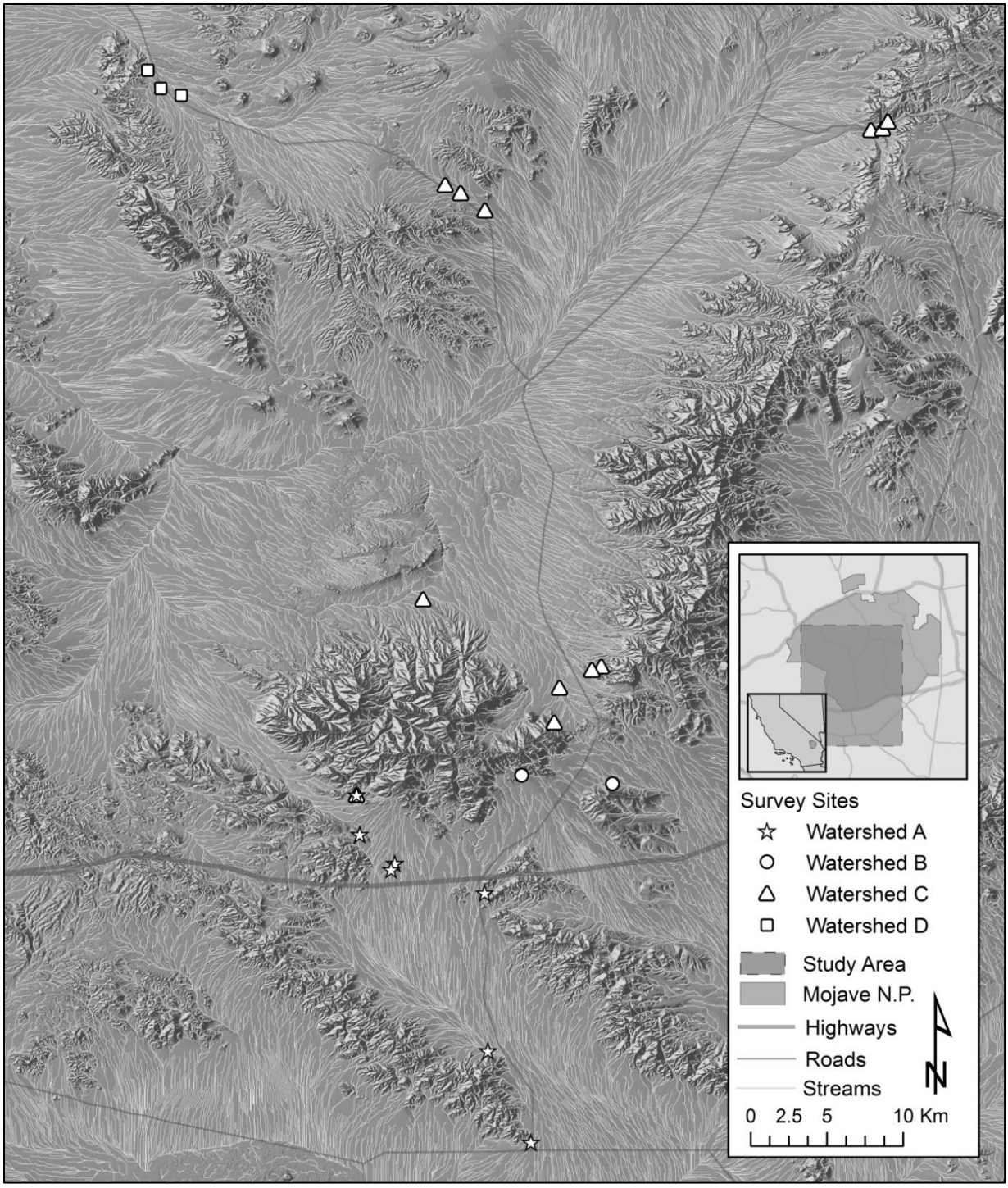

Figure 4-2: Map of one-hectare sample sites symbol coded by drainage basin. Major waterways are shown as white lines. We sampled leaf tissue from 15 individuals in each of the 23 samples sites. One site (site 15) was ascribed to two watersheds.

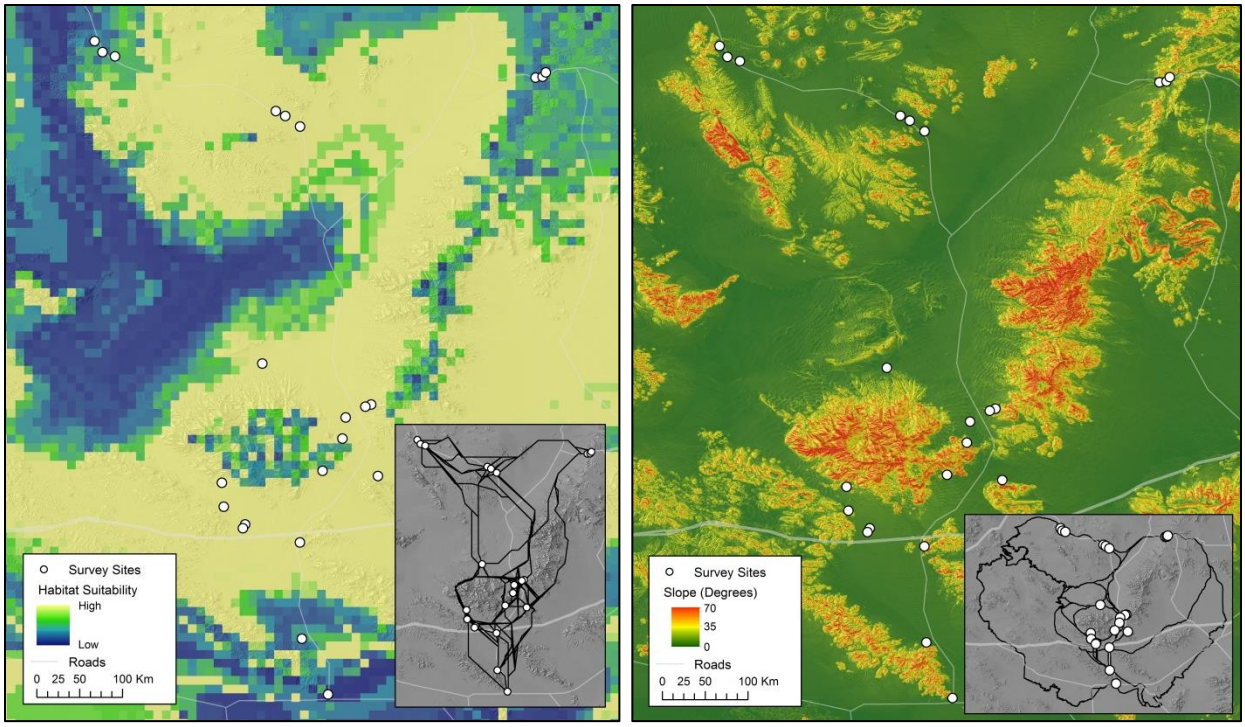

Figure 4-3: Maps of weighted surfaces and derived least-cost paths between sites based on habitat suitability (left) and slope distance (right). Inset maps show least-cost path distances between sites. The habitat suitability surface was modeled using 19 climate layers (Hijmans *et al.* 2005) and 1332 distribution records for *A. greggii* throughout the Southwest with a maximum entropy algorithm, MAXENT 3.3.3 (Phillips *et al.* 2004, 2006; Elith *et al.* 2011). The slope surface was calculated using a DEM for the area.

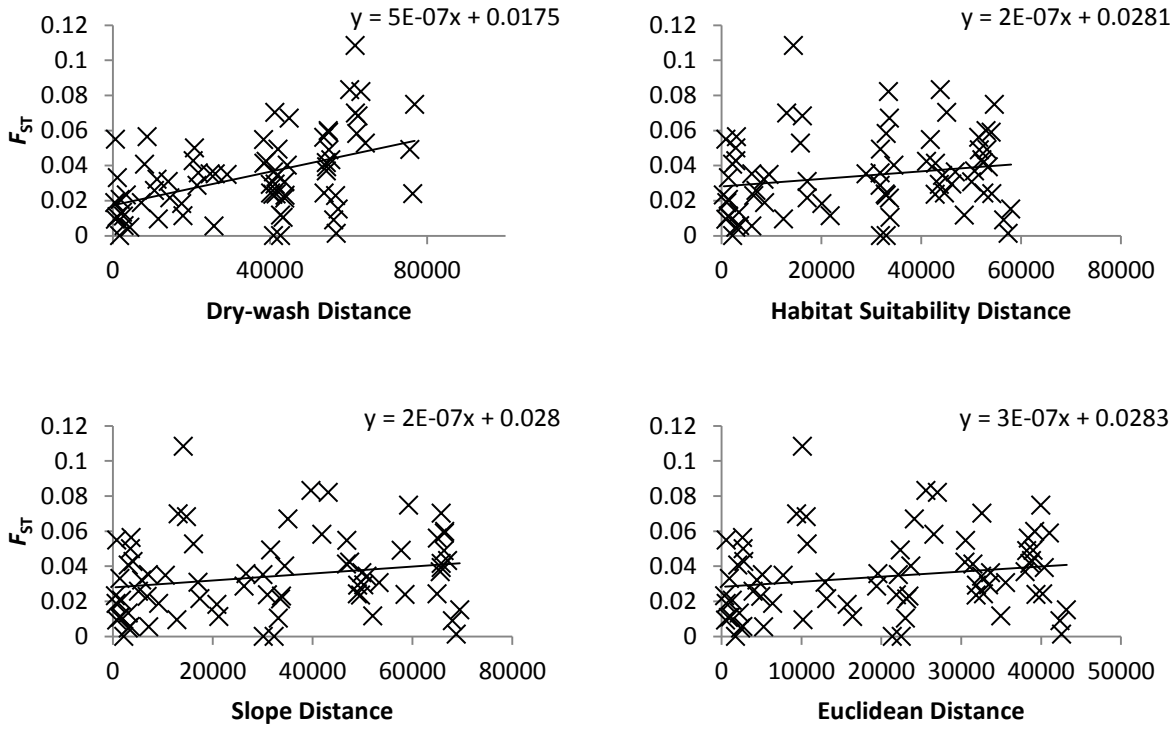

Figure 4-4: Relationship between genetic distance and four spatial distance measures. Euclidean distance is the direct map distance between locations. Dry-wash distance is the physical distance between sites measured following the path of a dry-wash. The other two distances are measured as least-cost path distances based on a slope and habitat suitability surface. All distance measures are in meters. Only within watershed comparisons are shown.

Table 4-1: Hierarchical AMOVA examining the relationship among watersheds and sampling sites of *A. greggii* in the Mojave National Preserve.

Source	*df*	MS	Est. Var.	Percent of Variance	Value	*P*
Among regions (F_{RT})	3	5.271	0.014	0%	0.000	0.529
Among populations w/in regions (F_{SR})	18	5.326	0.072	3%	0.031	0.001
Among individuals	301	2.765	0.038	1%		
Within individuals	323	2.709	2.704	96%		
Among all populations (F_{ST})					0.031	0.001

Table 4-2: Correlation between four geographic distance measures with genetic distance (F_{ST}) between sites. We report the correlation coefficient of the relationship (*r*), determined by the Mantel test, and *P*-value (*P*) determined through 100,000 randomizations. Asterisks (*) indicate significance at $P \leq 0.05$. The top table (a) compares all sites, and the bottom table (b) looks at only comparisons between sites within a watershed.

a. All Data	*r*	*P*
Slope Distance	-0.0291	0.4152
Habitat Suitability Distance	-0.0174	0.4372
Euclidean Distance	-0.0093	0.4713

b. Only within watershed	*r*	*P*
Dry-wash Distance	0.4736	0.0269*
Slope Distance	0.2111	0.1728
Habitat Suitability Distance	0.1934	0.1828
Euclidean Distance	0.1893	0.2138

Table 4-3: Results of AIC model selection to identify site level landscape effects on genetic diversity. Relatedness was measured using the Lynch and Ritland's measure (LRM), and genetic diversity was measured using both observed (H_O) and expected heterozygosity (H_E). Watershed area is the total area from which upstream dry-washes draw drainage. Slope, density, and habitat suitability were measured within the one-hectare sample sites. We report the significance (P) and fit of the linear model (R^2), as well as the AIC score (AIC) and improvement from next best model (Delta). Models are ordered according to AIC fit, with best models (lowest AIC score) at the top.

a. Relatedness (LRM)	P	R^2	AIC	Delta
Log(Watershed Area) + Log(Slope)	0.003905	0.3682	-89.283	1.96
Log(Watershed Area) + Density + Log(Slope)	0.01269	0.3361	-87.323	0.023
Log(Watershed Area) + Suitability + Log(Slope)	0.0128	0.3355	-87.3	0.42
Log(Watershed Area)	0.006337	0.2714	-86.88	1.412
Log(Watershed Area) + Density	0.02051	0.2542	-85.468	0.114
Log(Watershed Area) + Density + Suitability + Log(Slope)	0.03205	0.3002	-85.354	0.057
Log(Watershed Area) + Suitability	0.02209	0.2487	-85.297	1.635
Log(Watershed Area) + Density + Suitability	0.05187	0.2216	-83.662	4.028
Density	0.3209	0.001532	-79.634	0.931
Suitability	0.6931	-0.03969	-78.703	0.147
Log(Slope)	0.875	-0.04636	-78.556	0.45
Density + Suitability	0.5036	-0.02708	-78.106	0.472
Density + Log(Slope)	0.6184	-0.04838	-77.634	0.756
Suitability + Log(Slope)	0.859	-0.08341	-76.878	0.661
Density + Suitability + Log(Slope)	0.6985	-0.07595	-76.217	

b. H_O	P	R^2	AIC	Delta
Log(Slope)	0.02673	0.1753	-74.024	0.111
Suitability + Log(Slope)	0.0402	0.2023	-73.913	1.29
Density + Log(Slope)	0.07043	0.1563	-72.623	0.368
Density + Suitability + Log(Slope)	0.08777	0.1727	-72.255	0.18
Log(Watershed Area) + Log(Slope)	0.08939	0.136	-72.075	0.161
Log(Watershed Area) + Suitability + Log(Slope)	0.09962	0.1604	-71.914	1.071

a. Relatedness (LRM)	*P*	R^2	AIC	Delta
Log(Watershed Area) + Density + Log(Slope)	0.1476	0.1204	-70.843	0.539
Log(Watershed Area) + Density + Suitability + Log(Slope)	0.1705	0.1286	-70.304	0.951
Log(Watershed Area)	0.3891	-0.01041	-69.353	0.754
Density	0.7926	-0.04409	-68.599	0.078
Suitability	0.9964	-0.04762	-68.521	1.055
Log(Watershed Area) + Suitability	0.663	-0.05572	-67.466	0.093
Log(Watershed Area) + Density	0.6904	-0.06	-67.373	0.77
Density + Suitability	0.9648	-0.09607	-66.603	1.135
Log(Watershed Area) + Density + Suitability	0.8489	-0.1112	-65.468	

c. H_E				
Log(Watershed Area) + Log(Slope)	0.01876	0.2609	-99.666	1.437
Log(Watershed Area) + Density + Log(Slope)	0.04171	0.2408	-98.229	0.544
Log(Watershed Area) + Suitability + Log(Slope)	0.0513	0.2226	-97.685	1.455
Log(Watershed Area) + Density + Suitability + Log(Slope)	0.09181	0.1986	-96.23	0.959
Log(Slope)	0.1174	0.07041	-95.271	1.368
Log(Watershed Area)	0.2671	0.01345	-93.903	0.41
Suitability + Log(Slope)	0.2748	0.03331	-93.493	0.22
Density + Log(Slope)	0.3023	0.02401	-93.273	0.607
Suitability	0.7203	-0.04109	-92.666	0.014
Log(Watershed Area) + Suitability	0.396	-0.00269	-92.652	0.023
Density	0.7573	-0.04275	-92.629	0.702
Log(Watershed Area) + Density	0.5429	-0.03482	-91.927	0.419
Density + Suitability + Log(Slope)	0.47	-0.01691	-91.508	0.78
Density + Suitability	0.9144	-0.0902	-90.728	0.063
Log(Watershed Area) + Density + Suitability	0.6116	-0.05489	-90.665	

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