Botanical Journal of the Linnean Society, 2022, **XX**, 1–15. With 6 figures.

Short-distance gene flow and morphological divergence in *Eschscholzia parishii* (Papaveraceae): implications for speciation in desert winter annuals

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Received 10 August 2021; revised 10 December 2021; accepted for publication 2 February 2022

Winter annuals comprise a large fraction of warm-desert plant species, but the drivers of their diversity are little understood. One factor that has generally been overlooked is the lack of obvious means of long-distance seed dispersal in many desert-annual lineages, which could lead to genetic differentiation at small spatial scales and, ultimately, to speciation and narrow endemism. If our gene-flow hypothesis is correct, individual winter-annual species should have populations with genetic spatial structures implying short distances of gene flow. To test this idea, we sampled six populations of *Eschscholzia parishii* (Papaveraceae) in three pairs of watersheds within a 28-km radius in southern California. We quantified genetic diversity and structure and inferred the distance of gene flow in these populations using single nucleotide polymorphisms derived from genotyping-by-sequencing. Estimated distances of gene flow were quite small (σ = 10.4–14.9 m), with strong genetic structure observed within and between populations. Kinship declined steeply with ln distance (r^2 = 0.85). Petal size and shape differed significantly between the northernmost and southernmost populations. These findings support the hypothesis that the high diversity of warm-desert winter annuals might result, in part, from genetic differentiation within species at small spatial scales driven by poor seed dispersal.

ADDITIONAL KEYWORDS: dispersal – diversification – genotyping-by-sequencing – morphometics – population genetics – spatial genetic structure.

INTRODUCTION

The diverse North American desert flora exhibits a wide range of adaptations to water shortage. Desert plants mostly use one of four strategies for coping with low, unpredictable levels of soil moisture and high potential rates of evaporation, involving drought resistance, endurance, avoidance or escape (Solbrig & Orians, 1977). Perennial plants can *resist* drought by having succulent photosynthetic tissues (e.g. cacti, *Agave* L.), storing water and experiencing relatively little water stress; *endure* drought by reducing water loss by having thick leaves, low stomatal conductance, C_4 or CAM photosynthesis, and/or highly reflective (or densely hairy) foliage; or *avoid* drought by holding deciduous leaves or living as phreatophytes (deep-rooted plants that tap shallow groundwater near washes or springs). Desert annuals *escape* drought by growing and reproducing quickly after rains, and then dying after setting seeds (Ehleringer, 1985; Smith, Monson & Anderson, 1997; Angert *et al.*, 2009).

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In the Mojave and Sonoran Deserts, roughly half of all native vascular species are annuals, of which the great majority bloom after winter rains (Venable, Pake & Caprio, 1993; André, 2014). These desert winter annuals are remarkably diverse in both species and lineages. In California, deserts occupy < 25% of the land area, but are home to 39% of all native vascular plant species (Baldwin *et al.*, 2012, 2021; André, 2014), including 975 winter annuals and 68 summer annuals. Desert winter annuals comprise 18% of the extraordinarily rich vascular flora of California and 39% of the native desert flora (Table 1). In Anza Borrego State Park in the Colorado Desert, the westernmost outpost of the Sonoran Desert in the USA, winter annuals comprise 57.7% of the native flora, and summer or biseasonal annuals another 6.7% (Table 1). Of the native desert angiosperms in San Bernardino County (California), 541 species and subspecies of desert winter annuals occur in 186 genera, 50 families and 19 orders (André & Roberts, 2022). Five genera (*Phacelia* Juss., *Gilia* Ruiz & Pav., *Eriogonum* Michx., *Cryptantha* Lehm. ex G.Don, *Mentzelia* Plum. ex. L) account for 22% of the total; 98 genera contain only one winter annual each. Desert winter annuals have clearly arisen many times independently and account for a substantial fraction of plant diversity in the south-western USA.

The ecological and evolutionary drivers of winter annual diversity probably include (1) the open nature of deserts, (2) the concentration of rain during the winter months in many deserts, (3) adaptation to variable environmental conditions, (4) reliance on animal pollinators, (5) short generation time, (6) patchy, low-frequency germination and (7) seed dispersal over short distances. These factors are outlined below:

Open nature of deserts: Coverage of perennials in deserts is generally low due to seasonal or multi-year droughts, operating alone or combined with herbivory (McAuliffe, 1988; Hastings & Turner, 1995; Turner *et al.*, 2003; Bowers, Turner & Burgess, 2004). Desert annuals can thus diversify as they occupy (during and immediately after a rainy season) the large areas left open among long-lived perennials, which otherwise have competitive advantages in leaf height and rooting depth.

Winter rainfall: In many deserts, especially those on the western edge of continents, rainfall is concentrated in winter due to the presence of cold water offshore (MacArthur, 1972). Winter rainfall in deserts should favour the evolution of winter annuals, just as summer rainfall would favour the rise of summer annuals. Indeed, in North American hot deserts, the ratio of winter to summer/biseasonal annuals declines eastward from the Mojave and Colorado Deserts with winter rainfall driven by the cold California current, through the eastern Sonoran Desert with biseasonal rains, to the Chihuahuan Desert with summer rainfall driven by monsoons off the warm Gulf of Mexico (Shreve, 1951; Ludwig, Cunningham & Whitson, 1988; Smith *et al.*, 1997). Both winter and summer annuals are far less common and diverse in the cold deserts of the Great Basin (Venable *et al.*, 1993; Smith *et al.*, 1997), which we believe reflects the lower intensity of drought at lower temperatures and the consequent ability of perennials to dominate more of the landscape.

Resource partitioning in time and space: Annual diversification should also be driven by adaptation to and partitioning of differences in moisture availability across years and sites. Winter annuals differ substantially in their inherent water-use

Table 1. Numbers and proportions of species (including subspecies and varieties in California) of desert winter and summer annuals in selected floras of US warm deserts. For simplicity, the few species that germinate in response to cool- and warm-season rains have been lumped into the summer annuals.

Flora*	Desert winter annuals		Desert summer annuals		Total species
	No. of species	Proportion	No. of species	Proportion	
California					
CA state flora[1,2]	975	14.2%	68	1.0%	6578
CA desert flora[1,2]	975	39.3%	68	2.3%	2484
Colorado Desert (Anza Borrego State Park)[4]	138	57.7%	16	6.7%	239
Arizona					
Sonoran Desert – South Mountain[5]	148	42.7%	31	11.3%	274
Sonoran Desert – Tucson Mountains[5]	264	27.9%	100	17.0%	578

*References: [1]Baldwin *et al.* (2021); [2]J. André (unpubl. data); [3]André (2014); [4]T. Chester (unpubl. data), for vegetation below 1000 ft elevation; [5]Venable *et al.* (1993).

efficiency (WUE, g CO_2 fixed g^{-1} water transpired), which should be inversely related to stomatal conductance, and thus inversely related to relative growth rate (RGR, g plant tissue g^{-1} plant tissue day^{-1}) (Angert *et al.*, 2009; Gremer *et al.*, 2013). Species with high RGR (and, presumably, high seed output) and low WUE should and do germinate in wet years, whereas those with low RGR and high WUE germinate in most years, allowing all to coexist based on between-year differences in rainfall, growth and storage in the seed bank (Angert *et al.*, 2009; Gremer *et al.*, 2013). Differences in the timing and extent of germination in desert winter annuals may reflect *predictive germination* (responses to thermal or moisture cues correlated with future conditions favourable to a species) and/or *bet hedging* (reduced germination of seeds with multi-year dormancy in response to greater stochasticity in rainfall, lower mortality in the seed bank, lower dispersal and greater adult density dependence) (MacArthur, 1972; Venable & Lawlor, 1980; Gremer & Venable, 2014). Desert annual species can differ systematically in their germination response to moisture, temperature and light (Clauss & Venable, 2000). In addition, winter-annual species can differ in their distributions along the shrub-opening gradient (Keeley & Johnson, 1977; Tielbörger & Kadmon, 1997).

Animal pollination: Species diversification of desert winter annuals is probably driven to some extent by their reliance on animal (mostly insect) pollinators. Despite the open, often windy nature of deserts, wind-pollinated annuals or perennials comprise only a small component of the species richness in desert communities (Regal, 1982). This is presumably due in part to the high diversity of many desert communities (Solbrig & Orians, 1977; Ehleringer, 1985), and therefore the low density of most species, and in part to spatiotemporal variations in flowering within species, such that random wind movement is unlikely to place pollen on conspecific stigmas. Plant speciation in deserts based on pollinator specialization is thus a possibility, especially given that deserts are especially rich in bee species (Moldenke, 1979) and that bees are prone to specialize on host plants. Several large genera of desert winter annuals (e.g. *Phacelia*, *Cryptantha*, *Camissonia* Link, *Sphaeralcea* A.St.-Hil., *Mentzelia*) are each associated with specialist bee species (Moldenke, 1979). Across growth forms, insect pollinators are likely to travel very short distances, so that zoophily should result in shorter distances of pollen-mediated gene flow than wind pollination (Friedman & Barrett, 2009). Whereas wind pollination and self-pollination are rare among desert winter annuals in California, they are rather common in desert summer annuals, occurring in 14 of 35 genera containing such plants. This might reflect the abundance of anemophily in lineages that have evolved C_4 photosynthetic pathways adapted to high temperatures [e.g. Aizoaceae, Amaranthaceae (including Chenopodiaceae), Poaceae] and, perhaps not independently, that rely on wind or self-pollination when conditions are so hot (Branch & Sage, 2018) that animal pollinators might be deterred. Based on comparisons of 16 pairs of sister lineages in angiosperms, shifts from wind to animal pollination are accompanied by a 5.7-fold increase in species number (Kay *et al.*, 2006). Presumably, this is due to the greater specificity of mating and reproductive isolation involving animal pollinators (Schiestl & Schlüter, 2009) and divergent selection for adaptive radiation in pollinators (Stebbins, 1974; Givnish, 2010).

Life history and short generation times: Herbaceous lineages possess four times as many species as their woody sisters across angiosperms (Ricklefs & Renner, 1994; Dodd, Silvertown & Chase, 1999). This difference probably reflects the ability of smaller plants to partition the environment at smaller spatial scales (Ricklefs & Renner, 1994). In this respect, annuals are an extreme version of herbs. Other things being equal, the short generation time of annuals should allow them to evolve, diverge and ultimately speciate more rapidly than perennials.

Patchy rainfall and saltational speciation: Repeated drought and patchily distributed rainfall can create repeated population bottlenecks and extrinsic mating barriers, accelerating genetic differentiation at small spatial scales. These processes, when combined with selfing, have been proposed to lead to saltational (or catastrophic) speciation (Lewis, 1962; Raven & Axelrod, 1978) in which chromosomal rearrangements or unique ecological variants are rapidly fixed in a few generations, presumably during a population crash, and leading to more-or-less immediate reproductive and ecological isolation. Drastic fluctuations in rainfall and population sizes of annual plants are characteristics of summer-dry, Mediterranean and desert climates in California. The origin of winter-wet/summer-dry conditions there 5–15 Mya (Vermeij, 1989; Jacobs, Haney & Louie, 2004) may have helped drive the explosive speciation of annuals in the California Floristic Province and deserts, where they comprise 26% of all native vascular plants (Raven & Axelrod, 1978), and constitute most or all taxa in such highly diverse lineages as the tarweed alliance (Madiinae), *Clarkia* Pursh, *Collinsia* Nutt., *Downingia* Torr., *Gilia*, *Lasthenia* Cass, *Lupinus* L., *Mentzelia*, *Mimulus* L., *Nama* L., *Nemophila* Nutt. ex W.P.C.Barton, *Phacelia* and *Streptanthus* Nutt. Analysis of these winter annual lineages is needed to see whether they show higher rates of diversification than their perennial sister groups.

Seed dispersal and gene flow over short distances: Most desert annuals have small seeds unspecialized

for long-distance transport (i.e. they lack fleshy fruits, winged or plumed seeds, or adhesive propagules), and they include several genera (e.g. *Atrichoseris* A.Gray, *Baileya* Harv. & A.Gray ex Torr., *Chaenactis* DC.) in families (e.g. Asteraceae Bercth. & J.Presl) that often have such traits. Desert plants in general usually lack adaptations for long-distance transport (Fllner & Shmida, 1981; Venable *et al.*, 2008; Gremer & Venable, 2014). Fllner & Shmida (1981) argued that this lack of telechory may reflect the cost of dispersal structures and the fact that, although the amount and timing of rainfall should have an overwhelming impact on plant growth and fitness in deserts, the spatial scale of rainfall events and the intervening areas without rain is so large that no plausible increase in dispersal distance would get seeds into more favourable areas. In deserts, it may be better to use dormancy to await favourable rains than it is to risk dispersal to them in a given year (Venable & Lawlor, 1980; Venable *et al.*, 2008). Small seeds, being less attractive to seed predators, should experience low rates of post-dispersal predation and so be more likely to evolve multi-year dormancy (Thompson, 1987). Desert annuals generally produce small seeds with multi-year seed dormancy; the lower their dispersal distance, the greater should be selection for such dormancy (Venable & Lawlor, 1980; Levin, Cohen & Hastings, 1984).

Limited seed dispersal, other things being equal, should lead to genetic differentiation within species at small spatial scales and, ultimately, speciation (Givnish, 2010). Indeed, herbaceous plants with small seeds that lack adaptations for long-distance dispersal often show genetic differentiation within species at the smallest spatial scales (Vekemans & Hardy, 2004). We propose that such limited dispersal should be especially true of desert winter annuals, leading to genetic differentiation at small spatial scales and high rates of speciation (hereafter, the 'gene-flow hypothesis'). The processes favouring high rates of speciation in herbs should be especially strong in desert annuals, because they have short generation times, are almost all animal-pollinated and often lack adaptations for long-distance seed dispersal. Limited seed dispersal could also facilitate local differentiation processes associated with saltational speciation. Venable *et al.* (2008) argued that limited seed dispersal should also promote local species coexistence via the storage effect.

Venable *et al.* (2008) used direct observations in a 1-year study to show that two Sonoran Desert winter annuals had mean seed dispersal distances of 0.4–0.7 m. However, over the long term, much greater distances of seed dispersal might occur in such species via sheet (overground) flow after rainstorms or aerial dispersal during windstorms (Reichman, 1984). Alternative approaches to characterizing plant dispersal over longer periods, e.g. by studying the spatial genetic structure of populations (Vekemans & Hardy, 2004; Theim, Shirk & Givnish, 2014), should thus be used to quantify the long-term spatial scale of gene flow and test for substantial genetic divergence between populations in different watersheds that might arise from rare sheet flow events.

To test the gene-flow hypothesis, here we examine the population genetic structure and apparent spatial scales of gene flow in Parish's poppy, *Eschscholzia parishii* Greene (Papaveraceae), a winter-annual endemic to the Mojave, Colorado and Sonoran Deserts and surrounding areas. *Eschscholzia parishii* is an insect-pollinated diploid ($2n = 12$) that disperses its dry, finely sculptured seeds short distances from its capsular fruits through explosive dehiscence (Clark & Jernstedt, 1978). The seeds can float, based on their sculptured ridges capturing air bubbles with water tension (Clark & Jernstedt, 1978), so longer-distance dispersal might occasionally occur within watersheds after rains by sheet flow and runoff in rocky washes and channels where the species often grows, and aerially during windstorms. Given limited seed and pollen dispersal, we expect short distances of apparent dispersal and strong genetic differentiation among populations in different watersheds. We also test whether populations exhibit morphological differentiation related to distance, to evaluate whether genetic isolation over small spatial scales is coupled to phenotypic differences, which might be viewed as a first step in speciation.

MATERIAL AND METHODS

Sampling location and protocols

We surveyed six populations of *E. parishii* under permit in Anza Borrego State Park (ABSP) in March 2016 following a strong El Niño event. ABSP encompasses a substantial portion of the Colorado Desert in California, at the western edge of the Sonoran Desert in the USA. Annual mean temperature was 24.4 °C for 2008–2019 (NOAA climatic data). Mean monthly temperatures varied from 11.4–15.0 °C for January to 31.3–35.0 °C for July. Mean annual precipitation was 134.9 mm for 2008-2019. Winter precipitation (November–March) represented 60–56% of the annual rainfall. Assuming a doubling of the maximum potential evaporation rate with every 10 °C increase in temperature, the ratio of precipitation to potential evaporation during winter would be 7.1–5.4 times that during summer, indicating how much moister conditions are during the winter at ABSP.

Six populations were selected for study, each in a rocky wash in a different watershed, and separated by 7–57 km (Fig. 1). Given the habitat preferences of *E. parishii* and the physiognomy of the washes, individual populations had discrete boundaries.

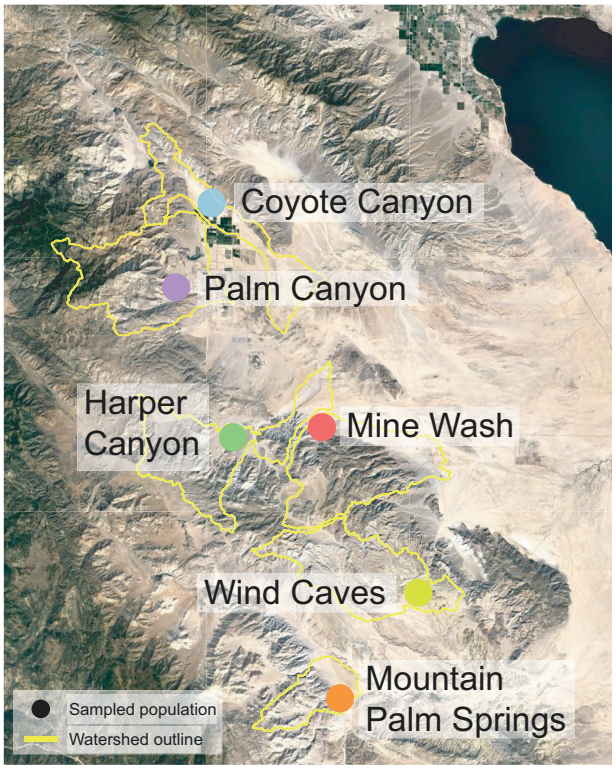

Figure 1. Map of sampled populations in Anza Borrego State Park, California, with the Salton Sea along the eastern edge. Yellow outlines depict distinct watersheds as delimited by the level-12 hydrological units database (data.ca.gov).

Two sites (Palm Canyon and Coyote Canyon; Fig. 1) had populations large enough for the extensive sampling needed to estimate the spatial scale of gene flow. In each population, we sampled a total of 100 plants in ten circular plots 40 m in diameter, placed to cover the population. Plants were collected using a protocol designed to obtain an approximately continuous logarithmic distribution of interplant distances (Supporting Information, Fig. S1). In each plot, we collected two individuals within 4 m of the centre point, three 4–10 m from the centre and three 10–20 m from the centre. An additional two plants, each within 1 m from a previously sampled individual, were also collected from each plot. Sample locations were recorded by capturing plot centroid locations using a high-precision GPS and then recording the distance and azimuth of all individuals to that centroid using laser rangefinders. An eleventh individual from within each plot was collected as a voucher specimen and lodged at the Wisconsin State Herbarium (WIS). To estimate population density, we established six parallel 100-m transects across each population and located four 4-m^2 quadrats on each transect using a random number generator. In each quadrat, we counted the number of individuals present and then used those data to calculate population size and density.

In the smaller populations at four other sites (Harper Canyon, Mine Wash, Mountain Palm Spring, Wind Cave; Fig. 1), we collected 26 individuals (13 at Wind Cave, which had a smaller population) across the extent of the continuous population. The location of each individual was recorded with GPS, except for at Mine Wash; individuals from Mine Wash were used for morphometric analyses, not analyses of spatial genetic structure. We collected leaf tissue for each individual sampled and immediately stored it in silica gel.

DNA EXTRACTION, SEQUENCING AND ASSEMBLY

Total genomic DNA was extracted from silica-dried leaf samples from 288 individuals using DNeasy 96 Plant Mini Kits (Qiagen, Valencia, CA, USA), and quantified with Quant-iT PicoGreen dsDNA assay kits. We then used AxyPrep Mag PCR Clean-Up beads (Axygen Scientific, Union City, CA, USA) to clean, concentrate and standardize the extractions, using double distilled H_2O for the final elution. DNA samples were then submitted to the University of Wisconsin-Madison Biotechnology Center, where genotype-by-sequencing (GBS) libraries were prepared using the *Eco*RI restriction enzyme and sequenced on an Illumina HiSeq 2000 Sequencing System (Illumina, San Diego, CA, USA).

Raw sequences were subsequently cleaned and assembled into loci. First, we used cutadapt v.1.3 (Martin, 2011) to remove Illumina adapter sequences. We then used the process-radtags program in the Stacks v.1.48 (Catchen *et al.*, 2013) pipeline to remove reads with ambiguously called bases (-c option) and those with < 90% probability of being correct and to truncate reads to 93 bp. Loci were then assembled in Stacks. We followed the approach of Paris, Stevens & Catchen (2017) and Spalink, MacKay & Sytsma (2019) to identify optimal parameters for discerning alleles and loci, which involved iteratively varying the allowable number of nucleotide differences in loci within samples (-M parameter) and then the number of differences within loci when merging across samples (-n parameter). We then selected the minimum values for these parameters that maximized the number of polymorphic loci, whereby no further increases to these values would yield significantly more heterozygous loci, using the 80% rule (Paris *et al.*, 2017). In our dataset, the optimal parameters were M = 3 and n = 2. Subsequent assembly followed the standard Stacks pipeline, including the use of the rxstacks module to correct insignificant genotype models, and the option in the populations program to remove loci below the log-likelihood limit of –50. Additional cleaning steps included removing loci that showed no variation among

all individuals, which are putative plastid markers (Kartzinel *et al.*, 2016). We eliminated samples with > 10% missing data, and we eliminated loci that were missing from > 10% of samples. These final steps were conducted using the hierfstat (Goudet, 2005) and adegenet (Jombart & Ahmed, 2011) packages in R v.3.5.1 (R Core Development Team, 2018).

Genetic diversity, spatial genetic structure and dispersal distance

We measured H_E, F_{IS} and pairwise population F_{ST} using SPAGeDi v.1.4 (Hardy & Vekemans, 2002). As a first measure of spatial genetic structure, we performed analysis of molecular variance (AMOVA). This process involves first measuring the proportion of genetic diversity contained within individuals, within populations and across populations. This is followed by a Monte-Carlo test with 1000 randomizations to test whether genetic diversity exhibits more partitioning in any of these categories than expected by chance. AMOVAs were performed using the R packages poppr (Kamvar, Tabima & Grünwald, 2014) and ade4 (Thioulouse *et al.*, 2018).

Next, we used SPAGeDi v.1.4 (Hardy & Vekemans, 2002) to test for significant isolation by distance (IBD) across all populations. Our calculations are for total gene flow; we did not distinguish the effects of pollen and seed movement. We calculated the haversine distances between all pairs of samples and binned these into ten logarithmic distance classes (0–3, 3–9, 9–27 m, etc.). We calculated average kinship coefficients (r) within each distance class and used 1000 random permutations and Mantel tests to assess significant departure from $r = 0$ within each class. We used Ritland's (1996) measure of kinship, which has been shown to outperform other metrics [e.g. Loiselle's kinship (Loiselle *et al.*, 1995)] with GBS data, where genotyping error, allelic dropout and missing data may be present in the dataset (Attard, Beheregaray & Möller, 2018). We then regressed average kinship against ln distance, using the average of the upper and lower bounds for each distance class, and least mean squares to determine the slope and significance of the relationship.

We performed principal components analysis (PCA) and spatial PCA (sPCA). sPCA explicitly integrates spatial autocorrelation and genetic variation to identify spatial genetic structure (Jombart *et al.*, 2008). This approach works like PCA, but the components are optimized by multiplying genetic variances by Moran's I (Moran, 1948, 1950), or the spatial autocorrelation between the georeferenced genotypes. To establish a spatial weighting matrix for sPCA, we identified sets of nearest neighbours based on Euclidean distances. PCA and sPCA were performed using the R packages ade4 (Thioulouse *et al.*, 2018) and adespatial (Dray *et al.*, 2021), respectively.

We used two approaches to measure fine-scale spatial genetic structure. First, we used fineRADstructure (Malinsky *et al.*, 2018), a clustering approach optimized for GBS-type datasets, to assess sample co-ancestry and population structure. Briefly, this method generates a 'co-ancestry matrix' based on haplotype relationships and uses this as input for the Bayesian clustering algorithm. Prior to analysis, loci were reordered according to their linkage disequilibrium, as recommended by the software developers for unmapped loci (Malinsky *et al.*, 2018). We used default parameters for the analysis, which included 100 000 Markov chain Monte Carlo generations sampled every 1000 generations with a burn-in of 100 000 iterations. We used 10 000 hill-climbing iterations to build the tree.

Second, we calculated Sp, a measure of the strength of spatial genetic structure, and (as our primary objective) σ, the square root of half the mean squared distance between parents and offspring, a metric of the spatial scale of total gene flow via pollen and seeds. We calculated these parameters independently for the Palm Canyon and Coyote Canyon populations, where we had sufficient sampling and estimates of population density. Sp, developed by Vekemans and Hardy (2004), is based on the slope of the regression between kinship and ln distance, and can therefore be compared amongst species and sampling designs. Within our two focal populations, we calculated average kinship among individuals within each of the distance classes defined above. Sp is calculated as $Sp = -b_F/(1-F_{(1)})$, where b_F is the regression slope and $F_{(1)}$ is the average kinship of individuals occurring within 3 m of each other (the first distance class). N_b was calculated for each population as the inverse of Sp. Error terms (± SD) for Sp correspond to those around the slope of this regression; those for N_b correspond to the inverse of those for Sp. The more noise around the regression of kinship against ln distance, the lower the estimate of Sp. Higher values of Sp indicate stronger spatial genetic structure at this scale. We used the iterative approach of Vekemans & Hardy (2004) to calculate σ, based on effective population density, the slope of the regression of kinship, and ln distance between σ and 20σ, and neighbourhood size N_b (the inverse of Sp). We calculated σ assuming effective population densities equal to 50% and 25% of flowering individuals per m².

Morphometrics

We analysed petal shape and size to test the hypothesis that populations differ significantly in floral morphology, as might be expected under local genetic differentiation. Flowers were photographed in the field from ten

individuals selected randomly from each population (one from each of the ten plots at Coyote Canyon and Palm Canyon). We placed a small glass micrometer on the top of each flower prior to photography, so that the petals were uniformly spread and allowed precise measurements from the resulting images.

We used geometric morphometrics to test for differences in petal shape. We placed two landmarks on the tip and centre bottom of the petals, and used elliptic Fourier analysis (Kuhl & Giardina, 1982) to extract and analyse 733 harmonics. We conducted PCA on the Fourier coefficients and tested for significant differences among populations using pairwise ANOVA. These analyses were conducted in R using Momocs (Bonhomme *et al.*, 2014) and RVAideMemoire (Hervé, 2020).

We used traditional morphometrics to test for differences in petal size. Petal length and width were captured for all petals using ImageJ (Schneider, Rasband & Eliceiri, 2012). Each petal was measured independently by two people, and all measures were used to calculate average petal length and width for each sample. We tested for significant differences among populations using pairwise ANOVA.

RESULTS

SEQUENCING AND ASSEMBLY

Illumina sequencing yielded 154 billion base pairs of data across 247 individuals. Forty-four billion base pairs were retained after removing reads that had ambiguous barcodes and RAD-tags, or were otherwise of low quality. The remaining sequences were assembled with ustacks into 12 million stacks across all samples, with an average depth of 37.7 reads. These stacks were then merged into 845 528 putative loci in cstacks. Of these, 827 885 loci were removed by populations because they were either below the log likelihood threshold of −50, or because they were not present in at least 75% of the individuals within any given population. Finally, we removed fixed loci, samples that had > 10% missing data and loci that were missing from > 10% of individuals. Our final dataset contained 232 samples and 1930 polymorphic loci.

GENETIC DIVERSITY, SPATIAL GENETIC STRUCTURE AND DISPERSAL DISTANCE

All populations generally had low heterozygosity (0.053–0.097) and gene diversity (0.068–0.11), and all exhibited inbreeding (0.128–0.271; Table 2a). Pairwise F_{ST} among populations ranged from 0.001 between Palm Canyon and Coyote Canyon (the two northernmost populations) to 0.025 between Palm Canyon and Wind Caves, one of the southernmost populations. AMOVA showed that 13% of the genetic diversity is partitioned between populations, 5% between samples and 82% within samples. Randomization tests indicated that significantly more genetic diversity was partitioned within and among populations and significantly less

Table 2. Summary statistics for (A) observed heterozygosity, gene diversity and inbreeding for all samples and each population; and (B) pairwise F_{ST} among populations.

A

	H_O (heterozygosity)	H_S (gene diversity)	F_{IS}
All samples	0.068	0.083	0.188
Coyote Canyon	0.097	0.11	0.127
Harper Canyon	0.054	0.072	0.255
Mine Wash	0.054	0.077	0.271
Mountain Palm Springs	0.053	0.068	0.218
Palm Canyon	0.091	0.1	0.128
Wind Caves	0.055	0.066	0.168

B

	Coyote Canyon	Harper Canyon	Mine Wash	Mountain Palm Springs	Palm Canyon	Wind Caves
Coyote Canyon	–	0.02	0.018	0.022	0.001	0.022
Harper Canyon	0.02	–	0.006	0.021	0.023	0.012
Mine Wash	0.018	0.006	–	0.023	0.022	0.014
Mountain Palm Springs	0.022	0.021	0.023	–	0.023	0.022
Palm Canyon	0.001	0.023	0.022	0.023	–	0.025
Wind Caves	0.022	0.012	0.014	0.022	0.025	–

genetic diversity within samples than expected from a panmictic metapopulation ($P < 0.01$; Fig. 2).

IBD analyses revealed that overall genetic kinship between individuals decreases as they become more geographically distant from each other (Fig. 3). The significant log-linear relationship between kinship and distance ($r^2 = 0.85$, $P < 0.01$) shows a steep decline in relatedness between 3 and 9 m in distance, but a smooth log-linear decline overall with essentially zero kinship after 3 km in distance, indicating that individual pairs of plants are no more or less related to each other than a random draw at this scale.

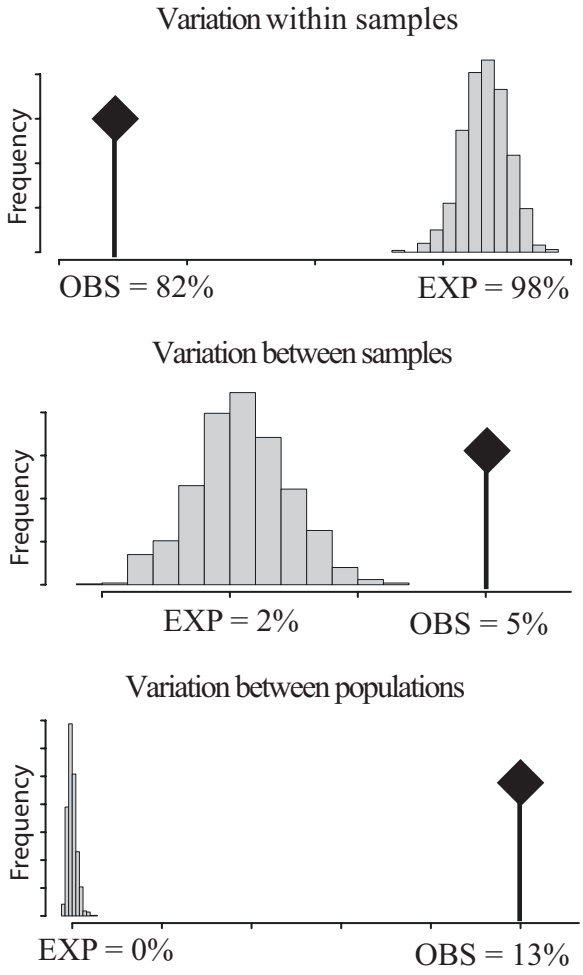

Figure 2. Analysis of molecular variance, depicting proportions of genetic diversity contained within individuals, within populations and across populations. Histograms show the distribution of expected values (EXP) for each metric, based on 1000 randomizations. Observed values (OBS) are indicated by diamonds. Variation within samples was significantly less than expected, whereas variation between samples and between populations was significantly greater than expected ($P < 0.05$).

Populations showed strong spatial–genetic segregation in both PCA and sPCA (Fig. 4). The first two components accounted for 2.8% and 1.6% of the overall genetic variance. In the standard PCA (Fig. 4A), the northern two populations (Coyote Canyon and Palm Canyon) overlap in ordinated genetic space, but are separate from the southern populations, which generally show more segregation. In the sPCA (Fig. 4B), all populations occur in separate clusters, indicating strong spatial autocorrelation and genetic segregation among populations. The fineRADstructure clustering was consistent with these patters (Fig. 5). All individuals were more closely related to individuals from the same population than they were to individuals from other populations. Likewise, the southern populations were more closely related to each other than they were to the northern populations, which formed a clade.

Sp was low but significantly greater than zero at both Coyote Canyon and Palm Canyon (0.003 and 0.005, respectively, $P < 0.01$). Estimates of N_b and σ converged on single values during the iterative estimation process. N_b ranged from 675 ± 238 to 689 ± 213 individuals in Coyote Canyon, and from 694 ± 1747 to 709 ± 1053 individuals in Palm Canyon, when effective population densities equalled 50% and 25% of flowering individuals per m², respectively. The size of the standard deviations of these estimates

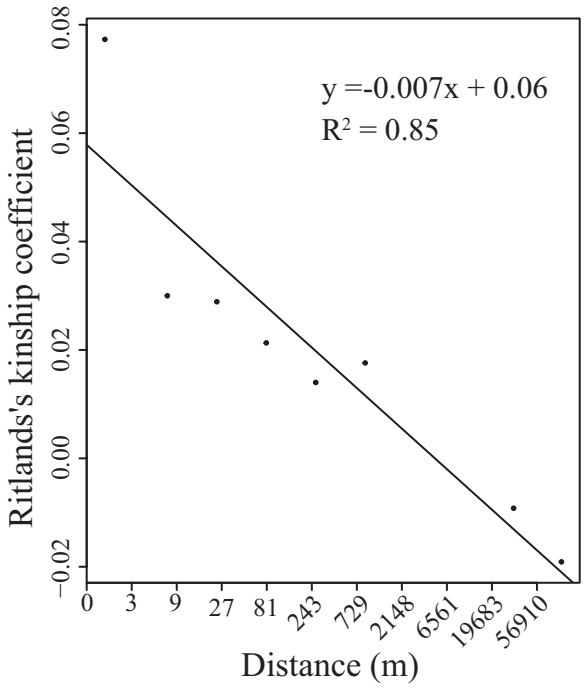

Figure 3. Kinship by distance regression, showing a near linear decline in relatedness among individuals with increased log-distance ($R^2 = 0.85$, $P < 0.05$).

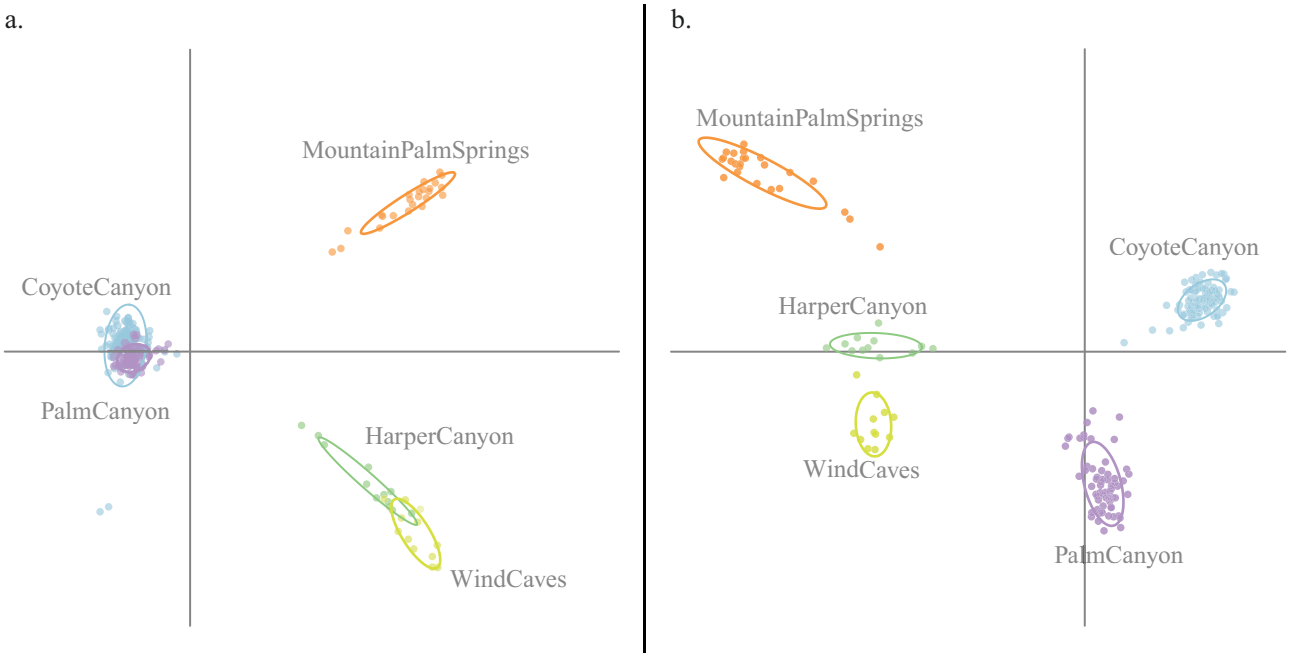

Figure 4. Principal component analyses. A, the first two axes from standard principal components analyses show strong genetic segregation between the northern and southern populations and segregation among southern populations. B, the first two axes from spatial principal components analysis, representing both genetic variance and spatial autocorrelation among individuals. All populations form cohesive and isolated clusters, indicating strong spatial autocorrelation and genetic segregation.

indicates substantial noise in the data relating kinship to ln distance between individuals, especially at Palm Canyon; the range of N_b values for the latter include values < 0, but in aggregate the distribution was significantly greater than zero. Estimated values of the root-mean dispersal distance ranged from σ = 10.4 ± 1.4 to 14.9 ± 2.2 m at Coyote Canyon, and σ = 11.0 ± 7.0 to 12.5 ± 20.5 m at Palm Canyon. In both populations, the larger estimations of dispersal distance correspond to assuming that the effective population density equalled 25% of that for flowering individuals. Note the smaller standard deviations for the estimate of σ than for *Sp* and N_b.

Morphometrics

In our geometric morphometric analysis, the first two principal components accounted for 85% of the variation in petal shape (Fig. 6A). The first component primarily captured variations in overall petal length/width ratio, whereas the second component captured variations in the location of the widest part of the petal. In plotting these components, we found substantial variation in petal shape among individuals, though all populations overlapped in morphospace. No significant differences in overall shape were observed among populations (Fig. 6B; $P < 0.05$).

Similar patterns were observed using traditional morphometrics. Both petal length and petal width varied substantially among individuals (0.87–1.97 and 0.71–1.82 cm, respectively; Fig. 6C, D). Petal lengths and widths were significantly larger in Coyote Canyon than Mountain Palm Springs ($P < 0.05$). Notably, these two populations are the two most geographically separated.

DISCUSSION

Gene flow in *E. parishii* occurs over small spatial distances, with mean estimates of σ ranging from 10.4 to 14.9 m at Coyote Canyon and from 11.0 to 12.5 m at Palm Canyon (see also Fig. 2). These values of σ are in the same low range as those seen in two other large, rapidly speciating genera, as estimated for one geophytic herb from Mediterranean scrub (mean σ = 16.7 ± 25.6 m; Henss *et al.*, 2013) in *Calochortus* Pursh and four understorey treelets from a tropical rain forest (mean σ = 36.9 ± 25.7 m; Theim *et al.*, 2014) in *Psychotria* L. Our estimates of total gene flow over only 10–15 m in *E. parishii* are thus consistent with our proposal that lack of adaptations for long-distance seed dispersal in most desert winter annuals, and thus seed dispersal only over small spatial scales, may be an important contributor to genetic divergence and

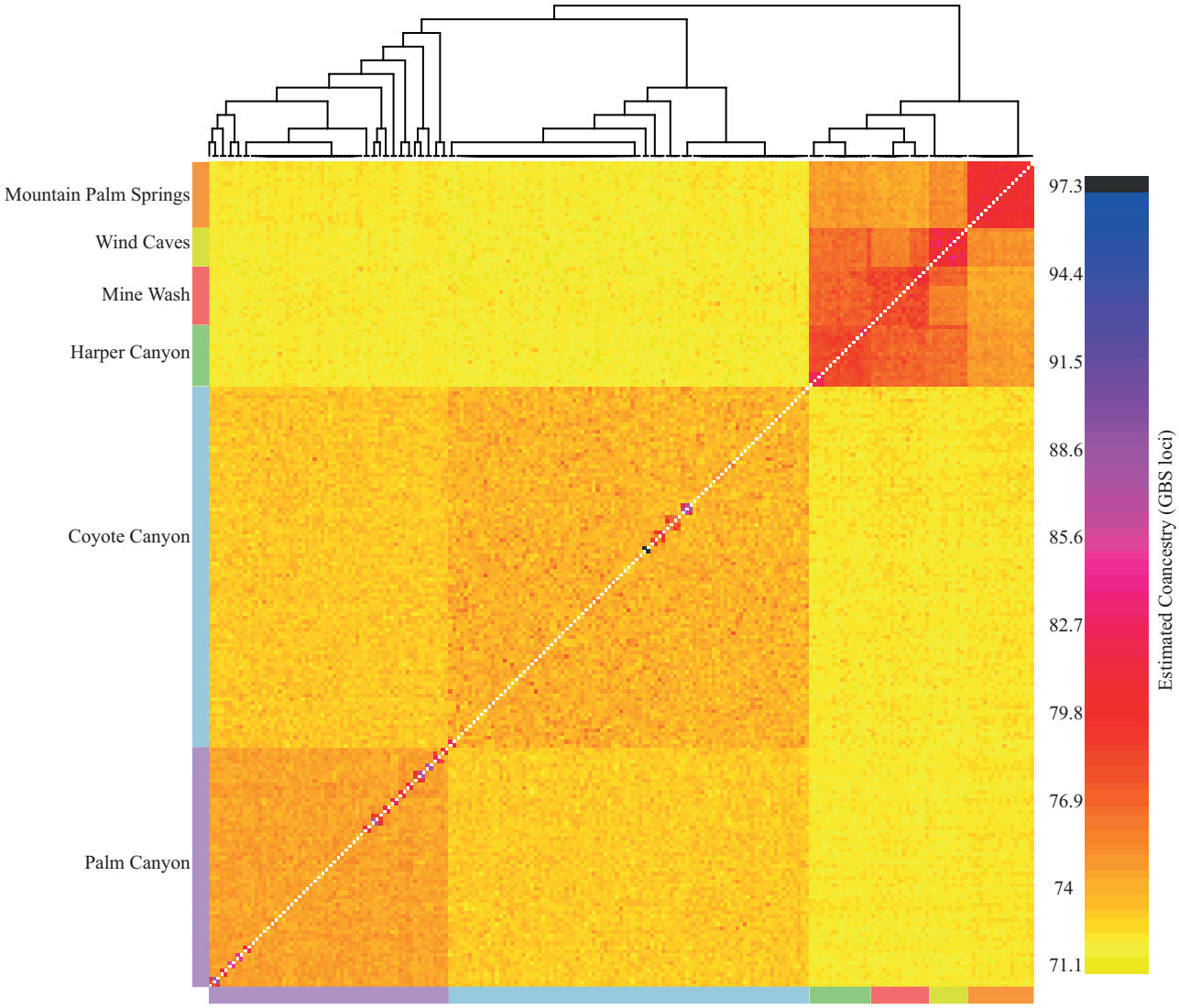

Figure 5. Co-ancestry matrix and dendrogram from fineRADstructure clustering analysis. The dendrogram shows that sampled individuals are more closely related to other individuals of the same population than they are to individuals from other populations. The co-ancestry matrix indicates that the southernmost populations share greater relatedness to each other than they do with the northern two populations. Populations are colour coded according to the map in Figure 1.

possibly speciation at small spatial scales in desert winter annuals. This hypothesis, in turn, is related to that advanced by Givnish (1998, 2010), Henss *et al.* (2013) and Theim *et al.* (2014) that limited seed dispersal (often associated with heavy seeds, lack of adaptation for long-distance dispersal and with dispersal of fleshy fruits by sedentary understorey birds) leads to genetic differentiation at fine spatial scales and can ultimately result in rapid speciation and narrow endemism. Given the small spatial scale of our study and that each population occurs in a different watershed, we expect that genetic divergence across our sampled sites might be the result of genetic isolation and drift. However, local selective pressures related to slope, aspect and soils may also be involved in this divergence, as even slight environmental differences could be important in these harsh, water-limited habitats. Indeed, selection would need to be invoked to explain these patterns if occasional gene flow is facilitated by sandstorms.

The occurrence of many large genera of desert and vernal-pool annuals in the California Floristic Province may partly reflect gene flow over short distances in these groups, which generally lack adaptations for long-distance dispersal. A general lack of telechory in desert plants may, in turn, reflect the cost of dispersal structures and the scale of distances between desert rainfall events being so large that no plausible increase

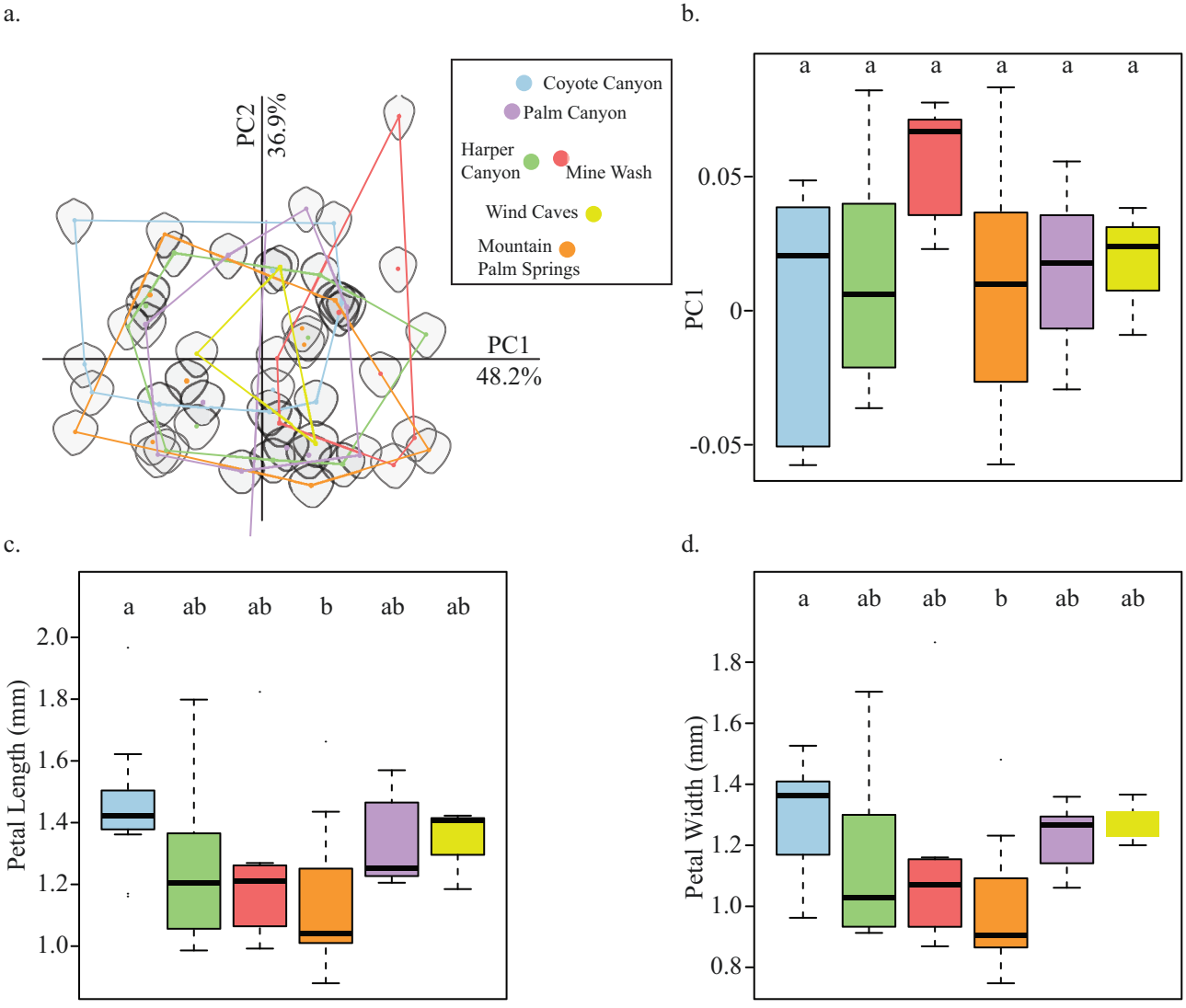

Figure 6. Morphometric analyses depicting petal shape and size differences between individuals and populations. Populations are colour coded according to the inset map, which shows the relative location of populations as indicated in Figure 1. A, first two axes of a principal components analysis of petal shape. Shape varies substantially among individuals, though all populations overlap in morphospace. B–D, boxplots depicting the population averages for shape PC1 (B), petal length (C) and petal width (D). Significant differences among populations as determined by ANOVA are indicated with lowercase letters above the boxplots. No significant differences in petal shape were observed between populations (B). Petals are significantly longer (C) and wider (D) in Coyote Canyon than Mountain Palm Springs ($P < 0.05$).

in dispersal distance would place seeds into more favourable areas (Fllner & Shmida, 1981). Raven & Axelrod (1974) attributed the large size of genera of desert and vernal-pool annuals mostly to saltational speciation, with patchy and unpredictable rainfall leading to rapid local fixation of any chromosomal rearrangements and, with that, rapid production of reproductive isolation between populations (Lewis, 1962). Our proposed mechanism of short gene-flow distances could facilitate such saltational speciation by restricting the spatial scale over which mutations involving chromosomal re-arrangements and/or ecological specialization occur. Rapid speciation, specifically in desert winter annuals in the hot deserts of California, Arizona and Sonora, would also be favoured by the other factors listed in the Introduction, involving the open nature of hot deserts, the large areas in these deserts not dominated by perennial plants, the concentration of rainfall and relatively low evaporation rates in the winter months, short life cycles and rapid evolution in annuals, extensive resource partitioning among annuals in space and time, near universal

occurrence of animal pollination in C_3 winter annuals vs. frequent occurrence of wind pollination in often C_4 summer-annual lineages, and the short generation times of annuals. Limited spatial scales of gene flow can, in general, lead to adaptation to environmental conditions at finer spatial scales within species (Endler, 1977). The frequent occurrence of large-scale gene flow via wind pollination in C_4 summer-annual lineages (e.g. in grass genera) may work against extensive speciation in summer-annual genera, even in the Chihuahuan Desert where summer rainfall is relatively frequent.

Gene flow over small spatial scales can permit differentiation within species at such scales, especially under directional selection (Endler, 1977; Leducq *et al.*, 2011), and may ultimately lead to speciation (Endler, 1977; Givnish, 2010). Indeed, Kisel & Barraclough (2010) found that the minimum island size required for speciation in various groups of organisms (ferns, flowering plants, bats, birds, lizards, carnivorous mammals and Lepidoptera) corresponded to parallel variation in genetic differentiation within species at small spatial scales. We must recognize, however, that limited gene flow alone will not automatically lead to speciation and that mating barriers must also arise between closely related populations, through incompatibilities born of multi-locus genetic divergence between populations driven by drift or selection (Dobzhansky, 1934; Muller, 1942; Orr, 1995; Orr & Turelli, 2001; Satokangas *et al.*, 2020) or through ecological or mating isolation resulting from selection on one or a few key loci (Schemske & Bradshaw, 1999; Savolainen *et al.*, 2006; Kostyun *et al.*, 2019; Nelson *et al.*, 2021). Either of these scenarios, however, would be made more likely by gene flow over small spatial scales.

The occurrence of low values of *Sp* (a measure of fine-scale genetic structure) in *E. parishii* suggests that, although gene flow at fine scales permits genetic differentiation at those scales, stochastic processes operating at a variety of scales (e.g. variation among nearby plants in fecundity, sheet flow within watersheds, sandstorm-driven aerial dispersal across watersheds) may be overwriting some of that differentiation. *Sp* values of 0.003–0.005 in *E. parishii* compare with a mean ± SD *Sp* value of 0.013 ± 0.009 across the 35 outcrossed and self-incompatible plant species surveyed by Hardy & Vekemans (2002). Therefore, although gene flow occurs over short distances in that desert annual, compatible with genetic differentiation over small spatial scales, especially under directional selection, the pattern of genetic differentiation is noisy. Our data show substantial differentiation among watersheds based on Ritland's kinship (Fig. 4), with relatedness showing the expected significant decrease with ln distance between sites (Fig. 3) and populations. Also, although all populations form clades (Fig. 5) and are genetically distinct (Figs 4, 5), genetic diversity is generally low within and among populations (Table 2). However, our lack of repeated sampling within individual watersheds makes it impossible to tease apart the putative roles of sheet flows vs. sandstorms. The morphometric differentiation of petal size among some populations (Fig. 6), based on admittedly limited sample sizes, is also consistent with gene flow and genetic differentiation at small spatial scales, leading to the potential for reproductive isolation.

Although our results are consistent with our stated hypotheses, this study should now be replicated for several winter-annual genera, including those with and without adaptations for long-distance dispersal (e.g. pappi and plumes in some Asteraceae) and those with and without high rates of net species diversification, with the latter inferred from time-calibrated molecular phylogenetic trees. The spatial scales at which genetic differentiation and gene flow take place should also be combined with the spatial scales of outcrossing depression (Waser, 1993), which can be seen as an initial, partial barrier to mating and a significant step linking short-term genetic differentiation within a species to processes that actually can lead to long-term reproductive barriers and speciation.

AUTHOR CONTRIBUTIONS

T.J.G. and D.S. conceptualized the study; D.S. coordinated the field and lab research; all participated in the field studies; D.S., N.K., J.H.R., E.E. and A.D. conducted the laboratory research; D.S. led the subsequent analyses; and D.S. and T.J.G. wrote the draft and incorporated comments from all participants.

ACKNOWLEDGEMENTS

We thank the Humboldt Fund of the Department of Botany at the University of Wisconsin for financial support for field work and DNA sequencing, Anza Borrego State Park for providing permission to collect tissue samples required for this study and J. André, B. Baldwin and T. Chester for tabulating California winter-annual diversity and reviewing the manuscript. This work was supported in part by the USDA National Institute of Food and Agriculture (McIntire Stennis Project 1018692 to D.S.).

DATA ACCESSIBILITY

DNA sequence data, SPaGeDi input files and R scripts for major analyses are deposited at Dryad under doi:10.5061/dryad.1vh.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Sampling design. Samples were collected to achieve a near logarithmic distribution of plant distance. Within each plot, we collected two individuals within 4 m of the centre point, three 4–10 m from the centre and three 10–20 m from the centre. These are depicted by the green dots in the figure below. An additional two plants, each within 1 m from a previously sampled individual, were also collected from each plot. These are depicted by red dots. Ten plots were sampled in each population.