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Climate structures genetic variation across a species' elevation range: a test of range limits hypotheses

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Abstract

Gene flow may influence the formation of species range limits, and yet little is known about the patterns of gene flow with respect to environmental gradients or proximity to range limits. With rapid environmental change, it is especially important to understand patterns of gene flow to inform conservation efforts. Here we investigate the species range of the selfing, annual plant, *Mimulus laciniatus*, in the California Sierra Nevada. We assessed genetic variation, gene flow, and population abundance across the entire elevation-based climate range. Contrary to expectations, within-population plant density increased towards both climate limits. Mean genetic diversity of edge populations was equivalent to central populations; however, all edge populations exhibited less genetic diversity than neighbouring interior populations. Genetic differentiation was fairly consistent and moderate among all populations, and no directional signals of contemporary gene flow were detected between central and peripheral elevations. Elevation-driven gene flow (isolation by environment), but not isolation by distance, was found across the species range. These findings were the same towards high- and low-elevation range limits and were inconsistent with two common centre-edge hypotheses invoked for the formation of species range limits: (i) decreasing habitat quality and population size; (ii) swamping gene flow from large, central populations. This pattern demonstrates that climate, but not centre-edge dynamics, is an important range-wide factor structuring *M. laciniatus* populations. To our knowledge, this is the first empirical study to relate environmental patterns of gene flow to range limits hypotheses. Similar investigations across a wide variety of taxa and life histories are needed.

Keywords: elevation gradients, gene flow, isolation by environment, *Mimulus*, species range limits, swamping gene flow

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Introduction

Patterns of abundance and genetic variation across a species' range can provide information about the processes that limit species distributions and can inform our understanding of how ecology, evolution and geography intersect (Hoffmann & Blows 1994; Bridle & Vines 2007; Sexton *et al.* 2009). The theoretical models proposed to explain range limitation incorporate a variety of mechanisms related to habitat quality, availability and connectivity (Gaston 2009; Sexton *et al.* 2009; Holt

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& Barfield 2011). Signatures can be detected using genetic markers that support or refute hypotheses concerning the maintenance of species borders (Eckert *et al.* 2008; Dawson *et al.* 2010; Moeller *et al.* 2011; Paul *et al.* 2011). Such information is important and timely for understanding distributions in the context of conservation and rapidly changing environments resulting from global climate change (Lesica & Allendorf 1995; Hampe & Petit 2005).

Perhaps the best-known conceptual framework to predict distribution limits is the Abundant Center Hypothesis (ACH). Declines in abundance from central to peripheral regions may be associated with spatial declines in habitat quality or availability (e.g. resource availability, abiotic stress, predation, etc.) such that populations ultimately fail to replace themselves or successfully establish (Brown 1984; Sagarin & Gaines 2002). Evolutionary causes of population decline at range limits under the ACH include what we term the *drift* scenario and the *swamping migration* scenario, both of which generate distinct expectations. In the drift scenario, peripheral populations may critically suffer from the effects of genetic drift due to small population sizes that result from reduced carrying capacities (e.g. Keitt *et al.* 2001; Case *et al.* 2005). This scenario predicts increased genetic isolation (differentiation) and lower genetic diversity towards margins (Eckert *et al.* 2008). The swamping migration scenario invokes a migration load from large, central populations of the species range (e.g. Haldane 1956; Mayr 1963; Antonovics 1976; Kirkpatrick & Barton 1997) and predicts low genetic differentiation and equivalent genetic diversity towards range limits (Barton 2001; Holt 2003; Dawson *et al.* 2010).

How gene flow interacts with spatial and environmental gradients may critically influence evolutionary and ecological outcomes towards range limits, yet we still have a limited understanding of the patterns of gene flow across species ranges (Orsini *et al.* 2013; Sexton *et al.* 2014; Wang & Bradburd 2014). Genetic isolation by distance (IBD) is expected to arise through the action of genetic drift and dispersal limitation (i.e. in the absence of natural selection) (Wright 1943). Alternatively, isolation by environment (IBE) is expected under the action of natural selection and/or environmentally influenced mating or migration (Crispo *et al.* 2006; Schluter 2009; Orsini *et al.* 2013; Sexton *et al.* 2014; Wang & Bradburd 2014). IBE has been shown to be the dominant geneflow pattern in animals, but not in plants, although much more research is needed in this area (Sexton *et al.* 2014). Additionally, although IBD and IBE often cooccur, IBE and swamping migration with respect to a particular environmental gradient are mutually exclusive (Sexton *et al.* 2014). Thus, a finding of IBE towards range limits allows the rejection of the swamping migration hypothesis with respect to that environmental gradient.

With climate change, species ranges could shift as they track their climate envelope across landscapes (Essl *et al.* 2015). We may observe 'leading' (at cold climate borders) and 'rear' or 'trailing' (at warm climate borders) range limits governed by different processes (Hewitt 2004; Hampe & Petit 2005). In warming climates, leading edges represent areas (i.e. the highest latitudes or elevations) where climate warming would cause population expansions as a result of rises in range-limiting low temperatures. Alternatively, rear edges (lowest elevations or latitudes) may represent older areas of the species range (i.e. past refugia), where climate warming may impose new, range-contracting stresses (Hampe & Petit 2005). Expectations at leading edges include more frequent long-distance dispersal, more founder events, greater population growth and, consequently, greater net immigration. In rear-edge areas, population stability, well-adapted populations and a larger ancestral pool of genetic diversity have been hypothesized, although these properties could erode with climate change (Hampe & Petit 2005).

Leading/rear-edge studies have mostly been framed in latitudinal contexts, with comparisons to regions that represent post-glacial expansions (leading areas) and refugia (rear areas) under new climate pressures (Hampe & Petit 2005); however, this phenomenon of shifting limits can also apply to elevation gradients (Parmesan 2006; Angert *et al.* 2011). Both latitude and elevation gradients are important sources of environmental heterogeneity that promote biodiversity, but there are biologically important differences between elevation and latitude and different responses can emerge (Halbritter *et al.* 2015). Temperature is strongly correlated between elevation and latitude, but photoperiod, precipitation and nonclimate factors (e.g. soils, habitats, ecological communities) may not be (Halbritter *et al.* 2013). Additionally, elevation-based climate gradients are steeper (occurring across shorter distances). Thus, gene flow is expected to be greater and dispersal limitation reduced across elevation gradients (Halbritter *et al.* 2013). The formation of species range limits via swamping gene flow has been hypothesized to be more likely across steep ecological gradients (Phillips 2012; Polechová & Barton 2015; but see Gomulkiewicz *et al.* 1999; Barton 2001), and yet very few empirical tests relating swamping gene flow to species range limits exist (but see Magiafoglou *et al.* 2002; Fedorka *et al.* 2012). In this vein, elevation gradients are convenient for testing hypotheses related to gene flow and the maintenance of species range limits. Elevation gradients have also been the recent focus of studies documenting important contemporary shifts in species ranges and its ecological

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and evolutionary consequences (e.g. Kelly & Goulden 2008; Rubidge *et al*. 2012).

We estimated population abundance, genetic diversity and contemporary gene flow across the species range of *Mimulus laciniatus* (Phrymaceae), an endemic plant to the Sierra Nevada within the diverse California Floristic Province. This species is convenient for testing hypotheses related to species range limits, climate gradients and centre-edge comparisons at both warm and cold climate range limits. First, it has clear habitat requirements, has an easily defined species range and elevation limits and is easily distinguished from closely related species. Second, *M. laciniatus* occurs across a wide altitudinal climate gradient and within a large enough range to allow replicate comparisons between central and peripheral areas at both low- and high-elevation limits. Finally, *M. laciniatus* is an autogamous (selfing), annual plant, with occasional outcrossing, which allows great colonization potential and short generation time, allowing for a relatively contemporary view of species range dynamics. We asked the following questions: (i) How do patterns of abundance, genetic diversity and connectivity (i.e. isolation) vary with regard to altitudinal climate and centre-edge gradients? (ii) Do the above patterns differ towards warm versus cold climate limits? and (iii) What is the pattern of gene flow across the range of *M. laciniatus* (IBD, IBE or swamping gene flow)? We discuss the implications of our findings for mechanisms generating range limits and for conservation considerations under rapid climate warming.

Methods

Study species and habitat

The cut-leaved monkeyflower (*M. laciniatus*) (Fig. 1) is endemic to the western slope of the Sierra Nevada of California. The dissected leaf margins of *M. laciniatus* set it apart from most other *Mimulus* species (but see Sexton *et al*. 2013), and this leaf shape is thought to be adaptive in the exposed, fast-drying environments in

Fig. 1 (a) *Mimulus laciniatus* flower. (b) *M. laciniatus* plants growing within moss. (c) Granite seep habitat.

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which it grows (Ferris *et al.* 2014, 2015). *Mimulus laciniatus* mainly grows within moss patches on ephemeral, slow-draining seeps on rocky outcrops (Fig. 1) and is strongly adapted to these habitats compared to its close relative, *Mimulus guttatus* (Peterson *et al.* 2013). *Mimulus laciniatus* develops much faster than *M. guttatus*, allowing it to complete its life cycle on the rocky seeps before they dry out in late spring or summer. *Mimulus laciniatus* has a high self-fertilization rate (estimated at 95% by Ferris *et al.* 2014), and it produces cleistogamous and chasmogamous flowers, the latter of which are visited by solitary bees (Sexton, personal observation). *Mimulus laciniatus* is considered to be part of the *Mimulus guttatus* species complex, which varies greatly in mating system and is generally known to be bee-pollinated (Ritland & Ritland 1989; Wu *et al.* 2007). Species within this complex have been shown to have passive, long-distance seed dispersal (≥1 km) by means of water, deer and migratory birds (Lindsay 1964; Waser *et al.* 1982; Vickery *et al.* 1986). *Mimulus laciniatus* has the same seed shape and size as *M. guttatus*, which occupies a much larger species range throughout western North America (Ferris *et al.* 2014). Thus, *M. laciniatus* has the potential for long-distance colonization and gene flow through seed dispersal (as in *M. guttatus*) and pollination. However, due to its unique habitat specialization on rocky seeps, *M. laciniatus* may be restricted from expanding its range into neighbouring habitats (Peterson *et al.* 2013).

The low-elevation limit of the species range of *M. laciniatus* occurs near the Sierran winter snow line where plant communities transition from mixed coniferous, montane woodlands with summer growing seasons to foothill woodlands with winter growing seasons (Barbour *et al.* 2007). The high-elevation limit occurs near the transition between subalpine forest and the treeless alpine zone with a short, frost-free growing season (Barbour *et al.* 2007). We sampled *M. laciniatus* across its full elevation extent, a wide climate gradient extending from chaparral to alpine zones, and across *ca.* 50% of its latitudinal and longitudinal extents (Fig. 2, Table 1). Seeds were collected from 23 populations along three elevation-based transects (one in Yosemite National Park and two in the Sierra National Forest), each having 7–9 populations spaced mostly at 200–400 m elevation-based intervals. Nearly, all populations within transects were greater than 1 km apart and most were several kilometres apart (Fig. 2). We chose this design to broadly sample habitats and climates and to include cold- and warm-elevation limits and interior populations (Fig. 2, Table 1). The three transects were approximately 20 km apart. Along each transect, potentially suitable habitats were exhaustively searched for 2–20 km beyond the upper and lower range limits to

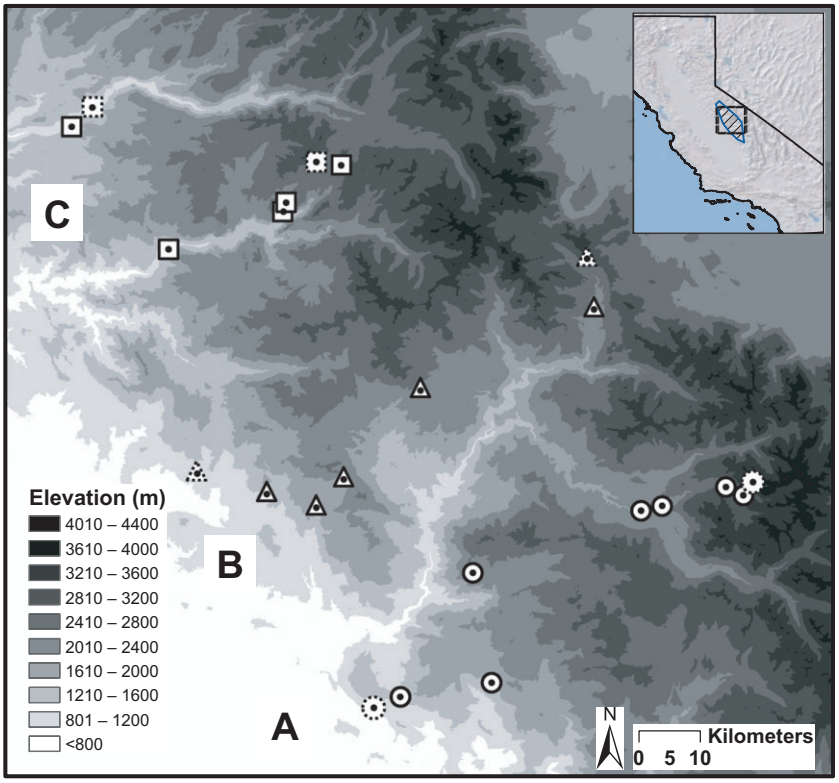

Fig. 2 Species range of *Mimulus laciniatus* (blue polygon in map inset) in the central Sierra Nevada of California. The elevation-based sampling design along three transects is shown. Circles = Transect A, triangles = Transect B and squares = Transect C. Elevation extremes within each transect are represented by dashed symbol outlines.

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Table 1 Population attributes and genetic summary statistics for 11 loci for 23 *Mimulus laciniatus* collection sites in the California Sierra Nevada. Site identifies transect (A, B, or C) and numbered elevation rank within a transect, including 'Low' and 'High' labels for edge populations

Site	Habitat	*N*	Lat., Long.	Elev. (m)	Area (ha)	F_{IS}	R_S	P_a	H_T
A-Low	F	31	37.03977, −119.40857	1000	0.869	0.922 ± 0.021	8.62 ± 2.21	1.27 ± 0.92	0.701 ± 0.259
A-2	F	43	37.04580, −119.35442	1220	0.270	0.919 ± 0.021	9.13 ± 2.41	2.00 ± 1.14	0.646 ± 0.318
A-3	MC	33	37.07225, −119.23030	1670	2.948	0.863 ± 0.021	11.43 ± 1.79	1.73 ± 0.62	0.779 ± 0.162
A-4	MC	41	37.32672, −119.01393	2012	0.160	0.924 ± 0.020	9.60 ± 2.15	0.55 ± 0.39	0.727 ± 0.171
A-5	MC	41	37.33572, −118.98323	2256	0.540	0.970 ± 0.012	8.65 ± 2.16	1.36 ± 0.64	0.708 ± 0.241
A-6	UM	46	37.23844, −119.25990	2473	0.330	0.945 ± 0.017	8.03 ± 1.40	0.55 ± 0.31	0.645 ± 0.212
A-7	UM	43	37.35800, −118.88063	2774	1.280	0.954 ± 0.012	8.99 ± 1.62	0.82 ± 0.38	0.631 ± 0.218
A-8	SA	48	37.35627, −118.86088	3095	0.358	0.950 ± 0.013	11.58 ± 1.55	1.64 ± 0.56	0.784 ± 0.120
A-High	SA	42	37.36328, −118.85703	3293	0.003	0.936 ± 0.016	9.13 ± 2.10	1.18 ± 0.42	0.642 ± 0.213
B-Low	F	49	37.38136, −119.66533	947	0.205	0.899 ± 0.033	6.97 ± 2.66	0.64 ± 0.36	0.576 ± 0.329
B-2	F	31	37.35265, −119.56288	1280	0.449	0.883 ± 0.031	8.29 ± 1.97	0.73 ± 0.38	0.701 ± 0.294
B-3	MC	39	37.33163, −119.49264	1585	0.677	0.913 ± 0.018	8.93 ± 2.53	1.45 ± 0.84	0.636 ± 0.370
B-4	MC	44	37.37436, −119.45190	1951	0.252	0.963 ± 0.013	9.81 ± 1.90	1.36 ± 0.49	0.652 ± 0.225
B-5	UM	41	37.50694, −119.33867	2200	1.901	0.913 ± 0.017	8.87 ± 2.31	0.73 ± 0.45	0.646 ± 0.330
B-6	UM	43	37.62173, −119.08628	2317	0.381	0.911 ± 0.019	8.54 ± 1.59	0.82 ± 0.38	0.677 ± 0.236
B-High	SA	30	37.69661, −119.09190	3049	1.340	0.957 ± 0.012	7.51 ± 1.42	0.45 ± 0.16	0.645 ± 0.234
C-Low	F	45	37.92159, −119.81907	1020	0.396	0.949 ± 0.018	8.95 ± 1.65	0.73 ± 0.47	0.691 ± 0.223
C-2	F	43	37.89388, −119.84903	1400	0.785	0.915 ± 0.016	11.60 ± 2.61	2.09 ± 0.92	0.750 ± 0.235
C-3	MC	40	37.71210, −119.70663	1555	0.142	0.895 ± 0.028	9.85 ± 2.66	1.55 ± 0.79	0.646 ± 0.312
C-4	MC	46	37.76630, −119.54213	1860	0.407	0.883 ± 0.016	10.68 ± 1.83	1.00 ± 0.47	0.749 ± 0.247
C-5	UM	48	37.77955, −119.53398	2165	0.150	0.950 ± 0.019	6.94 ± 1.58	0.18 ± 0.12	0.565 ± 0.234
C-6	UM	45	37.83630, −119.45562	2500	0.097	0.954 ± 0.023	10.92 ± 1.89	1.64 ± 0.49	0.745 ± 0.176
C-High	SA	38	37.84020, −119.49213	2774	0.167	0.983 ± 0.009	8.20 ± 1.30	0.45 ± 0.28	0.659 ± 0.148
Mean		41.3	— —	2011	0.613	0.928	9.18	1.08	0.678

Habitat categories are based on Storer *et al.* (2004 pp. 20–22) Sierran belts: F = Foothill; MC = Mixed Conifer; UM = Upper Montane; SA = Subalpine. *N* is the number of plants genotyped per population. Area is the estimated areal extent of a population in hectares. The following mean population genetics statistics, averaged across 11 loci, including standard errors, are presented: inbreeding coefficient (F_{IS}), mean allelic richness (R_s), mean number of private alleles rarefied from the smallest sample size (P_a) and overall gene diversity (H_T).

ensure sampling of populations at elevational extremes. No obvious geographic barriers to dispersal (e.g. large bodies of water, human-converted habitats) were observed at high- or low-elevation range limits, and similar habitats can be readily found in close proximity (<1 km) beyond current range limits.

The current elevation range of *M. laciniatus* appears to be stable since the early to mid-20th Century, and there is currently no evidence of contemporary, elevation-based range shifts. The Sierra Nevada were repeatedly glaciated in the Quaternary Period, and the last glacial advance ended ca. 13 000 years B.P. (Gillespie & Zehfuss 2004; Gillespie & Clark 2011). This glaciation covered much of the upper half of the current elevation range of *M. laciniatus*, an area that must have been recolonized since the late Pleistocene. Herbarium records for the current known elevation range limits date back to 1926 for the lowest elevations (e.g. below Hetch Hetchy Reservoir, 1005 m, by R. Bacigalupi, Record ID: POM 161240) and to 1954 for the highest elevations (e.g. at Mt. Hilgard, 3230 m, by P. Raven, Record ID: CAS 389948). In a seed-sowing experiment at field sites with habitats similar to those occupied by *M. laciniatus*, but beyond the current elevation limits, plants failed to establish self-sustaining populations during 5 years of post-experiment observation (Sexton & Dickman 2016), further indicating stable, contemporary range limits.

Seed collections, population area and plant density

All populations contained hundreds to thousands of seed-bearing individuals. At each site, the population margins were georeferenced and area was estimated in ArcGIS (Version 9) from minimum convex polygons. Seeds from each population were sampled in a stratified random fashion to maximize genetic representation across local habitat heterogeneity. Populations were first searched to identify habitat heterogeneity (i.e. varying aspects, slopes and codominant vegetation). Several transects, the number of which varied by areal extent of the population, were established to maximize spatial

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coverage and to represent local heterogeneity. Among these transects, seeds were randomly collected from at least 60 maternal families (dozens to hundreds of seeds per mother plant) at evenly spaced intervals, depending on transect lengths. Plants were selected at a minimum spacing of 5 m where possible to minimize genetic relatedness of the total sample. One plant per maternal family was raised in controlled environment chambers (grown in Sunshine Mix #1 media under 14-h days with 23 °C daytime and 4 °C night-time temperatures) for DNA extraction and genotyped separately (see Table 1 for final sample sizes of genotyped plants). Plant density was sampled in moss patches within 12 randomly assigned 0.5 m^2 plots at each site.

Elevation gradient and marginality measures

Elevation was used to estimate both position along the climate gradient and proximity to range limits (i.e. environmental marginality). The three transects varied moderately in upper range limit elevations (uppermost elevations = 3292 m, 3048 m and 2774 m for transects A, B and C, respectively), whereas lower limits were more similar (947–1020 m). A standardized continuous measure of gradient position, *Relative Elevation*, was defined as the proximity of a population to the low- or high-elevation limit of a transect relative to the transect's elevation midpoint. Thus, Relative Elevation values ranged between 1 and −1 for populations upslope and downslope, respectively, from the transect elevation midpoint:

$$\text{Relative Elevation} = \frac{E_{\text{population}} - E_{\text{centre}}}{E_{\text{centre-edge}}}$$

where $E_{\text{population}}$ is the elevation of a given population, E_{centre} is the midpoint of a given transect, and $E_{\text{centre-edge}}$ is the elevation breadth between a transect's midpoint and limits such that:

$$E_{\text{centre-edge}} = \frac{E_{\text{high edge}} - E_{\text{low edge}}}{2}$$

where $E_{\text{high edge}}$ is the elevation of the high-elevation range limit, and $E_{\text{low edge}}$ is the elevation of the low-elevation range limit for that transect. *Edge Index* was defined as the absolute value of Relative Elevation and provides a standardized continuous measure of elevation-based marginality. The elevation of one population, A-6, was high (2473 m), but in a geographically central location. This population also occupies a different substrate (volcanic soil) from other populations (mostly granite soil) and was one of the most genetically differentiated populations; it was therefore treated as an outlier and removed from marginality analyses.

Population climate values

To estimate climate values among populations, we downloaded the following four BIOCLIM variables (data for years 1950–2000 at ~1 km scale; Hijmans *et al.* 2005) for each population: annual mean temperature, annual precipitation, temperature seasonality (BIO4), and precipitation seasonality (BIO15) (Table S1, Supporting information). We then used principal components analysis (PCA in JMP version 9.0.2) to reduce climate dimensionality. We focused on the first two principal components that described 95.9% of the overall variance and had eigenvalues of 3.33 and 0.50 for components 1 and 2, respectively. Principal component 1 (83.3% of variation) was significantly positively correlated with the four BIOCLIM variables ($r > 0.846$, $P < 0.0001$) and negatively correlated with elevation ($r = -0.972$, $P < 0.0001$), whereas principal component 2 (12.6% of variation) was significantly positively correlated with annual precipitation ($r = 0.488$, $P = 0.018$), negatively correlated with temperature seasonality ($r = -0.439$, $P = 0.036$) and not significantly correlated with elevation ($r = 0.186$, $P = 0.395$). A climate distance matrix was calculated as the Euclidean distance between each population based on PC 1 and PC 2 values.

Population genetic diversity estimates

We extracted genomic DNA from leaf tissue raised from seed ($N = 950$) using a modified CTAB protocol (Lin & Ritland 1995) and obtained genotypes for 11 codominant markers – including 3 single-copy, nuclear-gene-intron-length markers (Fishman & Willis 2005; Sweigart *et al.* 2006; Lowry *et al.* 2008) and eight microsatellite markers (Kelly & Willis 1998) – for population genetic analyses (Table S2, Supporting information). These markers, including the microsatellites, do not follow a simple three/four base-pair (bp) mutation model as one-bp insertion–deletion mutations are very common in *Mimulus* in noncoding regions of the genome (Lowry *et al.* 2008; Oneal *et al.* 2014). Genotypes were obtained with an ABI 3730 DNA Analyzer, and alleles were scored visually in GENEMARKER (SoftGenetics LLC, State College, PA, USA). A random subset of individuals was reanalysed to verify repeatability of marker scores. Marker scores were consistent across repeatability tests. All loci occur within different linkage groups and can be considered genetically independent. We tested for Hardy–Weinberg equilibrium within populations using GENALEX version 6 (Peakall & Smouse 2006).

Null alleles, which occur when particular microsatellite alleles fail to amplify during PCR, could potentially introduce error into our analyses. Evidence for null alleles can be found in deviations from Hardy–Weinberg

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equilibrium, more specifically as an excess in the frequency of homozygous sites. However, given the highly inbreeding nature of *M. laciniatus* ($F_{IS} > 0.86$; Table 1), excess in homozygosity is expected due to the biology of the species. The software package INEST (Chybicki & Burczyk 2009) simultaneously estimates null allele frequencies and inbreeding. Using this method, we tested two models for each of our sampled populations: (i) a model including genotyping error, inbreeding and null alleles and (ii) a model including genotyping error and inbreeding. Comparison of deviance information criterion values revealed that these models were equivalent for 20 of 23 populations, providing little support for null alleles. In three populations (A-6, B-6, A-HIGH), the model including null alleles was slightly favoured, but frequency of null alleles was estimated to be, on average, <0.10.

Population genetic estimates were averaged across loci for all populations (Table 1). We used GenAlEx to calculate inbreeding levels (F_{IS}) and the number of private alleles from a randomized, rarefied sample based on the minimum population sample size ($N = 30$). Average allelic richness and gene diversity were calculated in FSTAT 2.9.3.2 (Goudet 2001).

Gene flow

Population connectivity and genetic distance estimates. We used a graph-theoretic approach (Dale & Fortin 2010) to visualize and estimate connectivity and genetic distances among populations across the species range. This method determines the minimum set of connections (termed 'edges' in graph theory) between populations that account for genetic covariance across the study set of populations. The strength and distribution of genetic covariance among populations are measured in multidimensional genetic space using a mapping procedure (Smouse *et al.* 1982). Significant connections between populations are identified simultaneously with correction for multiple tests (see Dyer & Nason 2004; Dyer *et al.* 2010). The resulting graph network (topology) represents the set of connections between populations that describes significant genetic covariance across the study area. We generated the graph network using the program Population Graphs (http://dyerlab.bio.vcu.edu/docs/popgraph.html) and visualized it with the program GRAPH (GENETICSTUDIO SUITE, Build 131, Dyer 2009). This process allowed us to assess which populations were significantly more or less genetically differentiated than expected by spatial distance alone. Under a model of isolation by distance (IBD), the spatial separation between populations is predicted to be inversely correlated with genetic covariance. As a consequence, the spatial separation of sampled populations can be used as a null hypothesis to examine deviance in the underlying dispersal process from a model of strict IBD. The deviance from this model can be identified in two ways: populations experiencing long-distance gene flow will be more geographically separated ('extended') than expected given the genetic topology, whereas populations whose connectivity (gene flow) is restricted in some way will be closer spatially ('compressed') than predicted by the genetic topology (Dyer *et al.* 2010). Compressed and extended connections are determined based on χ^2 tests (see literature accompanying the GENETIC STUDIO SUITE, Build 131, Dyer 2009). We also estimated centrality 'degree' values (Dyer 2009) for each population, which is simply the number of significant connections linking a given population to other populations in the graph network topology.

Genetic distances between populations were estimated from graph distances derived from the graph-theoretic approach. Graph distance, based on genotypic differences (Dyer & Nason 2004), can be a more accurate representation of genetic distance than allele frequencies (*e.g.* F_{ST}), which are influenced by between-population diversity differences (Charlesworth *et al.* 1997; Hedrick 2005). This is particularly true in highly inbreeding species such as *M. laciniatus*. To account for spatial distance in our estimates of genetic distance, we incorporated spatial distance between populations by first running a Mantel test with spatial distance as the predictive matrix and graph distance as the response matrix; residuals from this analysis were then used to calculate pair-wise graph distance estimates between populations (Table S3, Supporting information). We also calculated the mean distance between each population and all other populations, hereafter referred to as *mean graph distance*.

Gradient effects. We tested for range-wide associations between genetic distance, spatial distance (isolation by distance – IBD) and climate distance (isolation by environment – IBE) using three methods.

First, we compared population differentiation among elevation-based transects, among populations within transects and among all populations using AMOVA (Excoffier *et al.* 1992) in the GENO program (GENETICSTUDIO SUITE, Build 131, Dyer 2009). Additionally, we used Mantel tests and partial Mantel tests in the Manteller program in GENETICSTUDIO to test for range-wide associations between interpopulation spatial distances, genetic distances and climate distances (from BIOCLIM estimates; see previous section). Partial Mantel tests use residuals taken from an initial Mantel test between matrices X (e.g. spatial distance) and Y (e.g. genetic distance) to account for variation between X and Y in a subsequent Mantel test between matrices Y and Z (e.g. climate distance). Finally, we used a multiple matrix regression with randomization (MMRR) approach

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(Wang 2013) as an alternative method to the Mantel procedures, where climate distance (β_E) and spatial distance (β_D) were simultaneously regressed against genetic distance.

Contemporary gene flow. We used BAYESASS (Version 3.0, Rannala 2007) to estimate rates of recent immigration (over the last several generations) and directional gene flow to identify potential source–sink relationships across the range. BAYESASS does not assume Hardy–Weinberg or migration-drift equilibria, but it does assume that all potential sources of immigration have been sampled, an assumption that is easily violated. We proceeded based on the broad population representation of our sampling scheme across the elevation gradient and at range limits. We ran the BAYESASS MCMC for 10 000 000 iterations after an initial burn-in period of 5 000 000 iterations in which log likelihood values had peaked, sampling once every 2000 iterations. Allele frequency, migration rate and inbreeding rate parameters were optimized following Wilson & Rannala (2003) and Rannala (2007) and post-optimized MCMC runs started from different seeds showed consistent migration rates. Contemporary migration rate estimates between populations and their associated 95% credible sets are reported in Table S4 (Supporting information). To estimate the extent to which a given population received more immigrants or sent more emigrants, we first calculated the *net immigration rate* into a given population, A, from another population, B, from the BAYESASS contemporary immigration rate estimates:

$$\text{net immigration rate}_{B\Rightarrow A} = \text{IMM}_{B\Rightarrow A} - \text{IMM}_{A\Rightarrow B}$$

where $\text{IMM}_{B\Rightarrow A}$ is the contemporary immigration rate into Population A from Population B, and $\text{IMM}_{A\Rightarrow B}$ is the contemporary immigration rate into Population B from Population A. We then calculated the *mean net immigration rate* for a given population from all of its paired net immigration rate values between all other populations. Positive mean net immigration values indicate populations that receive more immigrants per generation than they produce as emigrants, whereas negative mean net immigration values indicate populations that contribute more emigrants to other populations than they receive as immigrants.

To test for immigration disparity at range limits, we estimated net immigration rates between each edge population and its nearest interior transect neighbour population. Finally, to test whether populations differ from one another in the degree to which they are composed of recent immigrants, *total immigration*, the sum of all estimated contemporary immigration rates (from all other populations) was estimated for each population. Net immigration, mean net immigration and total immigration rates were used in range-wide correlations and paired tests as described below.

Statistical tests of centre-edge patterns

We generated correlations and used two-tailed significance tests to detect associations of all genetic measures with Relative Elevation and the Edge Index. To test for the influence of transect on these associations, we included the effects of transect and the interaction between transect and Relative Elevation or the Edge Index for each respective model using an ANCOVA approach where transect was a categorical fixed effect, and Relative Elevation or the Edge Index were continuous variables. Akaike information criterion with correction for finite sample size (AICc) and Bayesian information criterion (BIC) statistics were used to evaluate full models (with transect effects) and reduced models (without transect effects). Additionally, we used matched-pairs tests to detect step-down changes in diversity at range limits (*sensu* Caughley *et al.* 1988; also see Hoffmann & Blows 1994), where edge populations were compared to the closest neighbouring interior population within their transect. The average spatial distances and altitude-based differences between these population pairs were 5.1 km and 356 m, respectively. Pairwise correlations were conducted in JMP (Version 12.0.1). Two-tailed, matched-pairs tests, where edge populations were compared to their closest neighbouring interior population within transects, were conducted as Wilcoxon signed-rank tests through randomized permutations. Mean graph distance and mean net immigration rate estimates derived from pairwise values are not statistically independent, and thus, we used permutations to generate null distributions (e.g. as in Yakimowski & Eckert 2008). Permutation-based correlations and signed rank distribution values were generated through the SPEARMAN and WILCOXON test procedures in the COIN package (Zeileis *et al.* 2008) using R statistical software (R Core Team 2014). We also tested for correlations between population area and plant density (two proxies for abundance) and population genetic estimates. Population area and plant density were square root and $\log_{10}$ transformed, respectively, to meet assumptions of normality. Edge Index and total immigration data were rank-transformed (Conover & Iman 1981) as other transformations failed to improve normality.

Results

Population area and density

Population area did not vary across elevation or centre-edge gradients (Tables 2 and 3). However, plant density

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increased towards both high- and low-elevation limits (Tables 2 and 3, Fig. 3a,b), contrary to central-marginal expectations. Population area and plant density did not significantly correlate with each other or any of the population genetic estimates (Tables 2 and 3).

Diversity

All genetic markers successfully amplified across populations and were highly polymorphic (Table S5, Supporting information). The overall inbreeding estimate (F_{IS}) was 0.93, and the among-population differentiation estimate (Φ_{ST}) was 0.177. This population differentiation level is lower than would be expected for highly selfing plants (e.g. mean G_{ST} = 0.553 for selfing annuals, Hamrick & Godt 1996; mean F_{ST} = 0.42 for selfing plants, Nybom 2004) and suggests moderate gene flow among populations. As expected for a selfing species, all loci in all *M. laciniatus* populations were not at Hardy–Weinberg equilibrium ($P < 0.001$). Inbreeding estimates (F_{IS}) increased significantly at higher elevations, but not towards marginal populations *per se* (Figs 3c,d, Tables 2 and 3). There was a slight, marginally nonsignificant increase (2.1%) in F_{IS} for edge populations compared to interior neighbouring populations ($P = 0.094$, Table 3).

Within-population genetic diversity did not significantly change along centre-edge gradients. Additionally, there were no significant transect, transect-by-Relative-Elevation or transect-by-Edge-Index interaction effects on genetic diversity and inbreeding rate estimates. Reduced models performed better than models including transect effects (see Table S6, Supporting information for ANCOVA results and AICc and BIC comparisons). Genetic diversity did not significantly change towards range limits for continuous correlations (Table 2). However, a significant reduction in genetic diversity in edge populations, compared to neighbouring, interior and transect populations was detected in mean allelic richness (mean drop of 17.8%, $P = 0.031$) and the number of private alleles per locus (mean drop of 46.6%, $P = 0.031$) using categorical, matched-pairs analyses (Table 3, Fig. 3e,f). Mean gene diversity tended to be lower (9.7% lower) in edge populations compared to interior neighbouring populations, but this pattern was not statistically significant at an alpha level of 0.05 ($P = 0.094$, Table 3). All populations contained private alleles (Table 1).

Gene flow

Connectivity. Connectivity did not differ significantly across the elevation gradient or with peripherality. Edge populations did not differ from central populations in the number of significant connections linking a given population to other populations (centrality degree) (Tables 2 and 3). This suggests that edge populations share genes with an equivalent number of populations to nonperipheral populations. Figure 4a shows the graph network superimposed onto the study area. When compressed and expanded edges (i.e. low and high connections of gene flow, respectively) are visualized (Fig. 4b), several notable patterns emerge: 1)

Table 2 Tests of continuous geographic variation among 23 populations of *Mimulus laciniatus* sampled across its geographic range in the Californian Sierra Nevada. Correlations were estimated from REML in JMP (version 9.0.2) or randomized permutation tests (spearman_test in the COIN package, Zeileis *et al.* 2008; R statistical software, version 3.2.0). See text for explanation of variables

	Correlation test statistics					
Variable	Mean	Range	Area (ha)	Plant Density	Rel. Elev.*	Edge Index*
Population size and density						
Area (ha)	0.61	0.0031–2.99	—	−0.01	−0.13	−0.11
Plant density (inds./m^2)	1278	433–2808	−0.01	—	0.03	**0.62****
Genetic diversity and differentiation						
Number of Private Alleles (rarefied)	1.08	0.18–2.09	0.10	0.23	−0.24	−0.04
Allelic Richness	9.18	6.94–11.60	0.17	0.05	0.02	−0.22
Gene Diversity	0.68	0.57–0.78	0.24	0.16	−0.00	−0.12
Inbreeding Coefficient (F_{IS})	0.93	0.86–0.98	−0.31	0.01	**0.56****	0.19
Mean graph distance	31.90	26.36–38.96	0.22†	1.00†	−0.82†	0.94†
Total immigration rate	0.21	0.11–0.33	0.27	0.42	−0.02	0.21
Mean net immigration rate	0.00	−0.02 to 0.01	−1.67†	0.62†	−1.00†	−0.16†
Centrality degree	6	3–9	−0.07	−0.012	**−0.38**	−0.31

Bold coefficients are significant (*$P \leq 0.05$, **$P < 0.01$) or marginally nonsignificant ($0.10 > P > 0.05$).
†Permuted asymptotic correlation tests. Values are Z values, and significance values (P values) are derived from the exact conditional distribution of Z (Zeileis *et al.* 2008).

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Table 3 Tests of categorical (edge vs. interior) geographic variation among 23 populations of *Mimulus laciniatus* sampled across its geographic range in the Californian Sierra Nevada. Two-tailed, matched-pairs tests, where edge populations were compared to their closest neighbouring interior population within transects, were conducted as Wilcoxon signed-rank tests through randomized permutations (wilcox. test in the COIN package, Zeileis *et al.* 2008; R statistical software, version 3.2.0). Statistics are *V* values from permuted signed rank tests. See text for explanation of variables

Variable	Edge mean	Interior mean	d.f.	*V*	*P**
Population size and density					
Area (ha)	0.50	0.39	5	12	0.844
Neighborhood density (inds./m^2)	1908	1314	4	15	**0.063**
Genetic diversity and differentiation					
Number of private alleles (rarefied)	0.79	1.48	5	0	**0.031**
Allelic richness	8.23	10.01	5	0	**0.031**
Gene diversity	0.65	0.72	5	2	**0.094**
Inbreeding coefficient (F_{IS})	0.94	0.92	5	19	**0.094**
Mean graph distance	32.49	32.75	5	8	0.688
Total immigration rate	0.22	0.24	5	10	1.0
Mean net immigration rate	0.004	0.005	5	5	0.313
Centrality degree	6.33	5.83	5	14.5	0.462

*Bold coefficients are significant ($P < 0.05$) and marginally non-significant ($0.10 > P > 0.05$).

cogradient gene flow (i.e. gene flow between transects at similar elevations) is common throughout the range, linking populations across great distances; 2) greater differentiation than expected by spatial distance is common between nearby populations within the same watershed (i.e. canyon or valley), suggesting reduced effective gene flow between differing elevations; 3) populations at the range limit are mostly (4 of 6 populations) highly differentiated ('compressed') from their nearest interior transect population, suggesting that elevation-based genetic structuring occurs even for peripheral populations.

Genetic distance. Edge populations were as differentiated as the average population within the study. Mean graph distance did not significantly differ at ($P = 0.688$) or towards ($P = 0.349$) range limits (Fig. 5a,b, Tables 2 and 3).

Gradient effects. The above connectivity patterns in graph analysis are consistent with a pattern of range-wide genetic isolation by environment (IBE) based on climate differences and not with a pattern of isolation by distance (IBD). IBE across the elevation gradient was further verified by three methods: AMOVA, whereby within-transect genetic variance was greater than between-transect variance; and partial Mantel and matrix regression tests, whereby IBE was significant and IBD was not when both effects were taken into account.

The AMOVA revealed significant genetic differentiation among transects, among populations within transects and among all populations ($\Phi_{ST} = 0.177$), but variance (i.e. population differentiation) was much higher (>6 times) within elevation-based transects ($\Phi_{SR} = 0.157$) than among transects ($\Phi_{RT} = 0.024$) that span several watersheds of the species range (Table S7, Supporting information). All test strata were significant at $P < 0.001$.

The isolation-by-genetic distance Mantel test was not significant across the species range ($Z(obs) = 8388.33$, $r = 0.023$, $P = 0.326$), whereas the climate distance-by-genetic distance Mantel test was significant ($Z(obs) = 40084.68$, $r = 0.117$, $P = 0.021$). These results were confirmed in the partial Mantel tests that accounted for covariance between climate and spatial distances. The partial climate distance-by-genetic distance Mantel test (based on residuals removed from spatial distance-by-genetic distance Mantel) remained significant ($Z(obs) = 39357.52$, $r = 0.110$, $P = 0.032$), whereas the partial spatial distance-by-genetic distance Mantel test (based on residuals removed from climate distance-by-genetic distance Mantel) remained non-significant ($Z(obs) = 7678.10$, $r = -0.018$, $P = 0.637$).

Using the MMRR approach, climate distance (IBE) was found to significantly correspond to genetic distance ($\beta_E = 0.154$, $P = 0.031$), whereas spatial distance did not significantly correspond to genetic distance ($\beta_D = -0.032$, $P = 0.656$).

Contemporary gene flow (Bayesian modelling). Bayesian analysis of recent gene flow detected no source–sink relationship between centre and edge populations. Contemporary gene flow rate estimates between specific population pairs varied considerably (0.004–0.178) as did estimates of total immigration for each population (0.110–0.328). Across the range (Table 2) and at range limits (Table 3), mean net immigration rates did not vary significantly (Fig. 5c,d). There was no difference between rates of recent immigration when comparing both geneflow directions (centre-to-edge versus edge-to-centre) when examining edge populations and the interior populations nearest to them along each transect. The average centre-to-edge immigration rate was 0.005, whereas the average edge-to-centre immigration rate was 0.047 (permuted paired signed-rank test, $V = 17$,

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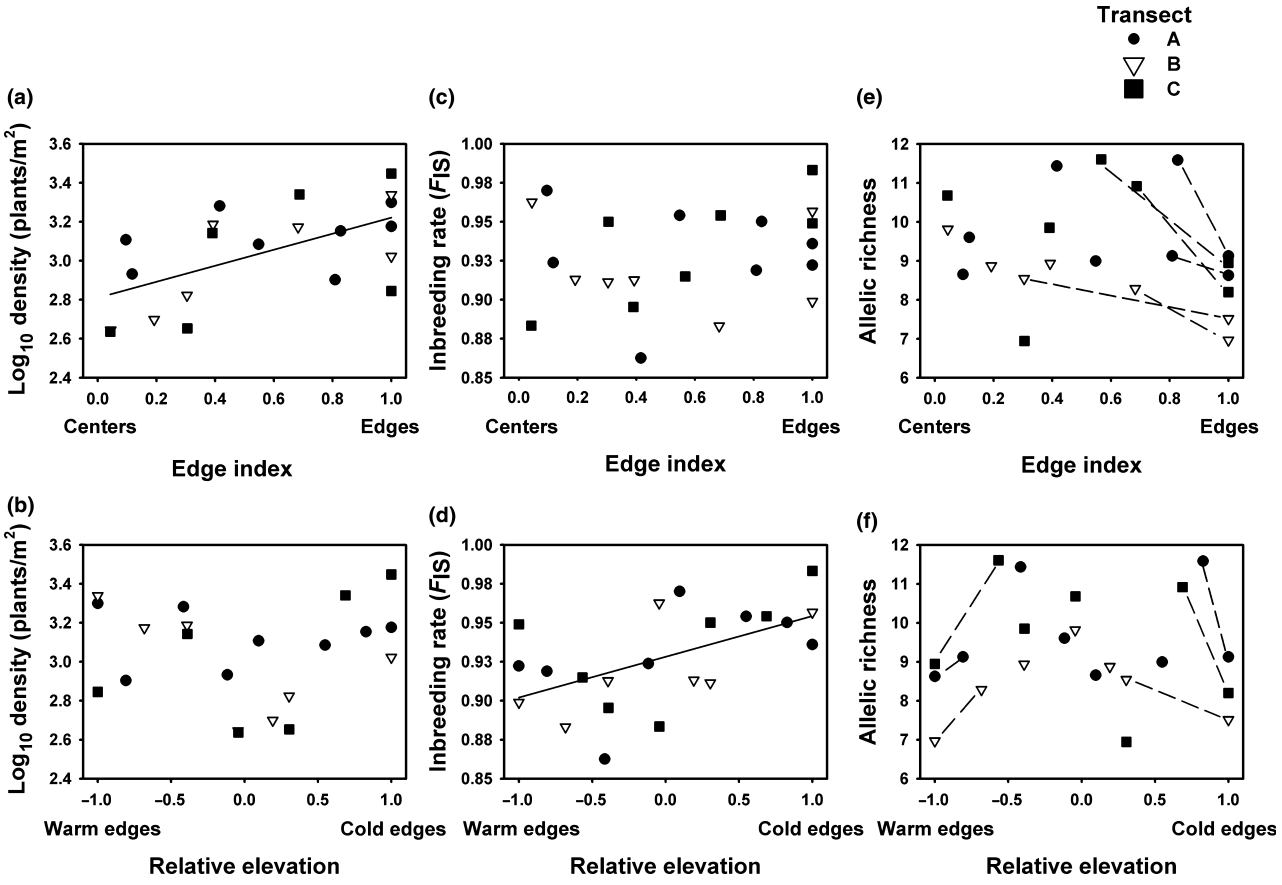

Fig. 3 Variation in plant density (a, b), inbreeding rates (F_{IS}) (c, d) and within-population allelic richness (e, f) with the Edge Index (top panels) and Relative Elevation (bottom panels). Linear fit of data is presented for reference only where variables are significantly correlated; see Table 2. Dashed lines identify edge populations, which had reduced allelic richness compared to their nearest interior neighbour within transects; see Table 3.

d.f. = 5, $P = 0.203$). Finally, the estimated proportion of individuals within populations that were recent immigrants did not vary significantly across the range (Table 2), or at range limits (Table 3), inconsistent with the hypothesis that edge populations are maintained or under heavy migration load (swamped) by contemporary gene flow.

Discussion

Centre-edge dynamics do not appear to contribute strongly to the current range limits of *Mimulus laciniatus*. Patterns of abundance, genetic variation and gene flow were not consistent with two common hypotheses of range limit formation: (i) failure of edge populations due to small population size (drift); (ii) failure to adapt to the conditions of range edges due to migration load from the centre of the range. Overall, peripheral populations appear to be similar to central populations with regard to diversity and connectivity. However, there was a surprising increase in plant density towards both warm and cold elevation limits. Although weak increases in inbreeding were detected towards higher elevations, genetic differentiation was fairly consistent and moderately high among all populations ($\Phi_{ST} = 0.177$). Further, although contemporary geneflow rates varied greatly among populations, no directional signals were detected between central and peripheral areas at either cold or warm limits. Isolation by environment (IBE) based on climate estimates, but not isolation by distance (IBD), was found across the species range. IBE implicates climate structuring as an important factor in range-wide processes through climate adaptation, nonrandom mating or both. We discuss these findings in the contexts of potential range limit mechanisms and conservation below.

Abundance, genetic diversity and connectivity towards warm and cold range edges

Genetic diversity and connectivity were consistent across the species range, whereas one proxy for

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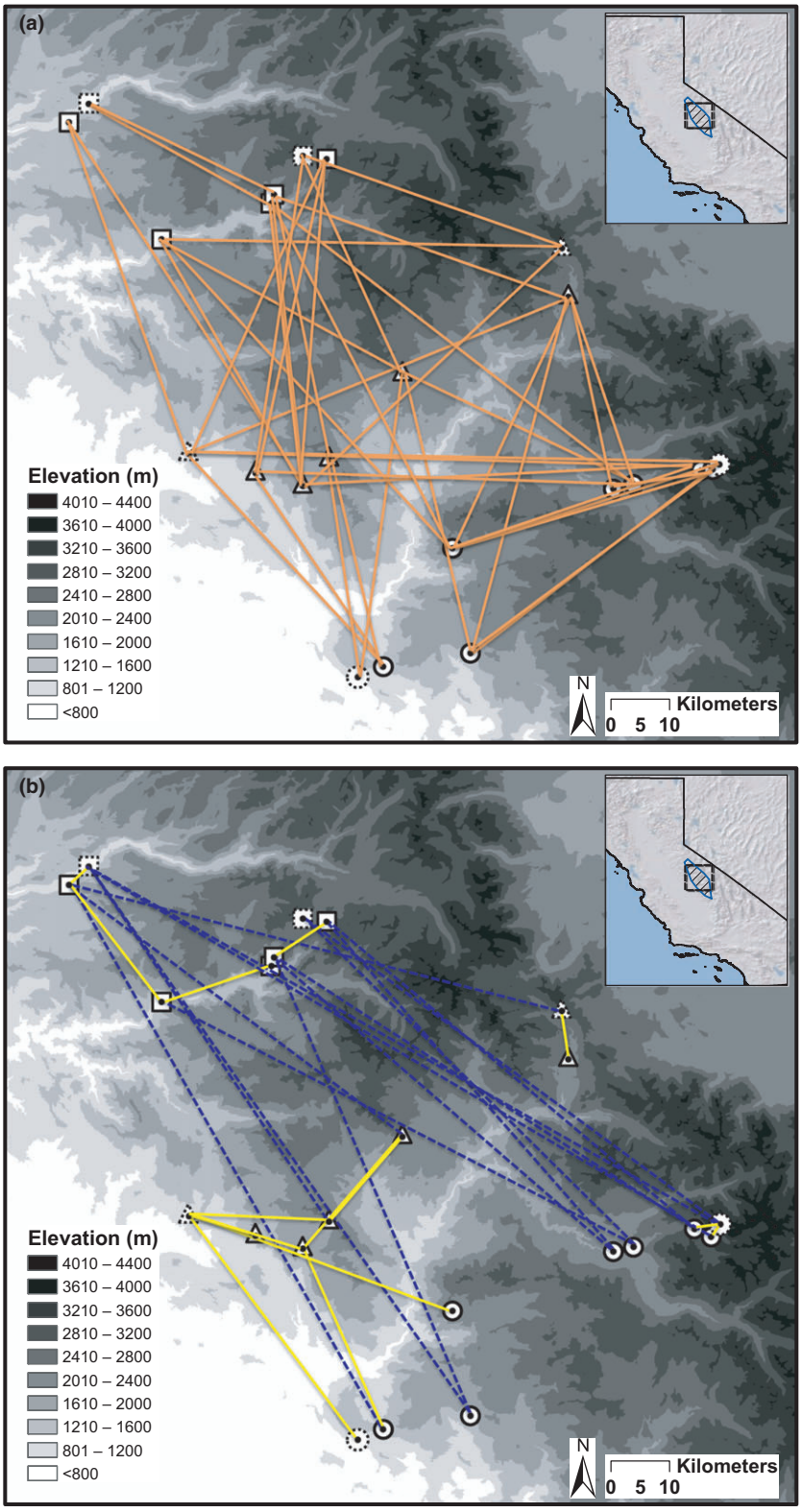

Fig. 4 (a) *Mimulus laciniatus* graph-theoretic topology (orange lines) superimposed onto the study area in the California Sierra Nevada. Lines ('edges') represent significant components of among-population genetic variation due to the connecting populations (nodes) (b) Population Graphs topology showing compressed (solid yellow) and extended 'edges' (dashed blue) – lines representing significant components of among-population genetic variation. Compressed edges represent greater differentiation than expected by spatial distance (i.e. reduced effective gene flow), whereas extended lines represent greater genetic similarity than expected by spatial distance (i.e. increased effective gene flow). Circles = Transect A, triangles = Transect B and squares = Transect C. Elevation extremes within each transect are represented by dashed symbol outlines.

abundance (plant density) increased towards range limits. Genetic diversity was reduced at both high- and low-elevation range limits relative to the nearest interior populations surveyed. However, edge population diversity was generally similar to that of central populations. Several interior populations that were compared to

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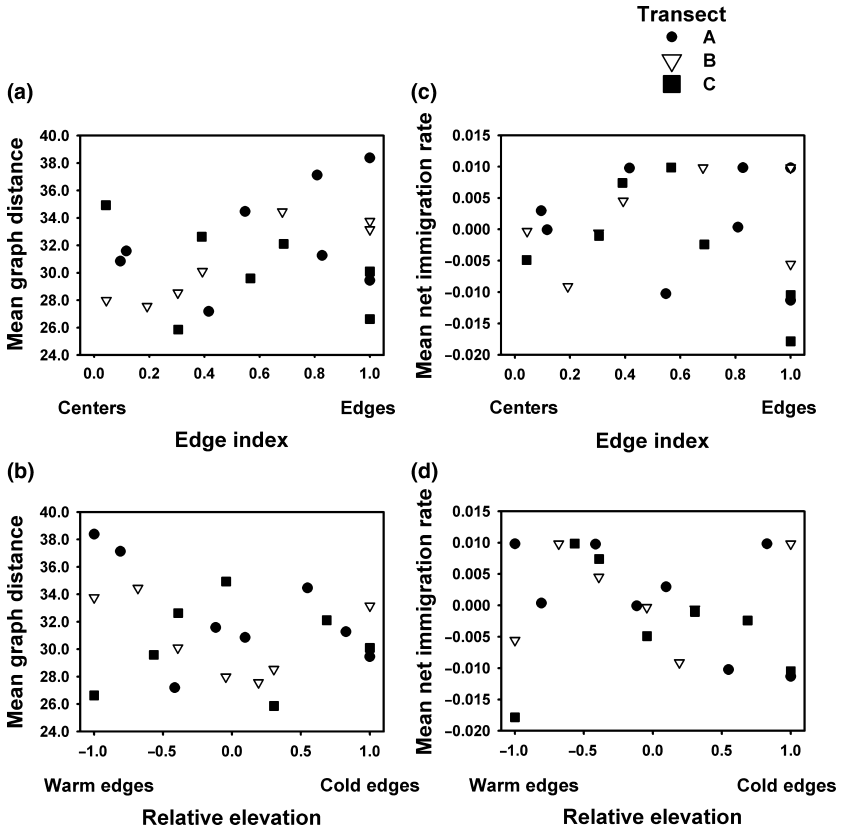

Fig. 5 Variation in mean genetic differentiation, estimated from mean graph distance (a, b) and contemporary mean net immigration rate (c, d) with the Edge Index (top panels) and Relative Elevation (bottom panels). Negative mean net immigration values indicate greater immigration, whereas positive values indicate greater emigration.

neighbouring edge populations had above-average genetic diversity (Fig. 3e,f). Most studies examining genetic diversity at range limits have focused on high-latitude limits (Eckert *et al.* 2008) – so it is unclear whether the observed increases in differentiation and decreases in diversity at range limits among studies are generally representative or unique to the history of cold climate latitudinal limits (e.g. post-glacial expansion). More investigations that examine low-elevation and low-latitude range limits are needed. We do not find general support for increased genetic drift towards elevation limits: genetic connectivity was similar and abundance actually increased towards high- and low-elevation limits. Support for this drift hypothesis associated with an abundant centre model has been mixed among different plant species, with contradictory evidence even within the same species (compare Samis & Eckert 2007; Yakimowski & Eckert 2008; Byars *et al.* 2009; Vaupel & Matthies 2012; Dixon *et al.* 2013; Stanton-Geddes *et al.* 2013; Griffin & Willi 2014). Such discordance is also common across nonplant species (Sagarin & Gaines 2002; Eckert *et al.* 2008; Sexton *et al.* 2009; Abeli *et al.* 2014) and may stem from the fact that many processes besides habitat quality can influence relative abundance, genetic diversity and genetic connectivity (Brown 1984). For example, patterns in neutral loci may approximate average genomewide levels of genetic variation influenced by past demographic events such as glacial refugia (e.g. Beck *et al.* 2008). Additionally, differences in life-history attributes, such as the high self-fertilization rate in *M. laciniatus*, may influence centre-edge pattern variation among species. Moreover, even when habitat quality does influence ecological and evolutionary processes, habitat quality may not change predictably across centre-edge gradients of a species range (Lira-Noriega & Manthey 2014).

Our elevation-based study was not consistent with expectations from leading-edge/trailing-edge frameworks in the context of global warming (*sensu* Hampe & Petit 2005). Population differentiation, connectivity and population diversity did not differ between low- (rear) and high-elevation (leading) climate limits. Further, we did not detect a net movement of individuals towards higher elevations as would be expected under a leading-edge scenario. We did detect a pattern of increased inbreeding with increased elevation, although inbreeding levels were very high for all populations examined ($F_{IS} > 0.86$). It is yet unclear whether this pattern has biological or ecological implications. Griffin & Willi (2014) found increased inbreeding in marginal areas of the *Arabidopsis lyrata* range in North America,

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consistent with the hypothesis of reproductive assurance in marginal environments (see Levin 2010; Hargreaves & Eckert 2014). However, other systems do not exhibit this pattern (e.g. de Waal *et al.* 2014). Increased plant density towards both climate limits found here remains unexplained, but could be related to the availability of suitable open-canopy environments in the lower and upper elevations of the Sierra Nevada (i.e. chaparral and subalpine habitats) where dense forests give way to the exposed rock faces, which are ideal habitats for *M. laciniatus* (Peterson *et al.* 2013; Ferris *et al.* 2014). It is a clear and important research need to understand under which conditions (e.g. elevation or latitudinal limits, varying life forms, varying mating systems, etc.) populations are contracting or expanding their ranges as a result of climate change (Angert *et al.* 2011). Warm and cold elevation limits do not show signs of range instability in this highly selfing annual plant.

Gene flow across the species range: isolation by environment

Isolation by climate environment (IBE) was evident across the species' range. This finding is consistent with studies of a close relative, *Mimulus guttatus*. Waser *et al.* (1982) found that climate (e.g. date of snowmelt) was the best predictor of differentiation and mating success among *M. guttatus* populations within and among different canyons in the Rocky Mountains of Utah. IBE is driven by environmentally mediated selection and/or nonrandom mating associated with environmental gradients, and the absence of IBD found in this system suggests that genetic patterns across the species range are unlikely to be the product of drift and dispersal limitation alone. *Mimulus laciniatus* populations, and populations within other species in the *Mimulus guttatus* species complex, exhibit genetically based differences among elevations in critical photoperiod to flowering (Friedman & Willis 2013). This coupled with evidence of elevation-based adaptation (Sexton *et al.* 2011) suggests that IBE is maintained at least partially by selection. Despite climate-based IBE in *M. laciniatus*, not all populations experiencing similar elevations are well connected. For instance, two warm-edge populations (A-Low and B-Low, Table 1) appear to be more isolated than expected by geographic distance (Table S3, Supporting information, Fig. 4a,b). The farthest low-edge populations in this study (A-Low and C-Low, Table 1) produced the fittest offspring when mated to each other and sown in a warm-edge garden study (Sexton *et al.* 2011), offering clues to how IBE may be favoured by selection: increased fitness from matings originating from similar environments versus flowering-time mismatches between disparate elevations.

Regarding causes of range limits

Although we cannot directly infer the causes of elevation range limits of *M. laciniatus* with these findings, we can point out several models of range limitation that are inconsistent with our study. As discussed above, it is unlikely that drift resulting from gradual declines in habitat quality or isolation is responsible for range limits in *M. laciniatus*. Changes in habitat quality and availability or biological interactions (e.g. competition) just beyond current limits may be important (Sexton & Dickman 2016). The change to chaparral environments at lower elevations, which lack winter snowpack, and to drier eastern Sierra environments beyond the crest at higher elevations (Barbour *et al.* 2007) may represent critical ecological transitions where the slow-draining, rocky seeps upon which *M. laciniatus* grow become rare or absent.

The results of our study (IBE with respect to climate) suggest that we can rule out swamping gene flow as a major factor regulating range limits in this system (Sexton *et al.* 2014). To our knowledge, a migration load effect has yet to be clearly demonstrated in maintaining species borders (but see Magiafoglou *et al.* 2002; Fedorka *et al.* 2012 for consistent patterns). Further, the theoretical basis for swamping gene flow limiting range limits may be tenuous. For example, Barton (2001) showed that if genetic variance is allowed to evolve across the range, stable range limits no longer form in the face of large migration loads (as in Kirkpatrick & Barton 1997). Our finding of IBE with respect to the relatively steep elevation gradient across the *M. laciniatus* species range demonstrates why swamping gene flow may be a rare cause of range limits.

Insufficient genetic variation to respond to natural selection at range limits may be a property of edge populations (Holt 2003; Blows & Hoffmann 2005; van Heerwaarden *et al.* 2009; Dawson *et al.* 2010). However, insufficient genetic variation to expand limits (i.e. the niche) may also be a species-wide constraint; that is to say, the genetic variation necessary to respond to selection beyond limits may not exist within a species (Blows & Hoffmann 1993; Kellermann *et al.* 2009). Deficient genetic variation to respond to conditions beyond the current range is likely to be an important cause of range limits in *M. laciniatus*, but requires further examination (e.g. Gould *et al.* 2014). A study by Sexton *et al.* (2011) using experimental crosses and subsequent common garden experiments suggests that there is climate adaptation in *M. laciniatus,* but that edge populations may also suffer from limited genetic variation. It is yet

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unknown whether these findings are specific to edge populations or apply generally across the range of *M. laciniatus*.

Concluding remarks and conservation implications

We conclude that populations of *M. laciniatus* are mainly structured by climate across the species range. Further, our study does not support several common centre-edge models for patterns of abundance, genetic variation and gene flow towards range limits. Elevation-based climate limits may be present due to a number of untested mechanisms, including evolutionary constraints on adaptation to conditions beyond the range limit. However, we do not find evidence that they are due to centre-edge patterns of swamping gene flow or low abundance. We found that all populations across the range, both central and peripheral, can have unique, elevation-based alleles. We therefore conclude that gene flow among populations is likely to be an important source of adaptive genetic variation during rapid environmental shifts (e.g. climate change).

Understanding adaptive constraints of organisms and the effects of varying patterns of gene flow in stressful and rapidly changing conditions (*sensu* Frankham *et al.* 2011; Aitken & Whitlock 2013) is an important future research need. Further, understanding natural patterns and directionality of gene flow across species ranges can inform our understanding of how we might expect populations to respond to rapid climate shifts. For instance, our findings of IBE with elevation give clues to potential management options for assisted gene flow under climate warming (see Aitken & Whitlock 2013; Wilczek *et al.* 2014). That is to say, we can identify environments between which assisted gene flow may be more important due to background geneflow patterns and rates, as well as population sources for that gene flow. This information is critical for assessing risk and managing populations in differing areas of species ranges, including in leading-edge and rear-edge contexts (Hampe & Petit 2005). As more studies are made across a wide variety of systems, we may be able to predict how various factors (i.e. life forms, life histories and geographic scales) will influence patterns of gene flow and whether these patterns are likely to be more or less adaptive with rapid environmental change.

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J.S., S.S. and K.R. designed the study. J.S. collected field data and plant materials. J.S., M.H., D.L. and H.M. tested genetic markers and J.S., M.H. and A.B. collected genetic data. J.S. and A.B. conducted analyses. J.S. wrote the first manuscript draft, and all authors contributed revisions.

Data accessibility

Site plant density estimates, microsatellite genotypes, and pairwise estimates of genetic, geographic and climate distances have been deposited at Dryad: doi:10.5061/dryad.8qc40.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Climate variables for each study population of *Mimulus laciniatus* generated from BIOCLIM (data for years 1950–2000 at ~1 km scale; Hijmans *et al*. 2005).

Table S2 Marker loci used for population genetic analyses for 950 plant samples.

Table S3 Pair-wise graph distance (genetic distance accounting for spatial distance) estimates among all *Mimulus laciniatus* study populations.

Table S4 Average recent immigration rate estimates (with 95% confidence intervals in parentheses) across populations of *Mimulus laciniatus* generated from the program BAYESASS (Rannala 2007).

Table S5 Allelic diversity for each locus across sampled study sites.

Table S6 Results of analysis of covariance (ANCOVA) including effects of Relative Elevation or Edge Index and sampling transect on genetic measures.

Table S7 Results of analysis of molecular variance (AMOVA) for the distribution of genetic marker variation across 23 sampled populations of *Mimulus laciniatus*.

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