Population Genetics and the Evolution of Geographic Range Limits in an Annual Plant

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Online enhancement: zip file.

abstract: Theoretical models of species' geographic range limits have identified both demographic and evolutionary mechanisms that prevent range expansion. Stable range limits have been paradoxical for evolutionary biologists because they represent locations where populations chronically fail to respond to selection. Distinguishing among the proposed causes of species' range limits requires insight into both current and historical population dynamics. The tools of molecular population genetics provide a window into the stability of range limits, historical demography, and rates of gene flow. Here we evaluate alternative range limit models using a multilocus data set based on DNA sequences and microsatellites along with field demographic data from the annual plant *Clarkia xantiana* ssp. *xantiana*. Our data suggest that central and peripheral populations have very large historical and current effective population sizes and that there is little evidence for population size changes or bottlenecks associated with colonization in peripheral populations. Whereas range limit populations appear to have been stable, central populations exhibit a signature of population expansion and have contributed asymmetrically to the genetic diversity of peripheral populations via migration. Overall, our results discount strictly demographic models of range limits and more strongly support evolutionary genetic models of range limits, where adaptation is prevented by a lack of genetic variation or maladaptive gene flow.

Keywords: coalescent, colonization, demographic history, gene flow, population genetic structure, metapopulation dynamics.

Introduction

The ecological and evolutionary causes of geographic range limits are a focus of increasing empirical and theoretical interest because range limits confront us with a fundamental problem in evolutionary biology—why, barring dispersal limitation, does a species not expand its range (Antonovics 1976; Geber 2011)? Distinguishing among the potential causes of geographic range limits requires some understanding of history, just as analyses of adaptive differentiation among populations require an assessment of historical processes and contingencies (Kawecki and Ebert 2004; Keller and Taylor 2008). Each of the major classes of models of range limits implicates population processes, many of which have taken place over long time periods or cannot be observed easily in the field. Insights into historical population dynamics—especially colonization history, population turnover, and population size changes—can be gained using molecular population genetics (e.g., François et al. 2008; Ross-Ibarra et al. 2008; Keller et al. 2010). In the first section of this article, we review the literature and highlight connections between theory on geographic range limits and the molecular population genetics of structured populations. In the remainder of the article, we examine the evidence for alternative models of range limits using population genetic data from *Clarkia xantiana* ssp. *xantiana* (Onagraceae), an annual plant endemic to the southern Sierra Nevada of California.

Nonequilibrium versus Stable Range Limits

Geographic range limits are not necessarily in equilibrium with current environments, especially during the process of biological invasions (Baker and Stebbins 1965; Sakai et al. 2001) or in landscapes where there has been historical or recent climate change. Dispersal-limited species in postglacial landscapes have often failed to recolonize suitable habitat (Svenning and Skov 2004), and current climate change has already caused range shifts for vagile organisms (Parmesan 2006). Ideally, an investigation of the causes of geographic range limits determines whether range limits are currently expanding, have recently been reached, or have remained stable over long time periods (the first two possibilities can be considered the recent arrival model of range limits; table 1). In many cases, ranges have expanded far too slowly to be observed, and we must turn to a fossil record, historical inference from population genetics, or field transplant experiments to identify range disequilibrium.

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Table 1: An overview of population genetic predictions under alternative models of range limits

Model of range limit	Molecular variation	Unique molecular variation	Frequency distribution of variants	Population structure among peripheral populations	Pattern of gene flow between center and edge
Recent arrival	Variable level of reduction depending on the age and rate of range expansion	Variable level of reduction depending on the age and rate of range expansion	Excess of rare variants in peripheral populations	Higher differentiation among peripheral populations under propagule-pool dynamics	Moderate to high rates of asymmetric gene flow from central to peripheral populations
Metapopulation dynamics at the range periphery	Strong reduction in diversity in peripheral populations	Strong reduction in unique genetic variation in peripheral populations	Excess of common variants in peripheral populations (e.g., elevated variance in Tajima's *D*)	Lower differentiation among peripheral populations under migrant-pool dynamics	No clear expectation
Source-sink dynamics	Strong reduction in diversity in peripheral populations	No unique genetic variants in peripheral populations	Excess of common variants in peripheral populations (e.g., elevated variance in Tajima's *D*)	Higher differentiation among peripheral populations under propagule-pool dynamics	High rates of gene flow from central to peripheral populations
Maladaptive gene flow	No clear expectation	No difference expected between central and peripheral populations	Excess of rare variants in the range center if there has been historical growth and export of individuals; no clear expectation for peripheral populations	Differentiation among peripheral populations will depend on the pattern of gene exchange between central and peripheral populations	Moderate to high rates of asymmetric gene flow from central to peripheral populations, with limited differentiation between them
Genetic constraints	No clear expectation	Low levels of unique variation may suggest limited opportunity for adaptation to marginal environments	No clear expectation	No clear expectation	No clear expectation

Demographic Models of Stable Range Limits

Stable geographic range limits can be caused by both demographic phenomena and evolutionary genetic limits on adaptation (Holt and Barfield 2011). Demographic mechanisms depend in part on whether range limits occur across an environmental gradient and whether the distribution of suitable habitat is continuous or spatially structured. For species with spatial structure and discontinuous habitat, range limits can result from (1) a lower frequency of suitable habitat, (2) higher rates of population extinction (e.g., due to Allee effects), and (3) lower rates of colonization (Holt and Keitt 2000). Each of these factors can, individually and in combination, produce range limits without an environmental gradient (Lennon et al. 1997; Holt and Keitt 2000; Holt and Barfield 2011). In continuous, homogenous landscapes, variation in extinction and colonization rates are not likely to be sufficient to prevent range expansion. For example, Keitt et al. (2001) found that Allee effects alone can cause range limits in a patchy, spatially structured landscape but that they are insufficient across continuous, homogenous landscapes (the metapopulation dynamics model of range limits; table 1). Because most species occur in subdivided populations distributed patchily across landscapes, it will often be necessary to consider how the dynamics of colonization and extinction affect the population dynamics of peripheral populations.

Even when demographic phenomena are not the cause of range limits, information on population dynamics is important for examining evolutionary models of range limits. For example, when range expansion is limited by adaptation across environmental gradients, peripheral populations may be maintained entirely by immigration when dispersal rates are high (the source-sink dynamics model of range limits; table 1). In these sink populations, genotypes are maladapted to the environments they occupy and chronically fail to respond to selection, but populations persist. From an empirical perspective, demographic models of range limits suggest that it is important to assess the frequency and timescale over which population extinction and colonization occur; however, these phenomena are difficult—if not impossible—to observe in most real populations. Extinction and colonization are unlikely to occur at regular intervals or within the time frame of most field studies, especially for organisms that are less vagile, are long-lived, or have dormancy. Because these demographic phenomena leave signatures at the molecular level, population genetic data can provide important insights into historical population dynamics.

Evolutionary Genetic Models of Stable Range Limits

Evolutionary genetic models of stable range limits address why populations fail to adapt to environmental circumstances at or beyond the range edge (Kirkpatrick and Barton 1997; Barton 2001). These models are based on the biogeographic premise of the abundant center hypothesis, where environments are more favorable and populations have higher growth rates in the center than at the periphery of a species' range (Andrewartha and Birch 1954; Whittaker 1975; Brown and Lomolino 1998), such that dispersal (gene flow) is biased from center to edge. Biased gene flow can hamper adaptive evolution at the edge of a species' range in some circumstances (the maladaptive gene flow model of range limits; table 1; Antonovics 1976; Kawecki and Holt 2002). Theoretical analyses by García-Ramos and Kirkpatrick (1997) and Kirkpatrick and Barton (1997) provide detailed predictions for how the steepness of environmental gradients and rates of dispersal can influence the likelihood of range expansion. One important prediction is that when environmental gradients are steep, gene flow can cause peripheral populations to become extinct (or to become demographic sinks), resulting in the contraction of the range, whereas along a shallower gradient or when there is no gene flow, the range can expand via local adaptation. Because dispersal across geographic scales is rarely possible to observe in nature, inferences about the directionality of gene flow will nearly always require population genetic analysis.

Finally, the limited potential for populations to adapt to conditions outside their current distributions might not be influenced by patterns of gene movement or any population process but simply by inherent genetic constraints, such as trade-offs or genetic correlations (the genetic constraints model of range limits; table 1). Assessing the importance of genetic constraints to adaptation beyond range limits is largely a quantitative genetic enterprise, which is not informed to a great extent by information on neutral genetic variation. Although the introduction of variants from central to peripheral populations might influence the nature of trade-offs or genetic correlations, there are few clear predictions about patterns of neutral genetic diversity or population structure under this model.

Historical Insights from Molecular Population Genetic Data

Distinguishing among range limit models is challenging and requires the integration of different research approaches. Molecular population genetics alone is unlikely to provide clear support for any single model. Instead, it can provide a first step toward understanding population dynamics and direct more time-consuming and costly experimental efforts. Here we describe how different classes of molecular population genetic analyses can be used in combination to evaluate aspects of alternative models of range limits. We emphasize that any single result is typi-

cally consistent with more than one range limit model (table 1) and that more than one of these simplistic models is likely operating in nature.

Sequence Polymorphism

If range expansion is limited by demographic phenomena, such as frequent extinction and recolonization, theory predicts reductions in absolute measures of diversity across the periphery of the species' range (e.g., θ_W or θ_π) and within populations (Pannell and Charlesworth 1999) as well as greater variance among populations (Wade and McCauley 1988; Pannell and Charlesworth 2000). Within-population diversity is reduced as a consequence of genetic bottlenecks associated with colonization, whereas regional (or specieswide) reductions in diversity arise from the high variance in reproductive success among lineages owing to the process of repeated colonization and population growth (Maruyama and Kimura 1980; Gilpin 1991; Whitlock and Barton 1997; Wang and Caballero 1999). The greater variance in diversity among populations results from stochasticity in colonization and variation in population age (Wade and McCauley 1988; Pannell and Charlesworth 2000). Observing reductions in neutral diversity in peripheral populations, however, does not exclude the possibility that evolutionary genetic models are important to explaining range limits but may indicate that peripheral populations are unstable (e.g., source-sink dynamics across environmental gradients) or young and have accumulated few new mutations.

The contribution of contemporary versus historical processes to rangewide patterns of molecular variation can be interpreted more clearly in light of field studies of population demography. Both molecular and demographic data provide information on effective population size (N_e)—albeit different types of N_e (Ewens 1982)—which can be used to understand the history and trajectory of populations through time (Crandall et al. 1999). Populations at equilibrium that have been large and stable are expected to show an association between measures of neutral genetic diversity and census population size (Whitlock and Barton 1997). However, backward-looking genetic estimates of N_e (e.g., θ_W; Watterson 1975) can differ strongly from demographic estimates describing current populations (Templeton 1980; Crow and Denniston 1988; Caballero 1994), particularly when populations are in decline (e.g., range contraction) or growing (e.g., the front of a range expansion). Therefore, estimates of different effective population sizes that describe historical (inbreeding N_e) versus current (variance or eigenvalue N_e) populations can provide key insights into the dynamics of geographic ranges.

Unique or Rare Genetic Variants

An additional insight provided by polymorphism data is the frequency and distribution of unique or rare genetic variants across a species' range (e.g., private alleles; Neel 1973). Holt and Keitt (2000) point out that if range limits form because of gradients in habitat availability or colonization rates, then we should not expect variation in population age across gradients, whereas if range limits form because of extinction gradients, then we should expect populations to be younger toward range limits. By extension, more novel genetic variants resulting from new mutations should accumulate in older central populations. This may be reflected in both neutral and beneficial mutations. Much as with total sequence polymorphism (described above), the number of unique alleles is expected to be lower in peripheral populations under nonequilibrium and demographic models of range limits. Under absolute source-sink dynamics, unique alleles are unlikely to be observed in peripheral sink populations. Holt and Keitt (2000) further suggest that this low rate of mutational input to range limit populations may render them less well adapted to range limit environments compared with central genotypes in their local environments. Limited mutational input may be one mechanism by which peripheral populations lack the ecologically important genetic variation necessary to respond to selection. These predictions have been rarely tested, but theory emphasizes the utility of a historical perspective for interpreting the results of field experiments.

Frequency Distribution of Sequence Variants

Statistics describing the frequency distribution of genetic variants (such as Tajima's *D* and Fu's *Fs*) are commonly used to examine whether individual genes conform to a neutral model of sequence evolution in an effort to detect the action of selection (Tajima 1989*b*) as well as to investigate whether demographic history has shaped genomewide patterns of sequence variation (Tajima 1989*a*; reviewed in Nielsen 2005). When these statistics are applied to local populations across species' ranges, they provide some insight into the history and variability of population dynamics (e.g., Moeller et al. 2007; Ross-Ibarra et al. 2008). If central populations exhibit higher historical rates of population growth and have been a source of colonists for peripheral populations, we expect a genomewide excess of rare variants (negative values of *D* and *Fs*) in central populations where population growth rates have been higher and from which individuals have been exported. This type of evidence would be consistent with predictions of the abundant center biogeographic model and the maladaptive gene flow range limits model.

Under rapid range expansion, by contrast, rare alleles (or new mutations) can rapidly increase in frequency at the front of a range expansion, where genetic drift is strong, and "surf" into newly occupied territories, resulting in strongly negative values of *D* and *Fs* (reviewed in Excoffier et al. 2009). Finally, if range limit populations have repeatedly experienced bottlenecks as a result of extinction/recolonization dynamics (metapopulation dynamics and source-sink models), theory predicts a high variance in *D* (including positive values), because rare variants will be lost due to genetic drift (Wakeley and Aliacar 2001; Wright and Gaut 2005). These predictions largely hold for stable populations with limited differentiation (and high rates of migration), but the situation can be considerably more complicated when population structure is stronger (Wakeley and Aliacar 2001).

Population Genetic Structure and Gene Flow

Both demographic and evolutionary genetic models of range limits make predictions about patterns of population genetic structure. Expectations for the effects of population turnover at the range periphery on patterns of population genetic structure (F_{ST}) depend in part on the source of new colonists for peripheral populations (Pannell and Charlesworth 2000). Populations could be maintained under a migrant-pool model of colonization, where individuals are a random sample from across the broader metapopulation, or under a propagule-pool model, where colonists come from a large single source, such as the central populations of the species' range (Slatkin 1977). In the former F_{ST} values are often reduced because migrant-pool dynamics facilitate gene flow among populations, whereas in the latter F_{ST} values are often expected to be elevated in the sink region because of variance in sampling colonists from the central source population (Wade and McCauley 1988; Whitlock and McCauley 1990).

Evolutionary models of range limits primarily make population genetic predictions about patterns of gene flow. To assess the contribution of nonneutral gene flow to the evolution of quantitative traits, the first problem is determining the rate at which gene combinations move among populations, especially asymmetrically from range center to edge. Historical rates and directional patterns of gene flow can be examined through the study of neutral genetic variation and by using coalescent-based models (e.g., Beerli and Felsenstein 1999; Hey and Nielsen 2004). Although these analyses can assess the plausibility that gene flow from central to peripheral populations could potentially influence adaptation in peripheral populations, they are inappropriate for judging whether gene flow influences components of fitness in peripheral environments. Instead, experimental analyses can ask whether there is a correspondence between observed phenotypes and predicted trait optima (Paul et al. 2011) or how the introduction of genes from central populations influences the lifetime fitness of individuals at the range edge.

In this study, we examined patterns of molecular genetic variation across six populations distributed along two geographic transects from range center to edge in the annual plant *Clarkia xantiana* ssp. *xantiana*. For population samples from these six geographic locations, we collected nuclear sequence data from nine genomic regions and microsatellite data from four loci. First, we examined the influence of geographic position (range center to edge) and contemporary demographic parameters (number of breeding individuals and population growth rates) on (1) levels of allelic richness and nucleotide polymorphism, (2) the frequency of private (or rare) alleles within populations, and (3) the demographic history of each population as inferred from the frequency distribution of genetic variants. Second, we examined patterns of population genetic structure and the directionality of historical migration among populations by means of a coalescent-based Bayesian approach. We interpreted our results in the context of the predictions of theoretical models on the causes of range limits and in relation to our field studies of this system (see Eckhart et al. 2011).

Methods

Study Species and Sample Collection

Clarkia xantiana ssp. *xantiana* (Onagraceae) is a winter annual endemic to the southern Sierra Nevada foothills and associated mountain ranges of California; its western range limit occurs at the San Joaquin Valley, and its eastern range limit occurs along an environmental gradient where no prominent physical barriers to dispersal are present (Eckhart and Geber 1999; Eckhart et al. 2011). Populations are typically discrete, occurring on steep slopes where competition from other herbaceous plants is moderate to low. Peripheral populations receive lower mean precipitation as well as less predictable precipitation than central populations (Eckhart et al. 2010, 2011). Seeds may remain dormant in the soil for at least 3 years, most likely longer; dormancy buffers populations from extinction during periods of drought. Seeds are dispersed passively, with no apparent biotic or abiotic dispersal mechanism other than gravity. All populations are primarily outcrossing but self-compatible (Runions and Geber 2000; Moeller 2006), with pollination effected primarily by solitary bees (Moeller 2005; Eckhart et al. 2006).

In August 2005, we collected seeds from six populations of *C. xantiana* ssp. *xantiana*. Two populations, Cow Flat and Delonegha, are located in the center of the geographic

distribution of the taxon (hereafter, "center"); two populations, Squirrel Mountain and Golf Course, are located within 5 km of the range limit (hereafter, "edge"); and two populations, Borel Road and Keyesville, are located roughly halfway between the center and edge populations (hereafter, "intermediate"). Populations ranged in area from 0.2 to 7.0 ha, all occurring in natural habitats. In each population we conducted random walks where we haphazardly sampled plants at predetermined intervals (e.g., every 10 m) and collected fruits from >25 maternal families scattered across the population without respect to plant size or any other plant characteristic. Seeds from each maternal family were germinated and grown in environmental chambers. DNA was extracted from newly expanded leaves using DNeasy Plant Mini kits (Qiagen, Valencia, CA).

As part of a larger field demographic study of *C. xantiana* ssp. *xantiana* populations from range center to edge (see also Eckhart et al. 2011), we estimated the total number of fruiting plants for each of the six populations over 4 years (number of breeding individuals, N_b). We used transect sampling across populations to estimate fruiting plant density from 1 × 0.5-m plots (35–128 plots/site), and populations were circumscribed in 2006 to estimate the area occupied. Populations were censused in 2006–2009, and means of the 4 years of estimates for each population were used in our analyses. Under the assumption of equal sex ratios and no reproductive skew, our estimates of the number of breeding individuals provide a proxy for variance effective population size. More detailed stage-specific demographic data were also collected in association with this study, providing estimates of current population growth rates (stochastic finite rate of increase, λ_S) based on 4 years of data (Eckhart et al. 2011).

DNA Sequencing

We used polymerase chain reaction (PCR) to amplify each of nine genomic regions (hereafter, "loci") from each of 135 *C. xantiana* ssp. *xantiana* DNAs (20–23 individuals from each of six populations). The PCR primers used to amplify these loci (provided in table A1 in a zip file in the online edition of the *American Naturalist*) were designed from EST sequences that came from *Clarkia breweri* flower buds (provided by Eran Pichersky, University of Michigan). We initially screened 40 arbitrarily chosen primer pairs for which BLAST searches revealed no evidence of duplication in the genome of *Arabidopsis thaliana*. Primer pairs were tested on two *C. xantiana* ssp. *xantiana* DNAs that were not included in the population sampling. The subset of primer pairs that resulted in successful PCR were further evaluated to be certain that primer pairs amplified single-copy genomic regions. For this, we screened populations of the highly selfing *Clarkia xantiana* ssp. *parviflora*, the sister subspecies of *C. xantiana* ssp. *xantiana*. Populations of *parviflora* contain little molecular variation, and individuals are typically homozygous. If the sequenced products from *parviflora* individuals revealed many polymorphic nucleotide sites or insertions/deletions, we assumed that the primers were not specific to a single locus and were excluded from further analyses. Nine primer pairs reliably amplified and were single copy, and these were used for data collection for *C. xantiana* ssp. *xantiana*. The selection of loci should not affect any estimates of diversity or migration given that the selection was based solely on amplification success of putatively single-copy regions and was not based on any estimates of polymorphism.

For all loci, PCR products were sequenced directly. PCR products with no heterozygous sites were assumed to come from homozygous individuals, and thus two identical sequences were included in analyses. For a subset of PCR products that had multiple polymorphic sites or that showed apparent indel polymorphism, loci were amplified a second time, and the resulting products were cloned into pGEM-T Easy vectors (Promega, Madison, WI). One to five cloned products were sequenced, and these allelic sequences were used to determine the phase of sequence variants. For the remaining polymorphic sequenced products, we inferred haplotypes using Phase v2.1 (Stephens et al. 2001). To avoid biasing results due to nucleotide misincorporation into cloned products, rare polymorphisms detected from cloned loci were confirmed by directly sequencing PCR products. Three genes (*a16*, *a23*, and *g2*) harbored multiple polymorphic indels, preventing us from obtaining a complete sequence for the entire PCR product. For these three loci, we used sequence data from only one end of the amplified region (i.e., forward or reverse) and truncated all reads when indels made inferring sequences difficult. Because we obtained sequence data from fewer individuals for locus *g2* than for other loci, we excluded it from population structure analyses. All sequences have been deposited in GenBank (JF290497–JF292254).

Microsatellite Genotyping

DNAs from the same 135 individuals were used to genotype four dinucleotide-repeat microsatellite loci. Information on the development of microsatellites, primer sequences, and annealing conditions can be found in table A2 in the zip file. PCR was conducted separately for each locus using four different fluorescent dyes (6-FAM, NED, PET, and VIC), and amplified products were combined for fragment separation on an ABI 3130xl analyzer, with LIZ used as a size standard. A subset of individuals were rerun using independent PCRs and independent fragment

analyses to confirm alleles. All fragment sizes were determined by directly examining each chromatogram.

Diversity Analyses

For each of the six population samples, we calculated haplotype richness for sequence data and allelic richness for microsatellite data. Because sample sizes were not equal, we rarefied haplotype and allelic richness using Contrib (Petit et al. 1998). Second, we examined the richness of private haplotypes and microsatellite alleles, those variants unique to a single population. Because private microsatellite alleles were uncommon, we also examined the richness of alleles unique to two populations. For sequence data, we estimated standard descriptive statistics of nucleotide polymorphism: the average number of segregating sites per site, θ_W (Watterson 1975), and the average number of pairwise differences between sequences, θ_π (Nei 1987). These two statistics provide estimates of historical and current effective population size, respectively (Crandall et al. 1999). To infer demographic history, we examined the frequency spectrum of mutations using Tajima's *D* (Tajima 1989*b*) and the frequency spectrum of haplotypes using Fu's *Fs* (Fu 1997). All of these statistics were calculated in DnaSP v5 (Librado and Rozas 2009).

We submitted estimates of all statistics described above to ANCOVA to test whether measures of population genetic diversity differed among geographic regions and whether they were associated with field estimates of N_b and λ_S. Each ANCOVA model included three independent variables: genetic locus, geographic region (center, intermediate, and edge), and the log of N_b or λ_S. When the geographic region term was significant, we used Tukey's test to assess the significance of differences among the three regions. Private sequence haplotype richness was square-root transformed to improve the homoscedasticity of residuals.

Population Genetic Structure Analyses

We used three approaches to characterize population structure. First, we examined the partitioning of genetic variation among regions, among populations within regions, and within populations using analysis of molecular variance (AMOVA) separately for DNA sequences and microsatellites (Excoffier et al. 1992). Second, we estimated genetic differentiation between pairs of populations for each sequenced locus separately using F_{ST} (Hudson et al. 1992). We examined whether values of pairwise F_{ST} differed depending on whether population comparisons were made within regions, between neighboring regions, or between range center and edge. We used an ANOVA that included two independent variables: the type of pairwise comparison (within region, between neighboring regions, or between center and edge) and the genetic locus. The results of this analysis provide more detail about the pattern of population differentiation than does AMOVA but should be viewed with caution because pairwise F_{ST} values are not independent.

Third, we used both microsatellite alleles and sequence haplotypes to examine the distribution of genetic lineages among geographic populations using the Bayesian clustering approach of Structurama (Huelsenbeck and Andolfatto 2007). Rather than fixing the number of genetic lineages (K) for each analysis and determining K on the basis of marginal likelihoods—the approach of Structure (Pritchard et al. 2000)—Structurama estimates K by allowing the number of lineages to be a random variable following a Dirichlet process prior (Pella and Masuda 2006). We performed a series of analyses to explore the sensitivity of the results to conditions of the model. First, we conducted an analysis where the number of lineages follows a Dirichlet process prior with the parameter α set as a random variable with a gamma probability distribution (shape and scale parameters were set to 1). Second, we ran a series of analyses with the prior mean $E(K) =$ 2, 3, 4, 5, 10, 15, or 20. Third, we fixed K to specified levels (2, 3, 4, or 5), as would occur in a standard Structure analysis. Each of the Markov chain Monte Carlo (MCMC) analyses was run for a total of 100,000 cycles. Posterior probability distributions were used to determine K.

We used Bayesian MCMC coalescent models in Migrate-n (Beerli 2006, 2008) to examine asymmetric patterns of gene flow among populations using sequence data. These analyses estimate the mutation-scaled effective population size ($\theta = 4N_e\mu$) and the mutation-scaled effective immigration rate between pairs of populations ($M = m/\mu$). Uniform priors were set for θ[0, 0.25] and M[0, 1,000] divided into 1,000 bins, and the transition-to-transversion ratio was set to 2. Multiple MCMC runs were conducted, which produced similar results. Each run was conducted with a long chain of 1×10^7 steps, where four chains were simultaneously run with adaptive heating (temperatures of 1, 1.5, 3, and 20), and sampling occurred every 20 steps. We examined asymmetric rates of immigration using M (the mode of the posterior distribution across all loci) and the number of immigrants per generation ($4Nm$), the product of M and θ of the recipient population.

Results

Microsatellite Diversity

Microsatellite allelic richness was significantly greater in central populations than in edge populations ($F_{2,17} =$ 5.1, $P = .02$; fig. 1). Private alleles (unique to a single

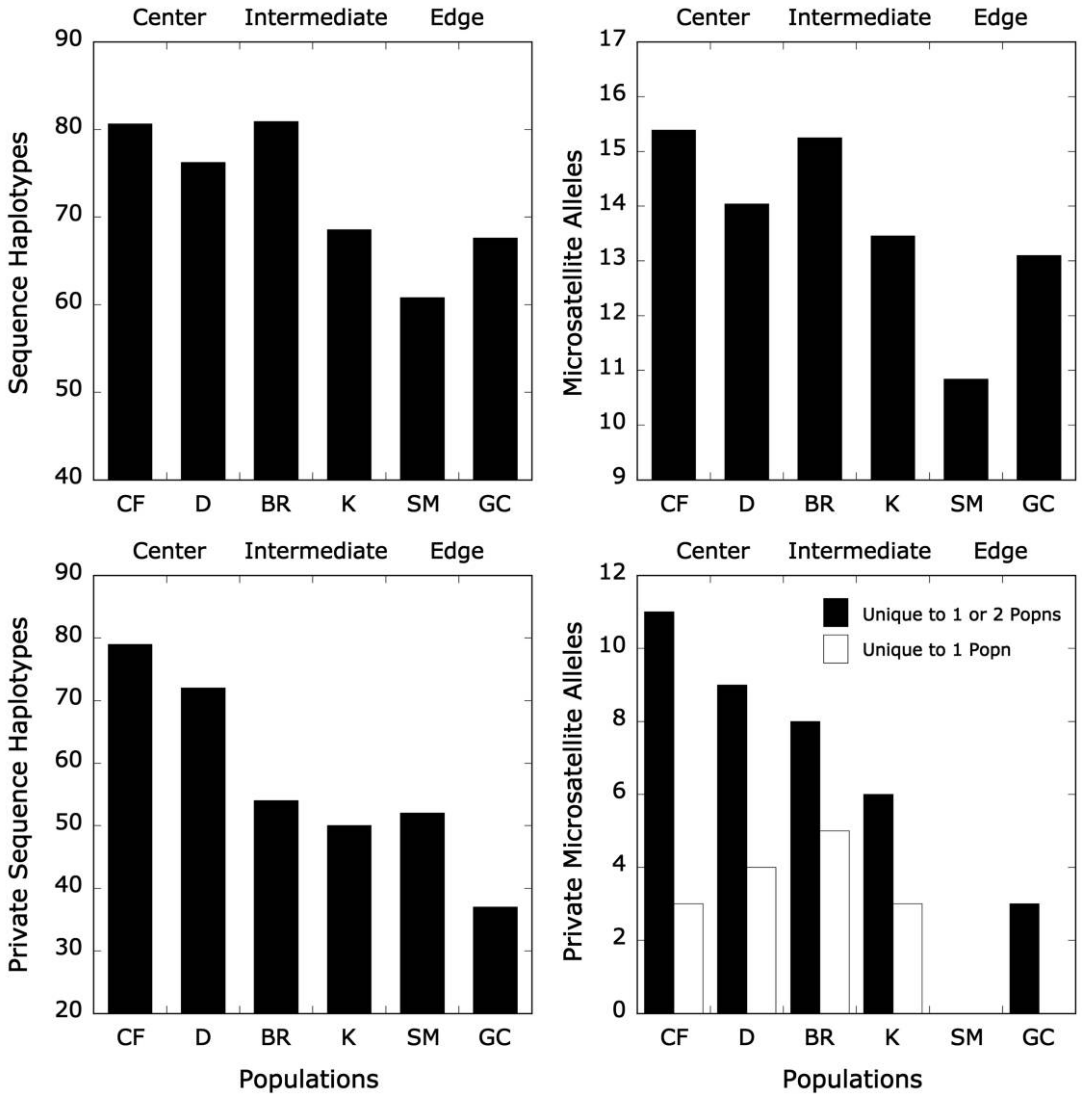

Figure 1: Sequence haplotype and microsatellite allelic richness in each of the six geographic populations from the center, intermediate, and edge portions of the species' range. Rarefied total allelic richness is shown in the upper panels, and private allelic richness is shown in the lower panels. Central populations are Cow Flat (CF) and Delonegha (D), intermediate populations are Borel Road (BR) and Keyesville (K), and edge populations are Squirrel Mountain (SM) and Golf Course (GC).

population) were not found in range edge populations, but all other populations harbored three to five private alleles; the effect of region was marginally significant ($F_{2,17} = 3.2$, $P = .065$; fig. 1). Alleles unique to one or two of the six populations were also significantly more common in central populations than in edge populations ($F_{2,17} = 17.6$, $P = .006$). Census population size (N_b) and the stochastic population growth rate (λ_S) were not significantly associated with any of the measures of microsatellite allelic richness ($P > .09$) or observed heterozygosity ($P > .13$). Unlike allelic richness, we found no evidence that observed heterozygosity differed among geographic regions ($F_{2,17} = 1.3$, $P = .30$).

Sequence Diversity

Haplotype richness was significantly greater in central populations than in intermediate or edge populations ($F_{2,32} = 3.4$, $P = .045$; fig. 1). Similarly, private sequence haplotype richness was significantly greater in center populations than in edge populations ($F_{2,32} = 5.6$, $P = .008$). We did not find a significant association between N_b (total:

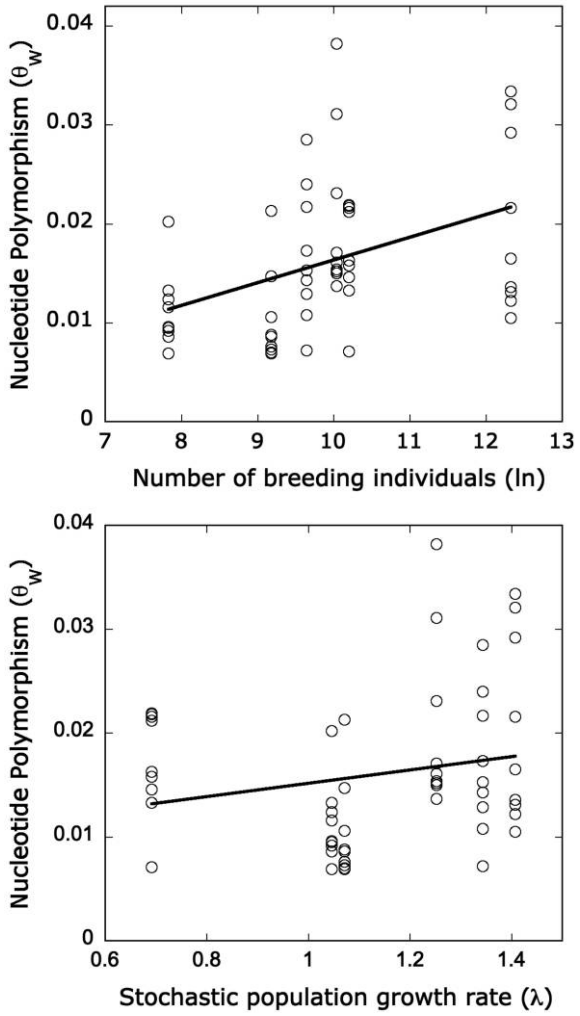

Figure 2: Relationship between estimates of total nucleotide polymorphism (θ_W) from nine sequenced genomic regions and the estimated number of breeding adult plants (N_b; *top*) or the stochastic population growth rate (λ_S; *bottom*) estimated from field demographic studies in 2006–2009.

$F_{1,32} = 2.5$, $P = .121$; private: $F_{1,32} = 0.6$, $P = .433$) or λ_S (total: $F_{1,32} = 0.5$, $P = .468$; private: $F_{1,32} = 0.2$, $P = .688$) and the number of total or private sequence haplotypes.

Nucleotide polymorphism was very high across populations for all sequenced sites ($\theta_W = 0.025$, $\theta_\pi = 0.015$) and for silent sites alone ($\theta_W = 0.044$, $\theta_\pi = 0.030$). Individual population estimates were also high (mean $\theta_W = 0.016$ for all sites and 0.030 for silent sites; table A4 in the zip file). Total nucleotide polymorphism was positively associated with N_b ($F_{1,37} = 4.2$, $P = .046$; fig. 2), but we found no significant differences among geographic regions ($F_{2,37} = 0.6$, $P = .544$). We also found no association between nucleotide polymorphism and λ_S ($F_{1,37} = 0.0$, $P = .998$). We found the same patterns for analyses based only on silent sites. Overall, data on sequenced loci and microsatellites revealed remarkably similar patterns of modestly greater allelic richness (particularly of private alleles) in central compared to peripheral populations but no association of microsatellite heterozygosity or nucleotide polymorphism with geography.

Demographic History

Fu's *Fs* ranged from −4.7 to −139.1 across the nine loci for the specieswide data set (mean = −68.0), with individual population estimates generally negative (45 of 54 estimates; table A4 in the zip file). Fu's *Fs* differed significantly among geographic regions, with central populations having more negative values than edge populations ($F_{2,42} = 3.7$, $P = .034$; fig. 3); we found no significant association of Fu's *Fs* with N_b ($F_{1,42} = 2.5$, $P = .120$) or λ_S ($F_{1,42} = 3.7$, $P = .651$). Tajima's *D* ranged from −0.5 to −1.9 across the nine loci for the specieswide data set (mean = −1.1), and individual population estimates were also generally negative (32 of 54 estimates; table A4 in the zip file). Tajima's *D* was not significantly associated with N_b ($F_{1,42} = 1.4$, $P = .245$) or λ_S ($F_{1,42} = 0.1$, $P = .732$) and did not differ significantly among regions ($F_{2,42} = 1.4$, $P = .268$; fig. 3); however, the pattern of variation for *D* was similar to that for Fu's *Fs*, with a tendency for more negative values in central populations.

Population Genetic Structure

AMOVA showed that most variation (>91%) was harbored within populations for both DNA sequences and microsatellites (table 2). We detected significant partitioning of molecular variation among regions for DNA sequence data (3%) but not microsatellites (1%) and significant partitioning of variation among populations within regions for both data sets (5%–7%; table 2). Values of F_{ST} estimated from sequence data were greatest between central and edge populations ($F_{5,107} = 4.7$, $P < .001$; fig. 4; table A5 in the zip file). We also found differentiation between the two center and the two edge populations, although it was less pronounced than the center versus edge comparison (fig. 4).

Results from Structurama analyses largely paralleled the F_{ST} results. We found the highest posterior probability ($\Pr(K|X)$) for $K = 3$, except when the prior mean was set to unrealistically high values—that is, when $E(K) = 10$, 15, or 20, the highest ($\Pr(K|X)$) was for $K = 4$ (fig. 5). In addition, the assignment of individuals to lineages was nearly the same for every analysis (fig. 6), including those where values of *K* were fixed (not shown). The three

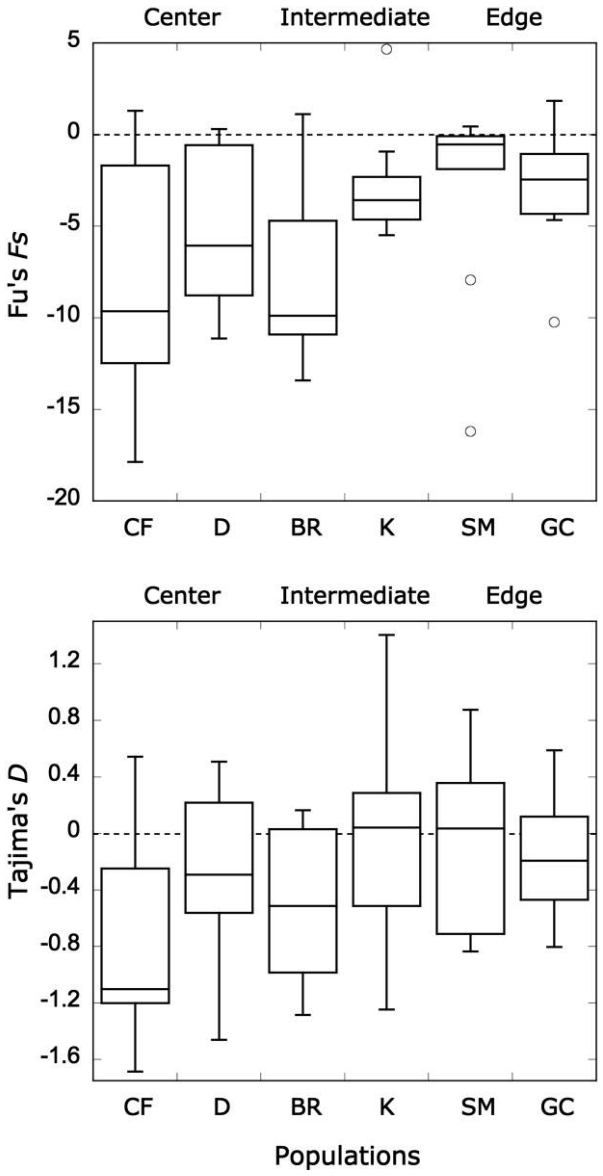

Figure 3: Variation among the six geographic populations in the frequency spectrum of haplotypes and mutations as described by Fu's *Fs* and Tajima's *D*, respectively. Box plots represent variation among the nine sequenced loci for each population. Abbreviations for populations are given in figure 1.

major lineages were differentiated across geographic populations (fig. 6). Lineages 2 and 3 predominated in the central populations, Delonegha and Cow Flat, respectively. Lineage 1 was primarily found in the intermediate populations, Keyesville and Borel Road. The edge populations only contained lineages that were also found in the center and intermediate populations: Squirrel Mountain harbored only lineage 1, and Golf Course had a mixture of the three lineages (fig. 6). Interestingly, the two edge populations showed limited overlap in genetic composition (also reflected by the high F_{ST} values between edge populations). No lineages were found in the edge populations that were not also found elsewhere, even when priors were set to unrealistically high levels.

Patterns of Migration

Levels of migration from center to intermediate and from intermediate to edge outweighed migration in the opposite direction, suggesting an overall asymmetry in migration patterns across the taxon's geographic range. On the basis of the populations sampled, migration more strongly affected the genetic diversity of the edge and intermediate populations, whereas immigration contributed comparatively less to the genetic diversity of the central populations. Figure 7 shows estimates of *M*, the relative importance of migration over mutation in the introduction of new variants into populations (for more details, see table A6 in the zip file). We found some evidence of asymmetric migration from central populations to both intermediate and edge populations but little contribution of migrants to the diversity of central populations (fig. 7). Similarly, values of *M* were greater for the contribution of intermediate-to-edge populations than edge-to-intermediate populations for both pairs of populations. The patterns of migration inferred from *4Nm* (number of immigrants per generation $= M \times \theta$) were similar, but the higher effective population sizes of intermediate populations than edge populations caused inferred migration rates to be high for edge-to-intermediate populations (table A6 and fig. A1 in the zip file).

Discussion

Although species' distributional boundaries have been a source of great interest among biogeographers (Elton 1927; Brown and Lomolino 1998), we have only begun to understand the ecological and evolutionary forces that cause them. Population genetic studies across species' ranges have lent some insight by testing predictions about how genetic variation within and among peripheral populations is expected to differ from that within and among central populations. For example, it is often argued on the basis of the abundant center hypothesis that peripheral populations are smaller or less dense and occur less frequently across landscapes (although support for this is not often found; Sagarin and Gaines 2002). In turn, rates of genetic drift have been predicted to be higher—leading to less genetic variation and greater differentiation—among peripheral populations than among central populations (Soulé 1973; Brussard 1984; Barton 2001; Eckert et al.

Table 2: Analysis of molecular variance (AMOVA) for six *Clarkia xantiana* ssp. *xantiana* populations from three geographic regions (center, intermediate, and edge) for DNA sequences and microsatellites separately

	DNA sequences		Microsatellites	
	% variation[a]	Φ[b]	% variation[a]	Φ[b]
Among regions (Φ_{CT})	3.4*	.03	1.2	.01
Among populations within regions (Φ_{SC})	4.7**	.05	7.3**	.07
Within populations (Φ_{ST})	91.8**	.08	91.5**	.09

Note: AMOVA results shown are weighted averages across all loci.

[a] The percentage of total variance explained by each hierarchical grouping, including the probability of having a more extreme variance component and Φ statistic than the observed values assessed by permutation tests.

[b] Fixation indices describing the correlation of haplotypes for each level of subdivision relative to a higher-level grouping.

* $P < .05$.

** $P < .001$.

2008). Tests of these predictions have provided a useful start but often remain isolated from both field investigations and the diverse predictions of theoretical models.

Our results suggest that range limit populations have significant but modest reductions in allelic richness relative to central populations and are similar to central populations in having very large effective population sizes (inbreeding N_e). The frequency distribution of genetic variants (*Fs* and *D*) and the correspondence between historical N_e (based on molecular data) and current N_b (based on field data) suggest that peripheral populations have been largely stable in size rather than having been recently founded or subject to bottlenecks. Central populations appear to have experienced historical population expansion and have disproportionally exported migrants to peripheral populations. These results, along with Eckhart et al.'s (2011) finding that there is limited suitable habitat beyond the current range limit, cast doubt on strictly demographic models of range limits. Instead, they lend stronger support to evolutionary models, where genetic constraints or maladaptive gene flow limit responses to selection at the range edge.

Patterns of Genetic Variation

The assumption that peripheral populations are smaller and of lower density has led to the prediction that genetic diversity is often reduced in peripheral populations as a consequence of random genetic drift. A recent review showed that 64% of studies comparing central and peripheral populations have detected a decline in diversity at range limits (Eckert et al. 2008). This decline could arise from elevated rates of genetic drift owing to smaller (or highly fluctuating) population sizes (Mayr 1963; Lewontin 1974), from frequent population turnover (metapopulation dynamics; Pannell and Charlesworth 2000), or because peripheral populations are newly founded at the front of a range expansion (Le Corre and Kremer 1998; Austerlitz et al. 2000). These reasons for lower genetic diversity have very different implications for why range limits form or become unstable.

In *Clarkia xantiana* ssp. *xantiana*, we found significantly lower levels of allelic richness in range limit populations, but reductions were small. Range limit populations harbored 82% of the sequence haplotype richness and 88.4% of the microsatellite allelic richness found in central populations. Intermediate populations showed virtually no reduction in richness, harboring 95% of both the sequence and the microsatellite allelic richness found in central populations. Our results are inconsistent with the common assumption that fewer alleles in peripheral populations result from higher rates of drift due to chronically small population size and low density. Our field studies have suggested that peripheral populations are not currently smaller or of lower density than central populations (Eckhart et al. 2011). Instead, it is possible that the greater stochasticity of range limit environments and greater demographic fluctuations (Eckhart et al. 2011) have led to episodes where strong drift causes the loss of some alleles.

Unlike patterns of allelic richness, we found no evidence that nucleotide polymorphism or microsatellite heterozygosity differed regionally. In addition, our estimates of historical effective population size ($N_e = \theta/4\mu$, where $\mu = 7 \times 10^{-9}$; Ossowski et al. 2010) are among the largest discovered in plants for both central and peripheral populations (696,428–1,298,214). These similar and large estimates of N_e for all populations discount the possibility that range limit populations have been recently founded, are at the edge of a rapidly advancing expansion front, or have experienced frequent population turnover. The frequency distribution of genetic variants (as described by Tajima's *D* and Fu's *Fs*) similarly suggests no evidence for

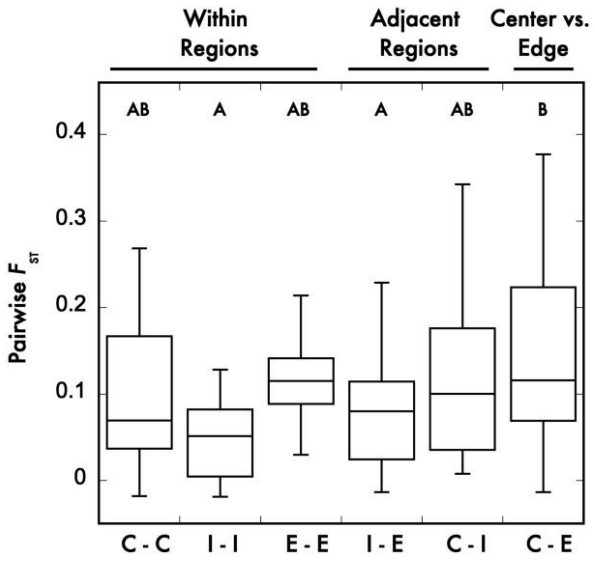

Figure 4: Patterns of population genetic divergence, as estimated by pairwise F_{ST} based on DNA sequence data from eight loci. Pairwise F_{ST} values were pooled according to whether pairs of populations came from within geographic regions (center [C], intermediate [I], or edge [E]), between adjacent regions, or between range center and edge.

population size changes at the range edge. Our results for *C. xantiana* ssp. *xantiana* on molecular diversity are similar to those found by Paul et al. (2011) using DNA sequence data for central and marginal populations of *Mimulus cardinalis*. These patterns have also been observed in comparisons of allozyme richness and heterozygosity for central and peripheral populations of 15 *Drosophila* species (Soulé 1973; Brussard 1984). Rather than varying regionally, our estimates of nucleotide polymorphism were associated more closely with variation in N_b, consistent with predictions for populations at mutation-drift equilibrium (Crow and Kimura 1970; Kimura 1983).

The maintenance of genetic diversity in *C. xantiana* ssp. *xantiana* populations, especially at the range edge, is likely influenced by the fact that seeds can remain dormant in the soil for at least 3 years and that seed banks affect population dynamics. In years of strong drought some populations have had no survivors, but in subsequent years individuals emerged from the seed bank across the entire site. Dormancy and seed banks not only should dampen the effect that environmental stochasticity has on long-term demographics (Kalisz and McPeek 1992, 1993) but also should slow the loss of genetic variation from populations (Templeton and Levin 1979; Hairston and De Stasio 1988). In *Clarkia springvillensis*, for example, McCue and Holtsford (1998) found that seed banks contained more genetic variation than vegetative adults and that differentiation among populations was reduced among seeds relative to adults. It is likely similar that *C. xantiana* ssp. *xantiana* seed banks contribute not only to the persistence of range edge populations but also to large effective population sizes that greatly exceed our field estimates of N_b. Because annual plants such as *C. xantiana* typically have seed banks, variance N_e is elevated by the average time to germination for seeds (Nunney 2002).

Although our data on putatively neutral genetic variation appear to discount the possibility that metapopulation dynamics and rapid population turnover are the likely causes of limits to range expansion, they say little about the role of genetic constraints in adaptation. Because additive genetic variation for quantitative traits is expected to decline as a result of drift as much as neutral molecular variation does (Wright 1969; Lande 1980), evidence for the presence of somewhat fewer alleles in range limit populations could be interpreted as suggesting that peripheral populations also contain less quantitative genetic variation for ecologically important traits (e.g., after range expansion; Pujol and Pannell 2008). Unfortunately, evidence of such a correlation is not often supported (Spitze 1993; Pfrender et al. 2000), in part because quantitative traits are often affected by nonadditive genetic variation, which can be converted to additive effects via genetic drift (Robertson 1952; Goodnight 1987; Willis and Orr 1993; Armbruster et al. 1998). Examining the role of genetic constraints in adaptation requires classical quantitative genetic approaches, especially in nature, rather than indirect inferences from neutral molecular markers. Ongoing field experiments in *C. xantiana* seek to estimate quantitative genetic parameters for these same populations to investigate whether genetic constraints limit responses to selection.

Historical Demography and Gene Flow

The abundant center hypothesis has served as the basis for theoretical models examining the role of gene flow in structuring range limits. This hypothesis has been examined primarily through field studies of population size and within-population density, which are limited to a few years. Because long-term changes in population size influence levels and patterns of genetic diversity (Tajima 1989*a*; Ramos-Onsins and Rozas 2002), molecular studies have the potential to complement field studies by providing a historical perspective on population dynamics. In *C. xantiana* ssp. *xantiana*, we found elevated levels of rare variants (negative values) across loci in central populations, a pattern suggesting demographic expansion, consistent with abundant center predictions. Both statistics describing the frequency distribution of variants (Tajima's *D* and

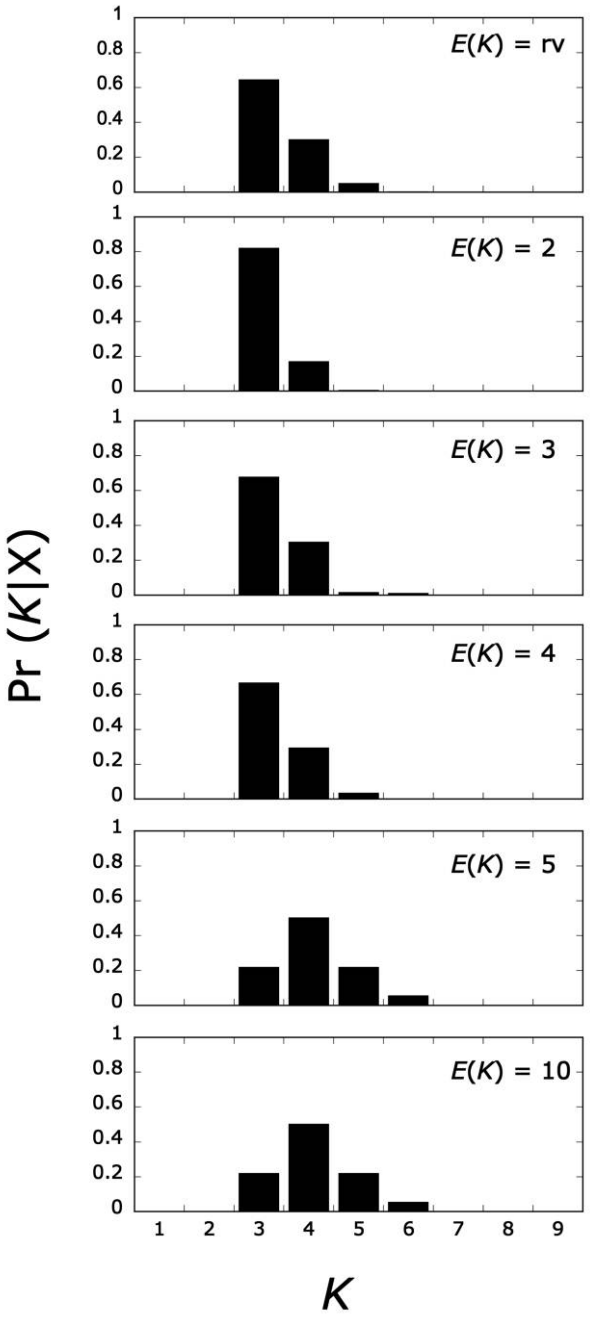

Figure 5: The distribution of posterior probabilities ($\Pr(K|X)$) for the number of inferred genetic lineages (K) from Structurama analyses with a range of Dirichlet process prior means ($E(K)$ = random variable [rv], 2, 3, 4, 5, and 10 lineages).

Fu's *Fs*) tended to be near 0 for range edge populations, providing little support for the hypothesis that peripheral populations represent a recent or ongoing range expansion.

On the basis of the abundant center assumption, population genetic studies across ranges have often evaluated the idea that genetic differentiation (typically estimated by F_{ST} or G_{ST}) is elevated in peripheral regions of a species' range as a consequence of the greater physical isolation of populations and reduced gene flow among them. Most studies (70.2%) that have tested for an increase in differentiation among peripheral populations have found it (Eckert et al. 2008). Although these findings are interesting, it is unclear how to interpret them in the context of models of range limits. Population genetic models have made strikingly contrasting predictions about whether population genetic structure should be elevated or reduced across ranges. Under stable conditions, populations after a range expansion are expected to have elevated levels of population genetic differentiation as a consequence of founder events and drift (Austerlitz et al. 1998; Le Corre and Kremer 1998). When populations are subdivided, levels of genetic differentiation can be elevated or reduced depending on whether colonization follows migrant-pool dynamics, where colonists come from across the metapopulation (F_{ST} often reduced), or propagule-pool dynamics (F_{ST} often elevated), where colonists come from a neighboring population (e.g., a central population). The specific outcome for a particular taxon depends on the rates of colonization versus gene flow among demes (Wade and McCauley 1988; Whitlock and McCauley 1990; Pannell and Charlesworth 1999). These differing signatures of population genetic differentiation are complex but may assist in differentiating whether metapopulation dynamics or evolutionary constraints on adaptation are more important for limiting range expansion.

Bayesian clustering analyses of neutral genetic differentiation in *C. xantiana* ssp. *xantiana* with Structurama revealed different dominant lineages in central populations compared with intermediate populations and even between the two central populations, which are separated by only 4 km. Peripheral populations were not strongly differentiated from intermediate populations and appear to be different "draws" of neutral variation from the range center, which could arise either because of differences in the sources of colonists from the range center or as a result of ongoing patterns of gene flow. We examined the prediction that range center populations asymmetrically contribute to the genetic diversity of intermediate and range edge populations using coalescent analyses implemented in Migrate. Our results largely support this hypothesis, as estimates of the migration parameter, M, from center to edge and intermediate to edge outweighed estimates for the opposite direction. Because the migration parameter describes the contribution of immigration to genetic variation found within local populations, it is relevant to questions about how central populations may influence the evolution of range edge populations. Our results pro-

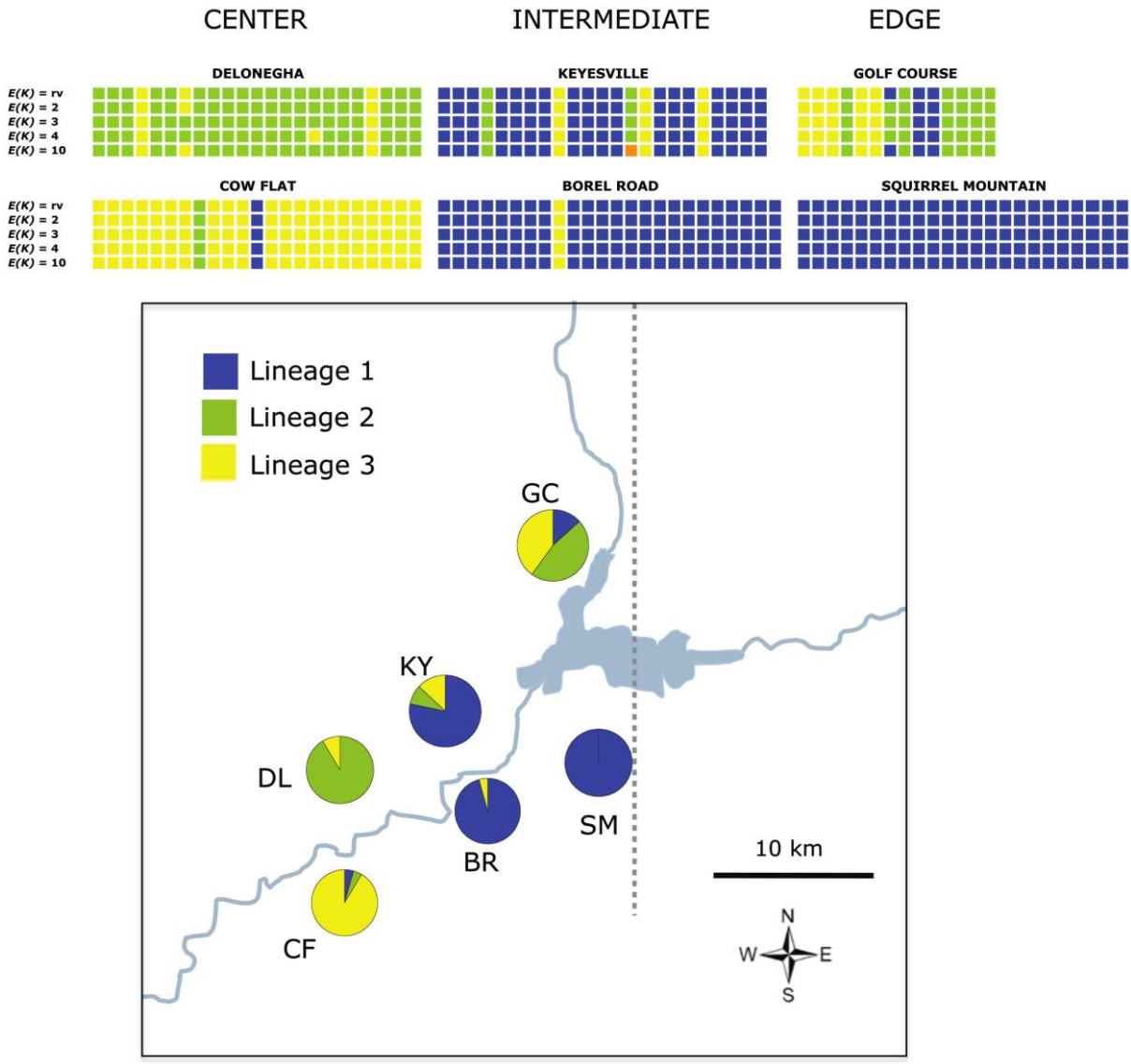

Figure 6: Assignment of individuals to genetic lineages (*top*) from five runs of Structurama and the distribution of lineages across geographic populations (*bottom*). rv = random variable.

vide little indication that edge or intermediate populations have contributed significantly to the genetic diversity found in the central populations studied here, but it appears that a fraction of diversity in edge populations can be explained by immigration from other populations rather than from local mutational input.

Implications, Problems, and Prospects

We have discovered a steep environmental gradient from range center to edge (Eckhart et al. 2010, 2011) along with evidence that central populations have both higher current population growth rates (Eckhart et al. 2011) and higher historical rates of population growth. Population genetic analyses also revealed some evidence that central populations have asymmetrically affected the genetic composition of peripheral populations. These results indicate the possibility that maladaptive gene flow could limit adaptation at the range limit. The important next step is to examine the fitness consequences of gene flow at and beyond the range limit via transplant experiments (e.g., Geber and Eckhart 2005; Griffith and Watson 2006; Angert

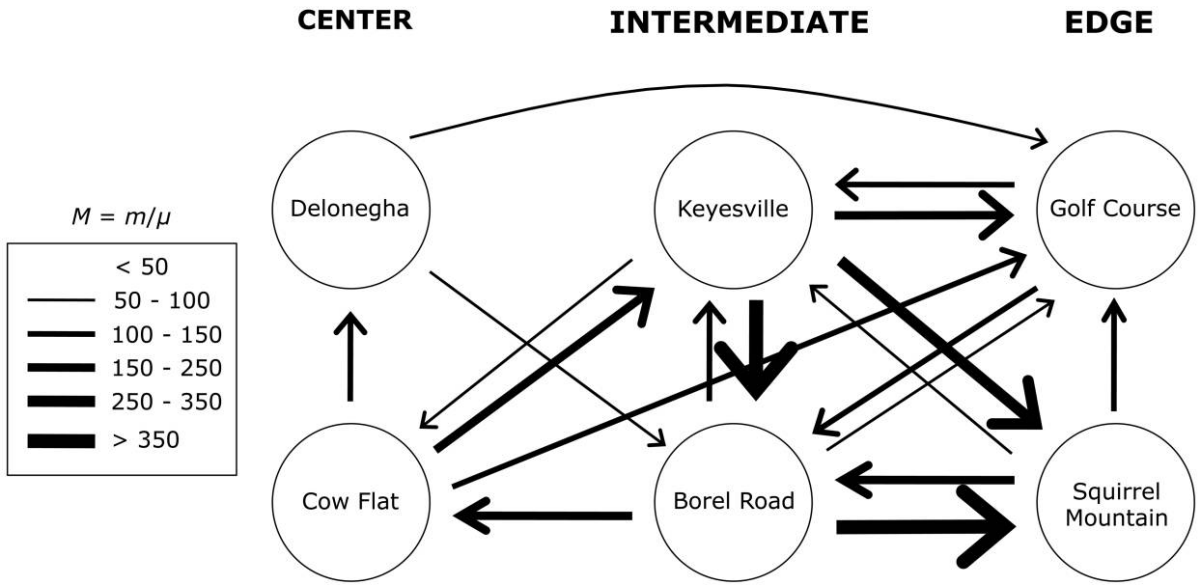

Figure 7: Estimates of historical asymmetric patterns of migration as inferred from Migrate analysis. Arrows represent the migration parameter, *M*, where the width of arrows is proportional to the contribution of migration to variation in the recipient population. Estimates (with 95% confidence intervals) of *M* can be found in table A6 in the zip file in the online edition of the *American Naturalist.*

et al. 2008). In other systems, evidence for maladaptive gene flow as a cause of distributional limits comes chiefly from studies of local population boundaries, where gene flow is likely to occur at very high rates (Antonovics 1968; McNeilly and Antonovics 1968), or from comparative studies, suggesting that the evolution of asexuality facilitates adaptation and range expansion in marginal environments (Lynch 1984; Bierzychudek 1985; Peck et al. 1998). At geographic scales, little experimental work has established whether and how gene flow may antagonize or facilitate responses to selection.

Our results also suggest that range edge populations contain less unique neutral genetic variation than populations in the range center. Although indirect, these patterns are consistent with the hypothesis that a lack of novel genetic variation may limit responses to selection. Preliminary results from a reciprocal transplant experiment in *C. xantiana* ssp. *xantiana* suggest that whereas central genotypes strongly outperform foreign genotypes in the range center, genotypes from the range periphery show no evidence of outperforming other genotypes in their resident sites or beyond the range edge (Geber and Eckhart 2005; M. A. Geber, D. A. Moeller, V. M. Eckhart, and P. Tiffin, unpublished data). Although more analysis is needed, these field results are consistent with the hypothesis that responses to selection may be hindered by genetic constraints.

In sum, molecular population genetic studies can provide important historical insights into population dynamics and patterns of range expansion. In our system, molecular data have cast doubt on strictly demographic models of range limits and suggest that evolutionary constraints are more likely. Despite these insights, there are considerable limitations to the use of population genetic studies alone for distinguishing among range limit models. Because many predictions about population genetic parameters are not unique to a single model and because some models make few clear predictions about patterns of neutral genetic variation (table 1), molecular studies are insufficient for any evaluation of the causes of range limits. Instead, our analyses suggest that molecular population genetics can be effectively combined with field studies to eliminate less likely models and direct intensive and costly field studies toward evaluating more plausible models of range limits.

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Table A1. Single-copy nuclear loci from which sequences were obtained. The annealing temperature for PCR was 55˚C for all loci.

Locus	Primer sequence (5- 3')	bp
a9	F: GTTGTATAATCACTGCTGCCC R: TTACACCGTCTTCTCAGCC	244
a16	F: AGATGATAGAATGATGCCC R: TTTCTTCGGTTTCCTAGGC	661
a23	F: AAGCACTTGGTTCAGACGATCCG R: ATGGAAGACATTGAGGATGG	498
d5	F: ATGTCTACTTGGCTAAGTTGGC R: TTGGCAAGAGTGCAAGCACG	598
d13	F: TTGTCAACACTGATCCTGAGG R: TGAGCAATAGTCCGATCGCTG	608
f9	F: ACAAACGGAGACATGTCCTGC R: ATATCTCAAATGGCTATGGCG	538
g2	F: TGTAAGATGCATACAGATCC R: CTTGCAGAGATTCGCC	284
i11	F: ATCACGAGTGTGACCCAGAGG R: AGACCATTGAGTCGACCC	523
k22	F: TAGCAGGCAGCTAGGACTCG R: TCAATGTTGAGAATGTGGAGG	670

Table A2. Microsatellite loci. The table reports the repeated sequence motif, primer sequences, annealing temperatures for PCR reactions (T_m), the size range of alleles genotyped in this study, the number of individuals genotyped across populations (N), and the number of alleles discovered for each locus across the six populations. Microsatellite loci were developed using a library enriched for dinucleotide repeats using a protocol modified from Hamilton et al. (1999)[a]. We selected loci that amplified reliably and that were variable based on a preliminary panel of DNAs that were screened.

Locus	Repeat motif	Primer sequence (5' – 3')	T_m	Size range (bp)	N	No. alleles
CX3	$(CT)_3TT(CT)_7$	F: ATATAAGCCCCCTTCCACTCCAACT R: GAGGATTCGTCTTCTACAATTAACCCTAAAA	50	243-263	131	10
CX7	$(TG)_2TT(TG)_8$	F: TTTCCCATGCAATGTG R: AGCGTGATATAGAGTCAAGACC	50	95-111	130	9
CX9	$TACA(TA)_4\ (TG)_8\ (TA)_2GA$	F:TATAGTTGTGCCACTGTTCTC R: TCTGCAGTTTTCATAAAGGG	55	150-218	129	23
CX11	$(TC)_{10}$	F: TCCCTAAATATTCAACAACAC R: TTCTTTTCTATCTCACAC	50	88-134	129	13

[a] Hamilton, M. B., E. L. Pincus, A. DiFiore, and R. C. Fleischer. 1999. Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques 27:500-507.

Table A3. Microsatellite variation. For each population by locus combination, the table shows the number of individuals genotyped, number of alleles, number of private alleles, number of alleles unique to one or two populations, expected heterozygosity, observed heterozygosity, and the P-value from tests of Hardy-Weinberg equilibrium.

Population	Locus	N	No. Alleles	No. Private Alleles	Unique to 1 or 2	H_E	H_O	P
Central								
Cow Flat	cx3	23	7	0	2	0.304	0.730	0.0000
	cx7	23	6	1	2	0.522	0.522	0.2666
	cx9	23	13	1	4	0.565	0.873	0.0000
	cx11	23	10	1	3	0.696	0.850	0.0401
	Total		36	3	11			
Delonegha	cx3	23	8	1	4	0.696	0.843	0.4144
	cx7	23	2	0	0	0.391	0.372	1
	cx9	23	12	3	5	0.652	0.756	0.4770
	cx11	23	8	0	0	0.522	0.790	0.0024
	Total		30	4	9			
Intermediate								
Borel Road	cx3	24	6	1	1	0.375	0.743	0.0001
	cx7	23	6	1	1	0.435	0.654	0.0000
	cx9	23	13	2	4	0.652	0.877	0.0045
	cx11	23	9	1	1	0.652	0.814	0.1465
	Total		34	5	7			
Keyesville	cx3	23	4	0	0	0.391	0.588	0.0017
	cx7	23	6	1	1	0.304	0.578	0.0011
	cx9	23	10	2	3	0.435	0.814	0.0000
	cx11	22	8	0	2	0.773	0.769	0.0474
	Total		28	3	6			
Edge								
Golf Course	cx3	15	5	0	1	0.733	0.651	0.2169
	cx7	15	4	0	1	0.400	0.524	0.0040
	cx9	15	6	0	1	0.467	0.763	0.0011
	cx11	15	7	0	0	0.733	0.763	0.6065
	Total		22	0	3			
Squirrel Mtn	cx3	23	3	0	0	0.435	0.563	0.0016
	cx7	23	4	0	0	0.391	0.592	0.0395
	cx9	22	5	0	0	0.364	0.563	0.0188
	cx11	23	7	0	0	0.869	0.780	0.6381
	Total		19	0	0			

Table A4. Sequence variation. For each population by locus combination, the table shows the number of sequences obtained, the number of haplotypes, θ_W for all sequenced sites (Total) and silent sites alone, θ_π for all sequenced sites (Total) and silent sites alone, Tajima's *D*, and Fu's *Fs*.

Popn	Locus	N	H	Total θ_W	Silent θ_W	Total θ_π	Silent θ_π	*D*	*Fs*
Central									
Cow Flat	a9	29	22	0.038	0.052	0.026	0.037	-1.2	-12.47
	a16	27	5	0.016	0.019	0.017	0.028	1.71	1.30
	a23	40	7	0.015	0.043	0.014	0.029	-1.10	0.23
	d05	34	29	0.031	0.044	0.015	0.045	-1.13	-17.28
	d13	44	13	0.015	0.020	0.006	0.012	-0.30	-1.68
	f9	42	24	0.017	0.049	0.011	0.022	-1.68	-9.63
	g2	14	11	0.023	0.031	0.015	0.02	-0.25	-5.93
	i11	44	23	0.015	0.020	0.009	0.025	0.54	-10.54
	k22	38	29	0.014	0.042	0.011	0.023	-1.31	-17.86
Delonegha	a9	27	11	0.011	0.017	0.028	0.04	-0.56	0.30
	a16	23	7	0.008	0.019	0.012	0.019	-0.29	-1.90
	a23	30	6	0.009	0.015	0.013	0.025	-1.34	0.32
	d05	34	23	0.021	0.034	0.016	0.049	0.24	-6.05
	d13	41	23	0.007	*	0.008	0.016	-0.31	-11.13
	f9	38	18	0.007	0.013	0.008	0.01	-1.46	-6.62
	g2	15	7	0.015	0.023	0.014	0.02	0.51	-0.57
	i11	40	24	0.009	0.021	0.012	0.033	-0.09	-9.60
	k22	32	24	0.007	0.014	0.014	0.028	0.22	-8.77
Intermediate									
Borel Rd	a9	30	20	0.022	0.036	0.022	0.032	-1.01	-9.88
	a16	27	10	0.017	0.034	0.013	0.021	-1.29	-4.70
	a23	29	8	0.013	*	0.027	0.061	0.16	1.12
	d05	38	27	0.028	0.06	0.015	0.044	-0.39	-10.81
	d13	37	24	0.015	0.029	0.008	0.016	-0.98	-13.40
	f9	42	22	0.007	0.008	0.011	0.013	-0.97	-7.67
	g2	15	10	0.024	0.04	0.016	0	0.03	-3.59
	i11	48	22	0.014	0.028	0.007	0.020	0.04	-10.91
	k22	42	28	0.011	0.015	0.013	0.027	-0.51	-11.27
Keyesville	a9	29	18	0.022	0.061	0.028	0.039	-0.51	-5.49
	a16	20	5	0.033	0.045	0.009	0.014	-1.24	-0.92
	a23	38	6	0.012	0.017	0.032	0.072	1.41	4.67
	d05	41	22	0.016	0.046	0.014	0.043	-0.63	-4.31
	d13	35	17	0.032	0.043	0.007	0.015	0.04	-5.22
	f9	40	17	0.013	0.018	0.008	0.011	0.44	-4.63
	g2	16	10	0.014	0.039	0.013	0.019	0.16	-3.58

	i11	41	16	0.029	0.043	0.010	0.03	0.29	-2.31
	k22	30	19	0.010	0.015	0.016	0.033	0.01	-2.73
Edge									
Golf Course	a9	23	11	0.021	.	0.029	0.029	0.12	0.17
	a16	15	9	0.022	0.038	0.016	0.020	-0.80	-4.66
	a23	20	5	0.013	0.025	0.021	0.022	-0.19	1.85
	d05	18	14	0.015	.	0.013	0.014	-0.21	-3.78
	d13	27	12	0.022	0.046	0.008	0.007	0.59	-1.45
	f9	23	12	0.016	0.031	0.009	0.011	-0.79	-2.45
	g2	11	6	0.007	0.006	0.011	0.011	0.08	-1.05
	i11	29	19	0.022	0.050	0.008	0.010	-0.47	-10.23
	k22	19	16	0.016	0.031	0.016	0.016	0.12	-4.31
Squirrel Mtn	a9	28	11	0.007	0.012	0.029	0.041	0.88	0.450
	a16	28	7	0.013	0.021	0.011	0.017	-0.79	-1.874
	a23	35	10	0.012	0.030	0.022	0.051	-0.04	-0.391
	d05	44	29	0.012	0.020	0.010	0.031	-0.83	-16.190
	d13	34	12	0.009	0.015	0.010	0.018	0.36	-0.050
	f9	43	14	0.010	0.026	0.008	0.009	0.57	-1.745
	g2	18	8	0.009	0.015	0.016	0.022	0.17	-0.533
	i11	46	21	0.020	0.032	0.009	0.025	0.04	-7.935
	k22	26	12	0.010	*	0.012	0.024	-0.71	-0.075
Species-wide	a9	167	77	0.049	0.078	0.029	0.042	-1.22	-71.87
	a16	140	17	0.021	0.035	0.014	0.022	-0.90	-7.23
	a23	192	22	0.033	0.060	0.022	0.050	-0.89	-4.73
	d5	209	114	0.022	*	0.014	0.045	-1.02	-125.06
	d13	218	65	0.014	0.022	0.008	0.015	-1.29	-54.30
	f9	228	86	0.026	*	0.010	0.013	-1.86	-89.29
	g2	284	95	0.013	*	0.011	0.030	-0.54	-106.66
	i11	242	118	0.027	*	0.015	0.033	-1.37	-139.06
	k22	284	27	0.019	0.026	0.016	0.023	-0.56	-14.22
Sum		1867	621						
Mean				0.025	0.044	0.015	0.030	-1.07	-68.05

* θ could not be calculated because of three segregating bases

Table A5. Pairwise F_{ST}s for the eight sequenced loci.

Pop 1	Pop 2	a9	a16	a23	d5	d13	f9	i11	k22	mean	
Within Region											
CF	D	0.05	0.12	-0.02	0.08	0.04	0.03	0.21	0.27	0.099	
K	BR	0.02	-0.02	0.06	0.04	-0.01	0.07	0.10	0.13	0.048	
GC	SM	0.13	0.03	0.13	0.10	0.08	0.09	0.15	0.21	0.117	
Center vs. Intermediate											
CF	BR	0.02	0.07	0.03	0.01	0.01	0.11	0.24	0.13	0.076	
CF	K	0.04	0.08	0.16	0.08	0.03	0.28	0.24	0.13	0.130	
D	BR	0.10	0.03	0.04	0.06	0.02	0.15	0.19	0.24	0.104	
D	K	0.13	0.08	0.18	0.17	0.02	0.34	0.10	0.21	0.153	
Edge vs. Intermediate											
GC	BR	0.07	0.02	0.08	0.03	0.01	0.18	0.23	0.21	0.105	
GC	K	0.08	0.08	0.20	0.11	0.01	0.11	0.10	0.16	0.107	
SM	BR	0.12	0.00	-0.01	0.05	0.02	0.10	0.13	0.03	0.054	
SM	K	0.08	0.02	0.10	0.03	0.04	0.00	0.12	0.10	0.061	
Center vs. Edge											
CF	GC	0.06	0.13	0.11	0.05	0.02	0.34	0.30	0.24	0.156	
CF	SM	0.11	0.13	0.08	0.07	0.08	0.32	0.22	0.11	0.140	
D	GC	0.13	-0.01	0.10	0.12	0.05	0.37	0.06	0.12	0.119	
D	SM	0.21	0.04	0.09	0.22	0.04	0.38	0.13	0.25	0.171	

Table A6. Results of the MIGRATE analysis including the source and recipient populations, the mode of the posterior distribution of the migration parameter *M* across all loci, the lower and upper bounds of the 95% confidence interval, the recipient population's θ, and 4*Nm* (the product of *M* and θ).

Source	Recipient	M Mode	M 2.5%	M 97.5%	Recipient θ	4*Nm*
CF	BR	0.5	0	125	0.0891	0.0
D	BR	65.5	15	147	0.0891	5.8
GC	BR	170.5	79	267	0.0891	15.2
K	BR	782.5	570	992	0.0891	69.7
SM	BR	321.5	212	626	0.0891	28.7
BR	CF	149.5	55	283	0.0046	0.7
D	CF	35.5	1	86	0.0046	0.2
GC	CF	15.5	0	60	0.0046	0.1
K	CF	59.5	2	145	0.0046	0.3
SM	CF	0.5	0	41	0.0046	0.0
BR	D	0.5	0	228	0.0173	0.0
CF	D	124.5	36	212	0.0173	2.2
GC	D	36.5	0	114	0.0173	0.6
K	D	30.5	0	100	0.0173	0.5
SM	D	15.5	0	67	0.0173	0.3
BR	GC	70.5	0	180	0.0046	0.3
CF	GC	102.5	42	335	0.0046	0.5
D	GC	67.5	0	233	0.0046	0.3
K	GC	216.5	40	383	0.0046	1.0
SM	GC	74.5	11	217	0.0046	0.3
BR	K	122.5	31	199	0.0136	1.7
CF	K	156.5	43	266	0.0136	2.1
D	K	42.5	2	113	0.0136	0.6
GC	K	125.5	34	210	0.0136	1.7
SM	K	84.5	0	232	0.0136	1.2
BR	SM	448.5	208	776	0.0056	2.5
CF	SM	0.5	0	106	0.0056	0.0
D	SM	30.5	0	98	0.0056	0.2
GC	SM	33.5	0	108	0.0056	0.2
K	SM	336.5	183	666	0.0056	1.9

Figure A1. Estimates of historical asymmetric patterns of migration as inferred from the MIGRATE analysis. Arrows represent the number of immigrants per generation, $4Nm = M \times \theta$, where the width of arrows is proportional the rate of immigration into a population.