JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12845

Pollinator-mediated assemblage processes in California wildflowers

R. BRISCOE RUNQUIST*†[1], D. GROSSENBACHER*‡[1], S. PORTER*§, K. KAY¶ & J. SMITH***

*Department of Evolution and Ecology, University of California, Davis, Davis, CA, USA
†Department of Plant Biology, University of Minnesota, 250 Biological Sciences, St. Paul, MN, USA
‡School of Biological Sciences, Washington State University, Pullman, WA, USA
§School of Biological Sciences, Washington State University, Vancouver, Vancouver, WA, USA
¶Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, Santa Cruz, CA, USA
**Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA

Keywords:

Clarkia;
community assemblage;
community phylogenetics;
congener;
Limnanthes;
mating system;
Mimulus;
null models;
phylogeny;
species coexistence.

Abstract

Community assembly is the result of multiple ecological and evolutionary forces that influence species coexistence. For flowering plants, pollinators are often essential for plant reproduction and establishment, and pollinator-mediated interactions may influence plant community composition. Here, we use null models and community phylogenetic analyses of co-occurrence patterns to determine the role of pollinator-mediated processes in structuring plant communities dominated by congeners. We surveyed three species-rich genera (*Limnanthes*, *Mimulus* and *Clarkia*) with centres of diversity in the Sierra Nevada of California. Each genus contains species that co-flower and share pollinators, and each has a robust phylogeny. Within each genus, we surveyed 44–48 communities at three spatial scales, measured floral and vegetative traits and tested for segregation or aggregation of: (i) species, (ii) floral traits (which are likely to be influenced by pollinators), and (iii) vegetative traits (which are likely affected by other environmental factors). We detected both aggregation and segregation of floral traits that were uncorrelated with vegetative trait patterns; we infer that pollinators have shaped the community assembly although the mechanisms may be varied (competition, facilitation, or filtering). We also found that mating system differences may play an important role in allowing species co-occurrence. Together, it appears that pollinators influence community assemblage in these three clades.

Introduction

Coexistence of species within a community is the outcome of multiple ecological and evolutionary processes operating across the landscape (Jordan, 1905, 1908; Diamond, 1975; Armbruster, 1995; Sargent & Ackerly, 2008; Chesson, 2000). In co-flowering plant communities, pollinators play a pivotal role in assemblage, as they are often essential to reproduction, establishment and population persistence. Pollinators visit flowers based on specific cues (e.g. petal size, shape and colour) and may act as a sieve determining the species, traits and phylogenetic composition of plant communities (Armbruster, 1995; Sargent & Ackerly, 2008). Additionally, plant species may compete or mutually benefit from sharing pollinators, further influencing coexistence and trait evolution (Rathcke, 1983; Armbruster, 1986; Moeller, 2004; Morales & Traverset, 2008; Mitchell *et al.*, 2009; Cadotte *et al.*, 2009; Briscoe Runquist & Stanton, 2013; Grossenbacher & Stanton, 2014). Studies of community composition can shed light on how pollinators may influence community assemblage (Gotelli, 2000; Kraft *et al.*, 2007; Sargent & Ackerly, 2008; Cavender-Bares *et al.*, 2012).

Correspondence: Ryan Briscoe Runquist, Department of Plant Biology, 250 Biological Sciences, University of Minnesota, St. Paul, MN 55108, USA.
Tel.: +1 612 624 3124; fax: +1 612 625 1738;
e-mail: rbriscoe@umn.edu
[1]These authors contributed equally to this work.

© 2016 EUROPEAN SOCIETY FOR EVOLUTIONARY BIOLOGY. *J. EVOL. BIOL.* **29** (2016) 1045–1058
JOURNAL OF EVOLUTIONARY BIOLOGY © 2016 EUROPEAN SOCIETY FOR EVOLUTIONARY BIOLOGY

Community assembly is a dynamic process that integrates the tolerances of species to the abiotic environment, and biotic interactions including the presence or absence of mutualists, competitors and predators. Modern coexistence theory posits that species are able to coexist if niche differentiation exceeds differences in competitive abilities that would otherwise lead to competitive exclusion (Chesson, 2000; Mayfield & Levine, 2010; HilleRisLambers *et al.*, 2012). If plant species compete for pollinator services, differences in the ability to attract and maintain faithful pollinators can generate fitness differences that lead to exclusion of the less competitive species from a community (Rathcke, 1983; Geber & Moeller, 2006; Morales & Traverset, 2008; Mitchell *et al.*, 2009). A species' ability to attract a novel pollinator or to leverage traits that encourage pollinator constancy is an example of stabilizing niche differences that could prevent competitive exclusion (Rathcke, 1983; Waser, 1986; Geber & Moeller, 2006). Plant species may also facilitate each other through shared pollinator services (Johnson *et al.*, 2003; Moeller, 2004; Geber & Moeller, 2006; Ghazoul, 2006). Coexistence of multiple flowering species may support larger and/or more diverse pollinator assemblages that, in turn, will increase the fecundity of co-flowering community members, particularly in stressful environments (Bosch & Waser, 1999; Johnson *et al.*, 2003; Geber & Moeller, 2006; Ghazoul, 2006; Gross, 2008; but see Chesson & Huntley, 1997). Thus, the balance between species' interactions with their abiotic and biotic environment contributes to landscape-level patterns of species and trait composition within communities.

Strong and consistent pollinator-mediated interactions lead to the aggregation or segregation of particular species and traits within communities (Armbruster, 1995; Geber & Moeller, 2006; Sargent & Ackerly, 2008). Aggregation refers to the clustering of species and traits, whereas segregation (sometimes referred to as overdispersion or evenness) implies that species or traits are less likely to be found together than under random expectations. Surprisingly disparate processes lead to aggregation including pollinator-mediated filtering, competition or facilitation (Sargent & Ackerly, 2008; Mayfield & Levine, 2010). Filtering, due to the presence or absence of necessary pollinators, leads to aggregation of species that rely on the same pollinator assemblage (Geber & Moeller, 2006; Sargent & Ackerly, 2008). Competition leads to aggregation when differences in species' ability to attract pollinators lead to strong fitness differences (Mayfield & Levine, 2010; HilleRisLambers *et al.*, 2012). In this case, species that are highly attractive to pollinators (strong competitors) will aggregate. Lastly, facilitation leads to aggregation if species or traits positively feedback to support and promote the pollinator assemblage that is most beneficial to a particular subset of the community (Gotelli & Graves, 1996; Geber & Moeller, 2006; Sargent & Ackerly, 2008).

In contrast to aggregation (which may be due to multiple processes described above), segregation is the result of a single process: competition (Sargent & Ackerly, 2008; Mayfield & Levine, 2010). Segregation occurs when species or traits that are highly similar suffer from strong competition, leading to exclusion of particular species or traits from the community (Diamond, 1975; Roughgarden, 1976; Tilman, 1982; Stone & Roberts, 1990; Gotelli, 2000; Gotelli & McCabe, 2002; Muchhala & Potts, 2007; Muchhala *et al.*, 2014). In this scenario, competition for pollinator services reduces co-occurrence of species that share pollinators and may even favour co-occurrence of species that differ in mating system (predominantly selfing vs. predominantly outcrossing, Waser, 1978; Fishman & Wyatt, 1999; Bell *et al.*, 2005).

While the aggregation or segregation of floral traits is likely driven by pollinators, other biotic and abiotic pressures may influence vegetative trait patterns. For example, local herbivores, plant diseases, soil moisture and elevation gradients are well known to drive the aggregation of species with similar heights and leaf characteristics (Cadotte *et al.*, 2009; Mayfield & Levine, 2010; Cavender-Bares *et al.*, 2012; HilleRisLambers *et al.*, 2012). The simultaneous study of both floral and vegetative traits may thus provide greater context for species co-occurrence patterns in co-flowering communities. Contrasting patterns of co-occurrence among these different functional traits may implicate multiple and potentially antagonistic processes underlying community assemblage. Unfortunately, these have rarely been considered in the same study (but see Muchhala *et al.*, 2014).

Species and their traits are not independent, but are the result of speciation and shared ancestry. When the pattern of trait evolution across a clade (e.g. trait divergence or convergence) affects the strength of interactions between species, this may determine whether close relatives co-occur (Darwin, 1859; Jordan, 1905, 1908; Sargent & Ackerly, 2008; Cadotte *et al.*, 2009; Mayfield & Levine, 2010). For example, if a trait exhibits phylogenetic signal (i.e. close relative are similar) and influences pollinator-mediated competition, we might expect concurrent segregation of the trait and close relatives. In this case, the ecological process of competition and evolutionary pattern of phylogenetic signal interact to generate the patterns of segregation. It is therefore necessary to evaluate trait evolution and community assembly patterns within a phylogenetic context to understand the interplay between ecological and evolutionary processes in determining community composition (Mayfield & Levine, 2010; Cavender-Bares *et al.*, 2012; Godoy *et al.*, 2014; Kraft *et al.*, 2015).

In this study, we explore how pollinator-mediated interactions shape community assemblage of three

California wildflower genera: *Limnanthes*, *Mimulus* and *Clarkia*. Congeners may interact strongly due to shared ancestry (Darwin, 1859), making them ideal to disentangle the ecological and evolutionary influences on species and trait co-occurrence. These genera meet three basic requirements: congeners have high actual or potential co-occurrence (Mason, 1952; Lewis & Lewis, 1955; Grossenbacher & Whittall, 2011), congeners interact strongly and frequently through shared pollinator services (Lewis, 1961; Moeller, 2004; Moeller & Geber, 2005; Moeller, 2006; Briscoe Runquist & Stanton, 2013; Grossenbacher & Stanton, 2014), and we are able to construct a robust phylogeny for each genus. To infer the processes that influence patterns of community assemblage within these focal genera, we addressed the following specific questions at three spatial scales: (i) Are congeners spatially aggregated or segregated? (ii) What is the pattern of floral and vegetative trait evolution across the phylogeny? (iii) Do congeners within a community have similar or dissimilar traits relative to what would be expected by chance? (iv) Are congeners within a community more or less closely related than expected by chance?

Materials and methods

Study area

This study took place in the northern and central Sierra Nevada foothills of California, during the spring of 2009. This area, roughly 350 km long and 50 km wide, has a Mediterranean climate (characterized by cool, rainy winters and hot, dry summers) and contains among the highest diversity of annual wildflowers found in North America. In this region, the three focal plant genera, *Limnanthes, Mimulus* and *Clarkia*, have high native species diversity and similar spring flowering times. Conspecifics and congeneric individuals dominate many local communities and heavily rely on pollinators: primarily native specialist bees.

Taxa

Limnanthes contains four species within the study area (Fig. 1), occurring in ephemerally wet grasslands, most often vernal pools. Seeds often germinate and seedlings grow during a mostly aquatic phase. Flowers bloom as the water recedes in the mid-to-late spring. *Limnanthes* flowers are radially symmetric, cream coloured and range in size from 10 to 50 mm (Fig. 1). For the four taxa surveyed, flowers are self-compatible and range from outcrossing to mixed-mating (Mason, 1952). Flowers are visited predominantly by native specialist bees, which provision larvae with only *Limnanthes* pollen and occasionally by native generalist bees and honey bees (Thorp, 1976; Thorp & Leong, 1998). Floral displays can range from 1 to 150 flowers/plant, and populations can be very dense (> 100 plants per m^2) in wet years. The clade is endemic to the California Floristic Province.

Mimulus contains 26 species within the study area (Fig. 1), with large differences in habitat affinity, occurring in seeps, vernal pools and dry rocky outcrops. There are large differences among species in flower size (2–50 mm in length), flower colour (dark red to yellow and white) and flower shape (near radial symmetry to highly zygomorphic flowers) (Grant, 1924; Fig. 1). All species are self-compatible, but mating systems range from predominant selfing to outcrossing (Beardsley *et al.*, 2004; Ritland & Ritland, 1989). Many *Mimulus* species are bee pollinated and several bees appear to be restricted *Mimulus* specialists (Hurd, 1979; USDA-ARS Regional Bee Survey 2006). Several species are primarily hummingbird or hawk moth pollinated (Beardsley *et al.*, 2004). The clade is found worldwide but has a centre of diversity in western North America, particularly California (Beardsley *et al.*, 2004).

Clarkia contains 11 species within the study area (Fig. 1), occurring in pine–oak woodlands, chapparal, road cuts and rock outcrops in full sun to part shade and on a variety of soils (e.g. serpentine, granitic, metamorphic and volcanic). *Clarkia* flowers are always four petaled and radially symmetric and range in colour from cream to dark pink with variable petal speckling or spotting (Fig. 1). Flower shape ranges from bowl-shaped to rotate and petal length from 6 to 20 mm. All species are self-compatible, but mating systems range from predominantly selfing to almost completely outcrossing (Lewis & Lewis, 1955). *Clarkia* species are primarily visited by species of native specialist bees, which use only *Clarkia* pollen to provision offspring and occasionally by native generalist bees (MacSwain *et al.*, 1973). *Clarkia* is largely restricted to California with few exceptions (Lewis & Lewis, 1955).

Species-level phylogenies

We reconstructed phylogenies for all three clades because previously published phylogenies used disparate methods and did not always estimate relative divergence times. We used previously published gene partitions and alignments for *Limnanthes* and *Mimulus* (Meyers *et al.*, 2010; Grossenbacher & Whittall, 2011). For *Clarkia*, we obtained sequences at the ITS and trnL-F gene regions from GenBank for 14 taxa, obtained tissue samples and sequenced these gene regions ourselves for an additional 13 taxa (see Appendix S1) and created alignments with MUSCLE v. 3.8 followed by manual adjustments (Edgar, 2004). All species from our study area were included except *Mimulus laciniatus* and two allopolyploid species: *Clarkia purpurea* and *Clarkia rhomboidea*.

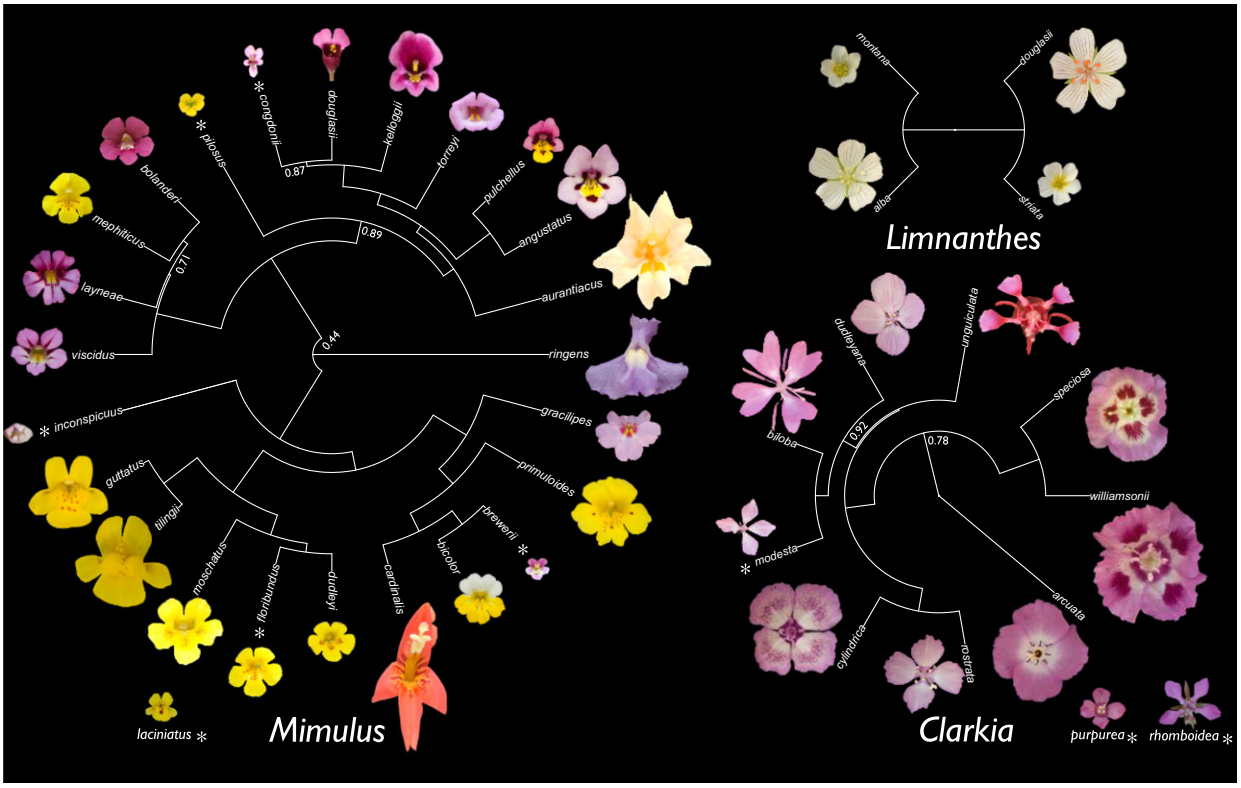


Fig. 1 Bayesian phylogenies with branch lengths proportional to time for *Limnanthes*, *Clarkia* and *Mimulus*, pruned to include only species observed in this study. Posterior probabilities are indicated below branches only when < 0.95. Asterisks indicate species that are predominantly selfing. All photos were taken at study sites in 2009. Note that *Mimulus laciniatus*, *Clarkia purpurea* and *Clarkia rhomboidea* were not represented in the phylogenies, yet occurred in our study area. They are thus excluded from downstream phylogenetic analyses.

We simultaneously estimated phylogenetic relationships and absolute divergence times among species within each clade in a Bayesian framework in BEAST v. 1.6.2 (Drummond *et al.*, 2006; Drummond & Rambaut, 2007). To accommodate heterogeneity in the molecular evolutionary rate among branches, we used an uncorrelated log-normal relaxed clock model. The prior model on branch lengths was set to a birth–death model. The prior model on substitutions, the number of Markov chain Monte Carlo (MCMC) generations and the number of MCMC runs varied by clade (Table S1). To determine whether the priors had unduly influenced posterior parameter estimates, we compared posterior parameter distributions to those obtained running BEAST on an empty alignment (i.e. without the DNA sequence data) using the same model. Posterior samples of parameter values were summarized and assessed for convergence and mixing using Tracer v. 1.6 (http://beast.bio.ed.ac.uk/Tracer), combined using LogCombiner v. 1.8.0 (http://beast.bio.ed.ac.uk/LogCombiner) and summarized using TreeAnnotator v. 1.8.0 (http://beast.bio.ed.ac.uk/TreeAnnotator).

All ensuing phylogenically informed analyses were performed on consensus trees, pruned to species found in the study area (Fig. 1).

Clade age estimates

Our study compares community assemblage patterns among three genera, each of which may have evolved over different amounts of evolutionary time. Older clades may assemble species that have diverged for greater lengths of time relative to younger clades, which may impact phylogenetic community patterns. Time since divergence may also affect species trait divergence, assembly and contemporary ecological processes (Pontarp *et al.*, 2015). To understand how clade age may influence patterns of co-occurrence, we estimated the time since the most recent common ancestor for each regional clade.

Fossils are not known for these clades; therefore, to estimate clade ages, we relied on the mean and range nrITS substitution rate for herbaceous plants (Kay *et al.*, 2006) using the data set from Grossenbacher *et al.* (2015). We used a kernel density function to estimate

the probability density function of clade ages using a sample of trees from the posterior distribution of trees from Grossenbacher *et al.* (2015) (pruned to species in our study area) and assessed whether the 95% confidence intervals were overlapping among clades.

Co-occurrence at three scales

To assess patterns of co-occurrence within a given plant community, we visited 50 predetermined GPS coordinates for each genus (N = 150 total sites) located within the study area (Fig. 2). Each site was treated as a separate community; thus, there were 50 replicate communities per genus and each community was assumed to have undergone independent assembly. GPS coordinates were selected randomly (irrespective of species) from a complete list of coordinates for each genus downloaded from the Consortium of California Herbaria database (http://ucjeps.berkeley.edu/consortium/) and were visited during spring 2009, each within 2 weeks of the date stated on the herbarium record. The average minimum distance between sites was 8.1 km for *Limnanthes* (range 0.6–34.7 km), 6.6 km for *Mimulus* (range 0.3–21.6 km) and 8.0 km for *Clarkia* (1.3–66.4 km). Starting at the centre of this patch, we assessed species presence/absence at three scales defined by increasingly larger community circles. The radii of the circles were 1 m, 30 m and 100 m. Previous study has shown the 1 and 30 m scales to be particularly relevant to pollinator-mediated interactions in these clades (e.g. Moeller, 2004; Briscoe Runquist & Stanton, 2013; Grossenbacher & Stanton, 2014). Voucher specimens were deposited at the UC Davis herbarium. In total, we surveyed 48 sites for *Limnanthes*, 48 sites for *Mimulus* and 44 sites for *Clarkia* with one or more species present that were used for further analysis.

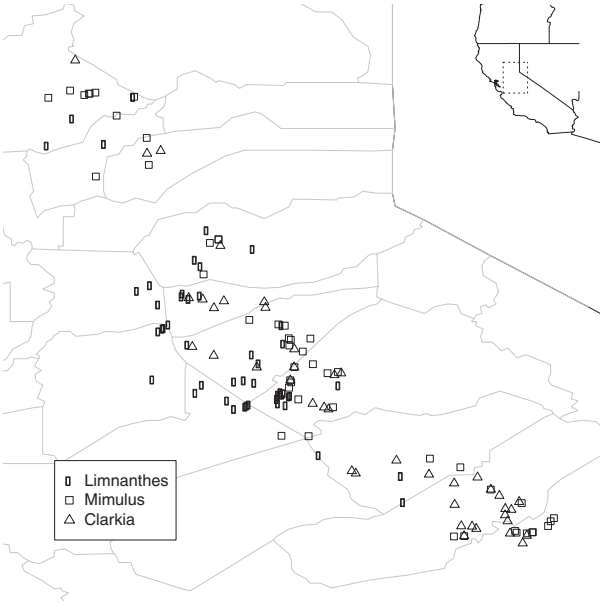


Fig. 2 Site locations of predetermined GPS coordinates in the northern and central Sierra foothills of California where co-occurrence was assessed (N = 48, 48 and 44 for *Limnanthes*, *Mimulus* and *Clarkia*, respectively).

Species traits

We calculated species mean trait values using material from the study area. At each site, we randomly chose 1–3 individuals of each species at that site to measure both vegetative and floral traits. To obtain species-level means, we averaged trait measurements of individuals from all the sites where they were found (mean of 3.7 *Mimulus* individuals/species, 4.6 *Clarkia* individuals/species and 7.4 *Limnanthes* individuals/species).

Floral traits

Pollinators use floral size, shape and colour to discriminate between species, making these potentially important trait axes for competition or partitioning among co-flowering species (van der Pijl, 1961; Chittka & Raine, 2006). To characterize overall flower size, we measured petal width, corolla diameter, petal length, flower depth, corolla aperture and corolla area on standardized photos in ImageJ (see Appendix S2; Fig. S1). Due to correlation among floral-trait measurements, we conducted a principal components analysis (PCA) including all floral traits within each genus to obtain fully orthogonal floral variables. For each genus, the first principal component (PC1) accounted for 60–80% of the variation in floral measurements and represented a measure of floral size, which we used in subsequent analyses.

We used a geometric landmark analysis to characterize the dimensions of flower shape for each species, which eliminates variation due to flower size and orientation (Adams *et al.*, 2004). We defined a number of easily identifiable landmarks (N = 75, *Limnanthes*; N = 50, *Mimulus*; N = 72, *Clarkia*) along the outline of the frontal view of the flower. We used the morphometric software tpsDIG2 v. 2.17 (http://life.bio.sunysb.edu/morph/) to capture the landmarks on up to five photographs per species in *Mimulus* and *Clarkia* (mean = 4) and up to 10 photographs per species for *Limnanthes* (mean = 7). We then used tpsRelw v. 1.53 (http://life.bio.sunysb.edu/morph/), as described in Rohlf & Slice (1990), to perform a generalized orthogonal least-squares Procrustes analysis (GPA) and thin-plate splines to describe the deformation in shape for each specimen's landmarks. Parameters of the thin-plate spline deformations are the partial warp scores. We then generated relative warp scores using a principle components analysis of the covariance matrix of the partial warp scores, which described the major axes of

shape variation among our specimens (Adams *et al.*, 2004). We used the first relative warp for each genus for further analysis of shape. RW1 explained 29–47% of the shape variation among specimens within a genus (see Appendix S3; Figs S2–S4).

We quantified flower colour from field-captured digital images with colour standards (see Appendix S4) using ImageJ software (Abramoff *et al.*, 2004). For each image, we recorded the average pixel values for the RGB colour channels within a defined floral region (Figs S5–S6) and for the colour standards. We then corrected the raw RGB colour values to compensate for nonlinear camera responses and variability in natural light conditions (Stevens, 2007; Stevens *et al.*, 2007; Westland & Ripamonti, 2004; see Appendix S4). As colour is processed independently of brightness in many vertebrate and invertebrate visual systems (reviewed in Endler & Mielke, 2005), we corrected colour reflectance values for brightness and analysed only chromasticity coordinates (a two-dimensional measure in triangular colour space) in downstream analyses.

Stigma–anther separation (herkogamy) is known to correlate with plant mating system and was measured as the distance between the tip of the stigma and the tip of the tallest anther on specimens in the field. Negative separation is possible when the anthers overtop the stigma.

Vegetative traits

We measured vegetative traits that reflect aspects of habitat affinity and competitive ability: plant height (cm), specific leaf area (SLA, cm^2 per mg) and leaf area (cm^2). Plant height is often correlated with shade tolerance or competitive ability and was measured as the length of the plant from the base to the uppermost leaf or flower. SLA is a common measure to characterize the ecophysiological strategy of a plant species; species with low SLA tend to have slower relative growth rates, longer-lived leaves and live in habitats that are nutrient poor or have low water availability (Pooter & Garnier, 1999). To measure SLA, we collected the youngest fully expanded leaf from any portion of the plant and immediately photographed it on a flat surface with a size standard; we measured area using ImageJ (Rasband, 1997–2014). The leaf was then dried at 60 °C for a week and weighed. SLA was calculated as the leaf surface area divided by the leaf dry weight. We also performed analyses on leaf area alone as this variable indicates light capture ability and correlates with water-use efficiency (Pooter & Garnier, 1999).

Analyses

Co-occurrence patterns among congeneric species

To assess whether congeneric species are aggregated, segregated, or randomly distributed across sites, we conducted null model analysis focusing on the C-score index of the presence/absence co-occurrence matrix for each genus separately (Stone & Roberts, 1990; Gotelli, 2000). The C-score assesses the number of 'mostly forbidden pairs' (i.e. pairs of species that only rarely co-occur in the same sites) in a co-occurrence matrix (Stone & Roberts, 1990). We used fixed species occurrence and fixed site occurrence simulations for *Mimulus* and *Clarkia*. For *Limnanthes*, due to the small number of species and the high number of sites with only one species present, we used a fixed species occurrence and equiprobable site occurrence model to assess significance. We used EcoSim v. 7 (Gotelli & Entsminger, 2011) to generate a distribution of test indices from 5000 null matrices created by permutation of the community co-occurrence matrix. Indices were considered significantly segregating if the value was > 97.5% of null index values and significantly aggregating if the value was < 2.5% of null index values (Stone & Roberts, 1990; Gotelli, 2000). Because the ranges of some species do not fully encompass the study area (n = 1, 3, 4 for *Limnanthes*, *Mimulus* and *Clarkia*, respectively), we also conducted null model analysis that accounted for these differences; this did not qualitatively change results and is not presented.

In the analysis of community co-occurrence patterns, it is possible that individual species pairs may exhibit patterns that are counter to overall community assemblage patterns and these specific pairs may highlight particular ecological mechanisms. To assess co-occurrence patterns of individual species pairs, we used the program Pairs (Ulrich, 2008; Ulrich & Gotelli, 2013) to ascertain if there were any significant co-occurrence patterns for specific species pairs within the co-occurrence matrix.

Phylogenetic signals of trait evolution

We assessed the degree of phylogenetic signal for floral and vegetative traits using Blomberg's K (Blomberg *et al.*, 2003). Blomberg's K is a measure of the observed variance of phylogenetically independent contrasts and ranges from 0 to infinity, with higher values indicating a greater degree of phylogenetic signal. We used the R phytools package to test the null hypothesis of no phylogenetic signal, that is $K = 0$. Significance was assessed at the $P = 0.05$ level by randomizing the tips of the phylogeny and comparing the observed K value to the null distribution.

Correlations between traits and the evolution of correlated traits can make it difficult to determine which traits influence community assemblage. We assessed trait correlations for each genus using phylogenetic generalized least squares (PGLS). We implemented a PGLS regression using the function corBrownian in the package ape (Paradis *et al.*, 2004) to obtain the phylogenetic correlation structure, assuming a Brownian motion model of evolution and the function gls in the nlme package (Pinheiro *et al.*, 2015). We note that we

also implemented a PGLS regression assuming an OU model of evolution using the function corMartins in the package ape (Paradis *et al.*, 2004) to obtain the phylogenetic correlation structure. This did not qualitatively change our results (not presented).

Patterns of trait aggregation and segregation

We tested for segregation or aggregation of trait values among co-occurring congeners. For each genus, we calculated a species-level phenotypic distance matrix for each trait individually. At each site, we calculate the phenotypic mean pairwise distance (MPD) of all species present. We then averaged across all sites to obtain an observed mean MPD and compared this to a mean MPD distribution from 10 000 randomly assembled communities for each trait. Null communities were generated using the independent swap algorithm in the picante R package, which preserves the species richness within sites and the relative frequency of occurrences of each species across all sites. We modified the original picante functions to allow for the inclusion of singleton sites (see Appendix S5 for code) because species may occur alone due to competitive exclusion and those species traits may also contribute to an overall pattern of trait segregation or aggregation. We calculated *Z*-values as the difference between the observed MPD and the null mean MPD. Positive *Z*-values suggest segregations. For a two-tailed test with a significance level of 0.05, the observed value had to be greater or < 97.5% of the null values. We conducted the same analyses using mean nearest-taxon distance (MNTD) and obtained similar results (not shown). Due to the possibility of false discovery from multiple comparisons, we employed a Benjamini–Hochberg correction when determining significance (Benjamini & Hochberg, 1995).

Co-occurrence and phylogenetic distance

We tested for a relationship between co-occurrence and phylogeny using a method developed for pairwise species' data sets (Fitzpatrick & Turelli, 2006). This method differs from the more commonly used MPD approach described above in that it accounts explicitly for shared ancestry, incorporates tree topology and more directly addresses how co-occurrence relates to node age (phylogenetic distance). We calculated pairwise species' co-occurrence indexes using Schoener's D (Schoener, 1968) in the R package picante, and calculated Fitzpatrick & Turelli (2006) weighted averages across all internal nodes of the phylogeny (using code from Brandvain *et al.*, 2014). We used linear regression to determine the slope of the relationship between co-occurrence and relative node age. To determine significance, we used Monte Carlo simulations to estimate the distribution of slopes under a null hypothesis of no phylogenetic signal as in Fitzpatrick & Turelli (2006). To be considered significant in a two-tailed test with a significance level of 0.05, the observed slope had to be greater or < 97.5% of the null slopes.

Excluding selfing species

Mimulus and *Clarkia* have many species that are predominantly selfing, which may limit pollinator-mediated interactions between species. Therefore, the co-occurrence analyses described above were also performed on reduced *Mimulus* and *Clarkia* data sets containing only outcrossing species that are more likely to interact through shared pollinators.

Results

Genus age estimates

The estimated time since the most recent common ancestor for *Mimulus* species in the study area was more than three times that of either *Limnanthes* or *Clarkia* (95% highest posterior density: *Mimulus* 30.9–53.9 Ma, *Clarkia* 4.2–9.3 Ma and *Limnanthes* 4.4–9.4 Ma). Thus, *Mimulus* assemblages, when compared to *Limnanthes* and *Clarkia* assemblages, may include species that are up to three times more distantly related. By contrast, potential divergence between congeners in *Limnanthes* and *Clarkia* assemblages are approximately equal.

Community-level co-occurrence

Congeners co-occurred at all three spatial scales, but there was striking variation in the degree of co-occurrence among genera. Species of *Limnanthes* rarely co-occurred—90% of sites contained only one congener at the coarsest spatial scale (Table S2) and only one species pair co-occurred (*L. alba* and *L. striata*). By contrast, species of *Mimulus* and *Clarkia* commonly co-occurred—60–80% of sites contained more than one congener and some with as many as 6–8 species co-flowering at the coarsest spatial scale (Table S2).

Are congeners aggregated or segregated?

Species of *Limnanthes* ($N = 4$) exhibited a significant pattern of segregation (C-score = 110.7–126.7; $P < 0.0001$). In an analysis designed to determine whether any of the six potentially co-occurring species pairs exhibited significant pattern, two pairs were significantly: *L. douglasii/L. striata* and *L. alba/L. striata* (Table S3).

Species of *Mimulus* ($N = 26$) did not exhibit significant genus-wide patterns of co-occurrence for the full data set (C-Score = 9.3–12; $P > 0.05$) or the reduced data set containing only outcrossing species (C-score = 9.9–13.6; $P > 0.05$). In an analysis designed to

determine whether any of the 325 potentially co-occurring species pairs exhibited significant pattern, just one pair was significantly segregating whereas seven pairs were significantly aggregating at the 100-m scale. At the 30-m scale, two pairs were significantly aggregating, one of which (*M. bicolor/M. laciniatus*$^{(selfer)}$) is shared between 100- and 30-m scales (Table S3). When restricting the analysis to just outcrossing species, there were two segregating pairs and three aggregating pairs at the 100-m scale and two aggregating pairs at the 30-m scale, one of which is shared between scales (*M. layneae/M. aurantiacus*) (Table S4).

In the full *Clarkia* data set ($N = 11$), species co-occurrence did not differ from random (C-score = 19.9–32.5; $P > 0.05$). However, when restricting the analysis to just outcrossing species, there was significant segregation at the 100-m scale (C-score = 35.6; $P < 0.05$). There was no significant pattern at the 30-m or 1-m scale (C-score = 22.6–29.3; $P > 0.05$). In an analysis designed to determine whether any of the 55 potentially co-occurring species pairs exhibited significant pattern, there were two aggregating pairs at the 100-m (*C. unguiculata/C. speciosa* and *C. willimsonii/C. modesta*$^{(selfer)}$) and 1-m scales (*C. purpurea*$^{(selfer)}$*/C. dudlyana* and *C. purpurea*$^{(selfer)}$*/C. cylindrica*) (Table S3). In an analysis of outcrossing species, only one pair is significantly aggregating at the 100-m scale (*C. unguiculata/C. speciosa)* (Table S4).

What is the pattern of trait evolution across the phylogeny?

Three traits in *Mimulus*, specific leaf area, plant height and flower shape, showed significant phylogenetic signal (Table 1), that is closely related species were more similar than would be expected than if species evolved completely independently. *Clarkia* showed significant phylogenetic signal for leaf area and stigma–anther separation (Table 1). We were unable to determine phylogenetic signal of species traits in *Limnanthes* because our data set contained too few species.

Table 1 Phylogenetic signal. Blomberg's *K* values.

	Limnanthes† *K*	*Mimulus K*	*Clarkia K*
Vegetative traits			
Plant height	1.489	0.300*	0.453
Specific leaf area	0.432	0.580***	0.618
Leaf area	0.733	0.268	0.876**
Floral traits			
Floral size	0.512	0.207	0.802
Floral shape	0.430	0.380**	0.826
Stigma–anther separation	0.462	0.159	1.824*

*Significant at $P < 0.10$, **significant at $P < 0.05$, ***significant at $P < 0.01$. †Not enough power to estimate significance in *Limnanthes*.

For all three genera, there were significant correlations among some of the measured traits (Table S5). In *Limnanthes*, floral traits were positively correlated with each other and negatively correlated with specific leaf area. In *Mimulus*, floral traits, plant height and leaf area were positively correlated. In *Clarkia*, there were fewer correlations; however, larger flowers had greater stigma–anther separation and were taller.

Do assemblages of congeners contain species that have similar or dissimilar traits relative to what would be expected by chance?

Limnanthes species exhibited significant segregation of floral shape and significant aggregation of floral size (Table 2; Table S6). Of the vegetative traits, leaf area was significantly segregated (Table 2).

In *Mimulus*, in the full data set, there were no significantly segregating or aggregating traits. By contrast, when restricting analysis to just outcrossing species, we found significant aggregation of floral shape and plant height (Table 2).

In the full *Clarkia* data set, stigma–anther separation was significantly segregated (Table 2). When restricting our analysis to just outcrossing species, there were no significantly segregating traits; there was significant aggregation of specific leaf area (Table 2).

Finally, within each genus, none of the significantly aggregating or segregating traits were correlated with one another (Table S5), suggesting that correlated evolution was not solely responsible for generating similar assemblage patterns across traits.

Are assemblages of congeners more or less closely related than would be expected by chance?

Only *Mimulus* showed a significant relationship between node age and co-occurrence, with phylogenetic segregation at the 30-m scale for the full data set, that is closely related species were less likely to co-occur than random expectation (Fig. 3). For *Limnanthes*, there was similarly a trend for phylogenetic segregation across all spatial scales; however, because our data set only contained four total species, the Fitzpatrick & Turelli (2006) permutation test is unable to assess the significance (we note that the slope of the relationship between node age and co-occurrence in *Limnanthes* was the greatest value possible with the data). In *Clarkia*, the relationship between co-occurrence and phylogenetic distance was nonsignificant across all spatial scales and data sets (Fig. 3).

Discussion

In this study, we explored the possibility that species interactions mediated through shared pollinator services influence the process of community assembly in three

Table 2 Trait segregation ('S') or aggregation ('A') in *Limnanthes, Mimulus* and *Clarkia* at three spatial scales.

	All species: outcrossers and selfers									Only outcrossers					
	Limnanthes N = 4			*Mimulus* N = 25			*Clarkia* N = 11			*Mimulus* N = 19			*Clarkia* N = 8		
	100 m	30 m	1 m	100 m	30 m	1 m	100 m	30 m	1 m	100 m	30 m	1 m	100 m	30 m	1 m
Floral traits															
Floral size (PC1)	**A**	**A**	**A**	–	–	–	–	–	–	–	–	A	–	–	–
Floral shape (RW1)	**S**	–	–	–	–	–	–	–	A	–	–	**A**	–	–	A
Stigma–anther separation	–	–	–	A	–	–	–	**S**	**S**	A	–	–	–	–	–
Whole flower colour				–	–	–	–	A	–	–	–	–			
Vegetative traits															
Plant height	–	–	–	–	–	–	–	–	–	–	–	**A**	–	–	–
Specific leaf area	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**A**
Leaf area	**S**	–	–	–	–	–	–	–	S	–	–	A	–	–	–

Traits significant at $P < 0.05$ are indicated by letters. Bolded letters indicate traits significant after Benjamini–Hochberg correction for false discovery rate at $P < 0.05$. All tests were performed on the full data set containing both outcrossers and selfers (left-hand data columns) as well as on a subsetted data set of only outcrossing *Mimulus* and *Clarkia* (right-hand data columns).

wildflower genera that are common subjects of ecological pollination studies. Although the results varied for genus and analysis, we found that congeners tend to be segregated across a range of spatial scales and display significant patterns of both aggregation and segregation of floral traits. Our results are largely consistent with other studies, finding that pollinator-mediated interactions, specifically competition, play at least a partial role in patterns of community species composition and floral traits (Armbruster, 1986; Armbruster *et al.*, 1994; Muchhala & Potts, 2007; McEwen & Vamosi, 2010; Eaton *et al.*, 2012; Heystek & Pauw, 2014; Muchhala *et al.*, 2014). While floral-trait aggregation could be due to a variety of pollinator-mediated processes, floral-trait segregation clearly suggests pollinator-mediated competition. Below, we discuss these in turn, as well as implications of vegetative trait patterns, and place our study in the broader framework of the community assemblage literature.

Aggregation of traits is consistent with competition, ecological filtering and facilitation (Mayfield & Levine, 2010; HilleRisLambers *et al.*, 2012). In our clades, pollinator filtering and facilitation may drive aggregation of similar species that attract and support a particular pollinator community. Competition may also play a role in aggregating traits that provide competitive advantages that lead to the exclusion of less competitive species (i.e. large flowers in *Limnanthes* or attractive flower shapes in *Mimulus*). We know of only one other study that found evidence for aggregation in floral traits in *Oxalis* (de Jager *et al.*, 2011). The authors of this study interpret the pattern of aggregation as support for facilitation among co-flowering species to attract a larger pollinator population, however, they do not exclude competition or filtering. Ultimately, experimental studies will be necessary to determine the processes structuring aggregating traits (e.g. Godoy *et al.*, 2014; Kraft *et al.*, 2015).

Segregation of traits and species is considered a signature of competition (Diamond, 1975; Gotelli, 2000; Mayfield & Levine, 2010). In this study, we found segregation of some floral traits and species in *Limnanthes* and *Clarkia* and mixed segregation patterns in *Mimulus*. The particular segregating floral trait within a clade may implicate different strategies of coexistence within a congeneric community structured by competition. Here, *Limnanthes* communities contained species with dissimilar floral shapes. This may indicate that co-occurring species avoid competition through attracting different pollinators within the communities or by having dissimilar traits that support pollinator constancy. *Clarkia* communities contained species dissimilar in their stigma–anther separation. This pattern implies that differences in mating system (i.e. autonomous self-fertilization vs. outcrossing) allow for greater congeneric coexistence. Likewise, in *Mimulus* communities, there was increased co-occurrence of outcrossing and selfing species, which was more common for more diverged species pairs. This may indicate that divergence in mating system is one of the important evolutionary mechanisms of co-occurrence in this genus but it interacts with other ecological and evolutionary processes in early divergence. Thus, across the three focal genera, co-occurring species may avoid competition through two common mechanisms: the attraction of different pollinators or differences in mating system.

Vegetative trait patterns did not contrast markedly with floral-trait patterns for the genera studied. Aggregation of SLA and plant height in *Clarkia* and *Mimulus*, respectively, is consistent with all ecological processes. Segregation of leaf areas in *Limnanthes* implicates competition. Given the rough concordance between floral

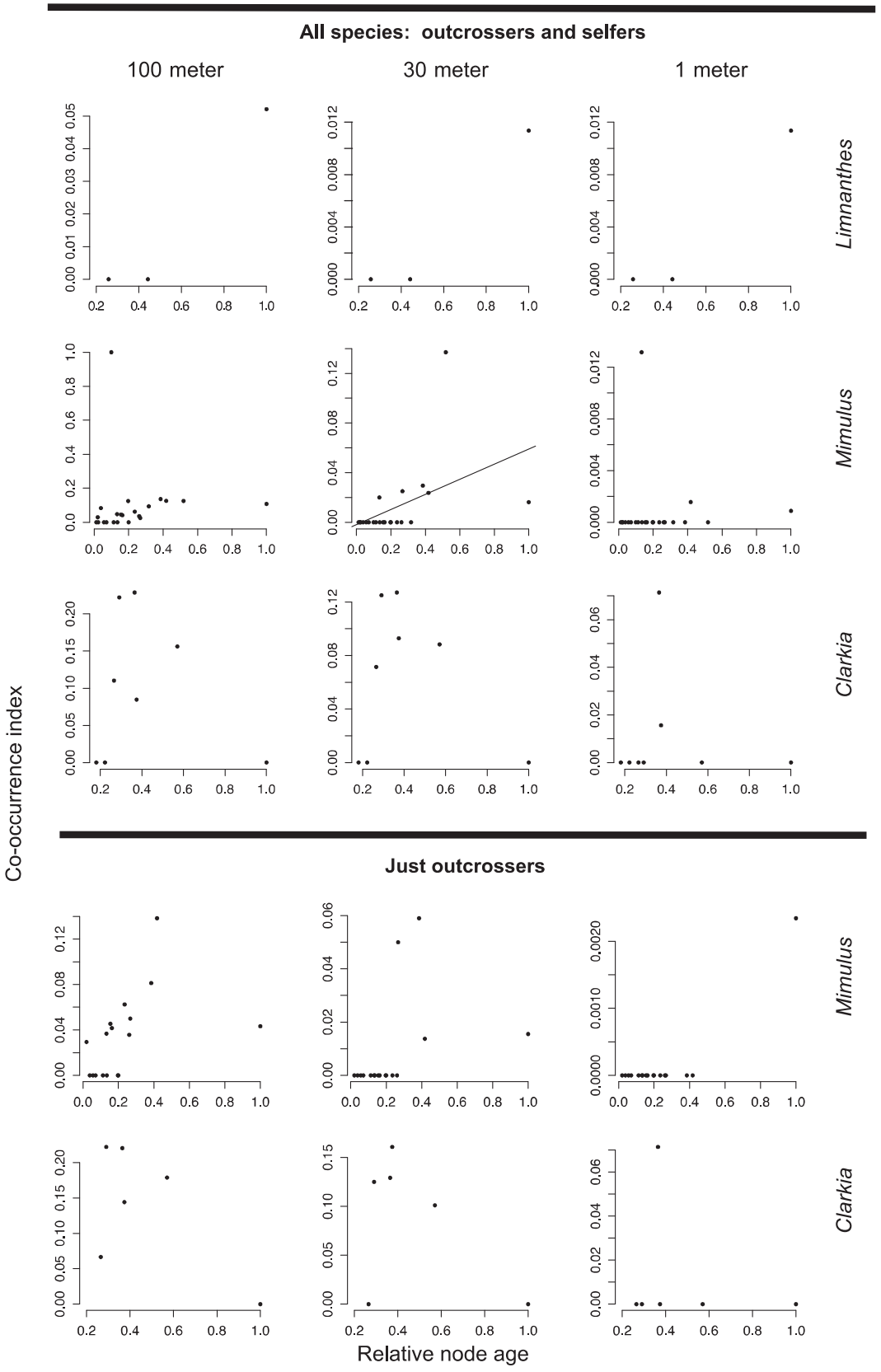

All species: outcrossers and selfers
100 meter
30 meter
1 meter
Limnanthes
Mimulus
Clarkia
Co-occurrence index
Just outcrossers
Mimulus
Clarkia
Relative node age

Fig. 3 Average co-occurrence (Schoener's D) by relative node age for *Limnanthes*, *Mimulus* and *Clarkia* at three spatial scales. Line segments represent significant slopes ($P < 0.05$) determined using the Fitzpatrick & Turelli (2006) permutation test. All tests were performed on the full data set containing both outcrossers and selfers (upper 3 figure rows) as well as on a subsetted data set of only outcrossing *Mimulus* and *Clarkia* (lower 2 figure rows).

and vegetative traits, similar ecological processes may be operating on these different sets of functional traits. It is also possible that the patterns we see in floral traits are due to community assembly by vegetative traits and correlations among floral and vegetative traits generating similar patterns of floral traits. We think this scenario is unlikely as traits with significant community assemblage patterns were uncorrelated in the present study. Furthermore, given the evidence of selection for independence of floral and vegetative traits in many specialized pollinator-dependent taxa, floral and vegetative trait correlation may not be generally true (Berg, 1960; Conner & Sterling, 1996), although it would need to be rigorously evaluated for other community assemblages (Armbruster *et al.*, 1999).

Co-occurrence of congeneric species implies there is a tension between filtering processes that aggregate species with similar niche preferences and competitive processes that exclude species that are too similar in niche. On evolutionary time scales, continued interactions among congeners may select for niche divergence that allow for greater co-occurrence. The time since divergence for a clade is a proxy for the potential for niche divergence. The strong negative relationship between node age and co-occurrence in *Limnanthes* (although significance was not assessable) may reflect competition and less time for adaptive amelioration of competitive interactions in this young clade. By contrast, the more equivocal relationships between phylogenetic distance and species and trait co-occurrence in *Mimulus* may reflect the longer time for adaptive niche divergence and amelioration of competitive effects in older clades.

Processes operating in contemporary communities may not reflect the historical processes that govern community assembly. Species of *Limnanthes*, *Mimulus* and *Clarkia* have all been the subject of experimental research that has implicated pollinator-mediated interactions as important processes in contemporary communities. Briscoe Runquist & Stanton (2013) found strong pollinator-mediated competition between two common *Limnanthes* species and a pattern of geographic segregation (Briscoe Runquist, 2012; Briscoe Runquist & Stanton, 2013) that are consistent with the patterns of segregation seen in this study. Likewise, research on floral colouration patterns in two species of *Mimulus* has also implicated pollinator-mediated competition operating in *Mimulus* communities (Grossenbacher & Stanton, 2014). Experimental work in *Clarkia* communities suggested that contemporary co-occurring species may experience facilitation (Moeller, 2004) but our study potentially implicates segregating as well as aggregating mechanisms (i.e. competition, filtering or facilitation). It is possible that competition and facilitation interact during community assembly to promote species coexistence but the balance of all interactions is competitive (e.g. Gross, 2008) or facilitation may only occur between species that have already been assembled due to filtering or competitive mechanisms. Studies of the patterns of community assembly, such as those in this study, are necessary to provide a basis for understanding the historical as well as contemporary community assembly mechanisms.

In this study, we focused on measures of traits at the species level that could be assessed from one visit to a plant community; however, there remain three factors that may be of particular importance in these genera that we did not assess: flowering phenology, reproductive character displacement and multivariate phenotypes. Divergence in flowering time is commonly viewed as a mechanism to allow species coexistence between otherwise competitive species pairs (Rathcke & Lacey, 1985; Ashton *et al.*, 1988; Gotelli & Graves, 1996). We only visited communities once and were unable to assess this trait, but there is evidence that flowering phenology may be important for our clades. For example, in communities of co-occurring *Clarkia*, congeners that share more similar pollinator communities have less flowering overlap than co-occurring species that have more divergent pollinator communities (Moeller, 2004; I. Singh *et al.* in prep). Future studies of wildflower community assembly may benefit from incorporating phenological traits. In studies of other clades, reproductive character displacement (RCD) in areas where species were sympatric as opposed to allopatric further implicated competition as the dominant ecological process affecting floral community composition (Armbruster, 1986; Armbruster *et al.*, 1994; Muchhala & Potts, 2007; Eaton *et al.*, 2012; Muchhala *et al.*, 2014). Testing for RCD requires that traits be assessed at a population level by measuring many individuals per site per species. We were unable to assess the potential for character displacement in this study because we did not adequately measure plants on a population basis. In future studies, explicitly incorporating population-level trait measurements to assess community-level patterns of RCD may allow us to distinguish between competition and facilitation in these clades. This could be especially revealing when species-level traits are aggregating. Lastly, pollinators perceive flowers as an integrated phenotype, and therefore, we may expect segregation and aggregation of some traits even when there is one dominant com-

munity assembly process. More studies of the functional relationships between species, traits and pollinators will help further our understanding of the primary drivers of assembly in particular communities.

Conclusions

Pollinator-mediated interactions are important drivers of patterns of species and trait diversity in native wildflower communities of *Limnanthes*, *Mimulus* and *Clarkia*. Furthermore, variation in mating system may influence patterns of species and trait co-occurrence and promote species co-occurrence. Future studies that assess patterns of character displacement in sites of species co-occurrence compared to where species do not co-occur, and niche filling in the context of species co-occurrence matrices could greatly enhance understanding of the ecological processes important to speciation and diversification.

Acknowledgments

We thank M. Stanton, S. Strauss, K. Moore and D. Harris for helpful discussion throughout the course of this study. We thank D. Moeller, N. Waser and three anonymous reviewers for comments on earlier versions of this manuscript. We also thank the Davis Botanical Society and the Northern California Botanists for providing funding. Max Lambert provided assistance in the field. Lastly, we thank all of the landowners who allowed us to conduct surveys on their properties.

References

Abramoff, M.D., Magelhaes, P.J. & Ram, S.J. 2004. Image processing with ImageJ. *Biophoton. Int.* **11**: 36–42.

Adams, D.C., Rohlf, F.J. & Slice, D.E. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. *Ital. J. Zool.* **71**: 5–16.

Armbruster, W.S. 1986. Reproductive interactions between sympatric *Dalechampia* species: are natural assemblages "random" or organized? *Ecology* **67**: 522–533.

Armbruster, W.S. 1995. The origins and detection of plant community structure: reprodutive versus vegetative processes. *Folia Geobot. Phytot.* **30**: 483–497.

Armbruster, W.S., Edwards, M.E. & Debevec, E.M. 1994. Floral character displacement generates assemblage structure of western Australian triggerplants (Stylidium). *Ecology* **75**: 315–329.

Armbruster, W.S., DiStilio, V.S., Tuxill, J.D., Flores, T.C. & Velásquez Runk, J.L. 1999. Covariance and decoupling of floral and vegetative traits in nine neotropical plants: a re-evaluation of Berg's correlation-pleiades concept. *Am. J. Bot.* **86**: 39–55.

Ashton, P.S., Givnish, T.S. & Appanah, S. 1988. Staggered flowering in the Dipterocarpaceae: new insights into floral induction and the evolution of mast fruiting in the aseasonal tropics. *Am. Nat.* **132**: 44–66.

Beardsley, P.M., Schoenig, S.E., Whittall, J.B. & Olmstead, R.G. 2004. Patterns of evolution in Western North American Mimulus (Phrymaceae). *Am. J. Bot.* **91**: 474–489.

Bell, J.M., Karron, J.D. & Mitchell, R.J. 2005. Interspecific competition for pollination lowers seed production and outcrossing in *Mimulus ringens*. *Ecology* **86**: 762–771.

Benjamini, Y. & Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. Roy. Stat. Soc. B* **57**: 289–300.

Berg, R.L. 1960. The ecological significance of correlation pleiades. *Evolution* **14**: 171–180.

Blomberg, S.P., Garland, T. & Ives, A.R. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.

Bosch, M. & Waser, N.M. 1999. Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (Ranunculaceae). *Am. J. Bot.* **86**: 871–879.

Brandvain, Y., Kenney, A.M., Flagel, L., Coop, G. & Sweigart, A.L. 2014. Speciation and introgression between *Mimulus nasutus* and *Mimulus guttatus*. *PLoS Genet.* **10**: e1004410.

Briscoe Runquist, R.D. 2012. Pollinator-mediated competition between two congeners, *Limnanthes douglasii* subsp. *rosea* and *L. alba* (Limnanthaceae). *Am. J. Bot.* **99**: 1125–1132.

Briscoe Runquist, R.B. & Stanton, M.L. 2013. Asymmetric and frequency-dependent pollinator-mediated interactions may influence competitive displacement in two vernal pool plants. *Ecol. Lett.* **16**: 183–190.

Cadotte, M.W., Cavender-Bares, J., Tilman, D. & Oakley, T.H. 2009. Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. *PLoS One*. doi: 10.1371/journal.pone.0005695.

Cavender-Bares, J., Ackerly, D.D. & Kozak, K.H. 2012. Integrating ecology and phylogenetics: the footprint of history in modern-day communities. *Ecology* **93**: S1–S3.

Chesson, P. 2000. Mechainisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.* **31**: 343–366.

Chesson, P. & Huntley, N. 1997. The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *Am. Nat.* **155**: 219–237.

Chittka, L. & Raine, N.E. 2006. Recognition of flowers by pollinators. *Curr. Opin. Plant Biol.* **9**: 428–435.

Conner, J.K. & Sterling, A. 1996. Selection for independence of floral and vegetative traits: evidence from five species. *Can. J. Bot.* **74**: 642–644.

Darwin, C. 1859. *On the Origin of Species by Means of Natural Selection*. J. Murray, London.

Diamond, J.M. 1975. Assembly of species communities. In: *Ecology and Evolution of communities* (M.L. Cody & J.M. Diamond, eds), pp. 342–444. MA, Harvard University Press, Cambridge.

Drummond, A.J. & Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**: 214.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* doi: 10.1371/journal.pbio.0040088.

Eaton, D.A.R., Fenster, C.B., Hereford, J., Huang, S.-Q. & Ree, R.H. 2012. Floral diversity and community structure in *Pedicularis* (Orobanchaceae). *Ecology* **93**: S182–S194.

Edgar, R.C. 2004. MUSCLE: multiple sequence alignments with high accuracy and high throughput. *Nucleic Acids Res.* **32**: 1792–1797.

Endler, J.A. & Mielke, P.W. 2005. Comparing entire colour patterns as birds see them. *Biol. J. Linn. Soc.* **86**: 405–431.

Fishman, L. & Wyatt, R. 1999. Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). *Evolution* **53**: 1723–1733.

Fitzpatrick, B.M. & Turelli, M. 2006. The geography of mammalian speciation: mixed signals from phylogenies and range maps. *Evolution* **60**: 601–615.

Geber, M.A. & Moeller, D.A. 2006. Pollinator responses to plant communities and implications for reproductive character evolution. In: *The Ecology and Evolution of Flowers* (L.D. Harder & S.C.H. Barrett, eds), pp. 102–119. Oxford University Press, New York.

Ghazoul, J. 2006. Floral diversity and facilitation of polliantion. *J. Ecol.* **94**: 295–304.

Godoy, O., Kraft, N.J.B. & Levine, J.M. 2014. Phylogenetic relatedness and the determinants of competitive outcomes. *Ecol. Lett.* **17**: 836–844.

Gotelli, N.J. 2000. Null model analysis of species co-occurrence patterns. *Ecology* **81**: 2606–2621.

Gotelli, N.J. & Entsminger, G.L. 2011. *EcoSim: Null Models Software for Ecology*. Aquired Intelligence, Inc. & Kesey-Bear, Jericho, VT.

Gotelli, N.J. & Graves, G.R. 1996. *Null Models in Ecology*. Smithsonian Institution, Washington, DC.

Gotelli, N.J. & McCabe, D.J. 2002. Species co-occurrence: a meta-analysis of J. M. Diamond's asembly rules model. *Ecology* **83**: 2091–2096.

Grant, A.L. 1924. A monograph of the genus *Mimulus*. *Ann. Mo. Bot. Gard.* **11**: 99–388.

Gross, K. 2008. Positive interactions among competitors can produce species rich communities. *Ecol. Lett.* **11**: 929–936.

Grossenbacher, D.L. & Stanton, M.L. 2014. Pollinator-mediated competition influences selection for flower-color displacement in sympatric monkeyflowers. *Am. J. Bot.* **101**: 1915–1924.

Grossenbacher, D.L. & Whittall, J.B. 2011. Increased floral divergence in sympatric monkeyflowers. *Evolution* **65**: 2712–2718.

Grossenbacher, D.L., Briscoe Runquist, R.D., Goldberg, E.E. & Brandvain, Y. 2015. Geographic range size is predicted by plant mating system. *Ecol. Lett.* **18**: 706–713.

Heystek, A. & Pauw, A. 2014. Does competition for pollinators contribute to structuring *Erica* communities? *J. Veg. Sci.* **25**: 648–656.

HilleRisLambers, J.P., Adler, B., Harpole, W.S., Levine, J.M. & Mayfield, M.M. 2012. Retihinking community assembly through the lens of coexistence theory. *Annu. Rev. Ecol. Evol. Syst.* **43**: 227–248.

Hurd, P.O. 1979. Superfamily Apoidea. In: *Catalog of Hymenoptera in America North of Mexico*, Vol. 2. (K.V. Krombein, P.D. Hurd, D.R. Smith & B.D. Burks, eds), pp. 1741–2209. Smithsonian Institution Press, Washington, DC.

de Jager, M.L., Dreyer, L.L. & Ellis, A.G. 2011. Do pollinators influence the assembly of flower colours within plant communities? *Oecologia* **166**: 543–553.

Johnson, S.D., Peter, C.I., Nilsson, L.A. & Ågren, J. 2003. Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology* **84**: 2919–2927.

Jordan, D.S. 1905. The origin of species through isolation. *Science* **22**: 545–562.

Jordan, D.S. 1908. The law of geminate species. *Am. Nat.* **42**: 73–80.

Kay, K.M., Whittall, J.B. & Hodges, S.A. 2006. A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: an approximate molecular clock with life history effects. *BMC Evol. Biol.* **6**: 36.

Kraft, N.J.B., Cornwell, W.K., Webb, C.O. & Ackerly, D.D. 2007. Trait evolution, community assembly, and the phylogenetic structure of ecological communities. *Am. Nat.* **170**: 271–283.

Kraft, N.J.B., Godoy, O. & Levine, J.M. 2015. Plant functional traits and the multidimentional nature of species coexistence. *Proc. Natl. Acad. Sci. USA* **112**: 797–802.

Lewis, H. 1961. Experimental sympatric populations of *Clarkia*. *Am. Nat.* **95**: 155–168.

Lewis, H. & Lewis, M.E. 1955. *The Genus Clarkia: University of California Publications in Botany*, Vol. 20. University of California Press, Berkeley & Los Angeles, CA.

MacSwain, J.W., Raven, P.H. & Thorp, R.W. 1973. *Comparative Behavior of Bees and Onagraceae. IV. Clarkia* Bees of the Western United States, Vol. 70. University of California Press, Berkeley & Los Angeles, CA.

Mason, C.T. 1952. *A Systematic Study of the Genus Limnanthes* R. Br.: University of California Publications in Botany, Vol. 25. University of California Press, Berkeley, CA.

Mayfield, M.M. & Levine, J.M. 2010. Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecol. Lett.* **13**: 1085–1093.

McEwen, J.R. & Vamosi, J.C. 2010. Floral colour versus phylogeny in structuring subalpine flowering communities. *Proc. Biol. Sci.* **277**: 2957–2965.

Meyers, S.C., Liston, A. & Meinke, R. 2010. A molecular phylogeny of *Limnanthes* (Limnanthaceae) and investigation of an Anomalous *Limnanthes* population from California, U. S. A. *Syst. Bot.* **35**: 552–558.

Mitchell, R.J., Flanagan, R.J., Brown, B.J., Waser, N.M. & Karron, J.D. 2009. New frontiers in competition for pollination. *Ann. Bot.* **103**: 1403–1413.

Moeller, D.A. 2004. Facilitative interactions among plants via shared pollinators. *Ecology* **85**: 3289–3301.

Moeller, D.A. 2006. Geographic structure of pollinator communities, reproductive assurance, and the evolution of self-pollination. *Ecology* **87**: 1510–1522.

Moeller, D.A. & Geber, M.A. 2005. Ecological context of the evolution of self-pollination in *Clarkia xantiana*: population size, plant communities, and reproductive assurance. *Evolution* **59**: 786–799.

Morales, C.L. & Traverset, A. 2008. Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. *Crit. Rev. Plant Sci.* **27**: 221–238.

Muchhala, N. & Potts, M.D. 2007. Character displacement among bat-pollinated flowers of the genus *Burmeistera*: analysis of mechanism, process and pattern. *Proc. Biol. Sci.* **274**: 2731–2737.

Muchhala, N., Johnsen, S. & Smith, S.D. 2014. Competition for hummingbird polliantion shapes flower color variation in Andean Solanaceae. *Evolution* **68**: 2275–2286.

Paradis, E., Claude, J. & Strimmer, K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289–290.

van der Pijl, L. 1961. Ecological aspects of flower evolution. II. Zoophilous flower classes. *Evolution* **15**: 44–59.
Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. & R Core Team 2015. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-120. http://CRAN.R-project.org/package=nlme.
Pontarp, M., Ripa, J. & Lundberg, P. 2015. The biogeography of adaptive radiations and the geographic overlap of sister species. *Am. Nat.* **186**: 565–581.
Pooter, H. & Garnier, E. 1999. Ecological significance of inherent variation in relative growth rates and its components. In: *Handbook of Functional Plant Ecology* (F.I. Pugnaire & F. Valladares, eds), pp. 81–120. Marcel Dekker Inc, New York, NY.
Rasband, W.S. 1997–2014. ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA. http://imagej.nih.gov/ij/.
Rathcke, B. 1983. Competition and facilitation among plants for pollination. In: *Polliantion Biology* (L. Real, ed.), pp. 305–325. Academic Press, Orlando, FL.
Rathcke, B. & Lacey, E. 1985. Phenological patterns of terrestrial plants. *Annu. Rev. Ecol. Syst.* **16**: 179–214.
Ritland, C. & Ritland, K. 1989. Variation of sex allocation among eight taxa of the *Mimulus guttatus*. *Am. J. Bot.* **76**: 1731–1739.
Rohlf, F.J. & Slice, D.E. 1990. Extensions of the Procrustes method for the optimal superposition of landmarks. *Syst. Zool.* **39**: 40–59.
Roughgarden, J. 1976. Resource partitioning among competing species – a coevolutionary approach. *Theor. Popul. Biol.* **9**: 388–424.
Sargent, R. & Ackerly, D.D. 2008. Plant-pollinator interactions and the assembly of plant communities. *Trends Ecol. Evol.* **23**: 123–130.
Schoener, T.W. 1968. The Anolis lizards of Bimini: resource partitioning in a complex fauna. *Ecology* **49**: 704–726.
Stevens, M. 2007. Predator perception and the interrelation between different forms of protective coloration. *Proc. Biol. Sci.* **274**: 1457–1464.
Stevens, M., Parraga, C.A., Cuthill, I.C., Partridge, J.C. & Troscianko, T.S. 2007. Using digital photography to study animal coloration. *Biol. J. Linn. Soc.* **90**: 211–237.
Stone, L. & Roberts, A. 1990. The checkerboard score and species distributions. *Oecologia* **85**: 74–79.
Thorp, R.W. 1976. Insect pollination of vernal pool flowers. In: *Vernal Pools, Their Ecology, and Conservation* (S. Jain, ed.), pp. 36–40. Institute of Ecology Publication, University of California, Davis, CA.
Thorp, R.W. & Leong, J.M. 1998. Specialist bee pollinators of showy vernal pool flowers. In: *Ecology, Conservation, and Management of Vernal Pool Ecosystems – Proceedings From a 1996 Conference* (C.W. Witham, E.T. Bauder, D. Belk, W.R. Ferren & R. Ornduff, eds), pp. 169–179. California Native Plant Society, Sacramento, CA.
Tilman, D. 1982. *Resource Competition and Community Structure*. Princeton University Press, Princeton, NJ.
Ulrich, W. 2008. Pairs – a FORTRAN program for studying pair-wise species associations in ecological matrices. http://www.uni.torun.pl/~ulrichw.
Ulrich, W. & Gotelli, N.J. 2013. Pattern detection in null model analysis. *Oikos* **122**: 2–18.
USDA-ARS. 2006. U. S. National Pollinating Insects Database. United States Department of Agriculture, Agriculture Research Service, Bee Biology and Systematics Laboratory, Logan, Utah (Accessed 2009-18-10).
Waser, N.M. 1978. Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. *Ecology* **59**: 934–944.
Waser, N.M. 1986. Flower constancy: definition, cause and measurement. *Am. Nat.* **127**: 593–603.
Westland, S. & Ripamonti, C. 2004. *Computational Colour Science Using MATLAB*. John Wiley & Sons Ltd, Chichester.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Assessing phylogeny and clade age.
Appendix S2 Floral-trait measurements.
Appendix S3 Assessing floral shape with relative warps.
Appendix S4 Assessing flower colour.
Appendix S5 Modification of mean pairwise distance algorithm to include singleton sites.
Table S1 Phylogenetic information for the three clades.
Table S2 Summary of co-occurrence at three spatial scales (100, 30 and 1 m radius) for three genera: *Limnanthes, Mimulus* and *Clarkia*.
Table S3 Significantly segregating and aggregating species pairs.
Table S4 Significantly segregating and aggregating species pairs for *Mimulus* and *Clarkia* including only outcrossing species.
Table S5 Trait regression slope values from PGLS.
Table S6 Numerical results of trait segregation or aggregation in *Limnanthes, Mimulus* and *Clarkia.*
Figure S1 Floral measurements for each genus (top to botom: *Limnanthes*, *Mimulus*, and *Clarkia*) include (a) petal width, (b) corolla diameter, (c) petal length, (d) flower depth, and (e) corolla aperture.
Figure S2 The consensus shape and pictorial descriptions of the axes of shape variation for *Limnanthes*.
Figure S3 The consensus shape and pictorial descriptions of the axes of shape variation for *Mimulus*.
Figure S4 The consensus shape and pictorial descriptions of the axes of shape variation for *Clarkia*.
Figure S5 Flower areas where color values were taken in *Mimulus* on an example flower.
Figure S6 Flower areas where color values were taken in *Clarkia* example flowers.

Data deposited at Dryad: doi: 10.5061/dryad.m03vf

Received 14 September 2015; revised 4 February 2016; accepted 5 February 2016