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Diversification of Trait Combinations in Coevolving Plant and Insect Lineages

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ABSTRACT: Closely related species often have similar traits and sometimes interact with the same species. A crucial problem in evolutionary ecology is therefore to understand how coevolving species diverge when they interact with a set of closely related species from another lineage rather than with a single species. We evaluated geographic differences in the floral morphology of all woodland star plant species (*Lithophragma*, Saxifragaceae) that are pollinated by *Greya* (Prodoxidae) moths. Flowers of each woodland star species differed depending on whether plants interact locally with one, two, or no pollinating moth species. Plants of one species grown in six different environments showed few differences in floral traits, suggesting that the geographic differences are not due significantly to trait plasticity. *Greya* moth populations also showed significant geographic divergence in morphology, depending on the local host and on whether the moth species co-occurred locally. Divergence in the plants and the moths involved shifts in combinations of partially correlated traits, rather than any one trait. The results indicate that the geographic mosaic of coevolution can be amplified as coevolving lineages diversify into separate species and come together in different combinations in different ecosystems.

Keywords: coevolution, complex traits, floral evolution, geographic mosaic.

Introduction

Coevolution between pairs of species is almost always embedded in a geographically varying network of interactions with other species. For example, coevolution between lodgepole pines and crossbills differs when red squirrels co-occur in the same community (Benkman et al. 2010), coevolution of woodland star (*Lithophragma*) plants and *Greya* moths is altered in a few localities by the presence of abundant solitary bees or bombyliid flies (Thompson and Cunningham 2002), and coevolution of wild parsnips and parsnip webworms differs when cow parsnips locally co-occur (Zangerl and Berenbaum 2003). These and other studies have indicated that the coevolutionary process does not always favor pairs of coevolving species (Thompson 2005, 2013; Nuismer et al. 2012; Poisot et al. 2012; Kagawa and Takimoto 2014). Although coevolution between pairs of interacting species can form geographic mosaics of traits and ecological outcomes (Lorenzi and Thompson 2011; Gibert et al. 2013; Vergara et al. 2013; Hague et al. 2016), coevolution within networks of interacting species has the potential to form even more complex geographic mosaics. Coevolution within local networks can act both directly and indirectly on each species as each evolutionary change cascades throughout the network. Mathematical models of coevolution have shown that the evolution of traits may differ when selection occurs within networks rather than between pairs of species (Guimarães et al. 2011; Nuismer et al. 2012; Bascompte and Jordano 2013).

Networks can form as coevolving lineages diversify. Species that originally coevolved with only one species in another lineage may expand their interactions in some regions to include other congeners within that lineage. What began as a globally pairwise interaction becomes a geographic mosaic of interacting species. Ongoing local loss or addition of species to an interaction, through range changes or other ecological processes, may continually alter this mosaic, as has been documented in multiple studies (Brodie et al. 2002; Parchman and Benkman 2002; Zangerl and Berenbaum 2003; Lankau 2012; Stouffer et al. 2014; Newman et al. 2015; Pérez-Méndez et al. 2016). How interactions assemble and evolve into local, regional, and global networks of different sizes and phylogenetic configurations has therefore become a major problem to understand in coevolutionary biology (Jordano et al. 2003; Strauss et al. 2005; Thompson 2005, 2013; Olesen et al. 2007; Hoeksema 2010; Jordano 2010; Nuismer et al. 2012; Bascompte and Jordano 2013; Wise and Rausher 2013; Heath and Stinchcombe 2014; Bronstein 2015; Parch-

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man et al. 2016). Addressing the problem requires large-scale analyses of how lineages of closely related species assemble and coevolve with other lineages in different environmental contexts.

In some coevolving interactions, the focus of reciprocal selection is sometimes on a particular trait in one species that is countered or matched by a particular trait in another species. Among the best-studied examples are the geographic differences in the levels of tetrodotoxin in *Taricha* newts and tolerance or detoxification of tetrodotoxin in *Thamnophis* garter snakes (Brodie et al. 2002; Hague et al. 2016) or the size of *Camellia* fruits and the length of camellia weevils used to pierce the fruits to reach the seeds (Toju et al. 2011). In some other coevolving interactions, however, the focus of selection may be on a set of traits that are partially correlated but evolve to similar outcomes when exposed to similar selection pressures. The now-classic example is the coevolution of the complex morphological traits of conifer cones and crossbill bills in different environments, in which the cones evolve toward more conical or cylindrical forms depending on whether selection is driven by squirrels or crossbills (Benkman and Mezquida 2015). In yet other interactions, selection could act on suites of partially correlated traits in ways that create multiple evolutionary solutions even within a single lineage. Previous work has suggested that the interactions between woodland stars (*Lithophragma*: Saxifragaceae) and *Greya* (Prodoxidae) moths have coevolved in this way (Thompson et al. 2013). Species and populations differ so widely in trait combinations involved in the interaction that no single coevolutionary solution is evident.

We therefore undertook an analysis of how multiple coevolutionary solutions are clustered within and among all species of interacting woodland stars and *Greya* moths. We predicted that the diversity of floral and moth morphology found within each species results in part from differences among ecosystems in the combination of locally interacting plant and moth species. This prediction follows from several past observations and results. First, populations of each woodland star species differ in whether they interact with one coevolving *Greya* moth species, two locally pollinating *Greya* species that differ in how they pollinate flowers, or, more rarely, no locally coevolving *Greya* moths. These interactions therefore have the potential to produce not only a geographic mosaic of coevolution between any one pair of interacting woodland star and *Greya* moth species but also a geographic and phylogenetic mosaic of coevolving traits in plants and the moths.

Second, *Greya* moth species differ in how they pollinate and lay their eggs in the reproductive parts of *Lithophragma* plants (fig. 1). *Greya politella* females pollinate flowers mostly while ovipositing through the corolla, as pollen adhering to the abdomen rubs off onto the stigma. In most populations of this species, females oviposit by piercing the base of the nectary disk with the ovipositor. While doing so, pollen adhering to the membrane of the extended ovipositor rubs onto the stigma. In contrast, *G. obscura* moths pollinate flowers only while nectaring. They then move to the base of the flower to oviposit into the outer ovary wall or the scape (Thompson et al. 2010). Experimental studies have shown that although *G. politella* is a much more effective pollinator than *G. obscura*, *G. obscura* is often more abundant (Thompson et al. 2010, 2013). The relative effects of these moth species on plant fitness could therefore vary among ecosystems.

Third, past studies have shown that fitness in the plants and the moths depends on their interaction in most localities. Not only are *Greya* species associated with *Lithophragma* specialized to feed as adults and larvae only on this plant genus in all communities in far western North America (Thomp-

Figure 1: *Greya* moths pollinating *Lithophragma* spp. *Far left*, *Greya politella* ovipositing into *L. bolanderi* and pollinating with pollen adhering to abdomen. *Middle*, *Greya obscura* nectaring on *L. cymbalaria*. *Far right*, *Greya politella* (*top*) and *G. obscura* (*bottom*) nectaring simultaneously on *L. cymbalaria*. Photos: John N. Thompson

son 2010) but also these moths are the major pollinators of their host plants. They also are the only insects that normally feed on these plants either as pollinators or as herbivores (Thompson and Cunningham 2002; Thompson and Fernandez 2006; Thompson et al. 2010). Both moth species impose a cost to the plants through larval feeding, but *G. politella* larvae eat only a small percentage of the developing seeds (Thompson et al. 1992), and *G. obscura* larvae usually feed on the ovary wall or the upper parts of the scape, although they sometimes also eat a small percentage of developing seeds. Past studies have found the interaction between the plants and the moths to be mutualistic in all but a few sites (e.g., Thompson and Cunningham 2002; Thompson and Fernandez 2006; Thompson et al. 2010). These few nonmutualistic sites are at the northern edge of the geographic ranges of plants and moths, where the mutualism is swamped in some sites by locally abundant bombyliid flies and solitary bees (Thompson et al. 1992). Otherwise, the plants and moths have been found to depend on each other throughout their geographic ranges.

Fourth, multiple floral and moth traits are involved in these interactions, generating a wide range of possible avenues for coevolutionary change. The differences among *Greya* species in pollination and oviposition mechanisms have the potential to favor the evolution of different combinations of floral traits associated with pollination, including ovary depth, floral width, floral flair, stigma size, pistil height style, and size of the floral petal platform that the moths use to position themselves while ovipositing or nectaring. Previous work has shown that these traits are phenotypically correlated to varying degrees and the absolute and relative values of the traits vary among population, species, and lineages within the genus (Thompson et al. 2013). On the moth side, the differences in oviposition behavior have the potential to affect the evolution of traits such as overall body size, haustellum length, abdominal segment lengths, and ovipositor length. As with the plants, past studies have shown that these traits vary considerably among populations and species (Davis et al. 1992; Thompson et al. 2013). As expected, then, previous experimental studies have shown that the trait combinations involved in pollination differ among plant and moth species and populations (Thompson et al. 2010, 2013; Friberg et al. 2014, 2016).

Based on this suite of previous results, we expected that trait combinations in woodland stars and *Greya* moths would vary geographically depending on whether local plant populations interacted with *G. politella*, *G. obscura*, or both moth species. We assessed the interactions in 90 ecosystems across the latitudinal range of *Lithophragma* in western North America (fig. 2; table A1; tables A1–A8 available online). In effect, our goal was to assess how coevolution of species is altered as pairwise interactions begin to diversify into small networks of interacting species. This region of North America is characterized by a wide range of levels of local adaptation and endemism in many taxa (Harrison 2013). The sites included all the named species and the full range of phylogeographic divergence within each plant clade and each moth species found in previous studies. In the zone of overlap between the two moth species, we then chose 37 sites to evaluate whether the moths differ in morphology when they occur together rather than alone within ecosystems. We also evaluated the extent to which plasticity may affect floral traits by growing *L. cymbalaria* in six environments that differed in light, soil, and water treatments.

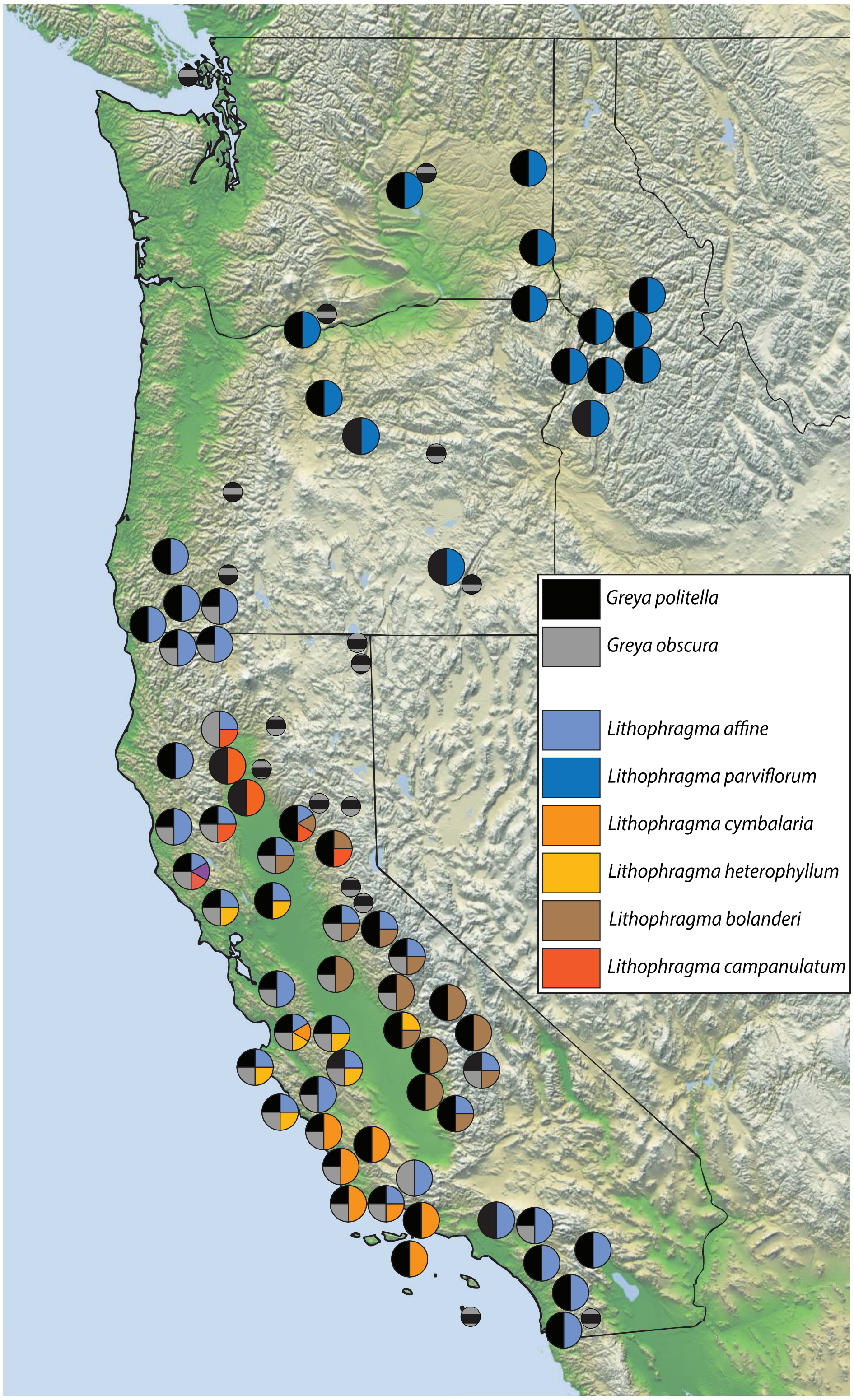
Material and Methods

Taxa

Lithophragma is a strongly supported monophyletic genus that is broadly distributed across the western United States and southwestern Canada (Taylor 1965; Soltis et al. 1992; Kuzoff et al. 1999; Deng et al. 2015). Two monophyletic clades within *Lithophragma* are used by *Greya* moths as adult and larval hosts. The two clades differ in multiple molecular and morphological characters (Kuzoff et al. 1999; Deng et al. 2015). The two *Greya* moth species that pollinate woodland stars are closely related but are not sister taxa. Molecular analyses have indicated that each of these moth species includes populations with varying degrees of genetic relatedness (Brown et al. 1997; Rich et al. 2008; Thompson et al. 2011). Both moth species are restricted to *Lithophragma* throughout their geographic range, except some divergent populations of *G. politella* in the northern Rocky Mountains that have shifted onto a closely related plant genus, *Heuchera*, and may be a separate species (Thompson et al. 1997; Nuismer and Thompson 2001). Both moth species show evidence of local adaptation in their behavioral responses to floral volatiles and in oviposition behavior on their hosts (Thompson and Cunningham 2002; Thompson et al. 2013; Friberg et al. 2014, 2016).

Sampling

Flowering begins and adult moths eclose between late February and June, depending on elevation and latitude. Pollination and oviposition occur at each site for only about 3 weeks each year. The sites sampled for *Lithophragma* flowers included a wide range of habitats, including Ponderosa pine woodlands, gaps in Douglas fir forests, open oak woodlands, rocky slopes of rivers, and meadow steppe (fig. 2; table A1). A few ecosystems had more than one *Lithophragma* species, but the species pollinated by *Greya* generally occurred in different habitats. Hence, we analyzed how each local *Lithophragma* population interacts with its local moth population(s). We surveyed flowering plants for presence of moths along transects up to 1 km through each site. Plants


Greya politella
Greya obscura
Lithophragma affine
Lithophragma parviflorum
Lithophragma cymbalaria
Lithophragma heterophyllum
Lithophragma bolanderi
Lithophragma campanulatum

are easily assessed, because each plant is about 20–40 cm tall with 1–10 flowers that open sequentially from bottom to top, starting about halfway up the scape.

An unusual feature of the interaction between woodland stars and *Greya* plants is that the moths can be reliably detected whenever flowering host individuals are present. Adult moths are active only during the day and remain on the host flowers throughout each day either resting, nectaring, mating, or ovipositing, moving only to find another host individual or mate. Males search for females by moving among woodland star plants, and pairs mate only on host flowers. Some of the 90 sites were visited for collection of plants and moths in multiple years as parts of other studies of interactions between woodland stars and *Greya* moths (Thompson and Cunningham 2002; Thompson et al. 2013; Friberg et al. 2014, 2016), but most were visited specifically for this study. For the *Lithophragma* populations in which we did not detect moths in the initial sampling year for that site, we returned to most in at least one more year to confirm that the moths were indeed not present at that site. These repeated visits confirmed that the initial scoring of the presence and absence of moth species was correct at all sites.

Floral Measurements

Across the 90 sites, 3,223 flowers were collected and measured (see appendix, available online, for details). At each site, one flower was measured from each plant, and each sampled plant was at least 1 m from other sampled plants. For consistency, we collected the second flower produced by a plant whenever possible. Samples included >90 flowers for each *Lithophragma* species, except for the two endemics with very small geographic ranges: *L. maximum* ($N = 30$), which is restricted to San Clemente Island off the coast of California, and the hybrid species *L. thompsonii* ($N = 28$), which is restricted to a narrow geographic band in central Washington State. Sample size for each population averaged 28.3 ± 10.97 SD.

Floral measurements included ovary depth, floral width, petal length, petal width, floral flair, stigma size, and pistil height (fig. A1; figs. A1, A2 available online). Prior experimental studies of the mechanics of pollination of woodland stars have shown that these morphological characters affect pollination efficacy by *Greya* moths (Thompson et al. 2013).

Moth Traits

We collected and measured 547 female moths (316 *G. politella* and 231 *G. obscura*) from 37 sites within the geographic region where the ranges of the two moth species overlap (table A2). These sites included 20 sites used for the analysis of floral traits and an additional 17 sites that increased the sampling density within the region. The sites encompassed populations from southwestern Oregon to southern California and east to the Sierra Nevada, including all local combinations of plants and moths commonly found in nature. All moths were collected directly from host flowers. Phylogeographic and phylogenetic studies have shown that *G. politella* and *G. obscura* are each monophyletic, but each includes a complex of populations that vary in degree of relatedness (Brown et al. 1994; Rich et al. 2008; Thompson et al. 2011). Sampling included all previously identified phylogeographic groups.

We measured wing length, haustellum length, seventh abdominal segment length, and ovipositor length on freshly dead moths. We chose these four moth characters, because prior time-lapse photographic analyses had indicated that they are important in how the moths interact with the plants, affecting pollination, oviposition, or both (Thompson et al. 2013). Wing length was used as an indicator of overall body size. It was measured as the combined length of each wing and the intervening thorax width. Haustellum (sucking mouthpart) length affects the ability of moths to reach nectar within the flower and was measured as the total length of the fully uncoiled haustellum. The length of the seventh abdominal segment is variable in both species and is especially elongated in *G. politella* females relative to females or males in all other species in the genus. The combination of the length of the seventh abdominal segment and the length of the ovipositor affects the ability of *G. politella* females to reach the ovary when ovipositing through the corolla. These two characters also affect the orientation of *G. obscura* females when ovipositing into the side of the ovary wall or the upper scape wall.

Statistical Analyses

Values for all floral and moth characters were initially measured in millimeters, which were then $\log_{10}$ transformed prior to analysis. We first evaluated how overall morphological variation was distributed within *Lithophragma* with

Figure 2: Geographic distribution of interactions between woodland star (*Lithophragma*) plants and *Greya* moths in the far western United States from Washington State in the north to California in the south. The pie diagrams include only plant species that interact with *Greya*. In central California, where some neighboring sites differ in species composition, overlapping pie diagrams are combined into a single pie to indicate the regional complexity of the interaction structure. Local sites, however, generally had one *Lithophragma* species and one moth species, two moth species, or no moths. Overlapping pie diagrams with the same combination of plant and moth species are shown as a single pie. Smaller pies with black and gray horizontal bars are sites at which *Lithophragma* plants occur without moths. Details of the sampled ecosystems are given in tables A1 and A2, available online.

respect to multiple floral characters associated with pollination by *Greya* moths, using quadratic discriminant analysis (QDA) to evaluate the traits that separate the species and clades (Thompson et al. 2013). We used quadratic linear analyses throughout, because the variance/covariance matrices were sufficiently variable that quadratic analyses were the more conservative choice. Linear analyses gave similar results with respect to statistical significance (not shown) and hence did not change conclusions. We evaluated whether floral trait combinations favored at sites where plants interact locally with one moth species differ from trait combinations at sites where plants interact with both moth species or no moth species. Hence, each plant population was characterized a priori as interacting with *G. politella* only, *G. obscura* only, both moth species, or neither moth species. Priors were set proportional to their occurrence in each data set. We also used QDA to analyze how moth species differed in morphology when they occur separately or together.

Canonical axes were scaled and displayed isometrically for the first two canonical axes. Separate absolute canonical scalings were used for plant and moth traits. Discriminant values are shown as multivariate means surrounded by an ellipse showing the 95% confidence limits. The standardized scoring coefficients were used to determine the partial contribution of each variable to each discriminant function. The structure coefficients (i.e., pooled within-canonical structure values) were used to interpret the discriminant function. Structure coefficient loadings <0.3 were not interpreted. Centroids for each group were used to evaluate the direction in discriminant space by which one group differed from the other(s). All analyses were performed using JMP Pro 12.

Evaluation of Plasticity in Floral Traits

We evaluated whether abiotic conditions could affect *Lithophragma* floral characters by growing *L. cymbalaria* plants from seed to flowering in growth chambers under six abiotic treatments: three light levels replicated for two soil and water conditions (see appendix for details). Field-collected seeds of *L. cymbalaria* were germinated in an incubator, placed into separate pots, and then transferred to growth chambers. For each of three light treatments, half the plants were grown in flat-bottomed rose pots and watered from above. The other half were grown in Cone-tainers and watered from below. The pots contained only slightly less soil than the Cone-tainers but had a substantially lower water column. These two treatments provided large differences in the soil and water environment in which the plants grew. The trays within each growth chamber were rotated weekly.

We counted the total number flowers per plant to assess whether the six environmental treatments were sufficiently wide to affect plant growth and reproduction overall. We collected and measured the second flower produced by each plant, using the same measurement protocol as in the field-collected plants. We included nine floral characters to increase the chance of finding any floral characters that vary with abiotic conditions: longest petal length, longest petal width, ovary depth, floral width at the nectary disk level, pistil height, maximum stigma lobe diameter, nectary thickness, maximum corolla opening diameter, and floral flair from the sepal tip to the nectary disk on the opposite side of the flower. Results for the number of flowers were exponentially distributed and therefore were analyzed with a generalized linear model based on an exponential distribution to evaluate the effects of the six different environmental conditions. Results for floral traits were log transformed and analyzed with ANOVA. Because the goal was to determine whether any of the six treatments affected these morphological floral characters, we report a one-way ANOVA for the effect of treatment for unbalanced data.

Results

Among the taxa that interact with *Greya*, the *L. campanulatum* clade (*L. bolanderi*, *L. campanulatum*, *L. cymbalaria*, and *L. heterophyllum*) formed a ring of populations around the central valley of California, as did *G. obscura* moths (fig. 2). The ranges of these species were, in turn, embedded within the broader geographic ranges of the *L. parviflorum* clade (*L. affine*, *L. parviflorum*) and *G. politella* moths. Consequently, the local assemblage of *Lithophragma* and *Greya* species varied geographically (fig. 2). Sites at the northern and southern edges of the species distributions had only one *Lithophragma* species pollinated by one *Greya* moth species. Sites near the center of the range of these interactions varied in whether the local *Lithophragma* species interacted with one *Greya* species, two *Greya* species, or, uncommonly, no *Greya* species.

Lithophragma clades and species differed in multiple floral characters and showed considerable multivariate variation in characters within species (fig. 3; table A3: Wilks's $\lambda = 0.0316$, $F = 226.969$, $\mathrm{df} = 63, 16{,}897$, $P < .0001$, no. flowers = 3,015). Species differed primarily along canonical axis 1 through a negative correlation between pistil height and ovary depth, with floral flair also contributing to a significant but lesser extent (table A3). Multiple characters contributed to the separation of species along canonical axis 2, driven partially by a negative correlation between increasing petal length and decreasing stigma size and floral flair (fig. 3; table A3). Some taxa never associated with *Greya* diverged strongly along this axis from taxa associated with *Greya*, whereas other taxa never associated with *Greya* had trait combinations intermediate between the two clades that interact with *Greya*. These results indicated that evaluation of trait shifts in response to selection imposed by *Greya* required separate analyses for each plant species, because each

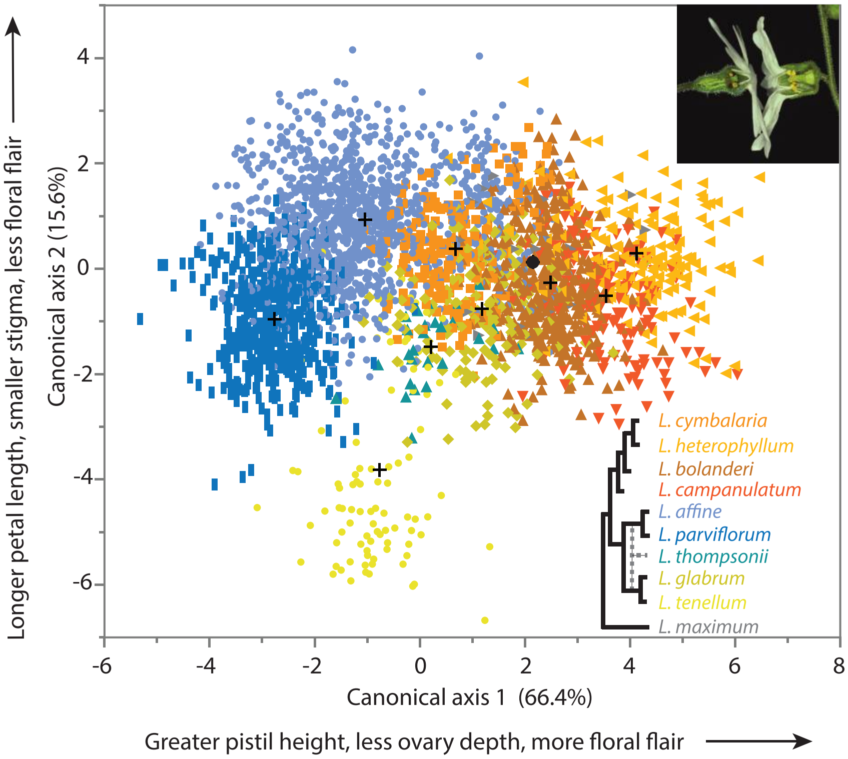


Figure 3: Variation in floral morphology among clades and species of *Lithophragma* as assessed with quadratic discriminant analysis. Crosses indicate the multivariate mean for each species. The black circle is the mean for the basal species of the genus, *L. maximum*. Phylogenetic affinities are based on Kuzoff et al. (1999) and Deng et al (2015). The dotted lines indicate the phylogenetic origin of a hybrid species. See table A1 for sample sizes for each species. Each axis shows the percentage contribution of that canonical axis to the overall discriminant analysis and up to three characters contributing to negative correlations among traits along that axis. The major characters contributing in each direction to negative correlations on that axis are shown along each axis. Photo shows the two extremes of floral morphology along canonical axis 1: longitudinally cut *L. parviflorum* on the left and *L. heterophyllum* on the right.

species occupied a range of morphological space that overlapped only partially with that of other species.

Four *Lithophragma* species had geographic ranges sufficiently broad that they differed in which *Greya* moths were present locally. *Lithophragma bolanderi* and *L. affine* occurred in all possible combinations with *Greya* moths. In both plant species, multiple floral characters contributed to divergence among populations, depending on which *Greya* species was present (fig. 4; table A4; QDA Wilks's $\lambda = 0.7433$, $F = 9.320$, $\mathrm{df} = 14, 816$, $P < .0001$ for *L. bolanderi*, no. flowers = 417; Wilks's $\lambda = 0.7274$, $F = 19.074$, $\mathrm{df} = 21, 3{,}471$, $P = .001$ for *L. affine*, no. flowers = 1,200). The relative effects of characters contributing to divergence among populations differed between the two species (fig. 4). These results corroborate and extend previous experimental studies showing that small differences among *Lithophragma* in multiple floral traits are important to the evolution of these interactions, because they affect which moth body parts touch the stigma and anthers during pollination (Thompson et al. 2013). *Greya obscura* usually co-occurred with *G. politella*, but when only *G. obscura* was present locally, the floral trait combinations in both *L. affine* and *L. bolanderi* differed from flowers in ecosystems in which *G. politella* was present (fig. 4; table A4). Few *Lithophragma* populations lacked *Greya* species, but those populations differed in floral trait combinations from conspecific populations that interact with *Greya* (fig. 4; table A4).

In *L. cymbalaria* and *L. parviflorum*, populations occur in nature under only a subset of the possible combinations of interactions with *Greya* moths (fig. 4; table A4). *Lithophragma cymbalaria* flowers in populations that interact with only *G. politella* differed from flowers in populations that interacted with both moth species (fig. 4; table A4; QDA Wilks's $\lambda = 0.6616$, $F = 14.910$, $\mathrm{df} = 7, 204$, $P < 0.0001$, no. flowers = 212). *Lithophragma parviflorum* flowers from populations that interact with only *G. politella* moths differed from those from populations that interacted with no *Greya* moths (fig. 4; table A4; QDA Wilks's $\lambda = 0.7526$, $F =$

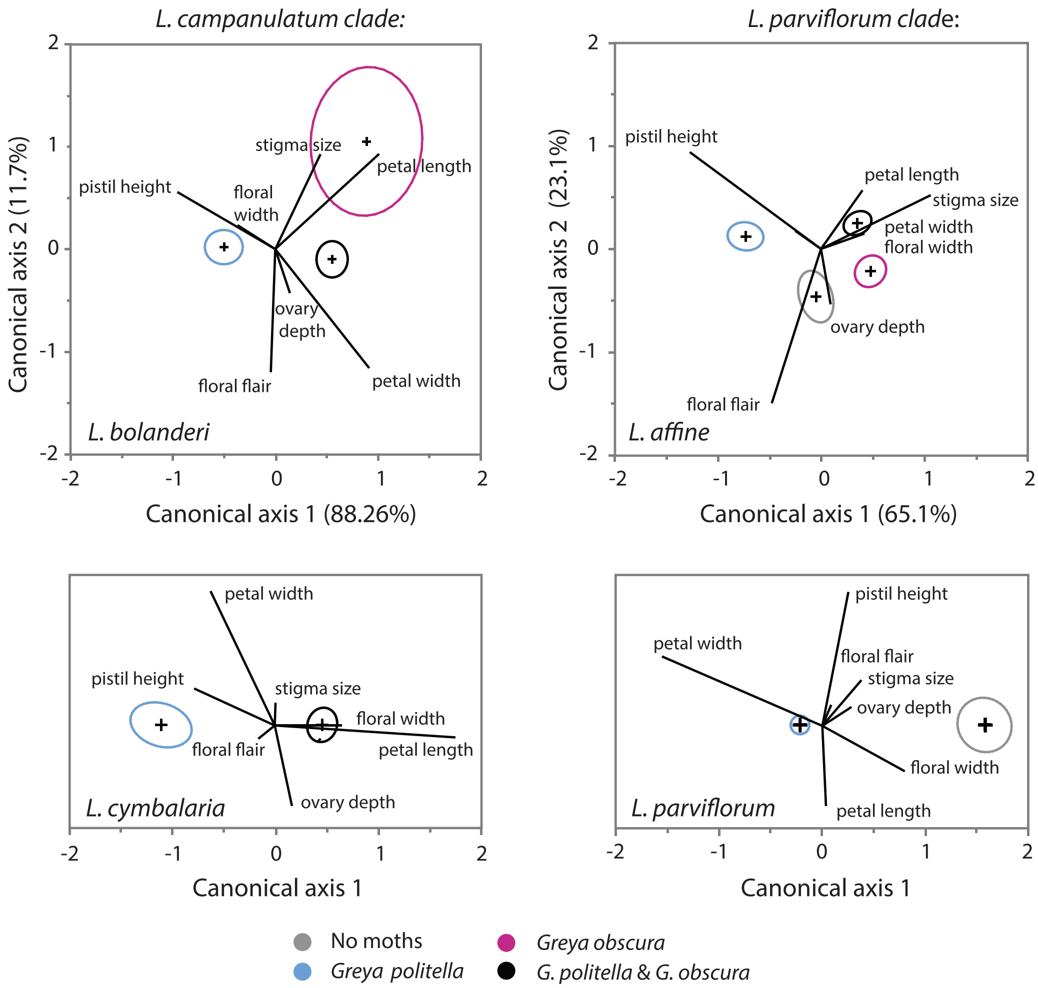


Figure 4: Differences in *Lithophragma* floral morphology among plants that interact with *Greya politella* only, *Greya obscura* only, both moth species, or neither moth species, using quadratic discriminant analysis. Crosses for each species are the multivariate means, and ellipses are the 95% confidence limits. The biplot rays for *L. bolanderi* and *L. affine* indicate the relative contributions of the floral characters to the observed differences for species in which multiple comparisons were possible. Panels on the left show species in the *L. campanulatum* clade, and panels on the right show species in the *L. parviflorum* clade. Each axis evaluating three or more groups shows the percentage contribution of that canonical axis to the overall discriminant analysis. For comparisons between two groups, loadings are shown vertically as well as horizontally to separate them visually, but their relative contributions are only their vertical projections downward along canonical axis 1. There is no canonical axis 2 for these two-group comparisons.

24.369, df $= 7, 519$, $P < .0001$, no. flowers $= 527$). In both species, flowers from populations that interact only with *G. politella* were narrower than in other populations. Otherwise, the two plant species differed in the floral traits contributing strongly to divergence mediated by interactions with *Greya* (table A4). In one additional species, *L. heterophyllum*, most populations interacted with both moth species, but a few populations interacted with only *G. politella*. Flowers from the few sites with only *G. politella* did not differ significantly in this species from those with both moth species (QDA Wilks's $\lambda = 0.8811$, $F = 1.587$, df $= 14, 340$, $P = .08$, no. flowers $= 179$).

We next assessed whether the moths, too, differ in morphology when they co-occur in the same ecosystem rather than isolated from each other. We evaluated morphological traits of the moths known from previous studies to be important during pollination of *Lithophragma* (Thompson et al. 2013). We focused this analysis on the geographic region of overlap between the two species, from southwestern Oregon to southern California. Both *Greya* species differed in morphology when occurring with the other moth species rather than alone (fig. 5; table A5; QDA Wilks's $\lambda = 0.0522$, $F = 235.754$, df $= 12, 1{,}379$, $P < .0001$, no. moths $= 528$). The differences were driven most strongly by shifts in ovipositor length and seventh abdominal segment length, although all four characters contributed somewhat to shifts along these two axes.

These overall differences in morphology between sympatric and allopatric moths included any direct effects of the moths on each other and any indirect effects mediated by coevolution of each moth species with its local host plant species. We were able to evaluate host-associated effects for one species in each of the two *Lithophragma* clades that are pollinated by *Greya* moths (fig. 6; table A6). These two plant species are sufficiently widespread to include populations that interact with both moth species and other populations that interact with only one moth species. For moths on *L. bolanderi*, *G. politella* differed in traits when co-occurring with *G. obscura*, but *G. obscura* did not differ, based on overlap of the 95% confidence limits (fig. 6; table A6; QDA Wilks's $\lambda = 0.0271$, $F = 80.329$, df $= 12, 331$, $P < .0001$, no. moths $= 132$). For moths on *L. affine*, the opposite pattern occurred: *G. obscura* differed in traits when occurring with *G. politella*, but *G. politella* did not differ, based on overlap of the 95% confidence limits (fig. 6; table A6; QDA Wilks's $\lambda = 0.0436$, $F = 102.995$, df $= 12, 545$, $P <$ .0001, no. moths $= 213$). Hence, divergence of *Greya* moths in ecosystems where they occur sympatrically is mediated in part by the particular *Lithophragma* species with which they locally interact. The differences in both moth species were driven mostly by divergence in ovipositor length and seventh abdominal segment length.

The experiment evaluating the effect of six abiotic growing conditions on floral traits showed that the proportion of *L. cymbalaria* plants that produced flowers among treatments did not differ significantly (χ^2 analysis, $\chi^2 = 4.36$, df $= 5$, $P > .499$), but the number of flowers per plant differed significantly on plants that produced flowers (GLM, $\chi^2 = 18.09$, df $= 5, 55$, $P > .003$), ranging among treatments from a mean of 14.1 to a mean of 53.9 (table A7). Typically, only some *Lithophragma* plants produce flowers in their first year of growth. Hence, these results indicate that the six treatments were sufficiently ecologically realistic that a similar numbers of plants in all treatments reached flow-

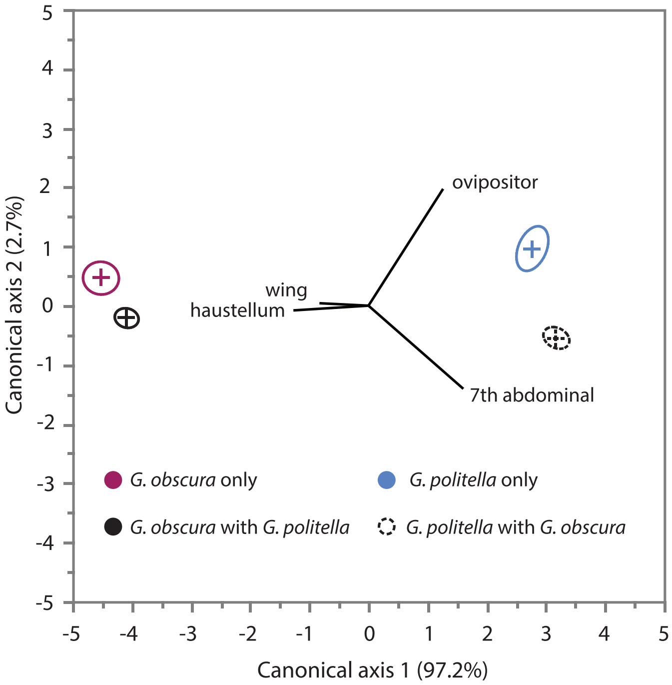


Figure 5: Differences in *Greya* morphology among ecosystems in which with the plants interact with *G. politella* only, *G. obscura* only, both moth species, or neither moth species, using quadratic discriminant analysis. Crosses for each species are the multivariate means, and ellipses are the 95% confidence limits. The biplot rays indicate the relative contributions of the morphological characters to the observed differences. Each axis shows the percentage contribution of that canonical axis to the overall discriminant analysis.

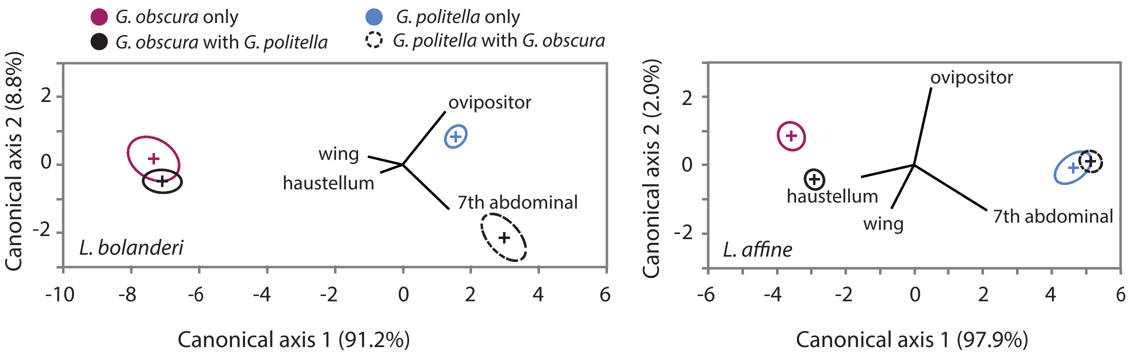


Figure 6: Effect of local *Lithophragma* host plant species and co-occurrence of *Greya* moths on divergence of morphological traits in each *Greya* species, using quadratic discriminant analysis. Crosses for each species are the multivariate means, and ellipses are the 95% confidence limits. The biplot rays indicate the relative contributions of the morphological characters to the observed differences. Each axis shows the percentage contribution of that canonical axis to the overall discriminant analysis.

ering, but the treatments were sufficiently different that some treatments allowed plants to produce many more flowers than other treatments.

The large differences in growing conditions, however, had little effect on floral size or shape (fig. A2). Eight of the nine floral characters did not differ significantly among any of the six treatments (ANOVA, all $P > .05$; table A8). Only the width of the widest petal differed among some treatments (ANOVA, $F = 3.64$, df $= 5, 52$, $P = .007$; table A8). Hence, floral size and shape characters associated with pollination were largely insensitive to a wide range of light, soil, and water conditions. Plants responded to variation in abiotic conditions mostly by altering the number of flowers rather than the sizes and shapes of flowers.

Discussion

The overall results indicate that the traits of woodland stars and *Greya* moths vary across the latitudinal range of the interaction depending on whether local woodland star populations interact with one or both *Greya* species. The trait combinations favored in the plants and the moths have expanded as both lineages have diversified in species and come together in different combinations in different ecosystems. The results therefore suggest that these interactions coevolve as a highly dynamic geographic mosaic that has been reshaped repeatedly over time as different combinations of plants and moths have assembled in different ecosystems. The overall lack of sensitivity of floral size and shape to the six experimental treatments suggests that the large differences in floral traits observed among *Lithophragma* populations are not environmentally induced. Instead, the analyses suggest a strong effect of selection imposed by *Greya* moths. Identifying these coevolved patterns was possible only through analysis of trait combinations among all the interacting species as they came together as different subsets in different ecosystems.

Within *Lithophragma* each species has shifted trait combinations in a unique way depending on which *Greya* species are present locally, but some general patterns emerge. Flowers from woodland star populations with only *G. politella* tend to have trait combinations that include shorter pistil heights or narrower flowers than found in other populations. These traits have the potential to increase the chance that an ovipositing female will contact the stigma with pollen that is adhering to the base or lower portion of her abdomen.

The analyses show that both *Greya* species have different trait combinations when they occur together rather than alone, but the selection pressures that may have driven these differences are not known. The results indicate that morphological shifts in the moths depend on the plant species on which they co-occur locally, but that effect could be either direct or indirect. There is little indication from previous studies of any direct competition between these moth species. Adult moths rest for long periods of time on flowers, potentially excluding visits by other moths, but a previous study indicated only in 1 of 2 years that resting on flowers may limit access to flowers (Thompson et al. 2010). Direct larval competition also seems unlikely, because larvae rarely eat more than a small proportion of developing seeds. Moreover, *G. politella* and *G. obscura* larvae only rarely co-occur in the same plant reproductive tissues.

More indirectly, parasitoids could to contribute to shifts in moth morphology and behavior when the moth species co-occur, and these shifts could depend on the plant species locally available to the moths. Braconid wasp parasitoids search for *G. politella* and *G. obscura* larvae on woodland stars and are common in some populations (J. N. Thompson, personal observation). Past studies suggest that braconid parasitoids commonly attack the larvae of some other *Greya* moths and impose selection on the moths (Althoff and Thompson 1999). Moreover, these studies have shown that some parasitoids differ among populations in how they search among plant parts when attempting to locate *Greya* larvae. Hence, selection imposed by parasitoids could affect where and how *G. politella* and *G. obscura* oviposit into woodland star tissues. That in turn could affect selection on morphological traits such as the length of the ovipositor or the length of the seventh abdominal segment. Tissue-dependent risk of parasitoid attack is one of the current working hypotheses to explain why *G. obscura* oviposits most often into the base of the floral ovary in some woodland star populations but often into the scape, away from the flowers, in some other populations (Friberg et al. 2016). It could also potentially explain why *G. politella* females in most populations oviposit by piercing the nectary disk to reach the ovary but females in at least one population slide through the unfused styles to lay eggs (Thompson et al. 2013). These differences in oviposition behavior affect where the eggs are deposited within the floral ovary and, consequently, could affect the ability of parasitoids to reach eggs or larvae. The possible role of parasitoids in shaping these interactions is therefore strong but not yet evaluated.

There is great potential for geographic and phylogenetic divergence in these coevolving interactions, because they have been diversifying for millions of years across a wide range of habitats. *Lithophragma* and the saxifrage-feeding *Greya* moths are both endemic to western North America (Davis et al. 1992; Thompson 2013). Molecular studies have indicated that the plants and the moths have been diversifying for at least 5–10 million years and have probably been interacting for much of that time (Rich et al. 2008; Thompson et al. 2011; Deng et al. 2015). Woodland stars are part of the *Heuchera* group (sometimes called the *Heucherina* group) of the Saxifragaceae, which has radiated widely in western North America over the past 10 million years (Kuzoff et al. 1999; Deng et al. 2015). During that time, taxa within the *Heuchera* group have become specialized to different pollinator taxa (Soltis and Hufford 2002; Okuyama et al. 2008; Thompson et al. 2013). The interactions between woodland stars and *Greya* moths have further diversified into interactions that range among species from parasitic to mutualistic (Thompson and Fernandez 2006; Thompson et al. 2010, 2013). A similar diversification in moth species and ecological outcomes has occurred in the closely related yucca moths, as they have coevolved with yuccas in western North America (Althoff et al. 2005; Segraves et al. 2005).

During their millions of years of diversification, different combinations of *Greya* moths and *Lithophragma* plants surely have come together repeatedly in different ecological settings as the geographic ranges of the species have expanded and contracted. Phylogeographic analyses of both *G. politella* and *G. obscura* suggest a complex past history of population subdivision, range expansions in some regions, and population stability in other regions (Rich et al. 2008; Thompson et al. 2011). The current geographic patterns in the local interactions between the moths and the plants, and the local differences in the combinations of plant and moth traits, probably reflect Pleistocene and post-Pleistocene changes in geographic ranges.

Woodland stars and *Greya* moths may be particularly strong agents of natural selection on each other. The adult moths take nectar only from the flowers on which they lay their eggs, mate only on host flowers, and rarely leave the flowers except to fly to another *Lithophragma* plant to search for nectar or mates. Individuals complete all stages of development on the host. Hence, fitness of these moths is tied directly to their survival and reproduction on their local host. In turn, the fitness of the plants depends on the moths' effects as pollinators in most populations that have been studied. No other specialist insects feed on these plants, and few generalists have been found to attack the plants in any population during several decades of study throughout the geographic range of these interactions.

More generally, the ability of woodland stars and *Greya* moths to locally fine-tune their coevolving adaptations may be a consequence of three aspects of how evolutionary and coevolutionary selection act on complex traits across ecosystems. Some mathematical models suggest that the degree of local adaptation within species increases with the number of traits exposed to spatially variable selection (MacPherson et al. 2015). Also, the coevolutionary process appears to be particularly adept at favoring and shaping the evolution of complex traits (Zaman et al. 2014) and diverse evolutionary outcomes (Thompson 2013). Evolutionary feedbacks resulting from reciprocal selection may therefore fuel the ongoing evolution of traits and the fine-tuning of local adaptation. In addition, studies of the evolutionary ecology and interactions between plants and other taxa have repeatedly shown that plants can adapt to interactions with other species across even small spatial scales. In a major review of studies of local adaptation in plant populations, Laine (2009) found that all reviewed species showed evidence of divergent selection among populations in the traits involved in interactions with other species.

Some floral and insect characters may be among the best candidates for local adaptation driven by coevolutionary selection. Although floral characters are correlated to varying degrees, there appears to be much opportunity for selection to favor new trait combinations. A review of phenotypic integration for morphological traits found that morphological traits in flowers are less tightly correlated than morphological traits in animals (Conner et al. 2014). Multiple studies have documented strong selection on floral traits mediated by interactions with pollinators (Anderson et al. 2010; Sletvold and Agren 2010; Agren et al. 2013; Schiestl and Johnson 2013; Campbell et al. 2014; Gómez et al. 2015) or the combined effects of selection imposed by pollinators and herbivores (Cariveau et al. 2004; Sletvold et al. 2015). Floral shapes often converge on similar trait combinations when under selection imposed by particular groups of pollinators (e.g., bees, moths, flies; Fenster et al. 2004, 2015; Rosas-Guerrero et al. 2014; Johnson and Raguso 2016), and multiple studies have shown that plant populations adapt to the traits of local pollinators (Pauw et al. 2009; Armbruster et al. 2011; Gowda and Kress 2012; Temeles et al. 2013; Anderson et al. 2014). Similarly, studies of experimental evolution in Lepidoptera and phylogenetic analyses have shown a response to selection in multiple directions even among partially correlated traits (Allen et al. 2008; Brakefield 2010). Hence, the quantitative morphological traits that have diverged in *Greya* and *Lithophragma* may be particularly responsive to subtle selective differences among populations.

The geographic mosaic of coevolution between woodland stars and *Greya* moths therefore appears to be driven in part by differences in how pairs or groups of species shape the partially correlated traits of each species in different ways in different ecosystems. No single plant or moth character drives the observed patterns. Divergence among sites in plant traits, moth traits, and the number of interacting species all contribute to the geographic and phylogenetic diversification of these interactions. Such geographic mosaics are likely common in coevolving interactions, but they are difficult to detect and evaluate without analyses of multiple populations and species.

The divergence in floral and moth morphology found when two, rather than one, mutualistic moth species pollinate a *Lithophragma* population is similar in some respects to that found in studies of the effects of antagonistic interactions among squirrels, crossbills, or both squirrels and crossbills on the morphology of conifer cones (Parchman and Benkman 2008; Benkman 2010; Mezquida and Benkman 2014). Regardless of conifer species, squirrels and crossbills differ in their selective effects on cone morphology. Co-occurrence of the squirrels and crossbills often results in a predictable shift in cone morphology, depending on which seed predator exerts the greater selection pressure on the local conifer population. In the interactions between woodland stars and *Greya* moths, co-occurrence of two *Greya* species results in trait combinations in the plants and the moths that differ from ecosystems in which only one or no *Greya* species occurs on these plants. The results hold for all *Lithophragma* species that interact with *Greya* moths. How the species respond when interacting with one or both *Greya* species, however, differs among woodland species. Reciprocally, the traits of the moths differ depending on whether they occur alone or together on different woodland star species. Overall, these results suggest that natural selection can shape traits of coevolving species in ways that are fine-tuned to the combination of locally interacting species. The combined geographic and phylogenetic patterns in these responses would be masked if all the plants and moths were lumped into a single analysis to assess the overall effects of *Greya* moths in general on woodland star plants. The species each evolve in slightly different ways, providing evidence of coevolution as a relentless and highly dynamic process. Amid ongoing fragmentation of habitats worldwide, the conservation of coevolving interactions may require increased focus on how best to conserve the multiple evolutionary and ecological solutions that arise as coevolving lineages diversify among ecosystems.

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