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Evolution of specialization in a plant-microbial mutualism is explained by the oscillation theory of speciation

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Specialization in mutualisms is thought to be a major driver of diversification, but few studies have explored how novel specialization evolves, or its relation to the evolution of other niche axes. A fundamental question is whether generalist interactions evolve to become more specialized (i.e., oscillation hypothesis) or if partner switches evolve without any change in niche breadth (i.e., musical chairs hypothesis). We examined alternative models for the evolution of specialization by estimating the mutualistic, climatic, and edaphic niche breadths of sister plant species, combining phylogenetic, environmental, and experimental data on *Acmispon strigosus* and *Acmispon wrangelianus* genotypes across their overlapping ranges in California. We found that specialization along all three niche axes was asymmetric across species, such that the species with broader climatic and edaphic niches, *Acmispon strigosus*, was also able to gain benefit from and invest in associating with a broader set of microbial mutualists. Our data are consistent with the oscillation model of specialization, and a parallel narrowing of the edaphic, climatic, and mutualistic dimensions of the host species niche. Our findings provide novel evidence that the evolution of specialization in mutualism is accompanied by specialization in other niche dimensions.

KEY WORDS: *Acmispon*, *Bradyrhizobium*, host specificity, *Mesorhizobium*, mutualist switches, niche evolution, rhizobia.

The evolution of specialization in biotic interactions is thought to be a major driver of diversification across the tree of life (Thompson 1989, 1994; Hembry et al. 2014). In particular, the establishment of taxon or genotype-specific relationships has been linked with adaptive radiations (Futuyma and Moreno 1988; Crepet and Niklas 2009; Hardy and Otto 2014; Hembry et al. 2014; Peay 2016). Intimate relationships between eukaryotes and microbial mutualists are ubiquitous and are among the most diverse kind of biotic interactions (Medina and Sachs 2010). For diverse animal hosts, changes in specificity of host-microbe associations can drive speciation events, and thus could be a major driver of host diversification (Brucker and Bordenstein 2012; Shropshire and Bordenstein 2016). The role of microbes in plant speciation is less studied. However, evidence suggests that specialized partnerships with mutualistic soil microbes have often allowed host plants to expand into novel habitats or ecological niches (Pirozynski and Malloch 1975; Remy et al. 1994; Humphreys et al.

2010), thus providing the divergent environments that could fuel diversification.

Two alternative models have been proposed to explain the evolution of novel specialization in species interactions. The oscillation hypothesis presumes that fitness benefits of specialization over generalism make the latter an ephemeral evolutionary state, and new specialist taxa are predicted to arise via speciation from transitory generalist populations (Janz and Nylin 2008; Hardy and Otto 2014). Species can experience oscillations in niche breath by expanding or contracting their ability to interact with other species, and generalism can be a preadaptation for shifts to novel associations (Janz and Nylin 2008). For instance, Nymphalini butterflies retain the capacity to feed on ancestral host plant species, regardless of specialization to current host-plant use (Janz et al. 2001), and thus are able to colonize novel hosts via gradual niche breadth expansion (Weingartner et al. 2006). Conversely, the musical chairs hypothesis predicts that specialist species can switch to a novel host or partner without changing niche breath, and thus without a generalist intermediary (Hardy and Otto 2014). Rapid switches in specialization can occur via mutations of major effect (Bradshaw and Schemske 2003). Many examples of specialization via partner switches have also been reported in lepidopteran clades, where switches among hosts without a change in niche breadth support the musical chairs theory (Hardy and Otto 2014). Most evidence for the oscillation and the musical chairs hypotheses has been generated using a phylogenetic perspective, but with less focus on microevolutionary processes or the multidimensional aspects of a species ecological niche.

The evolution of species interactions can have opposing effects on the breadth of other niche dimensions of a host species (Futuyma and Moreno 1988). When the acquisition of a new mutualist allows the host species to exploit a novel set of environmental conditions, the host species is then broadening its abiotic niche, such as when plant species are able to tolerate both mild and drier conditions via the association with endophytic fungi (Afkhami et al. 2014). However, novel mutualist interactions can also drive contraction of a host species abiotic niche, when the host adapts to conditions that are only accessed in the presence of the mutualist (Nuñez et al. 2009; Simonsen et al. 2017). Due to this intertwined nature of plant-microbe mutualisms in the evolution of the host plant niche, it is essential to understand how specialization of these different niche dimensions evolves, and if they evolve in parallel directions.

The legume-rhizobia mutualism exhibits substantial variation in specialization. The Leguminosae is the third most diverse family of angiosperms, is globally distributed (Bruneau et al. 2013), and associates with at least a dozen genera of rhizobia (Sawada et al. 2003). From the host perspective, specificity is modulated at two key steps of the mutualism, nodulation, and nitrogen fixation (Masson-Boivin and Sachs 2018; Poole et al. 2018). To initiate the association, legumes release specific flavonoids from roots that induce the expression of *nod* genes by compatible rhizobia, which in turn instigate developmental changes in the plant roots and lead to the formation of root structures called nodules (Masson-Boivin and Sachs 2018; Poole et al. 2018). However, not all rhizobia that instigate nodulation are compatible with the host to fix nitrogen; rhizobia that are specialized to one host often nodulate but then fail to fix nitrogen on related hosts (Ehinger et al. 2014; Pahua et al. 2018). When rhizobia form nodules but fail to fix nitrogen, legumes can target the ineffective strains for reduced proliferation within the host, a trait termed sanctioning (Kiers et al. 2003; Sachs and Simms 2008; Quides et al. 2017; Regus et al. 2017). Therefore, host plants have the ability to accept multiple rhizobial partners into the nodules but ultimately choose rhizobial partners that can fix nitrogen, by increasing the fitness of beneficial rhizobia within root nodules (Sachs et al. 2018).

Acmispon is a genus of legumes native to Western North America (Allan and Porter 2000; Sokoloff 2000; Brouillet 2008) with members that appear to exhibit multiple evolutionary shifts in rhizobial specialization. Field collections have identified two genera of rhizobia as symbiotic partners of *Acmispon* species, *Bradyrhizobium* spp. in nodules of *Acmispon strigosus* and *Mesorhizobium* spp. in its sister species *Acmispon wrangelianus* (Sachs et al. 2009; Sachs et al. 2010; Porter et al. 2019), suggesting recent evolution of specialization to these rhizobial lineages. However, symbiont specificity for other species is unknown, and this differentiation could be confounded by microhabitat preferences of both the plant and microbial species, as *A. strigosus* is commonly observed in sandy soils and drier locations, whereas *A. wrangelianus* is found in various grassland habitats spanning serpentine to nonserpentine soil types (Porter and Rice 2013), mainly in wetter locations. These host species also differ in chromosome number as *A. strigosus* has 14 chromosomes and *A. wrangelianus* has 12 chromosomes in the diploid state (Grant 1965, 1995). Genomic changes in chromosome structure can trigger evolutionary novelty and adaptive radiation in plants (De Storme and Mason 2014), and could be a mechanism driving the acquisition of novel symbionts.

Here, we evaluated alternative hypotheses for how specialization in mutualist partners evolved in *A. strigosus* and *A. wrangelianus*, specifically whether a generalized host evolved to become specialized to a rhizobial partner (i.e., oscillation hypothesis; Janz and Nylin 2008; Hardy and Otto 2014) or if a switch in rhizobial partners evolved without a change in niche breadth (musical chairs hypothesis; Hardy and Otto 2014). More broadly, we examined whether the mutualist niche evolved in concert with abiotic niche axes and reconstructed ancestral mutualist associations across the *Acmispon* host lineage. We predicted that

Table 1. Predictions of each evolutionary model of specialization in mutualism.

Description	Oscillation theory New specialist taxa arise via speciation from transitory generalist populations	Musical chairs New specialist species arise via speciation from switches in specialization, without a generalist intermediary
Effect in niche breadth	• Sister species will differ in their niche breadth	• Sister species will have similar niche breadths
Prediction 1	*A. strigosus* and *A. wrangelianus* form nodules and benefit from the association with both rhizobial genera(because species are predicted to retain the ability to return to their ancestral state, i.e., be generalists).	*A. strigosus* and *A. wrangelianus* form nodules and benefit only from the association with homospecific rhizobia(because species cannot switch back to their ancestral state, i.e., be specialized to previous partner).
Prediction 2	Substantial genetic variation in traits associated with rhizobial specificity is predicted to persist across the range of newly formed sister specialized species due to standing genetic variation from the ancestral generalist population	Given the lack of a generalist ancestor, minimal to no genetic variation in traits is predicted to be associated with rhizobial specificity
Prediction 3	Substantial phenotypic plasticity in plant traits associated with rhizobial specificity because periods of plant niche expansion are characterized by increased plasticity, which in turn facilitates transitions to novel symbiotic associations	No phenotypic plasticity in plant traits associated with rhizobial specificity, because no niche expansions are predicted.

under the oscillation hypothesis, *A. strigosus* and *A. wrangelianus* should retain the ability to associate with and benefit from the association with both rhizobial genera due to standing genetic variation from the ancestral generalist population, and thus substantial among-genotype variation in traits associated with specialization would persist across the range of newly formed specialized sister species (Table 1). Under the musical chairs hypothesis, we predict both species to be highly specialized to a rhizobial genus and with no ability to form associations with alternative rhizobial genera or to benefit from those associations (Table 1). Similarly, we would expect minimal among-genotype variation in specialization traits across the range of both sister species, given the lack of a generalist ancestor (Table 1). We tested genotypes of *A. strigosus* and *A. wrangelianus*, collected at multiple locations across California. To confirm plant species identity of genotypes and examine conservatism in specialization, we reconstructed their phylogenetic relationships using genome-wide single-nucleotide polymorphisms (SNPs) discovered through double-digest RAD-seq. We inoculated host genotypes with two strains each of *Mesorhizobium* and *Bradyrhizobium*, and also conducted mixed inoculations to examine partner choice under common climatic and soil conditions. Lastly, we formally estimated the breadth of both species' environmental niches and quantified the degree of overlap in their climatic and edaphic niches based on occurrence data. Our goals were to (i) evaluate the degree to which hosts specialize on rhizobia partners by testing whether host genotypes nodulate with and/or gain fixed nitrogen benefit from one or both bacterial genera, and by examining partner choice when both *Bradyrhizobium* and *Mesorhizobium* are present, (ii) test for host species genetic variation and plasticity in specificity traits, and (iii) evaluate whether the mutualist, climatic, and edaphic niche axes evolve in concert with each other.

Materials and Methods

SYMBIONT SPECIFICITY OF *Acmispon* spp. IN NATURE

To assess the rhizobia taxa that associate with *Acmispon* spp. in nature, we isolated and genotyped rhizobia from plant nodules across multiple locations in California (see SI). To infer how symbiont specificity has evolved within the genus, we reconstructed a phylogeny for *Acmispon* species with available sequence information from Allan and Porter (2000) and for which published data or our own field collections allowed us to genotype rhizobia (Fig. 1A; see SI for details). We included *Lotus corniculatus* (which is naturalized to California) and *Lotus arenarius*, which are Old World relatives of *Acmispon*, as an outgroup (Allan and Porter 2000). For the host plant phylogeny, sequences of the internal transcribed spacers (ITS1 and ITS2) and 5.8S region of the nuclear ribosomal DNA were aligned using MAFFT (Katoh et al. 2002) and a maximum-likelihood tree was

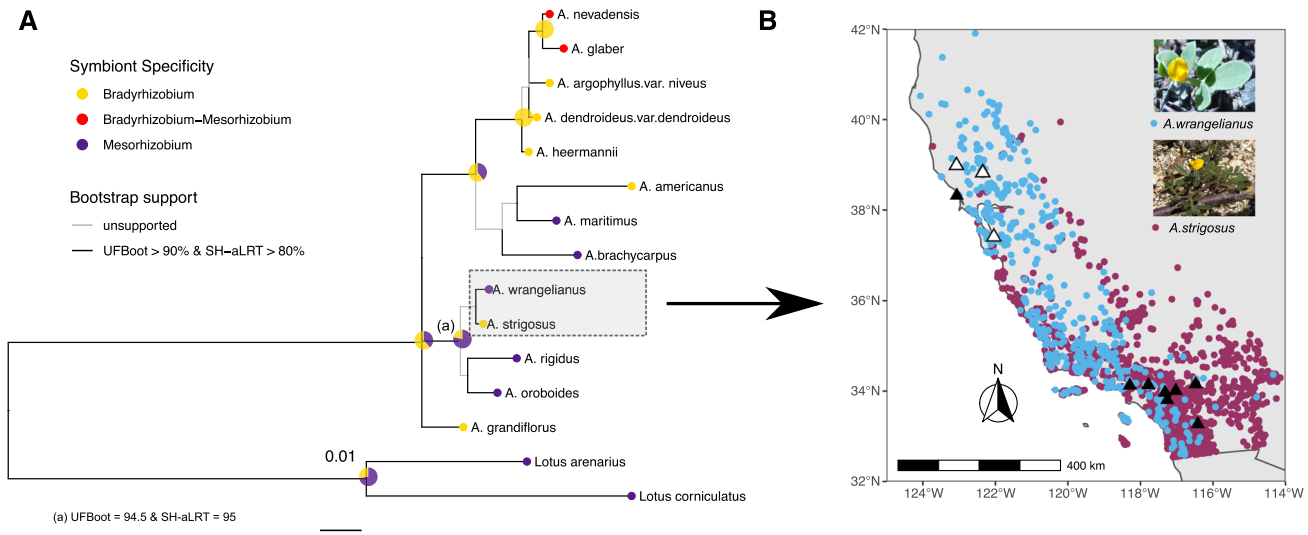

Figure 1. Symbiont specificity and distribution of *A. strigosus* and *A. wrangelianus* in California. (A) Reconstruction of phylogenetic relationships among *Acmispon* spp. with known rhizobial specificity information. Tip labels indicate the rhizobial taxa that associate with each host species. Marginal ancestral states are reported for nodes with strong support (SH-aLRT ≥ 80 and uBS ≥ 90), and Bayesian posterior probabilities for ancestral states are represented as pies. The dashed rectangle indicates the two sister species that are the focus of our study. (B) Locations where the different maternal lines of *A. strigosus* (black triangles) and *A. wrangelianus* (white triangles) were sampled. Colored dots represent the occurrences of each species extracted from the Consortium of California Herbaria Portal (CCH2, http://www.cch2.org/portal/).

constructed in IQ-TREE (Minh et al. 2020). Substitution models were estimated with ModelFinder (Kalyaanamoorthy et al. 2017) within IQ-TREE. Branch support was estimated with ultrafast bootstrap approximation (UFBoot; Hoang et al. 2017) and single-branch tests—SH-aLRT using 1000 replicates (Guindon et al. 2010). Ancestral trait reconstruction of symbiont specificity was performed using a continuous-time Markov chain model (Mk model) in *phylotools* (Revell 2012), using rhizobia genus as the character state. Marginal state reconstruction was performed at each node using an equal rate model. Stochastic character mapping was also performed to infer histories of trait evolution (Revell 2012, 2017). Ancestral state uncertainties were estimated based on 100 stochastic character maps and the average number of changes between states was calculated across the tree. Ancestral states were reported for nodes with more than 90% UFBoot support and >80% SH-aLRT.

SAMPLING OF HOST MATERNAL LINES

Acmispon strigosus and *A. wrangelianus* seeds were sampled from natural populations for which rhizobia were previously isolated (Sachs et al. 2009, 2010; Porter and Rice 2013; Hollowell et al. 2016). Eight maternal lines of *A. wrangelianus* were sampled across three sites (Fig. 1B and Table S1; for collection details, see Porter and Rice 2013) and 12 maternal lines of *A. strigosus* were sampled across seven locations (Fig. 1B and Table S1; Hollowell et al. 2016; Wendlandt et al. 2019). Plants were germinated from wild seeds and allowed to self-pollinate in a glasshouse for at least one generation.

RHIZOBIA STRAINS

Four rhizobia strains were tested on *A. strigosus* and *A. wrangelianus* host lines, including two *Bradyrhizobium* strains isolated from *A. strigosus* and two *Mesorhizobium* strains isolated from *A. wrangelianus*. *Bradyrhizobium* strain 05LoS23R7.12 (hereafter B1) was isolated at Bodega Marine Reserve (Sachs et al. 2009), and 13LoS15.1 (hereafter B2) was collected at Griffith Park (Table S1). Both strains nodulate and are beneficial to *A. strigosus* (Sachs et al. 2010; Regus et al. 2015). B1 has a haplotype that has been observed in multiple populations of *A. strigosus* including locations where *A. wrangelianus* is also reported, and B2 has a haplotype that was observed from a site where both *A. strigosus* and *A. wrangelianus* coexist (Fig. 1B; Torres-Martínez, L pers. obs.). *Mesorhizobium* strain #C120A (hereafter M1) was collected from McLaughlin Reserve and Strain #C265A (hereafter M2) was collected from Jasper Ridge Reserve (Porter and Rice 2013). Both *Mesorhizobium* strains are beneficial to *A. wrangelianus* (Porter et al. 2019). M1 was isolated from a nonserpentine location and is nickel sensitive, whereas M2 was isolated from a serpentine soil and it is nickel tolerant (Porter et al. 2019). No occurrences of *A. strigosus* have been reported near McLaughlin Reserve, whereas at Jasper Ridge, *A. strigosus* was reported ~2 km from *A. wrangelianus* (Fig. 1B).

COMMON GARDEN INOCULATION EXPERIMENT

Acmispon wrangelianus seeds were stratified for 2 weeks at 4 °C (Porter et al. 2019). Seeds of both species were surface sterilized (Sachs et al. 2009), planted in autoclave-sterilized soils (Pro League, Quickdry; Turface Athletics, Buffalo Grove, Illinois, USA) that provide negligible nutrients to plants, and hardened under greenhouse conditions for 2 weeks before inoculation on February 2019. Host lines were inoculated with clonal cultures of the four rhizobia strains (i.e., B1, B2, M1, and M2), equal concentrations (co-inoculation) of one *Bradyrhizobium* strain (B1) with each of the two *Mesorhizobium* strains (i.e., B1M1 and B1M2), or with water as an uninoculated control (i.e., C) for a total of seven inoculation treatments. B1 was used for co-inoculations because its haplotype is abundant across California (Hollowell et al. 2016) and expected to be encountered across the distribution of *A. strigosus* and *A. wrangelianus*. Plants were fertilized weekly with 5 ml of nitrogen-free Jensen's solution (Somasegaran and Hoben 1994).

Rhizobia cultures were grown on a modified arabinose gluconate medium (MAG; Sachs et al. 2009) until lawns formed (29 °C, ∼8 days), washed from plates, and resuspended in liquid MAG to estimate concentration via optical density. Washed cells were centrifuged (750 g, 20 min) to remove media and resuspended in sterile water to a concentration of 10^8 cells/ml. Inoculated plants received 5×10^8 rhizobia cells in 5 ml of sterile water and uninoculated control plants received 5 ml of sterile water (Fig. S1).

Maternal lines were arranged in size-matched groups based on the number of true leaves present on seedlings. Host line by treatment combination were randomly assigned with a total of five replicates. For host lines AcS031 and AcS075, only four and three replicates were assigned, respectively, due to seedling mortality, resulting in 679 plants in total.

PLANT HARVESTING AND NODULE CULTURING

Plants were harvested one block per week, from 6 to 11 weeks postinoculation. Soil was washed from root systems, shoot and root systems were separated, and nodules were removed, counted, and photographed to assess plant performance and specificity traits. Two and three nodules for the single and co-inoculated plants, respectively, were randomly selected for culturing rhizobia to confirm the effectiveness of the inoculation treatments and bacteria in planta abundance. The number of nodules selected per plant was chosen to complete harvest in a timely manner. Shoots, roots, and the remaining nodules were separately oven dried >3 days at 60 °C to measure dry biomass. The dry weight of nodules used for culturing was estimated using an empirically generated formula by Wendlandt et al. (2019) that correlates nodule area (mm^2) with nodule mass (mg).

To identify the rhizobial genus associating with each host plant under the single and co-inoculation treatments, nodules selected for culturing were surface sterilized with bleach, rinsed with ddH_2O, crushed, diluted to 10^{-3} and 10^{-5} concentration, and spread onto plates of glucose-based rhizobium-defined medium, where colonies of *Bradyrhizobium* and *Mesorhizobium* were differentiated based on color and size at 8 days after plating at 29 °C (Sachs et al. 2009). At this time point, *Bradyrhizobium* colonies were white and very small (∼0.5mm), and *Mesorhizobium* colonies were yellow and larger (2–3 mm; see Fig. S2). If overlap occurred, colonies from each genus were still distinguishable based on their coloration and shape.

HOST SPECIFICITY AND BENEFIT FROM MICROBIAL ASSOCIATION

To evaluate specialization, we estimated the probability of nodulation and the mean number of nodules formed under clonal inoculation treatments. Probability of nodulation was defined as the ability of the host-microbial combination to instigate formation of root nodules. The *Bradyrhizobium* strains were defined as "homospecific" on *A. strigosus* (i.e., previously isolated from the same species) and "heterospecific" on *A. wrangelianus* (i.e., isolated from a different species), and vice versa for the *Mesorhizobium* strains. Binary assessment (formed a nodule = 1, no nodule formed = 0) per maternal line was used to model the probability of nodulation. A mixed-effects logistic regression was used to test whether the probability of nodulation depends on inoculation with a homospecific or heterospecific strain, while accounting for the variation among host genotypes, that is, maternal families (Vermunt 2005). If only one plant replicate of a maternal line formed nodules across all treatments, it was removed from the trait estimates. Maternal lines AcW05 and AcW02 were removed. Total nodule number was modeled using a GLMM with a Poisson distribution. To overcome overdispersion, an observation-level random effect was included in our model (Harrison et al. 2018). The mixed-effects logistic regression evaluating the probability of nodulation and the GLMM model for the number of nodules included days postinoculation as a covariate, host species, rhizobia genus, and their interaction as fixed factors, and maternal line nested within species as a random factor.

We assessed benefits from mutualism by estimating relative host growth (RHG) for each replicate, calculated as the percentage of host growth under an inoculation treatment relative to uninoculated plants, modified from Regus et al. (2015):

$$\textit{Relative host growth} = \frac{\text{Shoot biomass of inoculated}_{ijk} - \text{Shoot biomass of uninoculated}_{\overline{X}_k}}{\text{Shoot biomass of uninoculated}_{\overline{X}_k}} \times 100,$$

where i = replicate, j = treatment, k = plant maternal line, and $\overline{X}_k$ = experiment-wide mean for uninoculated plants per maternal line.

Relative nodule biomass (RNB) was used to quantify host plant resources that were invested into rhizobia, calculated for each replicate (i) as the total nodule dry weight divided by total plant biomass. This trait estimates host preference and compatibility with a given rhizobial strain.

$$\text{Relative nodule biomass (RNB)} = \frac{\text{Nodule dry biomass of inoculated}_i}{\text{Total dry biomass of inoculated}_i}.$$

To test the degree to which host gained benefits and invested in mutualism, we fitted separate linear mixed models (LMMs) for RHG and RNB that included days postinoculation as covariate, host species, rhizobia genus, and their interaction as fixed factors, and maternal line nested within species as a random factor. RNB was arcsine square root transformed and RHG was log-transformed to meet the assumptions of analysis of variance.

To evaluate the independent effects of homospecific and heterospecific rhizobial genotypes on each host species, we performed separate GLMM and LMM models for each symbiosis trait (probability of nodulation, number of nodules, RHG, and RNB) where rhizobial genotype was used as a fixed factor. We assessed if relative host growth with each rhizobial genotype was greater than zero by performing a one-sample nonparametric bootstrap *t*-test, using *wBoot* (Weiss 2016).

All statistical models were fitted with the R package lme4 (Bates et al. 2015). We tested the significance of fixed effects of each model described above with marginal likelihood ratio tests using the *Anova* function in the *car* package (Fox and Weisberg 2019). Random factors were evaluated by comparing models with and without the factor and performing likelihood ratio tests with the function *anova*. Post hoc tests were conducted to identify differences among treatments for each species and among species for each treatment. Tukey–Kramer adjustment was used to control for multiple comparisons in the R packages *emmeans* (Lenth 2016) and *multcomp* (Hothorn et al. 2008). All analyses were performed in R version 3.6.1 (R Core Team 2019) with deviation coding ("contr.sum") for categorical variables.

GENETIC VARIATION AND PHENOTYPIC PLASTICITY IN HOST SPECIFICITY

Genetic variation and plasticity were evaluated separately for each host species for the probability of nodulation and the total number of nodules formed in response to homospecific and heterospecific inoculation. We compared generalized LMMs with different variance-covariance structures with log-likelihood tests. Our base model included rhizobial genus as a fixed factor, host maternal line as a random factor, and days since inoculation as a covariate. To test for significant genetic variation in each trait, we compared our base model with a model where the random factor was excluded. To test whether the genetic variation within a host species varied between the rhizobial treatments, a model where the among-line variance was constrained to be equal between rhizobial genera was compared to a model where the among-line variance was allowed to vary (Shaw 1991). To test for the presence of phenotypic plasticity, we evaluated the effect of rhizobial genus by comparing a model where this factor was removed with a model where the random factor was allowed to vary. A significant rhizobial lineage effect indicates host trait plasticity because it is comparing the probability of nodulation and total number of nodules under two separate biotic conditions averaged across maternal lines (Conner and Hartl 2004). When different plant lines express different trait values in the two rhizobia genera in the form of a crossing reaction norm, then a significant plant genotype-by-rhizobial genus is evident, indicative of genetic variation in phenotypic plasticity. To test for this interaction, a model where the intercept and slopes of the random factor were allowed to vary between rhizobial genera was compared with a model where only the intercept was allowed to vary (Lynch and Walsh 1998; Conner and Hartl 2004; Saxton 2004).

IN PLANTA RHIZOBIA ABUNDANCE

In planta rhizobia abundance was estimated to evaluate the performance of the rhizobia strains within the host plants and asses the level of host compatibility with each rhizobial genus. Proliferation of the strains within plant nodules indicates compatibility with the host and reduced sanctioning (Sachs and Simms 2008; Regus et al. 2017). Rhizobia abundance was measured as the average number of colony-forming units per nodule. Due to low number of colonies observed in nodules of plants inoculated with strains of the heterospecific rhizobial genus, differences of the in planta rhizobia abundance were only tested among host maternal lines inoculated with homospecific rhizobial strain treatments. A linear model was used that included maternal line, homospecific rhizobial strain, and their interaction as fixed effects and rhizobia abundance as the response variable. Rhizobia abundance was square root transformed to comply with assumptions of normality. Post hoc tests were performed using Tukey–Kramer adjustment for multiple comparisons.

PHYLOGENETIC RELATIONSHIPS OF MATERNAL PLANT LINES AND TRAIT CONSERVATISM

Plant maternal lines were genotyped with double-digest RADseq (ddRADseq) using the protocol of Brelsford et al. (2016). Genomic DNA of each sample was digested with *Eco*RI and *Mse*I and library was sequenced in the Illumina HiSeq 4000 at UC Berkeley.

Sequences were de-multiplexed, quality filtered, and truncated to 93 nucleotides using the *process_radtags* pipeline

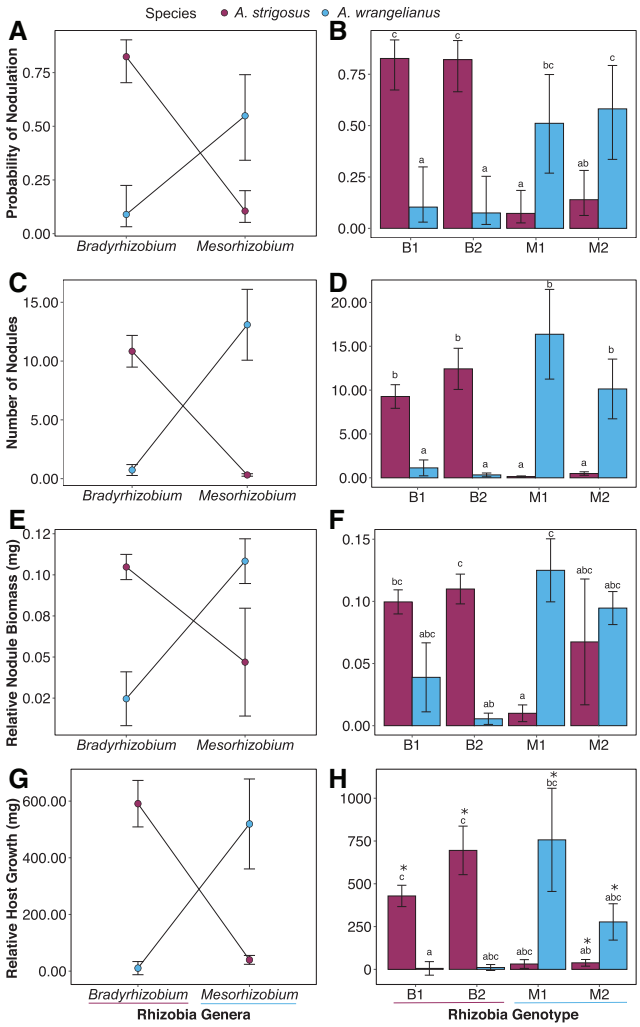

Figure 2. Specialization of sister *Acmispon* species to rhizobia genera and genotypes. Probability of forming a nodule (A and B), and total number of nodules formed (C and D) are used to infer levels of specificity of each host species to rhizobial taxa. Relative nodule biomass (E and F) and relative host growth (G and H) are used to quantify host rewards and benefits of the association, respectively. In panels A, C, E, and G, the dots represent the mean trait value. Bars represent mean trait values and error bars denote ± standard error of the mean (SEM). Letters above bars denote statistically significant pairwise differences among treatments and species based on Tukey's post hoc tests. Asterisk in panel D indicate a significant host growth benefit based on one-sample nonparametric bootstrap *t*-tests at $\alpha = 0.05$. B1 and B2 are the *Bradyrhizobium* genotypes. M1 and M2 are the *Mesorhizobium* genotypes.

in *Stacks version 2.5* (Catchen et al. 2011, 2013). We used reference-guided discovered SNPs after finding that a de novo and reference-guided SNP discovery approaches produced comparable relationships among species and genotypes (Table S2 and Figs. S3 and S4). Phylogenetic analyses were performed using Maximum likelihood with *RAxML* (Stamatakis 2014) with the GTR + Γ model of nucleotide evolution. The trees were visualized and edited in R version 3.6.1 using the packages *ggtree* (Yu et al. 2017; Yu et al. 2018), *treeio* (Wang et al. 2019), *phangorn* (Schliep et al. 2017), *adephylo* (Jombart et al. 2010), and *phylotools* (Revell 2012).

Phylogenetic signal was tested based on the null hypothesis that the probability of nodulation with an alternative rhizobia lineage is independent of the phylogenetic distance in the tree (Blomberg and Garland Jr 2002) using Moran's *I* (Moran 1950; Gittleman and Kot 1990) with the R package *phylosignal* (Keck et al. 2016). Significance of phylogenetic signal was tested with nonparametric randomizations.

QUANTIFICATION OF SPECIES' ABIOTIC NICHE

The abiotic niche of *A. strigosus* and *A. wrangelianus* was quantified using occurrence data from the Consortium of California Herbaria (http://www.cch2.org/portal/). Nine uncorrelated variables out of 19 bioclimatic variables from Worldclim version 2 (Fick and Hijmans 2017) were used to quantify the climatic niche (Fig. S5 and Table S5). The edaphic niche was quantified with 30 soil physicochemical properties (Table S6) extracted from the Harmonized World Soil Database version 1.2 (FAO 2012). For the climatic and edaphic niches, independent principal components analysis (PCA) was performed and calibrated on the entire environmental space of both species taking into account occurrence densities (PCA-env; Broennimann et al. 2012). Then a similar analysis was performed taking into account both climatic and edaphic variables to have an overall environmental abiotic niche estimate (Table S7). Niche overlap was estimated based on Schoener's *D* metric (Warren et al. 2008). The niche data were used to test two separate hypotheses of niche conservatism that can help estimate the prevalence of ecological divergence during speciation, niche equivalency, and niche similarity (sensu Warren et al. 2008). Niche equivalency tests whether the niches of two pairs of species are identical or effectively indistinguishable, whereas niche similarity tests whether the niche of one species predicts another's known occurrence better than expected by chance. Both tests were performed in *ecospat* (Warren et al. 2008; Di Cola et al. 2017) with 100 replications. To estimate niche breadth and position for each species, 1000 random points were sampled in proportion to the density of occurrence, and the median and variance among points were calculated along the first two environmental axes of the PCA-env, and the total niche breadth (area) was estimated as the product of the variances from PC1 and PC2 (Gómez et al. 2016). To corroborate patterns of niche evolution, we performed the same analyses but included global occurrences that were extracted from the Global Biodiversity Facility (GBIF 2020).

Table 2. Statistical models testing specificity and benefits from symbiosis of *A. strigosus* and *A. wrangelianus* with homospecific and heterospecific rhizobia genera.

		Probability of nodulation[a]		Total number of nodules[a]		Relative nodule biomass[b]		Relative host growth[b]	
Factor	*d.f.*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
Fixed factors									
Host species	1	2.224	0.136	0.781	0.377	0.341	0.559	1.777	0.182
Rhizobia genus	1	3.082	0.079	1.968	0.161	0.376	0.539	0.050	0.823
Host species × rhizobia genus	1	76.329	< 0.01***	148.344	< 0.01***	29.573	<0.01***	27.431	< 0.01***
Days since inoculation	1	0.075	0.786	1.809	0.179	1.755	0.185	14.460	< 0.01***
Random factor									
Host genotype (species)	1	11.229	< 0.01***	11.767	< 0.01***	1.522	0.217	0.613	0.434

Type III Wald chi-square tests are reported for the main effects of each GLMM and LMM model. *Significance at $\alpha = 0.05$;.**Significance at $\alpha = 0.01$; ***Significance at $\alpha = 0.001$

[a]Sample size, *N* = 338 (excluding controls); [b]Sample size, N = 140 (after removing plants that did not form nodules).

Table 3. Statistical models evaluating the specificity and symbiosis benefits of *A. strigosus* and *A. wrangelianus* with two homospecific and two heterospecific rhizobial genotypes.

		Probability of nodulation[a]		Total number of nodules[a]		Relative nodule biomass[b]		Relative host growth[b]	
Factor	*d.f.*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
Fixed factors									
Host species	1	0.099	0.753	0.060	0.808	5.019	0.025*	12.794	<0.01***
Rhizobia strain	3	15.013	<0.01***	23.432	<0.01***	20.397	<0.01***	19.841	<0.01***
Host species × rhizobia strain	3	75.944	<0.01***	150.066	<0.01*	32.606	<0.01***	27.956	<0.01***
Days since inoculation	1	0.070	0.791	1.908	0.167	1.903	0.168	14.401	<0.01***
Random factor									
Host genotype (species)	1	11.341	<0.01***	12.456	<0.01*	1.323	0.250	0.765	0.382

Type III Wald chi-square tests are reported for the main effects of each GLMM and LMM model. *Significance at $\alpha = 0.05$; **Significance at $\alpha = 0.01$; ***Significance at $\alpha = 0.001$

[a]Sample size, *N* = 338 (excluding controls); [b]Sample size, N = 140 (after removing plants that did not form nodules).

Table 4. Genetic variation and plasticity of host specificity traits.

		Probability of nodulation		Total number of nodules	
Species/Factor	*d.f.*	χ^2	*p*	*F*	*p*
A. strigosus					
Host line	1	2.417	0.059*	4.004	0.022*
Rhizobia genus	1	34.550	<0.01***	45.848	<0.01***
Host line × rhizobia genus	2	1.687	0.430	1.885	0.391
A. wrangelianus					
Host line	1	10.254	<0.01***	7.245	<0.01**
Rhizobia genus	1	9.001	<0.01**	11.884	<0.01***
Host line × rhizobia genus	2	4.260	0.119	8.399	0.015*

Days since inoculation was excluded from analyses of genetic segregation because they were not significant predictors in the statistical models.
*Significance at $\alpha = 0.05$; **Significance at $\alpha = 0.01$; ***Significance at $\alpha = 0.001$.

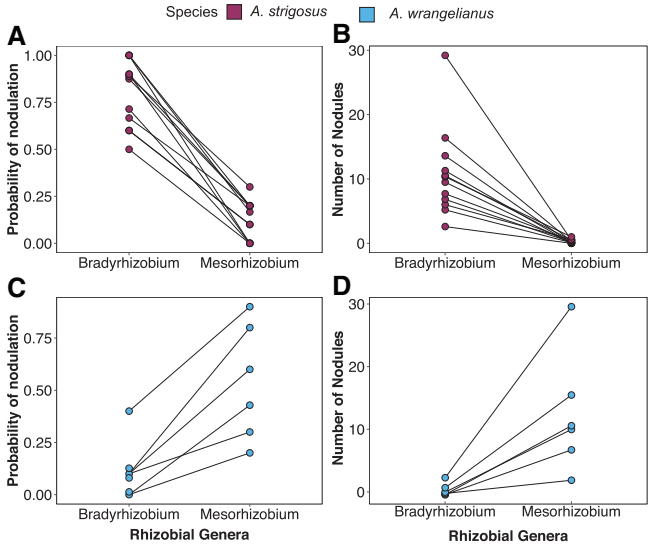

Figure 3. Genetic variation and plasticity of host specificity traits. Reaction norms of probability of nodulation and total number of nodules for *A. strigosus* (A and B) and *A. wrangelianus* (C and D) in response to inoculation with *Bradyrhizobium* and *Mesorhizobium*. Each point represents the mean phenotypic values of each inbred line. The spread of the points along the *y*-axis represents the species genetic variation for the trait under each rhizobial taxon. The lack of crossing lines indicates no significant genotype-by-rhizobial genus interaction.

Results

EVOLUTION OF SYMBIONT SPECIFICITY IN *Acmispon*

Most *Acmispon* species associated with only one rhizobia taxon in nature, with the exception of *Acmispon glaber* and *Acmispon nevadensis* that associated with strains of both groups (Fig. 1A; Tables S9 and S10), indicating a retention of the ability to associate with both rhizobia taxa and be generalists along the mutualistic niche axis. Multiple switches between *Mesorhizobium* and *Bradyrhizobium* specificity were inferred in the *Acmispon* lineage (Figs. 1A and S6). Marginal ancestral reconstruction inferred five changes between rhizobial taxa among *Acmispon* spp. (Fig. 1A), whereas stochastic character mapping indicated that on average, eight changes were observed over 100 trees (Fig. S6). The most recent ancestor of *A. strigosus* and *A. wrangelianus* was inferred to associate with *Mesorhizobium* (node *a* in Fig. 1A).

TESTS OF SYMBIONT SPECIFICITY IN *Acmispon* SPECIES

In *A. strigosus* and *A. wrangelianus*, the probability of nodulation and the number of nodules were higher with homospecific than with heterospecific rhizobial genera (Fig. 2A-D; Table 2). This pattern was consistent across rhizobial genotypes (Figs. 2B and 2D; Table 3). Nodulation of *A. strigosus* and *A. wrangelianus* with heterospecific strains produced plants with relatively few (<10) and small nodules (Fig. 2C and 2D). With the heterospecific strain M2, the probability of nodulation of *A. strigosus* was not significantly different from the probability of nodulation of *A. wrangelianus* when inoculated with homospecific strains M1 and M2 (Fig. 2B), suggesting an ability of *A. strigosus* to recognize a *Mesorhizobium* strain with similar odds as that of *A. wrangelianus*. Similarly, when host species were exposed to equal mixes of homo- and heterospecific strains, the probabilities of nodulation were higher for *A. strigosus* than *A. wrangelianus*, whereas the number of nodules was not significantly different between species (Fig. S7).

Investment into nodulation (i.e., RNB) was higher with homospecific than heterospecific rhizobia genera (Fig. 2E; Table 2). A significant Species-by-Rhizobia strain interaction indicated that RNB also varied with bacterial genotype (Fig. 2F; Table 3). In *A. strigosus*, investment into nodulation was 11 times higher with the homospecific strain B1, than with the heterospecific strain M1 ($t_{125} = 3.463$, $p = 0.0163$; Fig. 2F), whereas a similar investment was observed when inoculated with B1, B2, and M2. In *A. wrangelianus*, investment into nodulation was four times higher with both homospecific strains, than with the heterospecific strain B2 ($t_{128.2} = -3.199$, $p = 0.036$; Fig. 2F), and similar to strain B1 (Fig. 2F).

BENEFITS FROM MICROBIAL ASSOCIATIONS

Relative growth of hosts was 40 and 60 times higher with homospecific than with heterospecific rhizobia genera in *A. strigosus* and *A. wrangelianus*, respectively (Fig. 2G; Table 2), and varied depending on rhizobia strain (significant Species-by-Rhizobia genotype interaction; Table 3 and Fig. 2H). Overall, no growth benefit was observed in *A. wrangelianus* when inoculated with heterospecific rhizobial strains (i.e., relative host growth was not significantly higher than 0%; Fig. 2H and Table S3). Conversely, *A. strigosus* received a significant growth benefit from heterospecific strain M2 (Fig. 2H and Table S3). *Acmispon strigosus* plants inoculated with B2 experienced 20× growth compared to plants inoculated with M2 ($t_{126} = 3.187$, $p = 0.037$), and 10× higher growth when inoculated with B1 than with M2 ($t_{125} = 3.183$, $p = 0.038$), whereas significantly different growth was not observed among plants grown with B1, B2, and M1. In *A. wrangelianus*, relative growth of plants inoculated with M1 was 100× higher than with B1 ($t_{122} = -3.252$, $p = 0.031$), whereas no significant growth differences were observed among plants inoculated with M1, M2, and B2, and between M2 and B1 (Fig. 2H). Both host species received benefits from the association with homospecific strains (Fig. 2H). When host species were exposed to equal-mixes of homo- and heterospecific strains, the relative host growth was similar between host species and the mean values were not significantly different from the values ob-

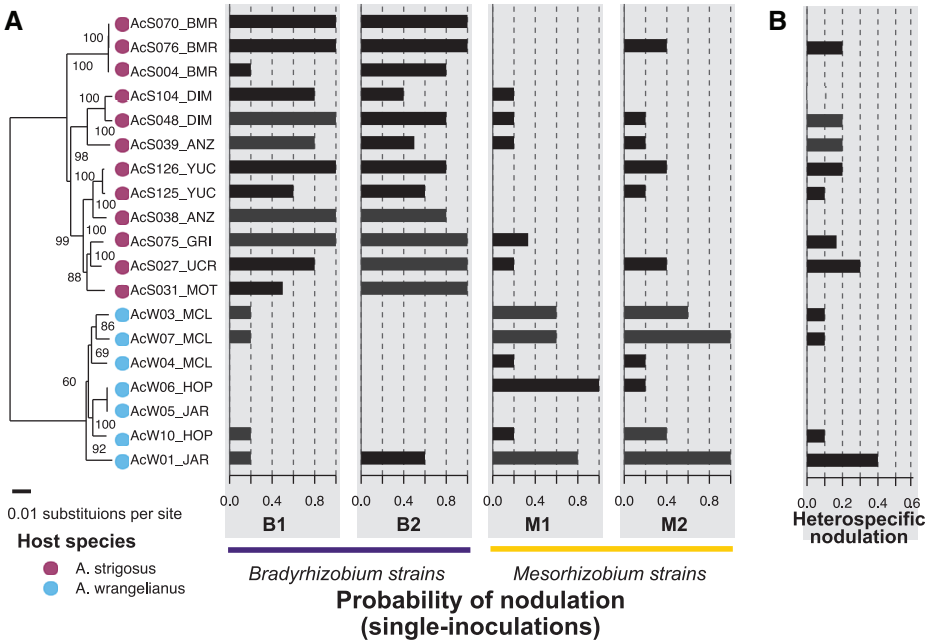

Figure 4. Phylogenetic signal of nodulation with heterospecific rhizobia. (A) Phylogenetic relationships among *A. strigosus* and *A. wrangelianus* genotypes are shown with bar plots representing probabilities of nodulation with the different rhizobial genotypes. (B) Average probability of nodulation with a heterospecific rhizobial genus.

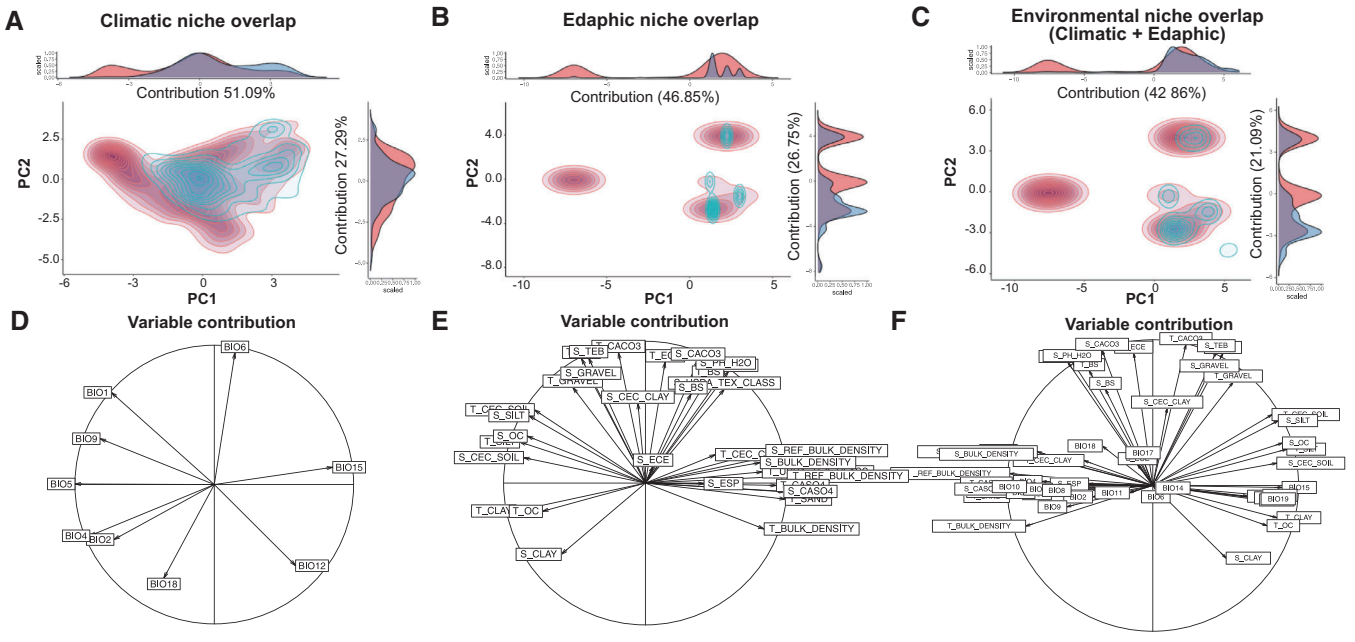

Figure 5. Abiotic niche overlap of *A. strigosus* and *A. wrangelianus*. Overlap of climatic (A), edaphic (B), and environmental niches (C) measured along two components of PCA-env. Magenta represents the niche of *A. strigosus*, whereas blue represents the niche of *A. wrangelianus*. High density of occurrences along the environmental space is represented by darker shades. Top and right panels in each plot highlight the differences (either light magenta or blue) and similarities (purple) in the niches along each principal component. Contribution of each environmental variable to the (D) climatic, (E) edaphic, and (F) environmental niches is depicted in a correlation circle. The closer a variable is located to each axis, the stronger is their contribution. The position indicates if the contribution is positive or negative along each axis (see Supporting Information for description of each variable).

served under clonal inoculations with homospecific strains (Figs. S7C and S7D).

GENETIC VARIATION AND PLASTICITY IN HOST SPECIFICITY TRAITS

Acmispon strigosus and *A. wrangelianus* each expressed somewhat similar patterns of genetic variation in specificity traits (Table 4 and Fig. 3). A significant host genotype effect indicated genetic variation in the probability of nodulation and the number of nodules in response to homospecific and heterospecific mutualists (Table 4 and Fig. 3). For *A. strigosus*, this variation was marginally significant for the probability of nodulation (Fig. 3). The significant rhizobial genus effect indicated that there is a change in the mean phenotypic value of each line when inoculated with different genera of rhizobia and suggest phenotypic plasticity in the probability and ability to form nodules in the presence of either *Bradyrhizobium* or *Mesorhizobium* strains (Fig. 3). No significant genotype-by-rhizobial genus was observed in *A. strigosus*, whereas in *A. wrangelianus* this interaction was significant in the total number of nodules indicating genetic variation in plasticity for the ability to form nodules (Table 4 and Fig. 3).

IN PLANTA RHIZOBIA ABUNDANCE

Heterospecific nodules formed by *A. strigosus* and *A. wrangelianus* had low abundance of in planta rhizobia ($<10^5$ cells per nodule) under both single and co-inoculation treatments (Fig. S8). In nodules harvested from the co-inoculation treatments, only homospecific rhizobia strains were recovered in both host species, with the exception of one nodule in each of two host genotypes, AcW03 and AcS070, where heterospecific strains were recovered at very low abundances (Fig. S8).

Rhizobia in planta abundance of homospecific genotypes varied among host maternal lines in *A. wrangelianus*, indicating host genotype by rhizobial genotype interaction with *Mesorhizobium* strains (Fig. S8; Tables 5 and S4). This pattern was not observed in *A. strigosus*.

PHYLOGENETIC RELATIONSHIPS AMONG MATERNAL LINES AND TRAIT CONSERVATISM

A total of 7702 high-quality SNPs were used to reconstruct the phylogenetic relationships of *A. strigosus* and *A. wrangelianus* maternal lines (Tables S2 and S3). *Acmispon strigosus* genotypes clustered in four distinct clades separately from the two clusters formed among *A. wrangelianus* genotypes (Fig. 4). AcW01 from Jasper Ridge Biological Reserve was the most divergent genotype of *A. wrangelianus* and the only one for which high nodulation with a heterospecific rhizobial lineage was observed (Figs. 4A and 4B). In *A. strigosus*, most genotypes had a nonzero probability of nodulation with a heterospecific rhizobial lineage (Figs. 4A

Table 5. Variation of in planta rhizobia abundance of homospecific rhizobial genotypes.

	A. strigosus			*A. wrangelianus*		
	Bradyrhizobium abundance[a]			*Mesorhizobium* abundance[b]		
Factor	*d.f.*	*F*	*p*	*d.f.*	*F*	*p*
Host line	11	4.993	$<0.01^{**}$	5	0.865	0.507
Homospecific rhizobia genotype	3	4.129	$<0.01^{**}$	3	0.890	0.445
Host line × homospecific genotype	33	1.105	0.324	112	3.380	$<0.01^{***}$

[a]Sample size, *N* = 208 nodules; [b]Sample size, N = 135 nodules.
*Significance at α = 0.05; **Significance at α = 0.01; ***Significance at α = 0.001.

Table 6. Environmental niche features.

Niche axis	*D*	Equivalency	Similarity	Species	Position PC 1	Niche breadth PC1	Position PC2	Niche breadth PC2	Total niche breadth
Climatic									
	0.659	0.990	0.059	*A. strigosus*	–0.132	4.582	–2.829	2.915	13.355
				A. wrangelianus	0.878	3.313	–2.933	2.285	7.570
Edaphic									
	0.675	0.990	0.009	*A. strigosus*	1.475	11.285	–2.810	8.753	98.779
				A. wrangelianus	1.872	2.643	–1.544	8.648	22.861
Total environmental									
	0.645	0.990	0.009	*A. strigosus*	1.427	16.241	–2.768	7.925	128.715
				A. wrangelianus	2.034	4.583	–1.409	7.532	34.517

and 4B). No phylogenetic signal was detected for the probability of nodulation with a heterospecific rhizobial lineage (Moran's $I = -0.05$, p-value $= 0.48$), indicating that the distribution of heterospecific nodulation is independent of the phylogenetic distance among genotypes across both species (Fig. 4B).

SPECIES ENVIRONMENTAL NICHE BREADTH AND OVERLAP

Niche breadth was broader for all abiotic axes in *A. strigosus* than in *A. wrangelianus* indicating asymmetric levels of specialization between these species. The first two components of the PCA explained on average more than 40% and 20% of the variation in the species occurrences within the climatic, edaphic, and total environmental spaces (Fig. 5A-C). In the climatic niche, the first principal component (PC1) was negatively associated with the maximum temperature in the warmest month and positively associated with precipitation seasonality (Fig. 5D; Tables S5 and S7), whereas the second component was positively associated by minimum temperature of the coldest month and negatively associated with precipitation of the warmest quarter (Fig. 5D; Tables S5 and S7). In the edaphic niche, the PC1 was negatively associated with the topsoil content of sand, the bulk soil density, and organic carbon content, and positively associated with the topsoil content of silt, clay, and gypsum (Fig. 5E; Tables S6 and S7), whereas the PC2 was positively associated with the topsoil and subsoil calcium carbonate content, total exchangeable bases, topsoil salinity, and pH (Fig. 5E; Table S6).

The *A. strigosus* climatic niche along PC1 was 1.5 times broader than *A. wrangelianus*, whereas similar breadths were obtained along PC2 (Fig. 5A; Table 6). Based on these components, *A. strigosus* occupies a wider range of temperatures over the warmest month and a wider variability in precipitation than *A. wrangelianus*, but a similar range of minimum temperatures over the coldest month and rainfall over the warmest quarter. Differences along PC1 of the climatic niche suggest that *A. strigosus* tolerates hotter and more seasonal precipitation conditions, whereas *A. wrangelianus* niche is restricted to colder and less variable precipitation conditions (Fig. 5A). The edaphic niche of *A. strigosus* was five times broader than the niche of *A. wrangelianus* along PC1, whereas along PC2 the niche breadth was similar (Table 6). This indicated that *A. strigosus* tends to occupy a variety of soil types characterized mainly by the high content of sand, variable concentrations of clay, gypsum, organic carbon, and high bulk density (soil compaction), whereas *A. wrangelianus* tends to occupy more restricted edaphic conditions with preference for soils with low sand content, high gypsum, silt, clay and organic carbon, and low soil bulk density. An overlap of 66%, 68%, and 65% were observed among the climatic, edaphic, and total environmental niches of *A. strigosus* and *A. wrangelianus*. All three aspects of the environmental niche (climatic, edaphic, and total) were divergent between these two sister species (i.e., rejection of the equivalency hypothesis; Table 6). The edaphic niche of either species can predict one another's known occurrence better than expected by chance (i.e., not rejection of similarity hypothesis), whereas this was not the case for the climatic niche where only a marginal significance of the similarity test was observed (Tables 6 and S8), suggesting a stronger divergence and less conservatism in the climatic than in the edaphic conditions occupied by species.

Discussion

An asymmetric pattern of ecological specialization was observed between two sister legume species. Across edaphic, climatic, and mutualistic niche axes, *A. strigosus* was more generalized than *A. wrangelianus*. The retention in the ability to associate with both rhizobial genera in some host genotypes and the ability to gain growth benefits from heterospecific rhizobia in some *A. strigo-*

sus lines suggest that specialization in mutualist choice evolved from transitory generalist populations, consistent with the oscillation hypothesis (Table 1). Additionally, the results that some *Acmispon* species associate with both rhizobia genera in nature, and that multiple shifts in symbiont specificity were inferred among *Acmispon* species, are consistent with the oscillation hypothesis.

Based on the oscillation hypothesis, we predicted that both host species should retain the ability to associate with and gain benefit from the association with both rhizobial genera due to standing genetic variation from the ancestral generalist population, and thus substantial genetic variation in traits associated with specialization would persist across the range of newly formed specialized sister species (Table 1). *Acmispon strigosus* and *A. wrangelianus* appear to be specialized to *Bradyrhizobium* and *Mesorhizobium*, respectively (Fig. 2). However, we observed that some, but not all, maternal lines of each host species have the ability to form nodules and gain modest benefit from the association with heterospecific rhizobial strains. Furthermore, significant genetic variation and phenotypic plasticity in specificity traits was observed, suggesting the presence of standing genetic variation in the capacity to associate with both rhizobia genera. Thus, some ability to form associations with heterospecific lineages is maintained in natural populations of both species, indicating a remnant capacity to be generalists. Our results align with an oscillation mode of evolution in specialization, where expansion in symbiotic niche breadth can occur via phenotypic plasticity or through standing genetic variation from a generalist ancestor (Janz and Nylin 2008). Further sampling of more populations across the species range of both host species could refine these current estimates of genetic variation and phenotypic plasticity, potentially uncovering more standing variation and genetic variation in plasticity in specificity traits.

Mutualist specificity comprises multiple evolved molecular signals from both the bacteria and the host plant (Perret et al. 2000; Yang et al. 2010). Therefore, even though heterospecific symbionts can trigger nodulation, this does not indicate that there will be a reciprocal exchange of benefits between host and mutualist. In our experiment, only some *A. strigosus* maternal lines obtained significant benefits in the interaction with a heterospecific lineage, whereas in *A. wrangelianus* this was not observed. Previous inoculations of *A. wrangelianus* with four different *Bradyrhizobium* strains reported similar findings where despite nodule formation, no host benefit was evident (Pahua et al. 2018). These results suggest that *A. strigosus* retains a broader ability to associate with and gain benefit from interacting with an alternative rhizobial genus, whereas *A. wrangelianus* is more restricted. However, the benefit gain in some *A. strigosus* genotypes when grown with a heterospecific lineage was only achieved with one out of the two *Mesorhizobium* strains tested (strain M2), suggesting a genotype-by-genotype interaction with heterospecific strains. It is possible that these genotype-specific compatibilities could result from historical co-existence of the host species in nature. Strain M2 was isolated from *A. wrangelianus* nodules in a nonserpentine soil at Jasper Ridge, where historical records indicate the presence of *A. strigosus* nearby (∼2000 m apart). Similarly, the one *Bradyrhizobium* strain (B1) for which *A. wrangelianus* relative nodule biomass was indistinguishable from that of *A. strigosus* was a strain collected in Bodega Marine Reserve, where both *A. strigosus* and *A. wrangelianus* are found (Sachs et al. 2009). Relative nodule biomass is a measurement of the magnitude of host investment into the association. In both *A. strigosus* and *A. wrangelianus*, investment in the association with a heterospecific partner depended on the rhizobial strain. Interestingly, similar investment in heterospecific and homospecific strains only occurred in populations where *A. strigosus* and *A. wrangelianus* co-occur (Fig. 1B). Even though we only have a small snapshot of the dynamics at local scales (e.g., at the population level), our data collected from multiple maternal lines across different populations suggest that the retention of generalism to a rhizobial partner might be a local process, further supporting the oscillation theory of speciation in this mutualism model.

Legume specificity traits are likely to have complex polygenic inheritance, given the importance of genotype-by-genotype and genotype-by-environment interactions in the legume-rhizobium mutualism (Argaw and Muleta 2017; Wood and Stinchcombe 2017; Porter and Sachs 2020). For instance, transcriptomic and phenotypic analyses of *Medicago truncatula* with two distinct species of *Ensifer* rhizobia revealed substantial variation among the host × mutualist combinations in gene expression profiles and symbiosis traits (Burghardt et al. 2017). Similarly, in both *A. wrangelianus* and *A. strigosus*, previous experiments have shown significant phenotypic and genetic variation in specificity traits among host genotypes when inoculated with different strains of either *Mesorhizobium* or *Bradyrhizobium*, respectively (Porter et al. 2019; Wendlandt et al. 2019). Our results suggest that a similar pattern occurs when different host maternal lines are exposed to heterospecific rhizobial genera, particularly in the recognition of a potentially compatible mutualist (i.e., probability of nodulation). However, variation was less evident in the number of nodules formed, suggesting a fixed ability in each species to discriminate the incompatible mutualist at later stages of the symbiosis process.

Acmispon strigosus and *A. wrangelianus* exhibit important differences in their climatic and edaphic niches, suggesting evolutionary divergence in key ecological requirements (Warren et al. 2008; Aguirre-Gutiérrez et al. 2015). A stronger difference in the ecological niches between species was observed in the edaphic conditions that they prefer because niche breadth was four times wider in *A. strigosus* than *A. wrangelianus* compared

to a twofold difference in niche breadth of the climate niche axis. However, in comparison to the edaphic niche, the climatic niche was not similar between host species, suggesting that the climate conditions experienced by one species cannot necessarily predict the conditions experienced by another. These findings suggest that the edaphic and climate niche axes are likely not evolving in concert, but further research will be required to assess the levels of conservatism of each of these axes among all *Acmispon* spp.

Patterns of symbiont specificity were not correlated with changes in chromosome numbers (see Table S9), and thus do not appear to underlie the rapid shifts in rhizobia partners we observe. Moreover, symbiont shifts among *Acmispon* spp. were mainly between rhizobia taxa inferred to have associated with hosts in the past because switches were mainly between *Mesorhizobium* and *Bradyrhizobium*, never with alternative rhizobia taxa such as *Ensifer* or *Rhizobium* that associate with *Lotus* spp. in the Old World (Andrews and Andrews 2017) and occur at high densities in the soils these *Acmispon* species occupy (Porter and Rice 2013). Although our reconstruction of *Acmispon* ancestral symbiont associations could be strengthened by a well-resolved and complete phylogeny of the host plants, our extensive field collection at multiple locations and assays testing the range of rhizobia taxa that can form association in two sister species provide multiple lines of evidence to support our inference that respecialization on ancestral symbiont taxa has been a common occurrence in *Acmispon* spp., supporting the oscillation theory (Janz et al. 2001; Janz and Nylin 2008).

In conclusion, the observed asymmetry in niche breadth between *A. strigosus* and *A. wrangelianus* in both biotic and abiotic niche axes suggests that a shift in mutualist specificity occurred along with climatic and edaphic specialization during the speciation process. Furthermore, shifts in rhizobial specificity across *Acmispon* spp. and retention of *Acmispon* species' ability to associate with ancestral rhizobial partner taxa suggest that mutualism specialization in this clade of native legumes can be explained by pulses of niche expansion and contraction as predicted by the oscillation theory. Our findings highlight the importance of assessing the evolutionary processes driving specialization in species interactions across both micro- and macroevolutionary scales, while accounting for multiple dimensions of the host species' ecological niche.

AUTHOR CONTRIBUTIONS

LTM, SP, and JLS conceived and designed the experiment. LTM, GB, JR, ML, WF, and TL implemented the experiment and collected the data. CW and JP produced the host plants genotyping data. LTM collected the rhizobia across *Acmispon* spp. AJW and JHC performed the sequencing of bacterial isolates from field collections and identification of the rhizobia genus. LTM, SP, and JLS analyzed the data. LTM and JLS wrote the first draft of the manuscript. All authors contributed to editing the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA ARCHIVING

All trait data and R codes are available in dryad (https://doi.org/10.6086/D1V68X). Host genotyping raw sequence data were archived at the Sequence Read Archive from the NCBI (BioProjectID: PRJNA663103). Gene sequence data of the rhizobial isolates were archived at NCBI (BankIt2430228: MW625102-MW625375).

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Host maternal lines field collection sites and geographic coordinates