ELECTRONIC SUPPLEMENTARY MATERIAL

Christie, K. and S.Y. Strauss. (2020). Frequency-dependent fitness and reproductive dynamics contribute to habitat segregation in sympatric Jewelflowers. *Proceedings of the Royal Society B: Biological Sciences.* DOI/10.1098/rspb.2020.0559.

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Experimental pollination

To assess the effects of heterospecific pollen transfer on seed viability we performed experimental crosses in the greenhouse using conspecific, heterospecific, and 50:50 pollen mixtures. We made crosses using individuals from six adjacent sites at McLaughlin, the same sites used in the field transplant experiment. We removed mature anthers from paternal plants with forceps, and manually applied pollen using anthers as paintbrushes, to the receptive stigmatic surfaces of bud-emasculated flowers of maternal plants. For the 50:50 pollen mixtures, we first completely saturated one half of the stigmatic surface with one species' pollen, and then completely covered the other half of the stigmatic surface with the other species' pollen in immediate succession. We alternated the order in which we applied pollen, and saturated stigmatic surfaces with pollen, far in excess of the number available ovules. In total, we made 291 crosses, including 97 intraspecific, 83 interspecific, 92 mixed-pollen, and 19 control crosses, using multiple maternal (n = 24) and paternal (n = 38) donors. Of these, 118 successfully set fruit; we used these crosses (n = 42 conspecific pollen, n = 42 mixed pollen, n = 34 heterospecific pollen) to determine if seed viability differed depending on pollen treatment.

We modeled seed viability (viable or inviable) using a binomial GLMM in R (g*lmer* function from the *lme4* package) with maternal species (*S. breweri, S. hesperidis*), pollen treatment (conspecific, 50:50 mixed, heterospecific), and a maternal species*pollen treatment interaction as fixed effects, with maternal and paternal individuals as random effects. We tested for differences among pollen treatments with Tukey's HSD tests using the *glht* function from the *multcomp* package in R.

Field soil moisture

To determine if soils at sites occupied by *S. breweri* and *S. hesperidis* differed in their water holding capacity, we measured gravimetric water content at six adjacent sites at three time points throughout the growing season (04/17/17, 05/14/17, 06/14/17). At each site, at each time point, we collected 10 soil cores from immediately beneath native plants at randomly selected microsites (n = 60 total samples per time point). Cores consisted of the top 15cm of the substrate, the predominant area of root growth for *S. breweri* and *S. hesperidis*. We passed soils through 4mm and then 2mm sieves, transferred 20mL aliquots of field-wet soil into aluminum boats, and dried the soils at 100 C° overnight. We used the weights of field-wet soils and dried soils to calculate gravimetric water content (Θ_d = [weight of wet soil - weight of dry soil] / weight of dry soil). We tested for differences in water-holding capacity between sites occupied by each species using t-tests (04/17/17 and 06/14/17 measurements) and Mann-Whitney tests (05/14/17 measurements) with Bonferroni correction.

Germination

Two hundred seeds, of a total of 1000 seeds planted in one S. *breweri* site and an adjacent *S. hesperidis* site, germinated in the field in 2015. In addition to assessing germination success in different habitats/soils in the field (see main text), we also conducted a follow-up experiment in the greenhouse, using seeds and soils collected from all six sites represented in the field transplant experiment. Here, we first collected field soil from immediately under native plants, then planted field-collected seeds (n = 240 total) into field soils in germination trays, and placed trays under a mist-bench in the greenhouse. The trays received automated mist for five minutes each hour, and ambient springtime light and temperature conditions in the UC Davis greenhouse. We assayed germination weekly for five weeks and counted the total number of germinants. We modeled germination success using a binomial GLM in R with species, soil type, and a species*soil type interaction as fixed effects.

Lathhouse soil transplant

We first collected 76 soil cores from six adjacent *S. breweri* and *S. hesperidis* sites at McLaughlin (n soil cores = 152 total). While keeping the soil structure as undisturbed as possible, we transferred soil cores into cylindrical greenhouse pots (D40 Deepots), and then transplanted greenhouse-grown germinants (n = 76 per species) into site-specific soils. We grew plants in a mesh-covered lathhouse at UC Davis, and used a conservative watering schedule, and ambient light and temperature conditions in the late spring to approximate field conditions. We did not hand pollinate plants, but allowed insect pollinators to freely visit the plants, entering and exiting the lathhouse through a wide mesh netting. We randomized the position of pots and racks every two weeks throughout the experiment. We scored survival and plant height at the end of the growing season as in our field experiment, and used flower number as a proxy for potential fruit production. We assessed patterns of local adaptation to specific soils using GLMs with species, soil type and a species*soil type interaction as fixed effects.

Additionally, to test the possibility that seed production and seed viability are associated with intrinsic properties of the soil (and unrelated to other ecological interactions in the field) we also scored seed production and seed viability of all survivors that produced fruit (n = 77), using a randomly-selected subset of five fruits per maternal individual. We modeled seed production (Gaussian) and seed viability (binomial) using GLMs in R with species, soil type, and a species*soil type interaction as fixed effects.

Supplementary Figures

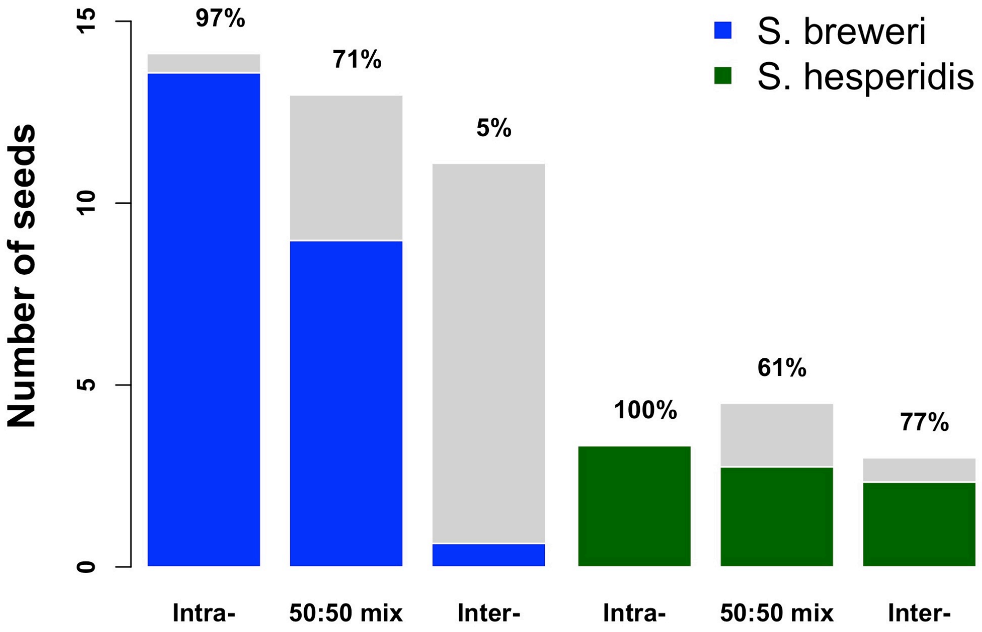


Figure S1: Seed viability in experimental crosses using conspecific, mixed (50:50), and heterospecific pollen. The total height of each bar represents the total number of seeds produced; the blue (*S. breweri*) and green (*S. hesperidis*) portions represents the average number of viable seeds, and the light gray portions represent the average number of inviable seeds. Text above each bar indicates median model predictions of seed viability from a GLMM.

Seed viability was reduced in both heterospecific ($p < 0.001$) and mixed pollen ($p < 0.001$) treatments compared to conspecific pollinations (Table S0; Fig. S1), and all cross types were significantly different from one another (Tukey's HSD, all p-values < 0.001). *S. breweri* produced 91-99% viable seeds in conspecific crosses (90% confidence intervals from model predictions), 47-87% viable seeds in mixed-pollen crosses, and 2-12% viable seeds in heterospecific crosses. *S. hesperidis* produced 100% viable seeds in conspecific crosses, 30-84% viable seeds in mixed-pollen crosses, and 43-100% viable seeds in heterospecific crosses, however the majority of *S. hesperidis* crosses (including intraspecific crosses) failed in this experiment, so these predictions are based on only 13 successful crosses. Potentially due to this small sample size, we did not observe a significant species*pollen source interaction (Table S0), however qualitatively, *S. hesperidis* suffered a less severe reduction in seed viability in heterospecific crosses compared to *S. breweri* (Fig. S1). This asymmetric reduction in seed viability following heterospecific pollen transfer is consistent with previous work showing less severe intrinsic postzygotic reproductive isolation at the seed production stage for *S. hesperidis*[57].

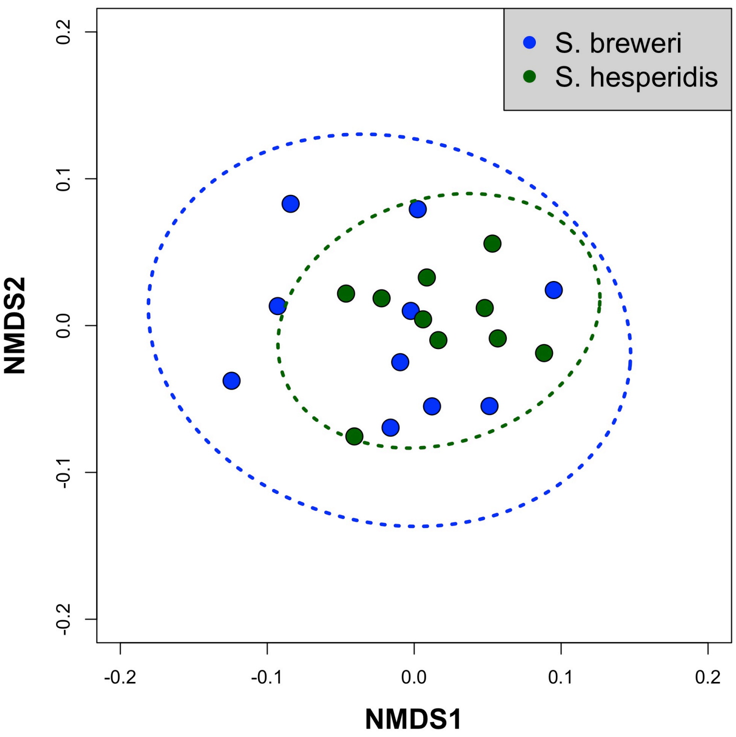


Figure S2: NMDS ordination of 33 physical site attributes, including soil texture and soil chemistry, for 20 *S. breweri* (n = 10) and *S. hesperidis* (n = 10) sites at McLaughlin. Points represent individual sites; ellipses represent 95% confidence intervals for abiotic niche breadth. We found no evidence for abiotic niche differences with respect to the combination of physical site attributes, and soil texture and soil chemistry variables (*adonis2* permutation test, p = 0.49).

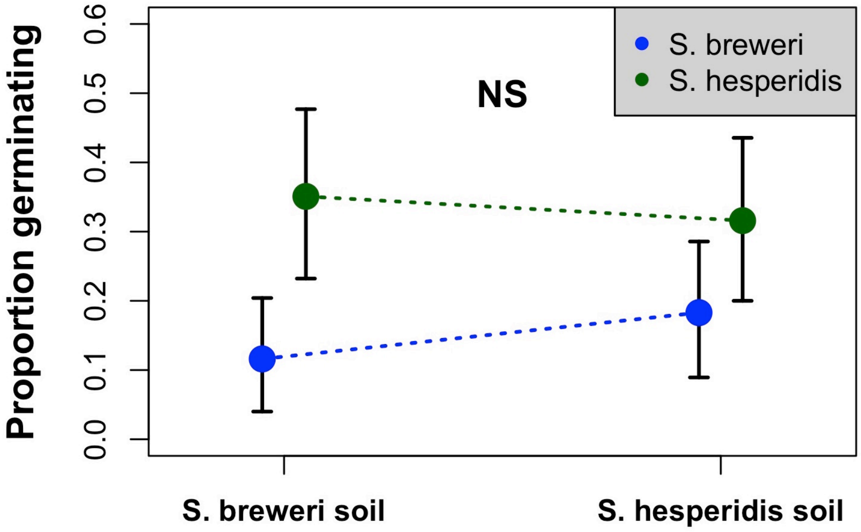


Figure S3: Germination success in site-specific soils in the greenhouse. Points indicate mean model predictions, and error bars show 95% confidence intervals. Similar to findings from the field (Fig. 2A), we found no evidence for a home-soil germination advantage when we planted seeds in site-specific field soils in the greenhouse (species*soil source interaction, p = 0.30). Seeds of both species germinated equally well in both soils.

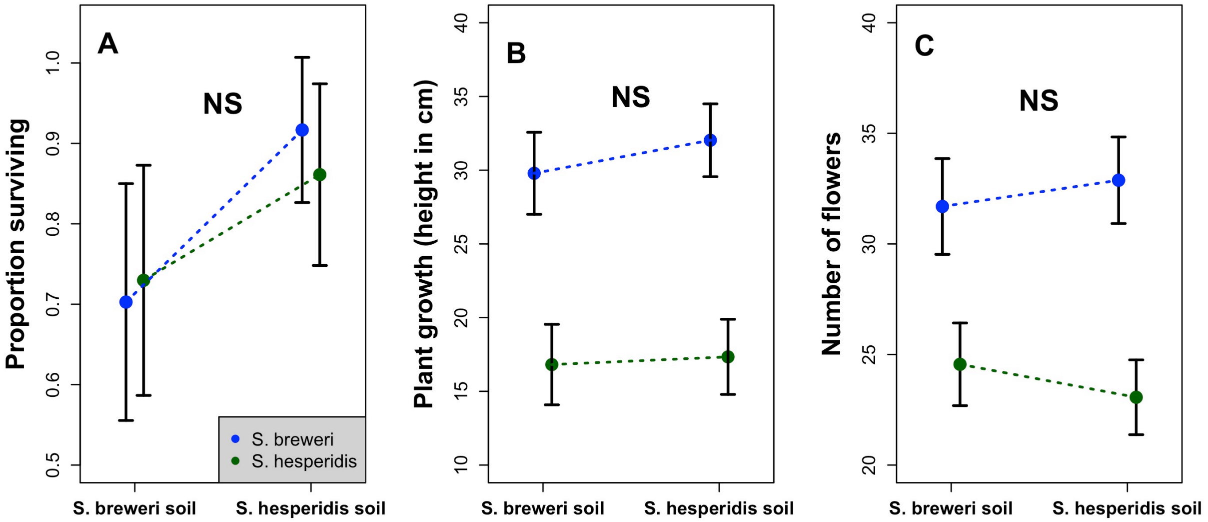


Figure S4: Average fitness associated with survival and growth in habitat-specific soils in the lathhouse. A.) Survival; B.) Plant height at the end of the growing season; C.) Flower production. Points represent mean model predictions; error bars show 95% confidence intervals; text depicts significance of species*soil type interactions.

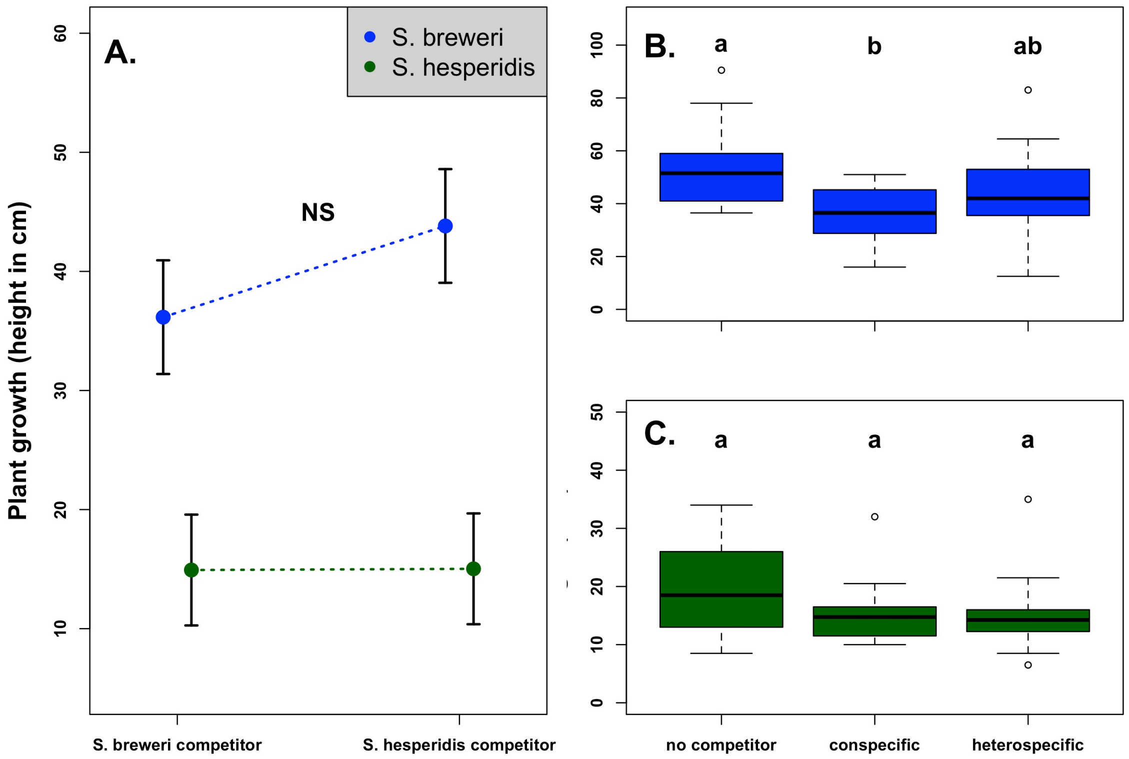


Figure S5: A. Plant growth when each species competed with conspecific and heterospecific competitors in single pots in the greenhouse. We found no differences in growth depending upon whether the competitor was a conspecific or a heterospecific (species*competitor interaction, p = 0.12). B. Plant growth for *S. breweri* (blue) when growing alone, with a conspecific competitor, and with a heterospecific competitor; treatments not sharing a letter are significantly different at the 95% confidence level using a Tukey's HSD test. C. The same data for *S. hesperidis* (green).

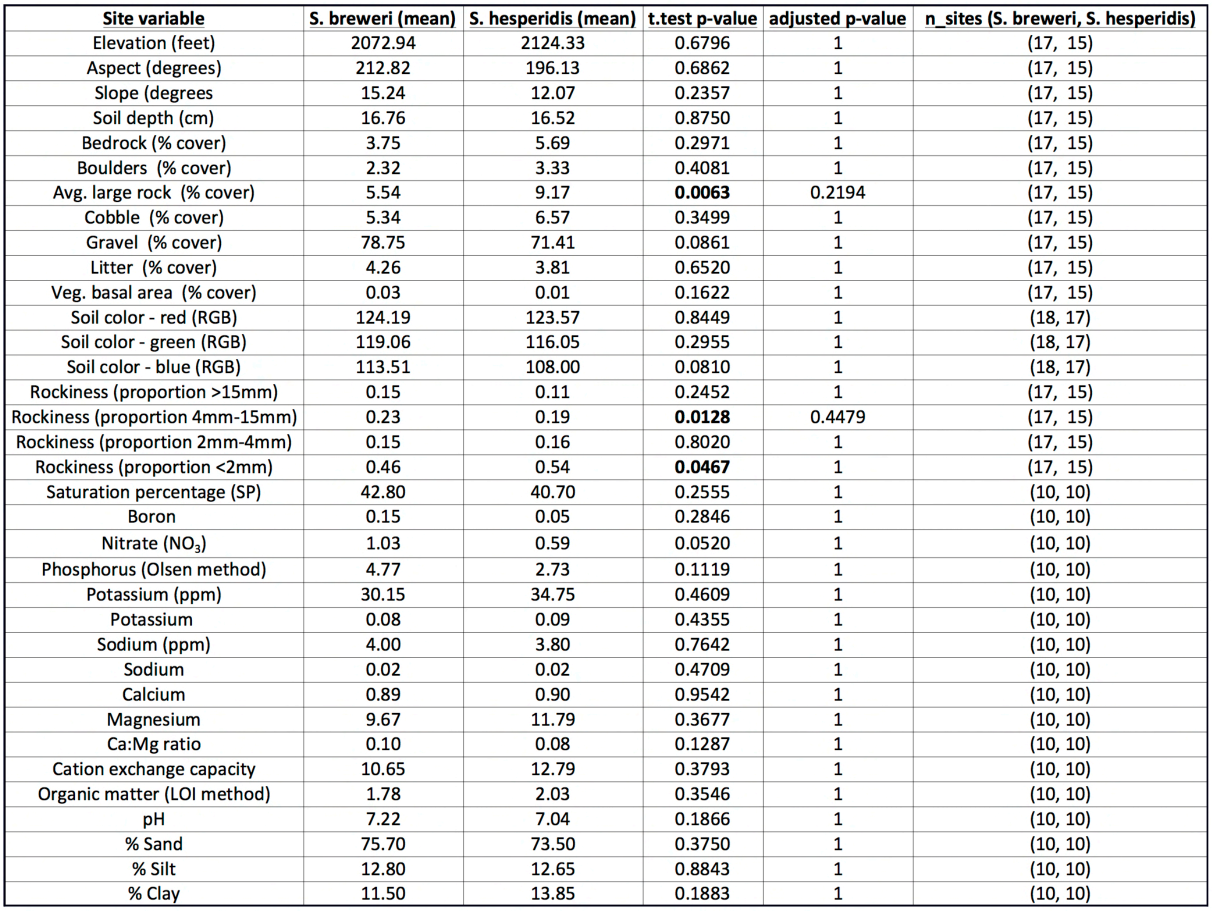
There were no differences in the relative effects of intra- compared to interspecific competition for either species (Fig. S5B; Fig S5C). Interestingly, the smaller *S. hesperidis* (often 50% smaller than *S. breweri*) showed no decrease in height when it competed with its larger congener, when it competed with a conspecific, and when it grew without a competitor (Fig. S5C).

Supplementary Tables

Table S0: Seed viability in experimental pollinations in the greenhouse
Model (binomial): seed viability ~ maternal species + pollen treatment + maternal species*pollen treatment + (1|maternal individual) + (1|paternal individual)

Factor	Estimate	SE	z value	p-value
maternal species (*S. hesperidis*)	15.7454	457.9476	0.034	0.973
treatment (mixed pollen)	-2.5196	0.2573	-9.792	**<2E-16**
treatment (heterospecific pollen)	-6.4463	0.3524	-18.294	**<2E-16**
maternal species (*S. hesperidis*) * treatment (mixed pollen)	-16.2169	457.9469	-0.035	0.972
maternal species (*S. hesperidis*) * treatment (heterospecific pollen)	-11.5811	457.9494	-0.025	0.98

Table S1: Summary of physical site attributes of *S. breweri* and *S. hesperidis* sites at McLaughlin.



Site variable	S. breweri (mean)	S. hesperidis (mean)	t.test p-value	adjusted p-value	n_sites (S. breweri, S. hesperidis)
Elevation (feet)	2072.94	2124.33	0.6796	1	(17, 15)
Aspect (degrees)	212.82	196.13	0.6862	1	(17, 15)
Slope (degrees	15.24	12.07	0.2357	1	(17, 15)
Soil depth (cm)	16.76	16.52	0.8750	1	(17, 15)
Bedrock (% cover)	3.75	5.69	0.2971	1	(17, 15)
Boulders (% cover)	2.32	3.33	0.4081	1	(17, 15)
Avg. large rock (% cover)	5.54	9.17	**0.0063**	0.2194	(17, 15)
Cobble (% cover)	5.34	6.57	0.3499	1	(17, 15)
Gravel (% cover)	78.75	71.41	0.0861	1	(17, 15)
Litter (% cover)	4.26	3.81	0.6520	1	(17, 15)
Veg. basal area (% cover)	0.03	0.01	0.1622	1	(17, 15)
Soil color - red (RGB)	124.19	123.57	0.8449	1	(18, 17)
Soil color - green (RGB)	119.06	116.05	0.2955	1	(18, 17)
Soil color - blue (RGB)	113.51	108.00	0.0810	1	(18, 17)
Rockiness (proportion >15mm)	0.15	0.11	0.2452	1	(17, 15)
Rockiness (proportion 4mm-15mm)	0.23	0.19	**0.0128**	0.4479	(17, 15)
Rockiness (proportion 2mm-4mm)	0.15	0.16	0.8020	1	(17, 15)
Rockiness (proportion <2mm)	0.46	0.54	**0.0467**	1	(17, 15)
Saturation percentage (SP)	42.80	40.70	0.2555	1	(10, 10)
Boron	0.15	0.05	0.2846	1	(10, 10)
Nitrate (NO_3)	1.03	0.59	0.0520	1	(10, 10)
Phosphorus (Olsen method)	4.77	2.73	0.1119	1	(10, 10)
Potassium (ppm)	30.15	34.75	0.4609	1	(10, 10)
Potassium	0.08	0.09	0.4355	1	(10, 10)
Sodium (ppm)	4.00	3.80	0.7642	1	(10, 10)
Sodium	0.02	0.02	0.4709	1	(10, 10)
Calcium	0.89	0.90	0.9542	1	(10, 10)
Magnesium	9.67	11.79	0.3677	1	(10, 10)
Ca:Mg ratio	0.10	0.08	0.1287	1	(10, 10)
Cation exchange capacity	10.65	12.79	0.3793	1	(10, 10)
Organic matter (LOI method)	1.78	2.03	0.3546	1	(10, 10)
pH	7.22	7.04	0.1866	1	(10, 10)
% Sand	75.70	73.50	0.3750	1	(10, 10)
% Silt	12.80	12.65	0.8843	1	(10, 10)
% Clay	11.50	13.85	0.1883	1	(10, 10)

Table S2.1: Germination success at two field sites in 2015

Model (binomial): germination ~ species + habitat + species*habitat + (1|experimental block)

Factor	Estimate	SE	z value	p-value
species (*S. hesperidis*)	1.165	0.2509	4.643	**3.43E-06**
habitat (*S. hesperidis*)	0.6917	0.387	1.787	0.0739
species (*S. hesperidis*) * habitat (*S. hesperidis*)	-1.035	0.3382	-3.06	**0.0022**

Table S2.2: Germination success in the greenhouse in field soils from six sites

Model (binomial): germination ~ species + soil type + species*soil type

Factor	Estimate	SE	z value	p-value
species (*S. hesperidis*)	1.4053	0.4847	2.8990	**0.0037**
soil type (*S. hesperidis*)	0.5305	0.5225	1.0150	0.3100
species (*S. hesperidis*) * soil type (*S. hesperidis*)	-0.6806	0.6506	-1.0460	0.2956

Table S2.3: Survival in a field transplant experiment

Model (binomial): survival ~ species + habitat + site + species*habitat + (1|experimental block)

Factor	Estimate	SE	z value	p-value
species (*S. hesperidis*)	-1.6101	0.5337	-3.0170	**0.0026**
habitat (*S. hesperidis*)	-0.0643	0.7249	-0.0890	0.9293
site (“Napa Junction”)	0.7055	0.6236	1.1310	0.2579
site (“Quarry View”)	-0.6572	0.5482	-1.1990	0.2306
species (*S. hesperidis*) * habitat (*S. hesperidis*)	3.0797	1.2550	2.4540	**0.0141**

Table S2.4: Plant growth in a field transplant experiment

Model (Gaussian): height (cm) ~ species + habitat + site + species*habitat + (1|experimental block)

Factor	Estimate	SE	t value	p-value
species (*S. hesperidis*)	-7.2230	0.9736	-7.4190	**1.5E-12**
habitat (*S. hesperidis*)	0.1420	1.6426	0.0860	0.9314
site (“Napa Junction”)	-2.9276	1.8512	-1.5810	0.1229
site (“Quarry View”)	-3.3036	1.8489	-1.7870	0.0826
species (*S. hesperidis*) * habitat (*S. hesperidis*)	-0.4161	1.5314	-0.2720	0.7860

Table S2.5: Fruit production in a field transplant experiment

Model (negative binomial): fruit number ~ species + habitat + site + species*habitat + (1|experimental block)

Factor	Estimate	SE	z value	p-value
species (*S. hesperidis*)	0.1467	0.1455	1.0080	0.3134
habitat (*S. hesperidis*)	-0.1591	0.3162	-0.5030	0.6149
site (“Napa Junction”)	-0.4074	0.3718	-1.0960	0.2731
site (“Quarry View”)	-0.8761	0.3738	**-2.3440**	**0.0191**
species (*S. hesperidis*) * habitat (*S. hesperidis*)	0.3254	0.2276	1.4290	0.1529

Table S2.6: Survival in a lathhouse soil transplant experiment

Model (binomial): survival ~ species + soil type+ species*soil type

Factor	Estimate	SE	z value	p-value
species (*S. hesperidis*)	0.1331	0.5161	0.2580	0.7966
soil type (*S. hesperidis*)	1.5377	0.7021	2.1900	**0.0285**
species (*S. hesperidis*) * soil type (*S. hesperidis*)	-0.7064	0.9286	-0.7610	0.4468

Table S2.7: Plant growth (height) in a lathhouse soil transplant experiment

Model (Gaussian): height in mm ~ species + soil type + species*soil type

Factor	Estimate	SE	t value	p-value
species (*S. hesperidis*)	-129.7400	19.8900	-6.5220	**2.02E-09**
soil type (*S. hesperidis*)	22.4200	18.9800	1.1810	0.2400
species (*S. hesperidis*) * soil type (*S. hesperidis*)	-17.1800	26.9000	-0.6390	0.5240

Table S2.8: Flower production in a lathhouse soil transplant experiment

Model (Poisson): flower number ~ species + soil type + species*soil type

Factor	Estimate	SE	z value	p-value
species (*S. hesperidis*)	-0.2551	0.0522	-4.8900	**1.01E-06**
soil type (*S. hesperidis*)	0.0368	0.0462	0.7950	0.4260
species (*S. hesperidis*) * soil type (*S. hesperidis*)	-0.0994	0.0710	-1.4000	0.1620

Table S3.1: Seed production in a lathhouse soil transplant experiment

Model (Gaussian): total seeds per fruit ~ species + soil type + site + species*soil type

Factor	Estimate	SE	t value	p-value
species (*S. hesperidis*)	-6.4900	1.6480	-3.9370	**0.0002**
soil type (*S. hesperidis*)	-2.8720	1.4190	-2.0240	**0.0467**
site ("Napa Junction")	3.9080	2.6510	1.4740	0.1449
site ("Quarry View")	-1.0110	2.8110	-0.3600	0.7202
species (*S. hesperidis*) * soil type (*S. hesperidis*)	4.4190	2.4590	1.7970	0.0766

Table S3.2: Seed viability in a lathhouse soil transplant experiment

Model (binomial): seed viability ~ species + soil type + site + species*soil type

Factor	Estimate	SE	z value	p-value
species (*S. hesperidis*)	-0.0600	0.1256	-0.4770	0.6330
soil type (*S. hesperidis*)	-0.0743	0.0891	-0.8340	0.4044
site ("Napa Junction")	0.7440	0.1893	3.9310	**0.0001**
site ("Quarry View")	-0.1077	0.2069	-0.5210	0.6027
species (*S. hesperidis*) * soil type (*S. hesperidis*)	-0.6408	0.1939	-3.3050	**0.0009**

Table S3.3: Seed production in a field transplant experiment

Model (Gaussian): total seeds per fruit ~ species + habitat + site + species*habitat + (1|experimental block)

Factor	Estimate	SE	z value	p-value
species (*S. hesperidis*)	-21.3540	3.5200	-6.0670	**8.48E-08**
habitat (*S. hesperidis*)	-19.3340	3.6650	-5.2760	**1.70E-06**
site ("Napa Junction")	5.2350	3.4150	1.5330	0.1300
site ("Quarry View")	1.6090	3.4140	0.4710	0.6390
species (*S. hesperidis*) * habitat (*S. hesperidis*)	36.5150	5.4050	6.7550	**5.07E-09**

Table S3.4: Seed viability in a field transplant experiment

Model (binomial): seed viability ~ species + habitat + site + species*habitat + (1|experimental block)

Factor	Estimate	SE	z value	p-value
species (*S. hesperidis*)	-2.9991	0.4363	-6.8740	**6.25E-12**
habitat (*S. hesperidis*)	-2.6314	0.4518	-5.8250	**5.72E-09**
site ("Napa Junction")	0.4748	0.4181	1.1360	0.2560
site ("Quarry View")	-0.0541	0.4151	-0.1300	0.8960
species (*S. hesperidis*) * habitat (*S. hesperidis*)	5.0882	0.6634	7.6700	**1.72E-14**

Table S4: Model selection table showing best-fitting models (delta AICc ≤2) explaining seed viability in experimental migrants.

n conspecifics	n fruits	n fruits per resident	site	species	n conspecifics *species	df	logLik	AICc	delta AICc	AICc weight
X			X			5	-939.934	1890.4	0	0.126
X						3	-942.629	1891.5	1.07	0.074
			X			4	-941.71	1891.8	1.37	0.064
X			X	X		6	-939.546	1891.8	1.44	0.062
						2	-943.965	1892	1.63	0.056
X				X	X	5	-940.844	1892.2	1.82	0.051
X	X			X		6	-939.836	1892.4	2.02	0.046

Table S5: Seed viability of experimental migrants in a field transplant experiment

Model (binomial): seed viability ~ number of conspecifics per block + site + (1|block)

Factor	Estimate	SE	z value	p-value
number of conspecifics	0.1903	0.1004	1.895	***0.0581***
site ("Napa Junction")	0.3706	0.4564	0.812	0.4168
site ("Quarry View")	-0.6467	0.4759	-1.359	0.1741