

***New Phytologist* Supporting Information**

Article title: Host investment into symbiosis varies among genotypes of the legume *Acmispon strigosus*, but host sanctions are uniform

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The following Supporting Information is available for this article:

Fig. S1 Host symbiotic efficiency of *A. strigosus* in each treatment

Fig. S2 Regression of rhizobial cells per nodule against nodule area

Fig. S3 Total nodule dry mass and total nodule number of *A. strigosus* in each treatment

Table S1 Collection information for *A. strigosus* host lines

Table S2 Genetic distance matrix of *A. strigosus* host lines at *nrITS* and *CNGC5* loci

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Table S4 Leaf %N and ¹⁵N content of *A. strigosus* during single-inoculations

Table S5 Total plant dry mass of *A. strigosus* in each treatment

Fig. S1 Test of the partner mismatch hypothesis based on host symbiotic efficiency of *A. strigosus* from different populations in (a) unfertilized and (b) fertilized conditions. Symbiotic efficiency was calculated as mg total plant dry mass (roots + shoots) gained from symbiosis per mg total nodule dry mass. Statistics were performed separately for singly-inoculated and co-inoculated plants. Only main effects were significant, and different letters indicate the significant main effects of strain, fertilization, and host population (Table 1). Different letters above strain treatments indicate significant differences among strains; different letters above fertilization treatments indicate significant differences between fertilization treatments. Different letters above populations indicate significant differences among populations; populations without letters did not differ from either extreme (i.e., ‘ab’). B = BMR (Bodega Marine Reserve), U = UCR (University of California, Riverside), C = Cla (Bernard Field Station of the Claremont Colleges), A = Anz (Anza-Borrego Desert State Park), G = Gri (Griffith Park), Y = Yuc (Burns-Pinyon Ridge Reserve near Yucca Valley). Bars represent \pm 1 SE.

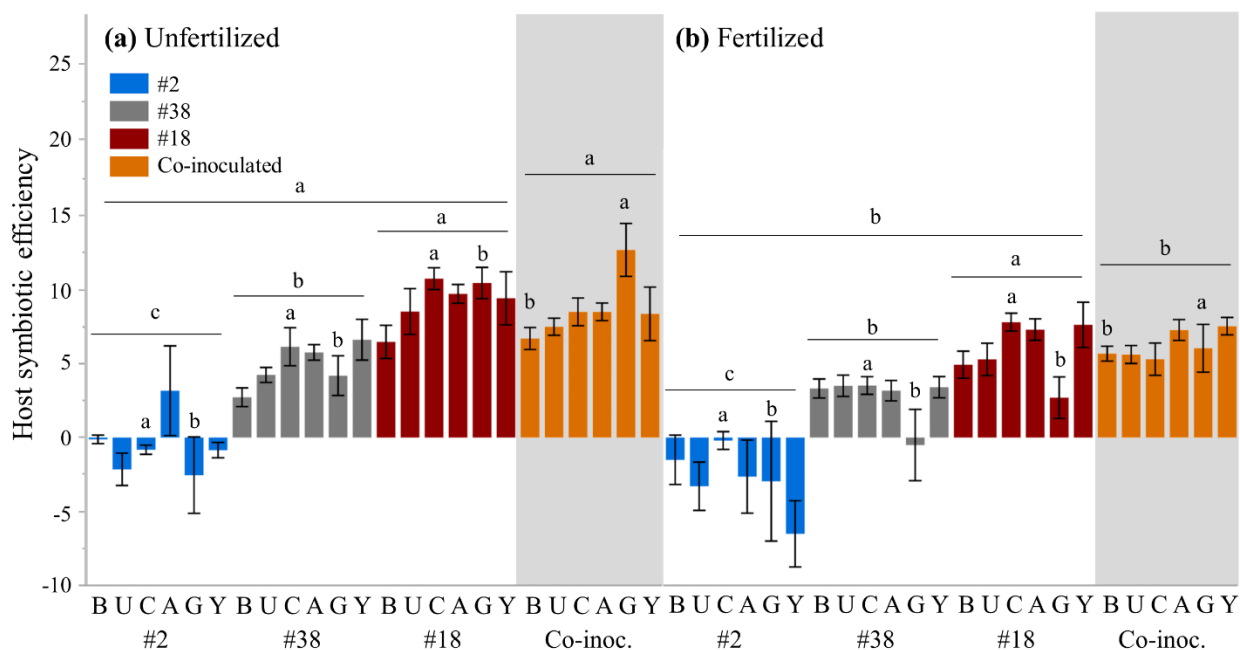


Fig. S2 Regression of rhizobia per nodule against nodule area for individual nodules cultured from *A. strigosus* singly-inoculated with each *Bradyrhizobium* strain, with data pooled among fertilization treatments and host lines. Strains #2, #38, and #18 are denoted by open gray circles, plus signs, and filled black circles, respectively. Regressions are significant for strain #2 and strain #18 but not for strain #38.

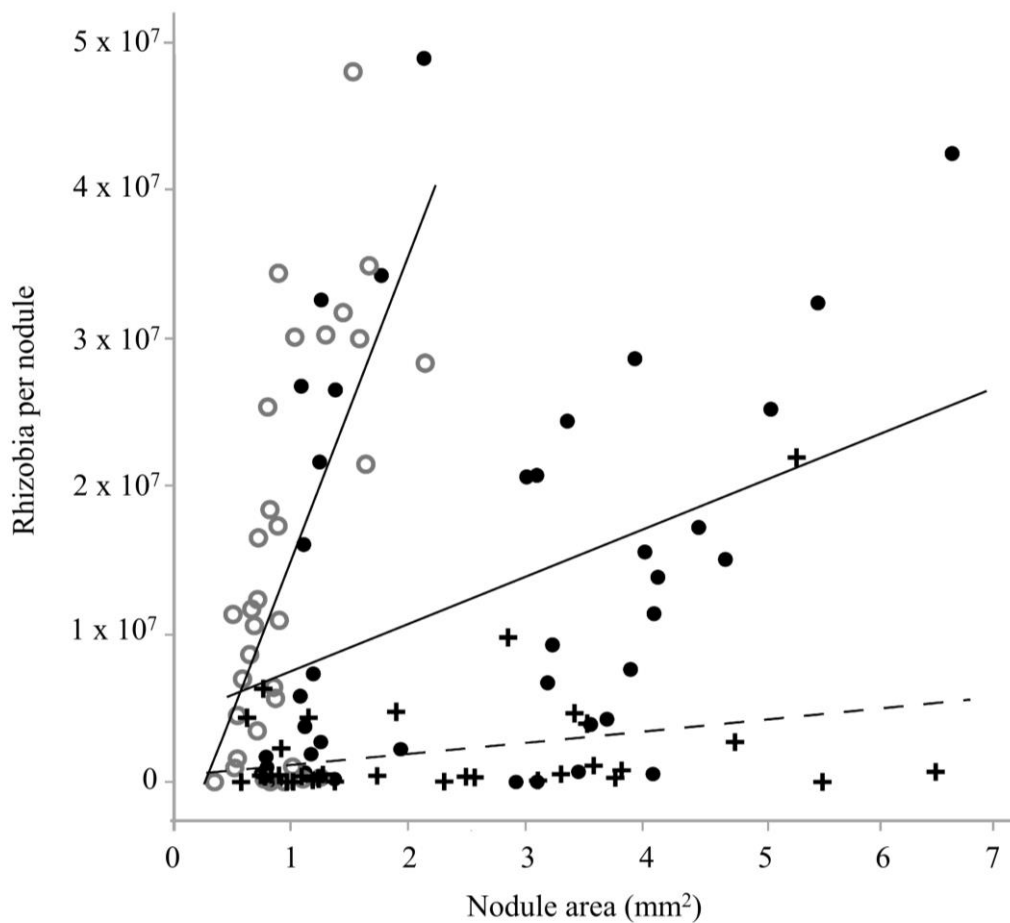


Fig. S3 Test of the resource satiation and host variation hypotheses using (a) total nodule dry mass and (b) total nodule number of *A. strigosus* lines from different populations in unfertilized and fertilized conditions. Statistics were performed separately for singly- and co-inoculated plants. (a) For total nodule dry mass of singly-inoculated plants, different letters above strain treatments indicate significant differences among strain and fertilization treatments (strain x fertilization effect; Table 2; note that the population x strain and the population x fertilization interactions were also significant). (b) For total nodule number of singly-inoculated plants, host populations denoted with daggers formed significantly fewer nodules with strain #2 than strains #38 and #18 (which did not differ) in the indicated fertilization treatment, whereas host populations without daggers did not differ in the number of nodules formed with the three strains in the indicated fertilization treatment (population x strain x fertilization effect; Table 2). (a,b) For co-inoculated plants, different letters indicate the significant main effects of host population and fertilization. Different letters above host populations indicate significant differences among populations; different letters above fertilization treatments indicate significant differences among fertilization treatments. B = BMR (Bodega Marine Reserve), U = UCR (University of California, Riverside), C = Cla (Bernard Field Station of the Claremont Colleges), A = Anz (Anza-Borrego Desert State Park), G = Gri (Griffith Park), Y = Yuc (Burns-Pinyon Ridge Reserve near Yucca Valley). Bars represent +/- 1 SE.

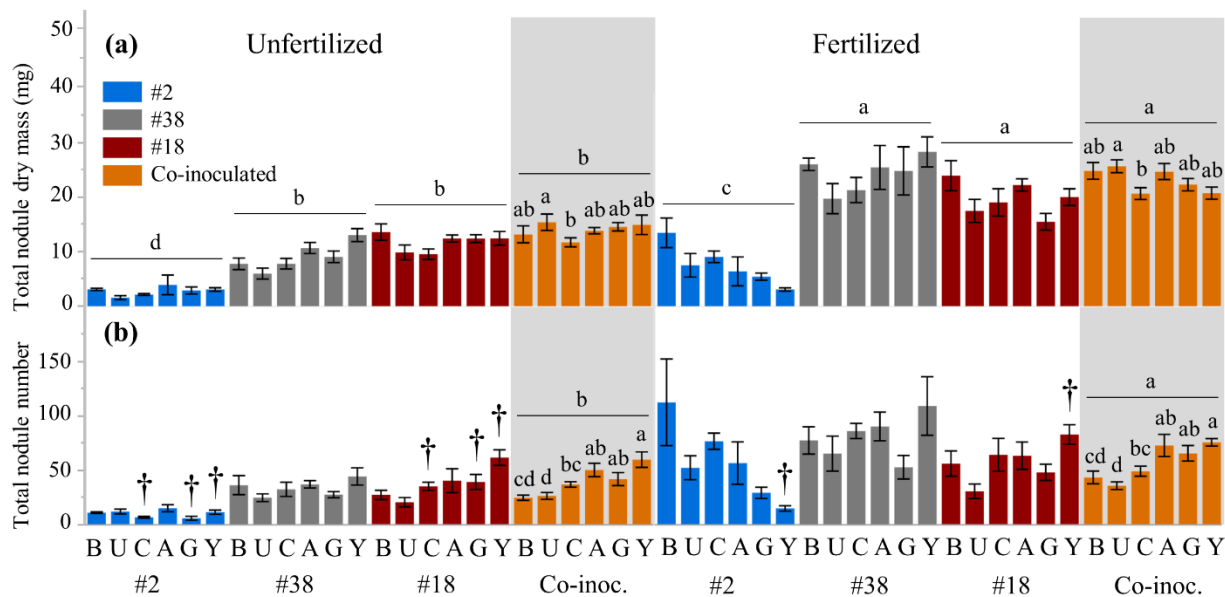


Table S1 Collection information for *A. strigosus* host lines.

<i>A. strigosus</i> host line	<i>A. strigosus</i> formal name	Collection year for wild seeds	Seed collection site (dec. deg.)	
			Lat.	Long.
Anz03	AcS046.Anz.m01.g1.r03	2011	33.2713	-116.4194
Anz11	AcS040.Anz.m01.g1.r06	2011	33.27068333	-116.4189333
BMR04	AcS074.BMR.u01.g1.r04	2011	38.31903333	-123.0642667
BMR07	AcS004.BMR.u01.g1.r01	2005	38.3193	-123.06365
Cla06	AcS047.Cla.m01.g1.r03	2011	34.11051667	-117.70845
Cla10	AcS047.Cla.m01.g1.r07	2011	34.11051667	-117.70845
Gri01	AcS075.Gri.u01.gwild	2012	34.12244444	-118.2930556
UCR03	AcS027.UCR.u01.g1.r01	2009	33.9659	-117.3227167
UCR10	AcS027.UCR.u01.g1.r03	2009	33.9659	-117.3227167
Yuc02	AcS052.Yuc.m01.g1.r01	2011	34.15315	-116.4751167
Yuc03	AcS052.Yuc.m01.g1.r02	2011	34.15315	-116.4751167

Table S2 Estimates of evolutionary divergence between *A. strigosus* lines for *nrITS* (466 nt, top) and *CNGC5* (441 nt, bottom). Positions containing gaps or missing data were deleted. Cell color intensity scales with the number of base substitutions per site between each pair of host lines. We genotyped 2-17 inbred progeny of the wild seed ancestor that defined each plant line, except for Gri01 (wild seed set), for which we genotyped 21 (*nrITS*) or 8 (*CNGC5*) plants grown from wild seeds.

<i>nrITS</i>	Anz03	Anz11	BMR04	BMR07	Cla06	Cla10	Gri01	UCR03	UCR10	Yuc02	Yuc03
Anz03											
Anz11	0.0021										
BMR04	0.0043	0.0065									
BMR07	0.0043	0.0065	0								
Cla06	0.0065	0.0086	0.0022	0.0022							
Cla10	0.0065	0.0086	0.0022	0.0022	0						
Gri01	0.0021	0.0043	0.0022	0.0022	0.0043	0.0043					
UCR03	0.0043	0.0065	0	0	0.0022	0.0022	0.0022				
UCR10	0.0043	0.0065	0	0	0.0022	0.0022	0.0022	0			
Yuc02	0	0.0021	0.0043	0.0043	0.0065	0.0065	0.0021	0.0043	0.0043		
Yuc03	0	0.0021	0.0043	0.0043	0.0065	0.0065	0.0021	0.0043	0.0043	0	

<i>CNGC5</i>	Anz03	Anz11	BMR04	BMR07	Cla06	Cla10	Gri01	UCR03	UCR10	Yuc02	Yuc03
Anz03											
Anz11	0.0137										
BMR04	0.0373	0.0325									
BMR07	0.0373	0.0325	0								
Cla06	0.0325	0.0277	0.0091	0.0091							
Cla10	0.0325	0.0277	0.0091	0.0091	0						
Gri01	0.0349	0.0301	0.0301	0.0301	0.0254	0.0254					
UCR03	0.0373	0.0325	0	0	0.0091	0.0091	0.0301				
UCR10	0.0373	0.0325	0	0	0.0091	0.0091	0.0301	0			
Yuc02	0.0137	0	0.0325	0.0325	0.0277	0.0277	0.0301	0.0325	0.0325		
Yuc03	0.0137	0	0.0325	0.0325	0.0277	0.0277	0.0301	0.0325	0.0325	0	

Table S3 Statistical model selection based on AICc (corrected Akaike's Information Criterion) of candidate models with different interaction terms. For each model, we tested all possible interactions among host population (P), strain (S), and fertilization (F) and then incrementally removed nonsignificant (non-bold) interactions. The AICc value of each chosen model (*) presented in Tables 1 and 2 was at least 1.96 units lower than other candidate models. We performed a retrospective power analysis for the highest-order nonsignificant interaction term tested for each response variable (generally the PSF interaction). Retrospective power analysis calculates ‘observed power’ (OP), which is the chance of detecting a significant effect using the given sample size to test a population with parameters estimated from the sample (i.e., with a true effect size equal to the sample effect size, and residual error variance equal to the model RMSE). We excluded the random effect of block from each model to facilitate the power analysis. A dagger indicates the highest-order nonsignificant interaction effect included in the model, for which OP was calculated.

Model	Interactions included	AICc	†OP
Single-inoculation			
Log ₁₀ (Plant relative growth)			0.7998
	PS, PF, SF, PSF†	119.255	
	PS, PF, SF	58.9152	
	* PS, SF	22.8121	
Symbiotic efficiency			0.6059
	PS, PF, SF, PSF†	1136.991	
	PS, PF, SF	1129.95	
	PS, PF	1124.962	
	PS, SF	1126.007	
	PF, SF	1126.236	
	PS	1121.395	
	PF	1121.869	
	SF	1123.463	
	* none	1119.428	
Log ₁₀ (Delta ¹⁵ N + 3)			0.2285
	PS†	171.1534	
	* none	24.9019	
Percent Ndfa			0.3315
	PS†	251.5754	
	* none	231.189	
Log ₁₀ (Mean nodule size)			0.5362
	PS, PF, SF, PSF†	164.1453	
	PS, PF, SF	99.0999	
	* PS, SF	65.87722	
Log ₁₀ (Total nodule dry mass)			0.6796
	PS, PF, SF, PSF†	157.8902	
	* PS, PF, SF	95.33293	
Log ₁₀ (Total nodule number)			
	* PS, PF, SF, PSF	221.708	
Co-inoculation			
Log ₁₀ (Plant relative growth)			
	* PF	62.68259	
Symbiotic efficiency			0.6853
	* PF†	635.1891	
	none	637.8435	
Log ₁₀ (Mean nodule size)			0.2341
	PF†	-26.9304	
	* none	-62.4644	
Total nodule dry mass			0.5034
	* PF†	680.2623	
	none	681.5416	
Log ₁₀ (Total nodule number)			0.1531
	PF†	-12.4138	
	* none	-48.6068	

Table S4 Nitrogen content of *A. strigosus* during single-inoculations with *Bradyrhizobium*. BMR = Bodega Marine Reserve, UCR = University of California, Riverside, Cla = Bernard Field Station of the Claremont Colleges, Anz = Anza-Borrego Desert State Park, Gri = Griffith Park, Yuc = Burns-Pinyon Ridge Reserve near Yucca Valley.

Fertilization, Host	Tissue %N by weight (SE)				%N derived from fixation (SE)		
	Uninoculated	Strain #2	Strain #38	Strain #18	Strain #2	Strain #38	Strain #18
Unfertilized							
Anz	0.65 (0.05)	0.60 (.)	3.95 (0.25)	5.25 (0.15)	.	.	.
BMR	0.55 (0.05)	0.50 (0.0)	3.6 (0.20)	4.7 (1.0)	.	.	.
Cla	0.60 (0.0)	0.70 (.)	3.65 (1.05)	4.75 (0.15)	.	.	.
Gri	0.55 (0.05)	0.65 (0.15)	2.95 (0.25)	3.85 (0.15)	.	.	.
UCR	0.55 (0.05)	0.60 (0.0)	2.9 (.)	5.4 (0.3)	.	.	.
Yuc	0.60 (0.0)	0.65 (0.05)	4.3 (0.10)	4.35 (0.45)	.	.	.
Fertilized							
Anz	0.85 (0.05)	1.1 (.)	3.0 (0.5)	3.9 (1.0)	-0.43 (1.01)	54.80 (14.77)	83.73 (6.41)
BMR	1.2 (0.2)	1.45 (0.15)	3.55 (0.25)	3.25 (0.05)	-3.67 (2.26)	75.58 (9.89)	81.55 (1.54)
Cla	1.15 (0.15)	1.25 (0.15)	2.8 (0.5)	4.6 (0.8)	0.76 (1.85)	73.56 (1.83)	88.20 (1.43)
Gri	0.9 (0.1)	0.75 (0.15)	1.65 (0.05)	3.15 (0.45)	5.19 (2.71)	71.14 (5.91)	76.79 (5.94)
UCR	1.7 (0.7)	1.7 (0.2)	3.75 (0.55)	4.05 (0.25)	2.01 (.)	62.81 (.)	83.00 (7.52)
Yuc	1.1 (0.0)	1.0 (0.2)	3.85 (0.15)	3.55 (0.35)	0.22 (1.35)	74.48 (0.56)	82.32 (0.98)

Table S5 Mean total plant (root + shoot) dry mass of *A. strigosus* plants from different populations within each fertilization and *Bradyrhizobium* strain treatment. BMR = Bodega Marine Reserve, UCR = University of California, Riverside, Cla = Bernard Field Station of the Claremont Colleges, Anz = Anza-Borrego Desert State Park, Gri = Griffith Park, Yuc = Burns-Pinyon Ridge Reserve near Yucca Valley.

Fertilization, Host	Single-Inoculation Plant Mass, mg (SE)				Co-Inoculation Plant Mass, mg (SE)	
	Uninoculated	Strain #2	Strain #38	Strain #18	Uninoculated	Co-Inoculated
Unfertilized						
Anz	10.4 (1.3)	11.8 (1.3)	71.3 (6.5)	130.8 (8.8)	15.4 (1.6)	133.2 (9.6)
BMR	6.3 (0.5)	6.0 (0.7)	29.5 (5.6)	86.7 (5.0)	7.6 (0.8)	102.7 (13.6)
Cla	5.4 (0.5)	3.9 (0.4)	50.3 (7.0)	108.7 (13.6)	7.7 (0.7)	107.3 (11.1)
Gri	13.2 (0.9)	11.5 (1.3)	57.6 (14.9)	145.2 (18.5)	19.8 (1.5)	190.8 (11.6)
UCR	7.2 (1.0)	5.0 (0.6)	33.8 (7.0)	83.4 (5.5)	7.5 (0.7)	118.9 (9.7)
Yuc	18.5 (2.7)	15.5 (2.4)	99.1 (9.9)	128.1 (16.4)	40.7 (12.8)	170.7 (21.9)
Fertilized						
Anz	119.0 (10.3)	111.7 (9.7)	208.6 (26.1)	275.1 (13.4)	124.3 (10.2)	301.2 (15.6)
BMR	92.8 (10.7)	82.0 (15.4)	180.6 (17.4)	212.1 (32.0)	105.0 (9.2)	245.4 (17.8)
Cla	91.6 (7.5)	90.6 (7.0)	171.3 (19.8)	240.9 (14.4)	130.7 (12.5)	238.7 (16.2)
Gri	172.7 (13.6)	161.6 (12.4)	207.2 (25.7)	208.8 (16.1)	196.8 (12.7)	341.3 (34.7)
UCR	83.2 (17.5)	61.9 (13.9)	151.3 (17.8)	176.4 (25.8)	106.2 (10.8)	249.8 (13.4)
Yuc	164.6 (10.3)	146.4 (8.9)	262.9 (20.5)	316.3 (23.3)	177.8 (6.4)	336.2 (14.2)