FIG. S1. *Ipomoea* Carolina morphotype and *I. grandifolia* interspecific crosses generally have higher fruiting success than many of the interspecific crosses made with either *I.* Carolina morphotype or *I. grandifolia* as one of the parents and the indicated species as the other parent. Bar plots have the percentage of fruit success indicated in white. Crosses between *Ipomoea* Carolina morphotype and *I. grandifolia* are in dark gray (on the left). Interspecific crosses including *I.* Carolina morphotype are in light gray. Interspecific crosses including *I. grandifolia* are in black. M = *I.* Carolina morphotype; G = *I. grandifolia*; C = *I. cordatotriloba*; La = *I. lacunosa*; Le = *I. leucantha*. Statistics found in Table 3. The same numbers are found in Table S4.

FIG. S2. Matrix of the number of seeds per fruit produced from each type of cross. Dashed lines indicate that there are no crosses that yielded that particular number of seeds. A within-individual cross (LaxS) resulted in 5 seeds per fruit, which is rarely seen in these *Ipomoea* species, which generally produce a maximum of 4 seeds per fruit. M = *I.* Carolina morphotype; C = *I. cordatotriloba*; G = *I. grandifolia*; La = *I. lacunosa*; Le = *I. leucantha*; S = within individual hand cross with anther removal the day before flower opening; X = selfed with no anther removal the day before flower opening.
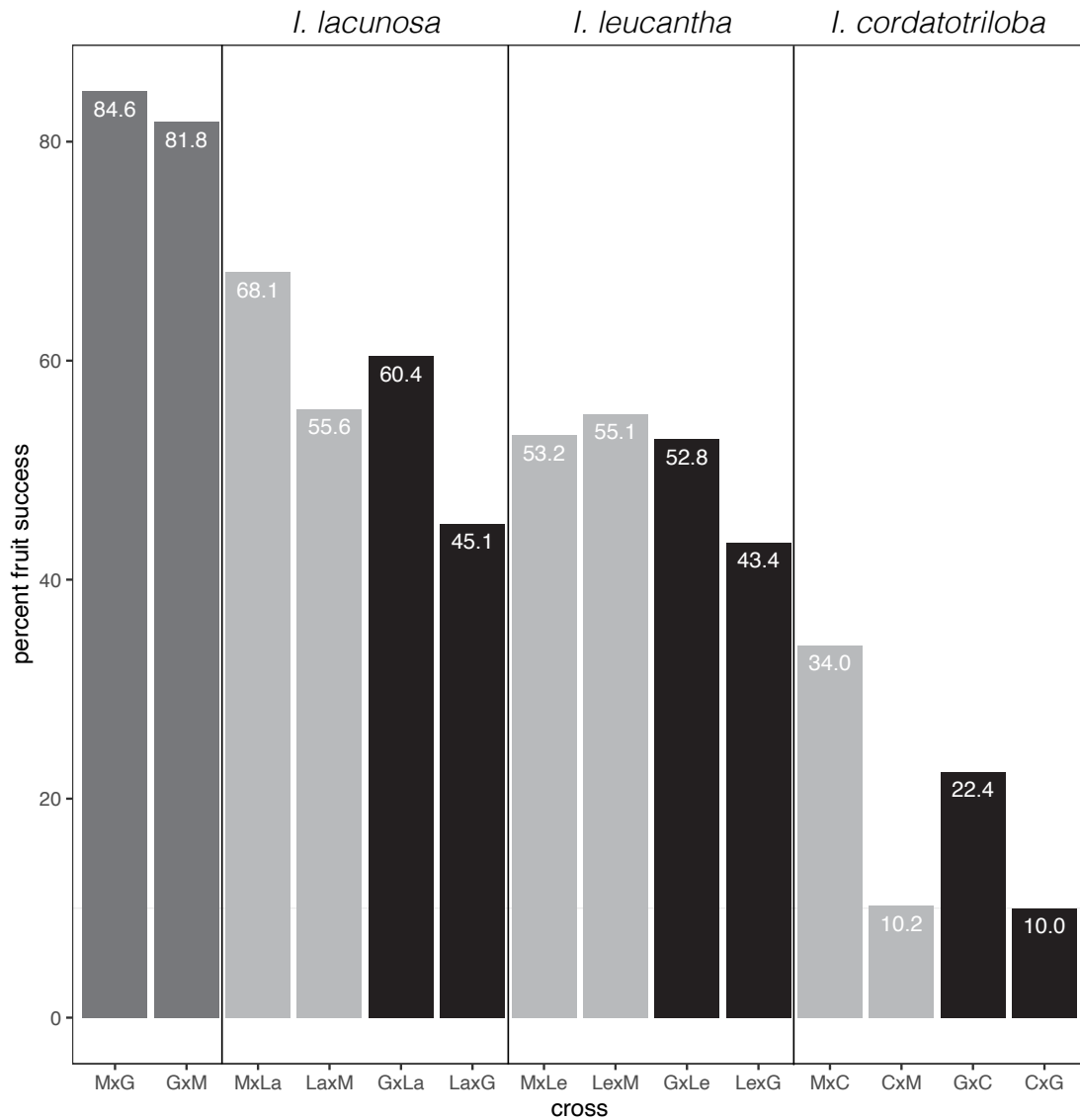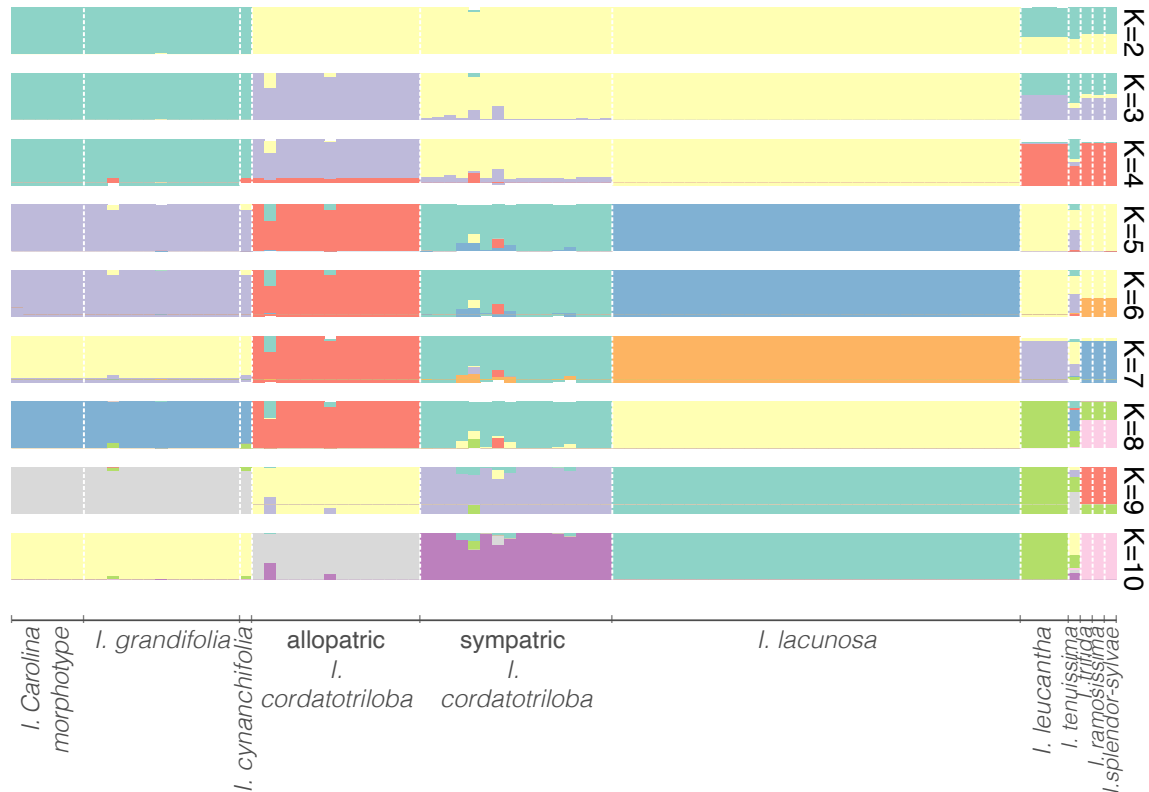
FIG. S3. fastSTRUCTURE and ADMIXTURE outputs for K2-10. Ten separate runs were done for each K with both fastSTRUCTURE and ADMIXTURE, and results from the runs were merged by K before plotting. *Ipomoea* Carolina morphotype and *I. grandifolia* are found on the left. The outgroup species, *I. ramosissima*, *I. trifida*, and *I. splendor-sylvae*, are found on the right side. A. fastSTRUCTURE results with K=6 and K=7 as the optimal numbers of groups (both 4/10 runs). B. ADMIXTURE results with K=5 as the optimal number of groups (7/10 runs); K=6 is the next optimal number (2/10 runs).
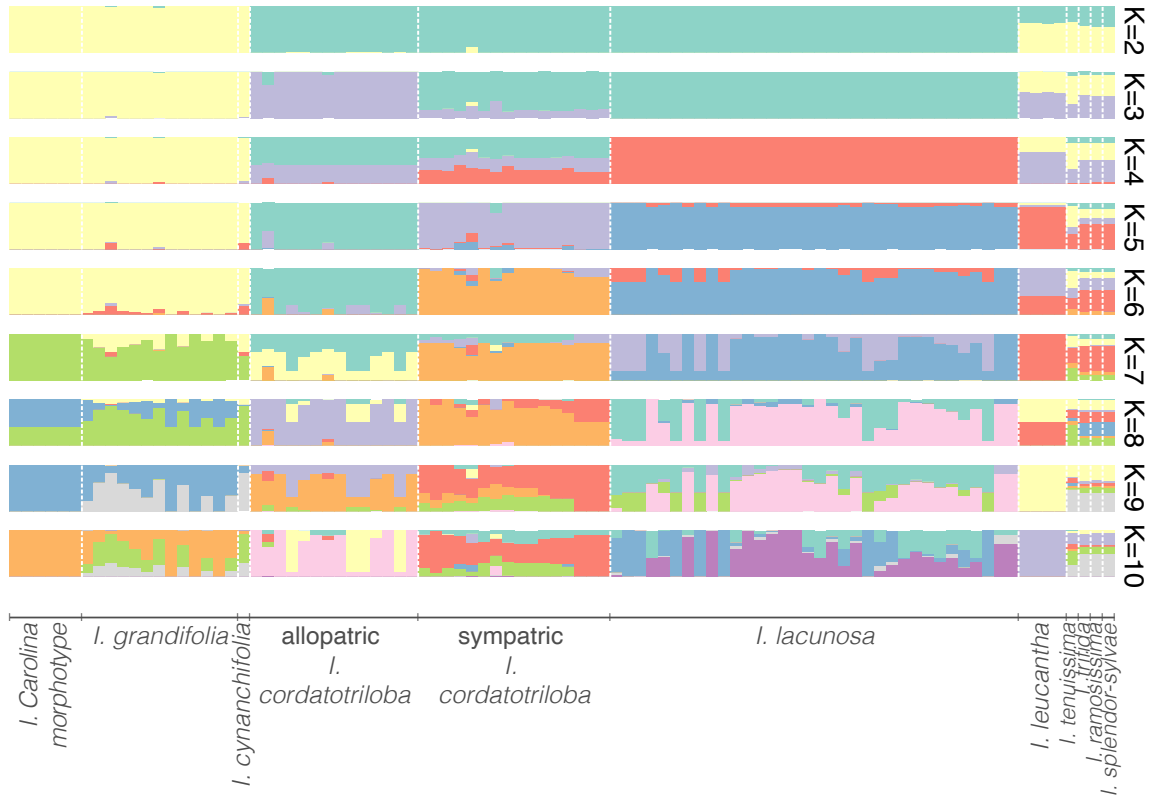
A. fastSTRUCTURE

B.   ADMIXTURE

FIG. S4. Pairwise PCA plots for the first 4 eigenvalues of the genetic variation among *Ipomoea* species. The clustering between *I.* Carolina morphotype (black dots, center) and *I. grandifolia* (dark blue dots, center) do not change even when examining the third and fourth eigenvalues.
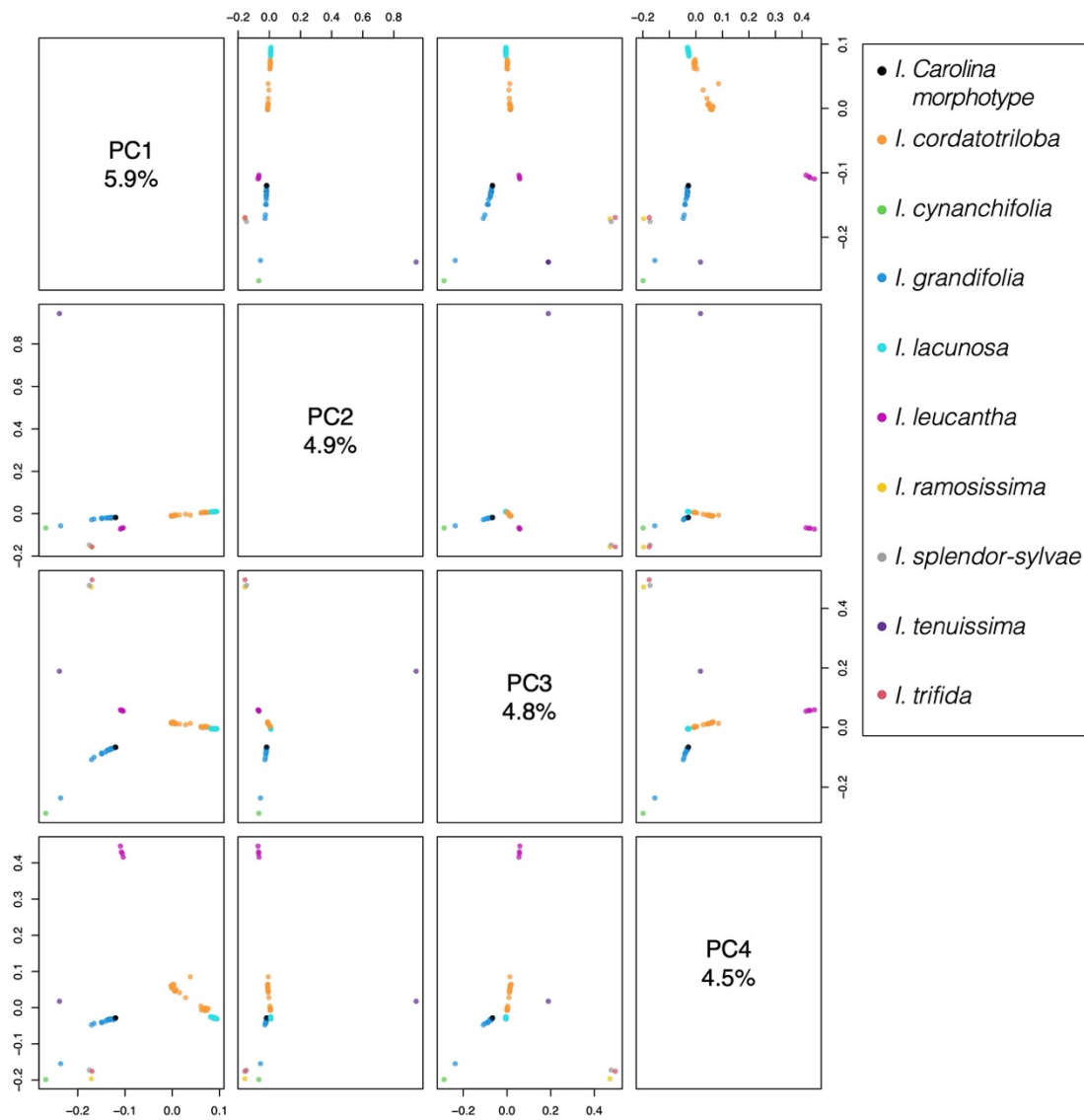
FIG. S5 Dendrogram from the cluster analysis of Euclidean distances shows similar patterns to those of the PCA (Figs. 5B, S4), neighbor-joining tree (Fig. 6), and fastSTRUCTURE and ADMIXTURE analyses (Figs. 5A, S3). *Ipomoea* Carolina morphotype forms a monophyletic cluster (bolded, black dots), but it is nested within the *I. grandifolia* cluster (in blue rectangular background), suggesting that *I.* Carolina morphotype is not genetically distinct from *I. grandifolia*.

FIG. S6. Dendrogram from the cluster analysis using identity-by-state pairwise distances also show similar patterns to those of the PCA (Figs. 5B, S4), neighbor-joining tree (Fig. 6), and fastSTRUCTURE and ADMIXTURE analyses (Figs. 5A, S3). *Ipomoea* Carolina morphotype forms a monophyletic cluster (black dots, bolded), but it is nested within the *I. grandifolia* cluster (dark blue dots; in blue rectangular background), suggesting that *I.* Carolina morphotype is not genetically distinct from *I. grandifolia*.

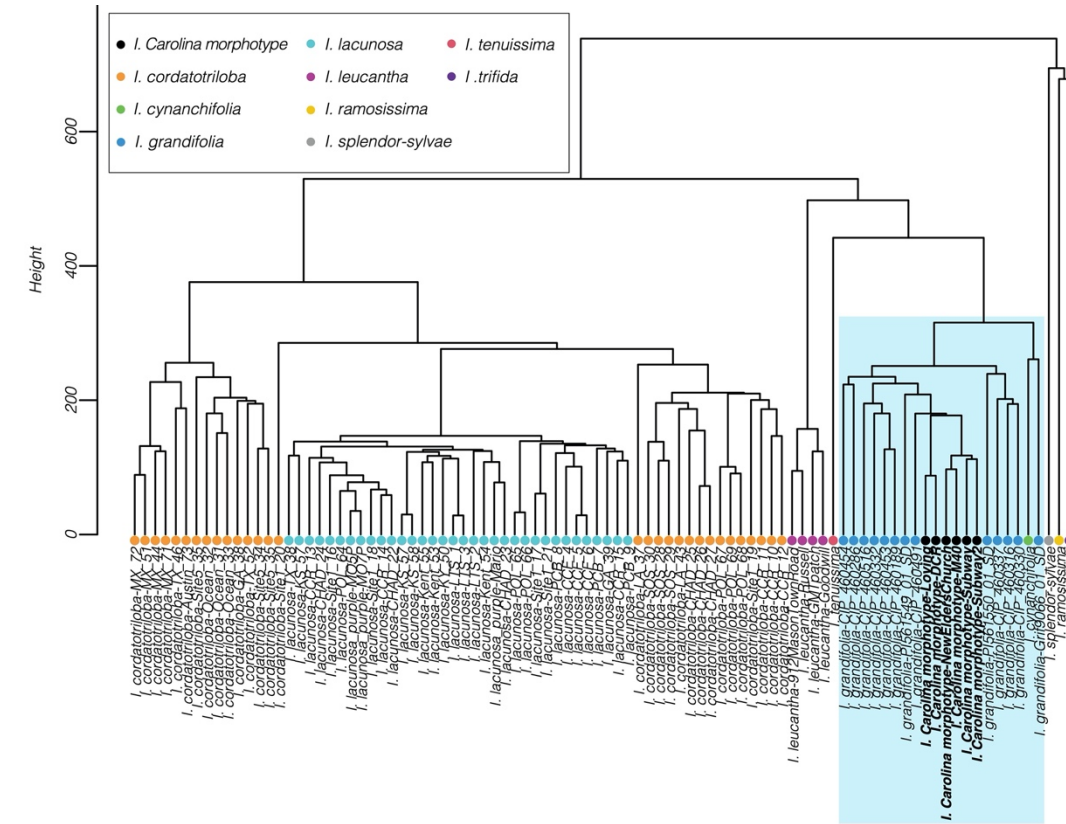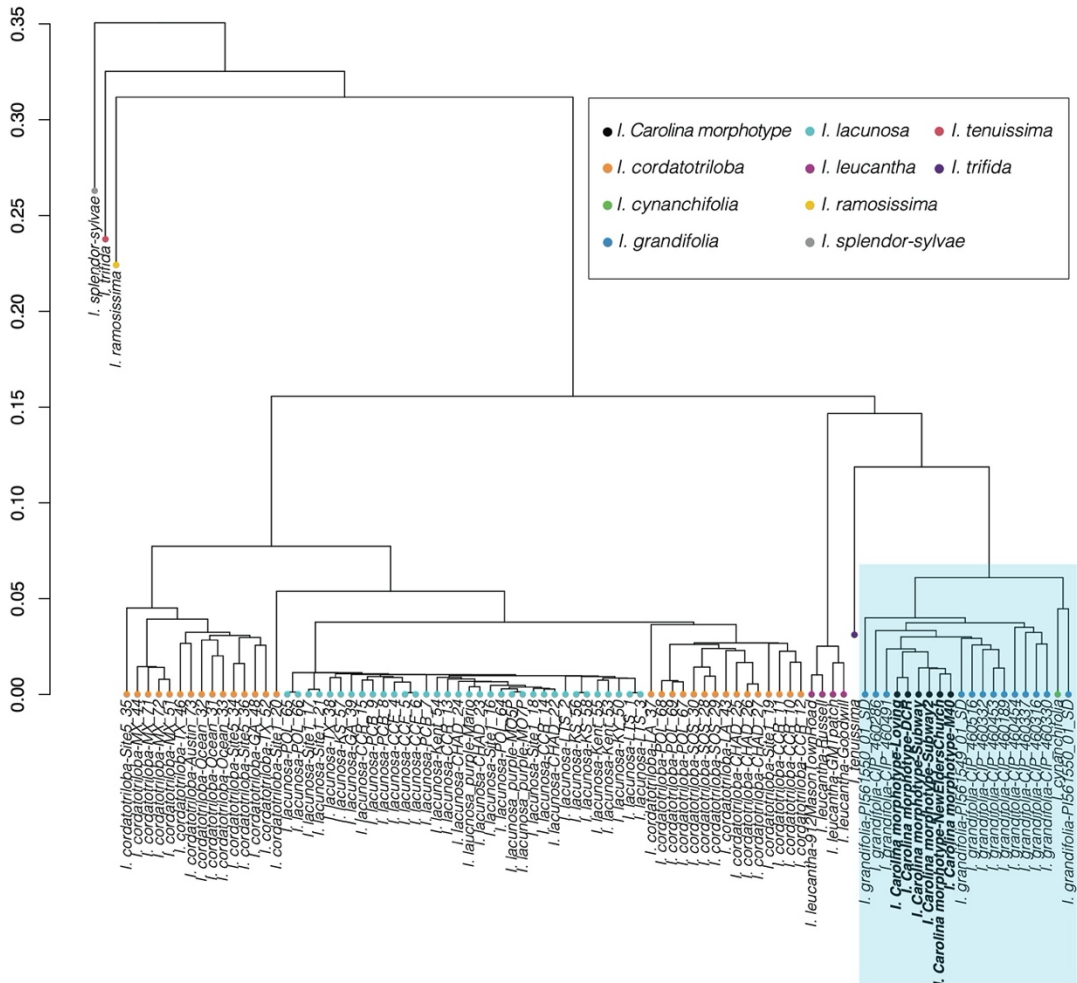Fɪɢ. S7. Possible cross compatibility between *I. lacunosa* and *I. leucantha.* Because within-species crosses were not done for *I. lacunosa* species, results from the *I. lacunosa* within individual crosses (LaxS) were used instead. While *I. leucantha* (LexLe) intraspecific crosses differed from that of *I. lacunosa* (fruit set: G=12.757, P=3.54E-4; seed set, all crosses: t=-4.645, df=36, P<0.0001), fruit and seed set from *I. leucantha* x *I. lacunosa* (LexLa and LaxLe) interspecific crosses were not significantly different (fruit set: G=2.093, P=0.148; seed set, all crosses: t=-0.535, df=36, P=0.596). Fruit set and seed set from both interspecific crosses were also not significantly different from *I. lacunosa* intraspecific crosses (fruit set: G=0.041, P=0.8395; seed set, all crosses: t=1.144, df=36, P=0.260). *Ipomoea lacunosa* intraspecific crosses yield an average 1.913 seeds per fruit (n=80, s.e.= 0.092, Ostevik et al. 2021). A. Pie chart of the percent of fruit set (e.g. crosses that resulted in at least one seed per fruit). Black indicates successful fruit set and white represents failed fruit set. B. Matrix of the number of seeds per fruit produced from each type of cross. C-D. Plots of the mean number of seeds produced per fruit for each type of cross. C: only crosses producing at least 1 seed. D: all crosses. Each point represents the mean number of seeds produced by approximately five crosses made between the same individuals, and the estimated marginal means and 95% confidence intervals from a linear mixed model are displayed. Light gray represents LexLe crosses, medium gray represents LexLa and LaxLe interspecfic crosses, and dark gray/black represents LaxS (*I. lacunosa* within individual crosses). Corresponds to Table S7.
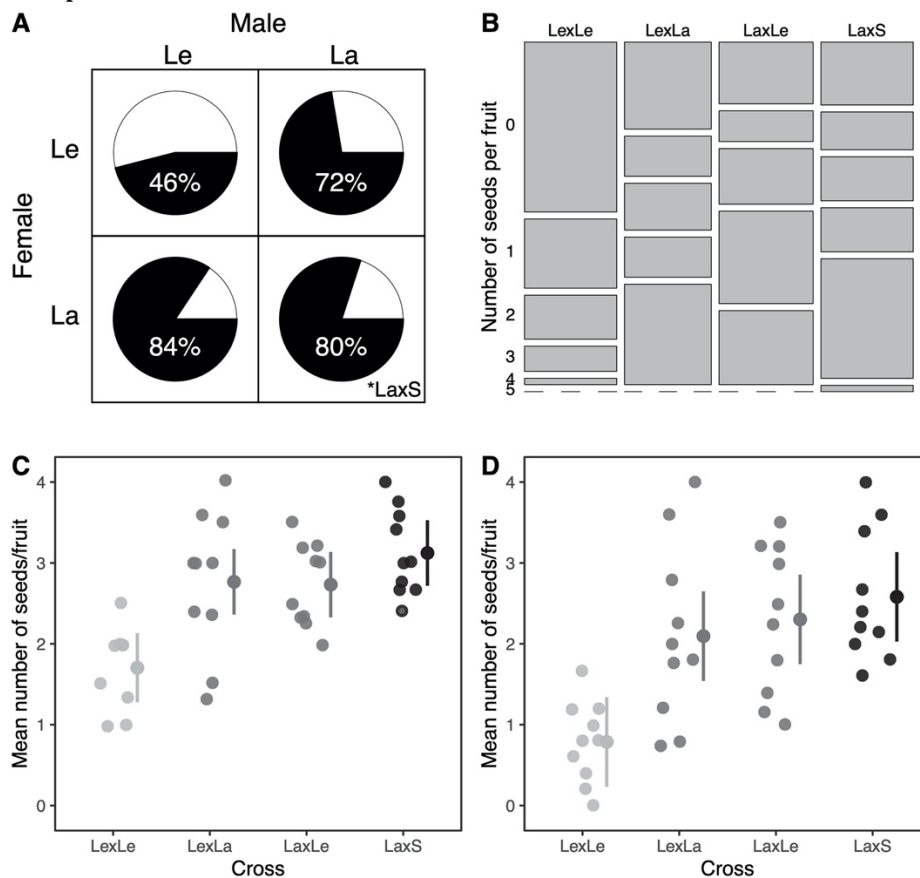
TABLE S1. List of species used in study, including the geographic location of the samples (if it is known), the source of the material (e.g. from seed banks), and whether the samples were sequenced in this study and the associated accession numbers.

Key/Descriptions of the columns

Accession	Sample accession name
Species	Species name
Sp	Species shorthand
General Location	General location of where the species was collected, if indicated (especially samples from the USDA)
Lat	Latitude coordinates of the species location
Long	Longitudinal coordinates of the species location
Source	Whether samples are from the USDA, CIP, or the Rauscher lab collections
Cr	Whether the samples were used in the crossing study
Family Notation	If used in the Crossing Study, shorthand for the individual
LfRNA_DNA	RNA or DNA reference name (name used for sequencing)
LfRNARef	Whether the samples were extracted and sequenced in this study or other previous studies.
SRA	Sequence Read Archive accession number
NCBI_Bioproject	NCBI Bioproject number

Accession	Species	Sp	Gen Locat	Lat	Long	Source	Cr	Family Notation	LfRNA_DNA	LfRNARef	SRA	NCBI_Bioproject
PC_Site5_1	*I. cordatotriloba*	C	USA, Carolinas	34.377	-77.897	Rauscher Lab	Y	18_01	C_34_Site5_1	Rifkin et al, 2019	SRR15373701	PRJNA769750
PI_518494_01_SD	*I. cordatotriloba*	C	Mexico	18.200	-93.083	USDA	Y	18_02	C_44_MX, PC_71_PI_518494_01_SD_1	Rifkin et al, 2019	SRR15373694, SRR15373677	PRJNA769750
L_CCF_1	*I. lacunosa*	La	USA, Carolinas	34.898	-79.802	Rauscher Lab	Y	18_04	L_4_CCF_1	Rifkin et al, 2019	SRR15373708	PRJNA769750
L_KY	*I. lacunosa*	La	USA, Kentucky	38.016	-84.505	Baskin Lab	Y	18_05	L_50_Spindeltop_Farm_KY	Rifkin et al, 2019	SRR15373672	PRJNA769750
L_Kent_7	*I. lacunosa*	La	USA, Kansas	37.193	-80.574	Rauscher Lab	Y	18_06	L_54_Kent_7	Rifkin et al, 2019	SRR15373667	PRJNA769750
L_KS_5	*I. lacunosa*	La	USA, Kansas	38.884	-95.321	Rauscher Lab	Y	18_07	L_57_KS_5	Rifkin et al, 2019	SRR15373686	PRJNA769750
CIP_460189	*I. grandifolia*	G	Argentina	-27.090	-55.567	CIP	Y	18_08	IpoGra8	this study	SRR14748296	PRJNA735523
CIP_460286	*I. grandifolia*	G	Argentina	-27.117	-54.917	CIP	Y	18_09	IpoGra9	this study	SRR14748318	PRJNA735523
CIP_460316	*I. grandifolia*	G	Paraguay	-25.367	-54.750	CIP	Y	18_10	IpoGra10	this study	SRR14748317	PRJNA735523
CIP_460330	*I. grandifolia*	G	Paraguay	-25.500	-55.080	CIP	Y	18_11	IpoGra11	this study	SRR14748316	PRJNA735523
CIP_460332	*I. grandifolia*	G	Paraguay	-25.500	-55.080	CIP	Y	18_12	IpoGra12	this study	SRR14748315	PRJNA735523
CIP_460337	*I. grandifolia*	G	Paraguay	-26.750	-55.267	CIP	Y	18_13	IpoGra13	this study	SRR14748314	PRJNA735523

CIP_460453	*I. grandifolia*	G	Argentina	-27.310	-54.160	CIP	Y	18_14	IpoGra14	this study	SRR14748313	PRJNA735523
CIP_460454	*I. grandifolia*	G	Argentina	-27.310	-54.160	CIP	Y	18_15	IpoGra15	this study	SRR14748312	PRJNA735523
CIP_460491	*I. grandifolia*	G	Argentina	-34.450	-58.800	CIP	Y	18_16	IpoGra16	this study	SRR14748311	PRJNA735523
CIP_460516	*I. grandifolia*	G	Paraguay	-24.783	-54.867	CIP	Y	18_17	IpoGra17	this study	SRR14748310	PRJNA735523
Subway	*I. Carolina morphotype*	M	USA, Carolinas	34.972	-78.188	Rausher Lab	Y	18_18	IpoAus18	this study	SRR14748320	PRJNA735523
Loving	*I. Carolina morphotype*	M	USA, Carolinas	35.319	-79.294	Rausher Lab	Y	18_19	IpoAus19	this study	SRR14748319	PRJNA735523
New Elder's Church	*I. Carolina morphotype*	M	USA, Carolinas	34.878	-77.972	Rausher Lab	Y	18_20	IpoAus20	this study	SRR14748308	PRJNA735523
DCR (IL21)	*I. Carolina morphotype*	M	USA, Carolinas	34.906	-77.920	Rausher Lab	Y	18_21	IpoAus21	this study	SRR14748299	PRJNA735523
M40	*I. Carolina morphotype*	M	USA, Carolinas	34.919	-78.028	Rausher Lab	Y	18_22	IpoAus22	this study	SRR14748298	PRJNA735523
912MasonTownRoad	*I. leucantha*	Le	USA, Carolinas	34.805	-76.876	Rausher Lab	Y	18_23	IpoLeu23	this study	SRR14748304	PRJNA735523
GMT_Patch	*I. leucantha*	Le	USA, Carolinas	33.986	-80.172	Rausher Lab	Y	18_24	Le_GMT	Liao et al, in prep	SRR14748429	PRJNA732922
Goodwill	*I. leucantha*	Le	USA, Carolinas	33.927	-80.154	Rausher Lab	Y	18_25	Le_Good	Liao et al, in prep	SRR14748428	PRJNA732922
Russell	*I. leucantha*	Le	USA, Carolinas	33.939	-79.105	Rausher Lab	Y	18_26	IpoLeu26	this study	SRR14748304	PRJNA735523
PC_CHAD_1	*I. cordatotriloba*	C	USA, Carolinas	34.310	-78.826	Rausher Lab	Y	18_28	PC_25_CHAD_1	Rifkin et al, 2019	SRR15373711	PRJNA769750
PC_CHAD_3	*I. cordatotriloba*	C	USA, Carolinas	34.310	-78.826	Rausher Lab	Y	18_28	PC_27_CHAD_3	Rifkin et al, 2019	SRR15373709	PRJNA769750
PC_Site1_2	*I. cordatotriloba*	C	USA, Carolinas	33.958	-78.992	Rausher Lab	Y	18_29	PC_19_Site1_2	Rifkin et al, 2019	SRR15373715	PRJNA769750
PC_Site1_3	*I. cordatotriloba*	C	USA, Carolinas	33.958	-78.992	Rausher Lab	Y	18_29	PC_20_Site1_3	Rifkin et al, 2019	SRR15373714	PRJNA769750
PC_POL_5	*I. cordatotriloba*	C	USA, Carolinas	34.947	-77.241	Rausher Lab	Y	18_30	PC_68_POL_5	Rifkin et al, 2019	SRR15373679	PRJNA769750
PC_POL_6	*I. cordatotriloba*	C	USA, Carolinas	34.947	-77.241	Rausher Lab	Y	18_30	PC_69_POL_6	Rifkin et al, 2019	SRR15373678	PRJNA769750
PC_SOS_2	*I. cordatotriloba*	C	USA, Carolinas	34.564	-77.937	Rausher Lab	Y	18_32	PC_29_SOS_2	Rifkin et al, 2019	SRR15373707	PRJNA769750
PC_SOS_3	*I. cordatotriloba*	C	USA, Carolinas	34.564	-77.937	Rausher Lab	Y	18_32	PC_30_SOS_3	Rifkin et al, 2019	SRR15373706	PRJNA769750
C_KKD_1	*I. cordatotriloba*	C	USA, Carolinas	33.686	-78.891	Rausher Lab	Y	18_33				
C_KKD_5	*I. cordatotriloba*	C	USA, Carolinas	33.686	-78.891	Rausher Lab	Y	18_33				

L_CCR_1	*I. lacunosa*	La	USA, Carolinas	33.482	-79.270	Rausher Lab	Y	18_34	L_13_CCR_1	Rifkin et al, 2019	SRR15373721	PRJNA769750
L_CCR_3	*I. lacunosa*	La	USA, Carolinas	33.482	-79.270	Rausher Lab	Y	18_34	L_15_CCR_3	Rifkin et al, 2019	SRR15373719	PRJNA769750
L_CHAD_3	*I. lacunosa*	La	USA, Carolinas	34.310	-78.826	Rausher Lab	Y	18_35	L_23_CHAD_3	Rifkin et al, 2019	SRR15373690	PRJNA769750
L_CHAD_4	*I. lacunosa*	La	USA, Carolinas	34.310	-78.826	Rausher Lab	Y	18_35	L_24_CHAD_4	Rifkin et al, 2019	SRR15373689	PRJNA769750
L_Site1_1	*I. lacunosa*	La	USA, Carolinas	33.958	-78.992	Rausher Lab	Y	18_36	L_16_Site1_1	Rifkin et al, 2019	SRR15373718	PRJNA769750
L_Site1_2	*I. lacunosa*	La	USA, Carolinas	33.958	-78.992	Rausher Lab	Y	18_36	L_17_Site1_2	Rifkin et al, 2019	SRR15373717	PRJNA769750
L_POL_1	*I. lacunosa*	La	USA, Carolinas	34.947	-77.241	Rausher Lab	Y	18_37	L_64_POL_1	Rifkin et al, 2019	SRR15373684	PRJNA769750
L_POL_4	*I. lacunosa*	La	USA, Carolinas	34.947	-77.241	Rausher Lab	Y	18_37	L_66_POL_4	Rifkin et al, 2019	SRR15373682	PRJNA769750
L_LTS_2	*I. lacunosa*	La	USA, Carolinas	35.336	-79.353	Rausher Lab	Y	18_38				
L_LTS_6	*I. lacunosa*	La	USA, Carolinas	35.336	-79.353	Rausher Lab	Y	18_38	L_3_LTS_6	Rifkin et al, 2019	SRR15373666	PRJNA769750
L_PCB_3	*I. lacunosa*	La	USA, Carolinas	34.222	-80.402	Rausher Lab	Y	18_39				
L_PCB_6	*I. lacunosa*	La	USA, Carolinas	34.222	-80.402	Rausher Lab	Y	18_39	L_9_PCB_6	Rifkin et al, 2019	SRR15373665	PRJNA769750
Subway	*I. Carolina morphotype*	M	USA, Carolinas	34.972	-78.188	Rausher Lab	N		A_2.2_Subway	Rifkin et al, 2019	SRR15373674	PRJNA769750
Grif_15931_01_SD	*I. cordatotriloba*	C	USA, TX			USDA	N		PC_52_Grif_15931_01_SD	Rifkin et al, 2019	SRR15373670	PRJNA769750
GRIF_6183_01_SD	*I. cordatotriloba*	C	USA, TX			USDA	N		PC_46_GRIF_6183_01_SD	Rifkin et al, 2019	SRR15373693	PRJNA769750
PC_CCR_1	*I. cordatotriloba*	C	USA, Carolinas	33.482	-79.270	Rausher Lab	N		PC_10_CCR_1	Rifkin et al, 2019	SRR15373664	PRJNA769750
PC_CCR_2	*I. cordatotriloba*	C	USA, Carolinas	33.482	-79.270	Rausher Lab	N		PC_11_CCR_2	Rifkin et al, 2019	SRR15373723	PRJNA769750
PC_CCR_5	*I. cordatotriloba*	C	USA, Carolinas	33.482	-79.270	Rausher Lab	N		PC_12_CCR_5	Rifkin et al, 2019	SRR15373722	PRJNA769750
PC_CHAD_2	*I. cordatotriloba*	C	USA, Carolinas	34.310	-78.826	Rausher Lab	N		PC_26_CHAD_2	Rifkin et al, 2019	SRR15373710	PRJNA769750
PC_Ocean_1	*I. cordatotriloba*	C	USA, Carolinas	34.390	-77.598	Rausher Lab	N		PC_31_Ocean_1	Rifkin et al, 2019	SRR15373705	PRJNA769750
PC_Ocean_2	*I. cordatotriloba*	C	USA, Carolinas	34.390	-77.598	Rausher Lab	N		PC_32_Ocean_2	Rifkin et al, 2019	SRR15373704	PRJNA769750
PC_Ocean_3	*I. cordatotriloba*	C	USA, Carolinas	34.390	-77.598	Rausher Lab	N		PC_33_Ocean_3	Rifkin et al, 2019	SRR15373703	PRJNA769750
PC_POL_3	*I. cordatotriloba*	C	USA, Carolinas	34.947	-77.241	Rausher Lab	N		PC_67_POL_3	Rifkin et al, 2019	SRR15373681	PRJNA769750
PC_Site5_4	*I. cordatotriloba*	C	USA, Carolinas	34.377	-77.897	Rausher Lab	N		PC_35_Site5_4	Rifkin et al, 2019	SRR15373700	PRJNA769750

PC_Site5_7	*I. cordatotriloba*	C	USA, Carolinas	34.377	-77.897	Rausher Lab	N	PC_36_Site5_7	Rifkin et al, 2019	SRR15373699	PRJNA769750
PC_SOS_1	*I. cordatotriloba*	C	USA, Carolinas	34.572	-77.945	Rausher Lab	N	PC_28_SOS_1	Rifkin et al, 2019	SRR15373708	PRJNA769750
PI_518495_02_SD	*I. cordatotriloba*	C	USA, Mexico	18.067	-92.917	USDA	N	C_51_MX, PC_72_MX	Rifkin et al, 2019	SRR15373676	PRJNA769750
PI_645624_01_SD	*I. cordatotriloba*	C	USA, Louisiana (approx)	30.413	-91.180	USDA	N	PC_37_PI_645624_01_SD_LA	Rifkin et al, 2019	SRR15373698	PRJNA769750
PI_645625_01_SD	*I. cordatotriloba*	C	USA, Louisiana (approx)	30.413	-91.180	USDA	N	WC_43_PI_645625_01_SD_LA	Rifkin et al, 2019	SRR15373695	PRJNA769750
PI_645627_02_SD	*I. cordatotriloba*	C	USA, Georgia (approx)	31.421	-83.550	USDA	N	PC_48_PI_645627_02_SD_GA	Rifkin et al, 2019	SRR15373673	PRJNA769750
PI_675061	*I. cordatotriloba*	C	USA, Texas	30.183	-97.876	USDA	N	PC_73_PI_675061_1	Rifkin et al, 2019	SRR15373675	PRJNA769750
PI549093_01_SD	*I. cynanchifolia*	Cyn	Brazil (CIP_460149)	-15.643	-56.127	USDA	N	IpoCyn_10	this study	SRR14748297	PRJNA735523
Grif9066_01_SD	*I. grandifolia*	G	Brazil (CIP_460106)			USDA	N	IpoGra_09	this study	SRR14748294	PRJNA735523
PI561549_01_SD	*I. grandifolia*	G	Argentina (CIP_460189)	-27.090	-55.567	USDA	N	IpoGra_07	this study	SRR14748296	PRJNA735523
PI561550_01_SD	*I. grandifolia*	G	Paraguay CIP_460190	-25.750	-55.660	USDA	N	IpoGra_08	this study	SRR14748295	PRJNA735523
L_CCF_2	*I. lacunosa*	La	USA, Carolinas	34.898	-79.802	Rausher Lab	N	L_5_CCF_2	Rifkin et al, 2019	SRR15373691	PRJNA769750
L_CCF_4	*I. lacunosa*	La	USA, Carolinas	34.898	-79.802	Rausher Lab	N	L_6_CCF_4	Rifkin et al, 2019	SRR15373680	PRJNA769750
L_CCR_2	*I. lacunosa*	La	USA, Carolinas	33.482	-79.270	Rausher Lab	N	L_14_CCR_2	Rifkin et al, 2019	SRR15373720	PRJNA769750
L_CHAD_2	*I. lacunosa*	La	USA, Carolinas	34.310	-78.826	Rausher Lab	N	L_22_CHAD_2	Rifkin et al, 2019	SRR15373692	PRJNA769750
L_Kent_6	*I. lacunosa*	La	USA, Carolinas	37.193	-80.574	Rausher Lab	N	L_53_Kent_6	Rifkin et al, 2019	SRR15373668	PRJNA769750
L_Kent_8	*I. lacunosa*	La	USA, Carolinas	37.193	-80.574	Rausher Lab	N	L_55_Kent_8	Rifkin et al, 2019	SRR15373688	PRJNA769750
L_KS_4	*I. lacunosa*	La	USA, Carolinas	38.884	-95.321	Rausher Lab	N	L_56_KS_4	Rifkin et al, 2019	SRR15373687	PRJNA769750
L_KS_8	*I. lacunosa*	La	USA, Carolinas	38.884	-95.321	Rausher Lab	N	L_58_KS_8	Rifkin et al, 2019	SRR15373685	PRJNA769750
L_LTS_3	*I. lacunosa*	La	USA, Carolinas	35.336	-79.353	Rausher Lab	N	L_1_LTS_3	Rifkin et al, 2019	SRR15373725	PRJNA769750
L_LTS_5	*I. lacunosa*	La	USA, Carolinas	35.336	-79.353	Rausher Lab	N	L_2_LTS_5	Rifkin et al, 2019	SRR15373724	PRJNA769750

L_PCB_1	*I. lacunosa*	La	USA, Carolinas	34.222	-80.402	Rausher Lab	N	L_7_PCB_1	Rifkin et al, 2019	SRR15373669	PRJNA769750
L_PCB_2	*I. lacunosa*	La	USA, Carolinas	34.222	-80.402	Rausher Lab	N	L_8_PCB_2	Rifkin et al, 2019	SRR15373713	PRJNA769750
L_POL_3	*I. lacunosa*	La	USA, Carolinas	34.947	-77.241	Rausher Lab	N	L_65_POL_3	Rifkin et al, 2019	SRR15373683	PRJNA769750
L_Site1_4	*I. lacunosa*	La	USA, Carolinas	33.958	-78.992	Rausher Lab	N	L_18_Site1_4	Rifkin et al, 2019	SRR15373716	PRJNA769750
L_Site1_4	*I. lacunosa*	La	USA, Carolinas	33.958	-78.992	Rausher Lab	N	L_21_Site1_4	Rifkin et al, 2019	SRR15373712	PRJNA769750
PI_645621_02_SD	*I. lacunosa*	La	USA. Georgia (approx)	31.421	-83.550	USDA	N	L_39_PI_645621_02_SD_GA	Rifkin et al, 2019	SRR15373696	PRJNA769750
PI_645623_01_SD	*I. lacunosa*	La	USA, Texas			USDA	N	L_38_PI_645623_01_SD_TX	Rifkin et al, 2019	SRR15373697	PRJNA769750
Mario_plac_selfed F1	*I. lacunosa (purple)*	La (p)	USA, Carolinas			Rausher Lab	N	IpoLacPur_01	this study	SRR14748309	PRJNA735523
MO5P_MebaneOaksP5	*I. lacunosa (purple)*	La (p)	USA, Carolinas (approx)	36.077	-79.262	Rausher Lab	N	IpoLacPur_04	this study	SRR14748307	PRJNA735523
MO7P_MebaneOaksP10	*I. lacunosa (purple)*	La (p)	USA, Carolinas (approx)	36.077	-79.262	Rausher Lab	N	IpoLacPur_06	this study	SRR14748306	PRJNA735523
PI552786_01_SD	*I. ramosissima*	Ram	Bolivia (CIP_460036)	-15.530	-67.460	USDA	N	IpoRam_02	this study	SRR14748303	PRJNA735523
PI561557_01_SD	*I. splendor-sylvae*	Spl	Mexico			USDA	N	IpoSpl_03	this study	SRR14748302	PRJNA735523
PI553012_01_SD	*I. tenuissima*	Ten	USA, Florida (approx)	25.400	-80.658	USDA	N	IpoTen_01	this study	SRR14748301	PRJNA735523
PI561543_01_SD	*I. trifida*	Tri	Venuzuela (CIP_460007)	10.095	-68.003	USDA	N	IpoTri_04	this study	SRR14748300	PRJNA735523

TABLE S2. Numbers of emasculated, unpollinated flowers that produced at least 1 seed ("fruited"). Species = species emasculated. Number emasculated = number of flowers that were emasculated but left to be unpollinated. Fruited = number of flowers that produced at least 1 seed. Proportion = proportion of emasculated, unpollinated flowers that resulted in fruit set.

Species	Number emasculated	Fruited	Proportion
I. Carolina morphotype	76	5	0.066
I. cordatotriloba	6	0	0.000
I. grandifolia	59	2	0.034
I. lacunosa	25	1	0.040
I. leucantha	56	3	0.054
Total	222	11	0.050

TABLE S3. Summary of the transcriptome sequencing output on one Illumina NovaSeq 6000 S2 150bp PE flow cell. These samples were multiplexed with other samples for another project. Lane = lane number. Sample name = name assigned to sequencing sample and corresponds with Table S1. Passed filter yield (b) = number of base pairs that passed filter. number of clusters that passed filter = number of sequencing clusters that passed the filter. Q30 % = percentage of sequencing quality with Q30. Average quality score = average sequencing quality score. Barcode = unique dual index barcodes for the sample used in the multiplexed run.

Lane	Sample Name	passed filter yield (b)	number of clusters that passed filter	Q30 %	Average quality score	Barcode
1	IpoAus18	1,878,974,842	6,221,771	92.97	35.79	CACTAGAC+AGTCCTCA
2	IpoAus18	1,876,944,194	6,215,047	92.58	35.71	CACTAGAC+AGTCCTCA
1	IpoAus19	3,476,095,198	11,510,249	93.33	35.87	GACAATTC+CATATCGT
2	IpoAus19	3,471,086,226	11,493,663	92.99	35.8	GACAATTC+CATATCGT
1	IpoAus20	3,445,039,934	11,407,417	93.34	35.87	ATCAGAGC+GAGCTCAG
2	IpoAus20	3,441,474,220	11,395,610	92.96	35.8	ATCAGAGC+GAGCTCAG
1	IpoAus21	4,076,911,514	13,499,707	93.47	35.89	ACGAGGAG+AGTTTAGG
2	IpoAus21	4,079,888,932	13,509,566	93.13	35.83	ACGAGGAG+AGTTTAGG
1	IpoAus22	3,377,721,718	11,184,509	93.26	35.86	TACGGCAG+TTCATATC
2	IpoAus22	3,380,007,556	11,192,078	92.84	35.78	TACGGCAG+TTCATATC
1	IpoCyn_10	4,176,320,250	13,828,875	93.22	35.86	TCAGCGCC+AGGAACAT
2	IpoCyn_10	4,172,397,572	13,815,886	92.83	35.78	TCAGCGCC+AGGAACAT
1	IpoGra_07	3,011,496,586	9,971,843	93.17	35.83	ACAAGGCA+TTGCGCGA
2	IpoGra_07	3,004,090,942	9,947,321	92.8	35.76	ACAAGGCA+TTGCGCGA
1	IpoGra_08	4,002,397,544	13,252,972	93.17	35.85	ATGCCGGT+TGGTCCTC
2	IpoGra_08	4,003,790,670	13,257,585	92.74	35.76	ATGCCGGT+TGGTCCTC
1	IpoGra_09	2,759,727,944	9,138,172	93.49	35.9	TGTTCGCC+TCCTACCT
2	IpoGra_09	2,755,093,452	9,122,826	93.13	35.83	TGTTCGCC+TCCTACCT
1	IpoGra10	3,634,936,930	12,036,215	93.15	35.83	TAATCTCG+GTGTGACA
2	IpoGra10	3,626,071,720	12,006,860	92.76	35.75	TAATCTCG+GTGTGACA
1	IpoGra11	4,918,856,408	16,287,604	93.37	35.87	AGTCACAT+CCATAATG
2	IpoGra11	4,931,108,850	16,328,175	93	35.8	AGTCACAT+CCATAATG
1	IpoGra12	3,895,806,342	12,900,021	93.47	35.89	TCTGGAAC+AGGTGCTA
2	IpoGra12	3,905,868,982	12,933,341	93.12	35.83	TCTGGAAC+AGGTGCTA
1	IpoGra13	1,407,310,940	4,659,970	93.48	35.9	GCCAATCC+GATCGGAC
2	IpoGra13	1,404,881,350	4,651,925	93.12	35.83	GCCAATCC+GATCGGAC
1	IpoGra14	2,379,034,596	7,877,598	93.29	35.86	CCTTTCAC+AAACAAGA
2	IpoGra14	2,374,180,852	7,861,526	92.91	35.79	CCTTTCAC+AAACAAGA
1	IpoGra15	2,185,166,602	7,235,651	93.09	35.83	CTTGCATA+GAAGAGGT
2	IpoGra15	2,182,227,538	7,225,919	92.67	35.75	CTTGCATA+GAAGAGGT
1	IpoGra16	4,567,442,866	15,123,983	93.27	35.85	CTTGACGA+TTGTGTGC

2	IpoGra16	4,570,982,306	15,135,703	92.92	35.78	CTTGACGA+TTGTGTGC
1	IpoGra17	5,129,895,518	16,986,409	93.1	35.82	CCTGTCAA+ACAGCCAT
2	IpoGra17	5,128,590,576	16,982,088	92.7	35.74	CCTGTCAA+ACAGCCAT
1	IpoGra8	2,924,659,506	9,684,303	93.36	35.88	CATACCGT+TAAGAGCG
2	IpoGra8	2,927,272,108	9,692,954	92.98	35.8	CATACCGT+TAAGAGCG
1	IpoGra9	5,466,269,460	18,100,230	93.29	35.86	ACCGGAGT+CCTTCCTT
2	IpoGra9	5,472,953,928	18,122,364	92.93	35.79	ACCGGAGT+CCTTCCTT
1	IpoLacPur_01	4,978,159,846	16,483,973	93.12	35.83	TACCGGCT+GCCAGCTA
2	IpoLacPur_01	4,982,102,456	16,497,028	92.72	35.76	TACCGGCT+GCCAGCTA
1	IpoLacPur_04	4,302,552,626	14,246,863	93.57	35.91	GCCACGAC+ACCCGAGG
2	IpoLacPur_04	4,306,302,560	14,259,280	93.25	35.85	GCCACGAC+ACCCGAGG
1	IpoLacPur_06	3,321,331,976	10,997,788	93.52	35.91	ACACGACT+CTGCGGAT
2	IpoLacPur_06	3,316,430,214	10,981,557	93.18	35.84	ACACGACT+CTGCGGAT
1	IpoLeu23	4,661,974,604	15,437,002	93.46	35.89	CCATCCGC+TACGCCTT
2	IpoLeu23	4,648,561,878	15,392,589	93.08	35.82	CCATCCGC+TACGCCTT
1	IpoLeu26	3,612,092,744	11,960,572	93.44	35.89	TGCCCATC+CCACACTT
2	IpoLeu26	3,604,678,342	11,936,021	93.05	35.82	TGCCCATC+CCACACTT
1	IpoRam_02	3,466,606,358	11,478,829	93.57	35.92	GGTGTGAG+GTGGTGTT
2	IpoRam_02	3,460,655,750	11,459,125	93.19	35.85	GGTGTGAG+GTGGTGTT
1	IpoSpl_03	3,511,434,634	11,627,267	93.24	35.86	CTTTCCCT+AGTAAGCC
2	IpoSpl_03	3,514,915,486	11,638,793	92.85	35.78	CTTTCCCT+AGTAAGCC
1	IpoTen_01	3,464,083,450	11,470,475	93.53	35.91	TTGCGTTA+TTCGGGAA
2	IpoTen_01	3,466,711,756	11,479,178	93.17	35.84	TTGCGTTA+TTCGGGAA
1	IpoTri_04	4,845,839,452	16,045,826	93.18	35.84	CAGCAGTC+ACGCCAAC
2	IpoTri_04	4,841,814,094	16,032,497	92.79	35.76	CAGCAGTC+ACGCCAAC

TABLE S4. Unweighted average seed set per fruit from all crosses made between the species pair. The average was calculated without factoring in crosses made between the same two individuals (approximately 5) representing a unique population pair. The number of crosses and standard error values are found within the parenthesis for each species pair. The species on the left represents the female parent, and the species on the top represents the male parent. No crosses were performed within and between *I. lacunosa* and *I. cordatotriloba* because these crosses were done in a previous study (notated with asterisks, Ostevik et al., 2021). Results from all cross combinations between *I.* Carolina morphotype and *I. grandifolia* are shaded in gray. M = *I.* Carolina morphotype; C = *I. cordatotriloba*; G = *I. grandifolia*; La = *I. lacunosa*; Le = *I. leucantha*; S = within individual cross with anther removal (a control); X = within individual self (without anther removal, for most cases, just self-pollination without manipulation unless there was some distance between the anther and the stigma).

		Male						
		M	G	C	La	Le	S	X
Female	M	1.868 (53, 0.224)	2.5 (52, 0.197)	0.640 (50, 0.153)	1.957 (47, 0.251)	1.553 (47, 0.247)	1.952 (21, 0.305)	3.5 (34, 0.148)
	G	2.545 (55, 0.209)	1.731 (52, 0.232)	0.306 (49, 0.0932)	1.896 (48, 0.251)	1.509 (53, 0.237)	2.269 (52, 0.220)	3.472 (53, 0.139)
	C	0.265 (49, 0.123)	0.260 (50, 0.124)	1.483 (445, 0.073) **	0.0724 (442, 0.0194) **	0.111 (45, 0.0726)	1.955 (22, 0.319)	2.344 (32, 0.272)
	La	1.333 (45, 0.216)	0.882 (51, 0.176)	0.0630 (476, 0.0123) **	1.909 (482, 0.0679) **	2.275 (51, 0.202)	2.44 (50, 0.229)	3.136 (66, 0.148)
	Le	1.184 (49, 0.193)	0.736 (53, 0.140)	0.149(47, 0.0679)	2.085 (47, 0.239)	0.820 (50, 0.153)	0.875 (16, 0.287)	3.346 (26, 0.241)

TABLE S5. Summary of the fruit set (at least 1 seed per fruit) for each cross type. A. All intra- and interspecific crosses with anther removal. B. Within individual crosses with anther removal (S) or without anther removal (X). Cross = the two species in the cross, where the female parent species is listed first in the cross, and the male parent species is listed second. fail = number of crosses resulting in no fruits. success = number of crosses resulting in at least 1 seed per fruit. sum = total number of crosses made. fail_prop = proportion of the cross resulting in no fruits. success_prop = proportion of the cross with at least 1 seed per fruit. M = *I.* Carolina morphotype; C = *I. cordatotriloba*; G = *I. grandifolia*; La = *I. lacunosa*; Le = *I. leucantha*; S = within individual cross with anther removal (a control); X = within individual self (without anther removal, for most cases, just self-pollination without manipulation unless there was some distance between the anther and the stigma).

A.

Cross	fail	success	sum	fail_prop	success_prop
MxM	19	34	53	0.358	0.642
MxC	33	17	50	0.66	0.34
MxG	8	44	52	0.154	0.846
MxLa	15	32	47	0.319	0.681
MxLe	22	25	47	0.468	0.532
CxM	44	5	49	0.898	0.102
CxG	45	5	50	0.9	0.1
CxLe	42	3	45	0.933	0.067
GxM	10	45	55	0.182	0.818
GxC	38	11	49	0.776	0.224
GxG	22	30	52	0.423	0.577
GxLa	19	29	48	0.396	0.604
GxLe	25	28	53	0.472	0.528
LaxM	20	25	45	0.444	0.556
LaxG	28	23	51	0.549	0.451
LaxLe	8	43	51	0.157	0.843
LexM	22	27	49	0.449	0.551
LexC	42	5	47	0.894	0.106
LexG	30	23	53	0.566	0.434
LexLa	13	34	47	0.277	0.723
LexLe	27	23	50	0.54	0.46
Total	532	511	1043	0.510	0.490

B.

Cross	fail	success	sum	fail_prop	success_prop
MxS	4	17	21	0.190	0.810
MxX	1	33	34	0.029	0.971
CxS	6	16	22	0.2723	0.727
CsX	7	25	32	0.219	0.781
GxS	13	39	52	0.25	0.75
GxX	1	52	53	0.019	0.981
LaxS	10	40	50	0.2	0.8
LaxX	5	61	66	0.076	0.924
LexS	9	7	16	0.563	0.438
LexX	2	24	26	0.077	0.923

TABLE S6. Comparison of fruit set and mean number of seeds for *I. leucantha* and *I. lacunosa* crosses. Because within-species crosses were not done for *I. lacunosa* species, results from the *I. lacunosa* within individual crosses (LaxS) were used instead. Asterisks indicate significant at overall P < 0.05 after a FDR adjustment for multiple comparisons. Le = *I. leucantha*; La = *I. lacunosa*. First row: comparison of species difference for intraspecific crosses. Second row: comparison of *I. lacunosa* within individual crosses and interspecific crosses. Third row: comparison of reciprocal interspecific reciprocal crosses. Fourth row: comparison of intraspecific vs. interspecific crosses. t.ratio is the test statistic used to calculate the p-value. Corresponds to Figure S7.

	fruit set			mean number of seeds per fruit						
				crosses with 0 seeds removed			all crosses			
	G	p.value		df	t.ratio	p.value		df	t.ratio	p.value	
LexLe vs LaxS	12.757	3.54E-04	*	35	-4.896	<.0001	*	36	-4.645	<.0001	*
LaxS vs (LexLa + LaxLe)	0.041	0.8395		35	1.531	0.135		36	1.144	0.260	
LexLa vs LaxLe	2.093	0.148		35	0.124	0.902		36	-0.535	0.596	
LexLe + LaxS vs LexLa + LaxLe	5.854	0.0155	*	35	-1.659	0.106		36	-1.884	0.068	

TABLE S7. Outputs for choosing the best K groups for fastSTRUCTURE and ADMIXTURE. Column names are in the format "run number: seed number." A. Output from fastSTRUCTURE chooseK.py script for 10 runs. Highlighted in gray are the numbers used to determine the optimal K groups for each set of runs. B. Cross-validation output from ADMIXTURE for 10 runs. Lowest values are inferred to be the optimal K groups. Highlighted in light gray and with one asterisk * are K groups with the lowest value. Highlighted in dark gray and with two asterisks ** are K groups with the second lowest value.

A.

	1: 100	2: 11755	3: 50410	4: 1139	5: 74050	6: 67373	7: 86590	8: 47830	9: 30294	10: 21114
Model complexity that maximizes marginal likelihood	6	6	7	6	7	9	7	7	8	6
Model components used to explain structure in data	5	6	5	6	6	5	6	6	5	6

B.

	1: 43	2: 12345	3: 33374	4: 6783	5: 10507	6: 67498	7: 42824	8: 98023	9: 17430	10: 89078
CV error (K=2)	0.48422	0.48345	0.48286	0.48362	0.48358	0.48359	0.48316	0.48306	0.483	0.48397
CV error (K=3)	0.35476	0.35497	0.35438	0.35514	0.35451	0.35529	0.35402	0.35397	0.35433	0.35477
CV error (K=4)	0.30599	0.31283	0.31522	0.31628	0.30764	0.31507	0.31322	0.31216	0.31386	0.30477
CV error (K=5)	0.26280 *	0.26215 *	0.26178 *	0.26111 **	0.2621	0.26179 *	0.26209 *	0.26174 *	0.3098	0.26438 *
CV error (K=6)	0.26892	0.3024	0.30156	0.26148	0.26052 *	0.26565	0.26246 **	0.26494 **	0.25942 *	0.26483 **
CV error (K=7)	0.26587 **	0.26592 **	0.26212 **	0.25937 *	0.26143 **	0.26340 **	0.27103	0.26721	0.26237 **	0.27477
CV error (K=8)	0.27305	0.26671	0.27382	0.29816	0.30299	0.27125	0.29719	0.26582	0.26303	0.26714
CV error (K=9)	0.29925	0.27285	0.26563	0.31007	0.31643	0.26652	0.26689	0.26941	0.27382	0.27216
CV error (K=10)	0.31753	0.27383	0.26822	0.30895	0.30043	0.30926	0.27222	0.26754	0.34918	0.2984