Supporting information:

Recipient and donor characteristics govern the hierarchical structure of heterospecific pollen competition networks

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The following Supporting Information is available for this article:

Table S1. Species names, common names, varieties and sources of the different seeds.

Table S2. Numerical values of all the traits measured for each species.

Table S3. Seed set in percentage for hand cross-pollination, hand self-pollination, natural selfing and apomixis for all species.

Table S4. Species x species matrix with the significance of effect "yes" or "no" of the different donors on the seed set of the different recipient species.

Table S5. Estimates, standard error, t-value and P-value of the effect of the different 9 donors on each recipient species.

Table S6. Number of seeds produced with 100% foreign pollen treatments for the different recipient species.

Table S7. Phylogenetic signal and significance for all the different traits

Table S8. Procrustes analysis results.

Figure S1. Total amount of pollen deposited per stigma.

Figure S2. Pollen ratios for the different recipient species.

- Figure S3. Pollen ratios for the different recipient species by family.
- Figure S4. Statistical comparison of the pollen ratios by family.
- Figure S5. Unipartite bidirectional network with asymmetrical effect.
- Figure S6. Correlation matrix for all the different traits.
- Figure S7. Species reproductive biology.
- Figure S8. Grouped effect sizes by family for each recipient species.

 ${\bf Table~S1.~Species~names,~common~names,~varieties~and~sources~of~the~different~seeds.}$

Species	Common names	Varieties	Source
Brassica oleracea	Wild cabbage	Capitata	https://www.mrfothergills.com.au/
Brassica rapa	Pak choi	Chinensis	https://www.mrfothergills.com.au/
Eruca sativa	Rocket		https://www.mrfothergills.com.au/
Sinapis alba	White mustard		https://www.mrfothergills.com.au/
Ipomoea aquatica	Water spinach		https://www.theseedcollection.com.au/
Ipomoea purpurea	Morning glory		http://www.shaman-australis.com.au
Capsicum annuum	Capsicum	California Wonder	https://www.edenseeds.com.au
Petunia integrifolia	Petunia		https://www.dianeseeds.com/
$Solanum\ ly coper sicum$	Tomato	Tommy Toe	https://www.mrfothergills.com.au/
Solanum melongena	Eggplant	Little Fingers	https://www.4seasonsseeds.com.au/

Table S2. Trait measurements ($\bar{X} \pm SD$) for the 13 different traits measured for each plant species. Detailed information of how pollen: ovule ratio, selfing rate and SI index were calculated is available in the plant functional traits section from the methods.

Species	Pollen size μm	Pollen grains per anther	Ovule number	Pollen:ovule ratio	Stigma area $\mu \mathrm{m}^2$	Stigma length (mm)	Stigma width (mm)	Style length (mm)	Style width (mm)	Ovary length (mm)	Ovary width (mm)	Selfing rate	SI index
Brassica oleracea	27.72	42033	29	8696.48	0.62	0.53	0.88	2.32	0.65	5.93	1.11	0.0	0.00
Brassica rapa	25.35	7133	26	1646.08	0.36	0.37	0.73	1.08	0.52	3.53	0.88	0.0	0.00
Capsicum annuum	32.46	30761	241	765.83	1.06	0.72	1.18	3.24	1.06	3.15	5.80	0.8	0.64
Eruca versicaria	24.95	22151	24	5537.75	0.35	0.73	0.67	6.60	0.73	4.42	0.94	0.1	0.02
$Ipomo ea\ aquatica$	70.10	858	4	1072.50	3.26	1.43	2.25	19.44	0.45	2.38	1.42	0.6	0.75
Ipomoea purpurea	97.59	654	6	545.00	2.27	1.24	1.88	28.23	0.58	1.06	1.57	1.0	2.74
Petunia integrifolia	24.74	34657	220	787.66	1.17	0.80	1.32	14.65	0.45	3.13	1.77	0.9	0.26
Sinapis alba	33.59	3507	6	3507.00	0.55	0.63	0.91	3.62	0.77	1.98	1.07	0.7	1.12
$Solanum\ lycopersicum$	22.00	28915	92	1885.76	0.09	0.19	0.35	6.47	0.31	1.16	1.13	0.7	0.48
Solanum melongena	25.18	166989	1010	992.01	1.14	0.96	1.33	11.33	0.94	4.02	3.55	1.0	1.45

Table S3. Results of the test of hand cross-pollination, hand self-pollination, spontaneous selfing and apomixis for all species. The values provided are the seed:ovule ratios in percentage.

Species	Hand cross- pollination	Hand self-pollination	Spontaneous selfing	Apomixis
Brassica oleracea	32.07	0.00	0.00	0
Brassica rapa	44.97	0.00	0.00	0
Capsicum annuum	80.00	56.47	19.34	0
Eruca sativa	23.75	0.42	0.00	0
Ipomoea aquatica	40.00	30.00	20.00	0
Ipomoea purpurea	31.67	86.67	31.67	0
Petunia integrifolia	80.16	24.77	0.00	0
Sinapis alba	41.67	48.33	5.00	15
Solanum lycopersium	85.65	41.20	68.48	0
$Solanum\ melongena$	60.48	74.87	21.56	0

Table S4. Species x species matrix with the significance of effect "yes" or "no" of the different donors on seed set from the linear models. The category of "yes" represents a significant effect of the pollen donor (columns) on the recipient species (rows) and the category of "no" represents a lack of significant seed set reduction in comparison with the control. Significance was tested for all species by comparing the different 50-50 CP-HP pollen treatments with the control treatment of hand cross-pollination with conspecific pollen.

	$Brassica\\oleracea$	Brassica rapa	$Eruca\ sativa$	Sinapis alba	$Ipomoea\\aquatica$	Ipomoea purpurea	$Capsicum \ annuum$	$Petunia\\integrifolia$	Solanum lycopersicum	Solanum melongena	% of $significant$ $effect$
B. oleracea		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	100
B. rapa	No		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	88.9
E. sativa	Yes	No		No	Yes	No	No	No	No	No	22.2
S. alba	No	Yes	No		Yes	Yes	Yes	No	No	No	44.4
I. aquatica	Yes	Yes	Yes	Yes		Yes	Yes	Yes	Yes	Yes	100
I. purpurea	No	No	Yes	No	No		Yes	No	Yes	No	33.3
C. annuum	Yes	Yes	No	Yes	No	Yes		Yes	Yes1	Yes	77.8
P. integrifolia	Yes	No	No	Yes	Yes	Yes	No		Yes	Yes	67.7
S. lycopersicum	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes		Yes	88.9
S. melongena	No	Yes	Yes	No	Yes	Yes	Yes	No	No		55.6
											$\bar{X} = 67.8$

Table S5. Results of the linear models conducted to test heterospecific pollen effect with the 50-50 CP-HP pollen treatments. We conducted 10 different linear models (M1, M2, M3, M4, M5, M6, M7 M8, M9 and M10) where the effect of the different 9 pollen donors was compared with the control treatment of hand cross pollination with conspecific pollen for each recipient species (intercept). The first species on the row names are the pollen recipient species and the second species the pollen donor species. Each treatment is compared with the control or reference treatment that is indicated with the recipient species names and the intercept term between parentheses

	Estimates	Standard error	t-value	P-value
M1 Petunia integrifolia - (Intercept)	4.63	0.49	9.36	0.00
M1 Petunia integrifolia - Brassica oleracea	-1.84	0.81	-2.27	0.03
M1 Petunia integrifolia - Brassica rapa	-0.97	0.81	-1.19	0.24
M1 Petunia integrifolia - Capsicum annuum	0.78	0.81	0.96	0.34
M1 Petunia integrifolia - Eruca vesicaria	-0.91	0.81	-1.12	0.26
M1 Petunia integrifolia - Ipomoea aquatica	-3.18	0.81	-3.92	0.00
M1 Petunia integrifolia - Ipomoea purpurea	-4.21	0.81	-5.19	0.00
M1 Petunia integrifolia - Sinapis alba	-2.99	0.81	-3.69	0.00
M1 Petunia integrifolia - Solanum lycopersicum	-2.40	0.81	-2.96	0.00
M1 Petunia integrifolia - Solanum melongena	-2.66	0.81	-3.27	0.00
M2 Solanum lycopersicum - (Intercept)	4.39	0.24	18.54	0.00
M2 Solanum lycopersicum - Brassica oleracea	-0.47	0.41	-1.14	0.26
M2 Solanum lycopersicum - Brassica rapa	-2.74	0.41	-6.67	0.00
M2 Solanum lycopersicum - Capsicum annuum	-4.39	0.41	-10.71	0.00
M2 Solanum lycopersicum - Eruca vesicaria	-2.97	0.41	-7.25	0.00
M2 Solanum lycopersicum - Ipomoea aquatica	-4.13	0.41	-10.06	0.00
M2 Solanum lycopersicum - Ipomoea purpurea	-3.80	0.41	-9.27	0.00
M2 Solanum lycopersicum - Petunia integrifolia	-1.82	0.41	-4.44	0.00
M2 Solanum lycopersicum - Sinapis alba	-3.72	0.41	-9.08	0.00
M2 Solanum lycopersicum - Solanum melongena	-1.84	0.41	-4.49	0.00
M3 Solanum melongena - (Intercept)	6.34	0.70	9.09	0.00
M3 Solanum melongena - Brassica oleracea	-0.67	0.99	-0.68	0.50
M3 Solanum melongena - Brassica rapa	-4.33	0.99	-4.39	0.00
M3 Solanum melongena - Capsicum annuum	-2.33	0.99	-2.36	0.02

M3 Solanum melongena - Eruca vesicaria	-3.12	0.99	-3.16	0.00
$M3\ Solanum\ melongena$ - $Ipomoea\ aquatica$	-6.34	0.99	-6.43	0.00
$M3\ Solanum\ melongena$ - $Ipomoea\ purpurea$	-2.71	0.99	-2.74	0.01
$M3\ Solanum\ melongena$ - $Petunia\ integrifolia$	-1.43	0.99	-1.45	0.15
M3 Solanum melongena - Sinapis alba	-1.11	0.99	-1.12	0.26
$M3\ Solanum\ melongena$ - $Solanum\ lycopersicum$	-0.84	0.99	-0.86	0.39
M4 Capsicum annuum - (Intercept)	4.59	0.52	8.78	0.00
M4 Capsicum annuum - Brassica oleracea	-1.80	0.74	-2.43	0.02
M4 Capsicum annuum - Brassica rapa	-2.51	0.74	-3.39	0.00
M4 Capsicum annuum - Eruca vesicaria	-0.63	0.74	-0.85	0.40
M4 Capsicum annuum - Ipomoea aquatica	-1.36	0.74	-1.84	0.07
M4 Capsicum annuum - Ipomoea purpurea	-4.00	0.74	-5.41	0.00
M4 Capsicum annuum - Petunia integrifolia	-4.59	0.74	-6.21	0.00
M4 Capsicum annuum - Sinapis alba	-3.63	0.74	-4.92	0.00
M4 Capsicum annuum - Solanum lycopersicum	-1.52	0.74	-2.06	0.04
M4 Capsicum annuum - Solanum melongena	-3.29	0.74	-4.46	0.00
M5 Brassica oleracea - (Intercept)	1.99	0.22	8.98	0.00
M5 Brassica oleracea - Brassica rapa	-1.37	0.31	-4.35	0.00
M5 Brassica oleracea - Capsicum annuum	-0.87	0.31	-2.76	0.01
M5 Brassica oleracea - Eruca vesicaria	-1.92	0.31	-6.13	0.00
M5 Brassica oleracea - Ipomoea aquatica	-1.91	0.27	-7.04	0.00
M5 Brassica oleracea - Ipomoea purpurea	-1.99	0.31	-6.35	0.00
M5 Brassica oleracea - Petunia integrifolia	-1.64	0.31	-5.21	0.00
M5 Brassica oleracea - Sinapis alba	-1.57	0.31	-4.99	0.00
M5 Brassica oleracea - Solanum lycopersicum	-1.47	0.31	-4.70	0.00
M5 Brassica oleracea - Solanum melongena	-1.61	0.31	-5.12	0.00
M6 Brassica rapa - (Intercept)	2.37	0.19	12.49	0.00
M6 Brassica rapa - Brassica oleracea	-0.54	0.29	-1.89	0.06
M6 Brassica rapa - Capsicum annuum	-1.42	0.29	-4.94	0.00
M6 Brassica rapa - Eruca vesicaria	-2.37	0.29	-8.24	0.00
M6 Brassica rapa - Ipomoea aquatica	-1.92	0.29	-6.67	0.00

M6 Brassica rapa - Ipomoea purpurea	-2.26	0.29	-7.86	0.00
M6 Brassica rapa - Petunia integrifolia	-2.37	0.29	-8.24	0.00
M6 Brassica rapa - Sinapis alba	-2.37	0.29	-8.24	0.00
M6 Brassica rapa - Solanum lycopersicum	-1.88	0.29	-6.55	0.00
M6 Brassica rapa - Solanum melongena	-2.37	0.29	-8.24	0.00
M8 Eruca sativa - (Intercept)	1.34	0.35	3.80	0.00
M8 Eruca sativa - Brassica oleracea	-1.34	0.50	-2.69	0.01
M8 Eruca sativa - Brassica rapa	0.66	0.50	1.32	0.19
M8 Eruca sativa - Capsicum annuum	-0.27	0.50	-0.55	0.58
M8 Eruca sativa - Ipomoea aquatica	0.87	0.50	1.75	0.08
M8 Eruca sativa - Ipomoea purpurea	-0.19	0.50	-0.39	0.70
M8 Eruca sativa - Petunia integrifolia	0.20	0.50	0.41	0.68
M8 Eruca sativa - Sinapis alba	0.32	0.50	0.65	0.52
M8 Eruca sativa - Solanum lycopersicum	-0.52	0.50	-1.04	0.30
M8 Eruca sativa - Solanum melongena	0.54	0.50	1.09	0.28
M9 Ipomoea purpurea - (Intercept)	0.82	0.20	4.05	0.00
M9 Ipomoea purpurea - Brassica oleracea	0.37	0.29	1.31	0.19
M9 Ipomoea purpurea - Brassica rapa	-0.41	0.29	-1.43	0.16
M9 Ipomoea purpurea - Capsicum annuum	-0.82	0.29	-2.86	0.01
M9 Ipomoea purpurea - Eruca vesicaria	-0.75	0.29	-2.62	0.01
$M9\ Ipomoea\ purpurea$ - $Ipomoea\ aquatica$	0.41	0.29	1.44	0.15
M9 Ipomoea purpurea - Petunia integrifolia	-0.37	0.29	-1.31	0.19
M9 Ipomoea purpurea - Sinapis alba	-0.33	0.29	-1.14	0.26
M9 Ipomoea purpurea - Solanum lycopersicum	-0.82	0.29	-2.86	0.01
M9 Ipomoea purpurea - Solanum melongena	-0.24	0.29	-0.82	0.41
M10 Ipomoea aquatica - (Intercept)	0.80	0.09	9.36	0.00
M10 Ipomoea aquatica - Brassica oleracea	-0.80	0.12	-6.62	0.00
M10 Ipomoea aquatica - Brassica rapa	-0.80	0.12	-6.62	0.00
M10 Ipomoea aquatica - Capsicum annuum	-0.80	0.12	-6.62	0.00
M10 Ipomoea aquatica - Eruca vesicaria	-0.80	0.12	-6.62	0.00
M10 Ipomoea aquatica - Ipomoea purpurea	-0.80	0.12	-6.62	0.00

M10 Ipomoea aquatica - Petunia integrifolia	-0.59	0.12	-4.89	0.00
M10 Ipomoea aquatica - Sinapis alba	-0.80	0.12	-6.62	0.00
M10 Ipomoea aquatica - Solanum lycopersicum	-0.69	0.12	-5.70	0.00
M10 Ipomoea aquatica - Solanum melongena	-0.80	0.12	-6.62	0.00

Table S6. Number of seeds produced with 100% foreign pollen (N=900). From 900 pollination events we found seed production in just 13 specific cases and 0 seed production in the other 887 pollination events. In this table we show just the treatments that lead to seed production.

Recipient	Donor (100% foreign pollen)	Seed number
Solnamum lycopersicum	Sinapis alba	3
$Solnamum\ melongena$	Petunia integrifolia	36
Capsicum annuum	Eruca sativa	3
$Capsicum\ annuum$	Sinapis alba	127
Brassica rapa	Brassica oleracea	2
Brassica rapa	Brassica oleracea	13
Sinapis alba	Brassica oleracea	7
$Sinapis\ alba$	Brassica oleracea	5
Sinapis alba	Brassica oleracea	7
$Sinapis\ alba$	Capsicum annuum	1
Sinapis alba	Capsicum annuum	6
Sinapis alba	Solanum lycopersicum	1
Eruca sativa	Petunia integrifolia	2

^{*} Note: There are some specific cases that we think that hybridization did not occur and that the recipient species were pollen contaminated, like the one of C. annuum with S. alba with 127 seeds. This seems to be the case for 5 other crosses that had very low seed production when they were pollinated with 100% foreign pollen with species of other family members. This represents very few isolated cases (0.67% of all the 100% foreign pollen crosses conducted) and we think that our results are not compromised by this fact.

 $\textbf{Table S7.} \ \ \text{Phylogenetic signal and significance for the 13 different reproductive traits measured for all species.}$

Lambda	P-value	Traits
0.95	0.20	Selfing rate
1.00	0.00	Pollen size
0.00	1.00	Pollen anther
0.00	1.00	Ovule number
0.00	1.00	Pollen-ovule ratio
0.89	0.01	Stigmatic area
0.70	0.05	Stigma length
0.77	0.03	Stigma width
0.93	0.01	Style length
0.00	1.00	Style width
0.47	0.30	Ovary length
0.00	1.00	Ovary width
0.00	0.02	SI index

Table S8. Procrustes correlation, sum of squares and significance from Procrustes analysis between the matrix of effect sizes (species x species matrix) and the distance matrix of each trait for all families, just Solanaceae family and just Brassicaceae family.

Correlation (r)	Sum of squares	P-value	Traits	Family
0.34	0.88	0.70	Selfing rate	All
0.36	0.87	0.42	Pollen size	All
0.35	0.88	0.57	Pollen per anther	All
0.33	0.89	0.55	Number of ovules	All
0.30	0.91	0.86	Pollen-ovule ratio	All
0.37	0.86	0.68	Stigmatic area	All
0.62	0.62	0.10	Stigma length	All
0.46	0.79	0.47	Stigma width	All
0.43	0.82	0.45	Style length	All
0.59	0.65	0.11	Style width	All
0.34	0.88	0.57	Ovary length	All
0.35	0.88	0.93	Ovary width	All
0.49	0.76	0.24	Self-incompatibility index	All
0.86	0.26	0.25	Selfing rate	Solanaceae
0.76	0.42	0.33	Pollen size	Solanaceae
0.42	0.82	0.79	Pollen per anther	Solanaceae
0.52	0.73	0.83	Number of ovules	Solanaceae
0.87	0.25	0.04	Pollen-ovule ratio	Solanaceae
0.82	0.33	0.17	Stigmatic area	Solanaceae
0.89	0.20	0.08	Stigma length	Solanaceae
0.84	0.29	0.12	Stigma width	Solanaceae
0.78	0.39	0.50	Style length	Solanaceae
0.52	0.73	0.71	Style width	Solanaceae
0.64	0.60	0.79	Ovary length	Solanaceae
0.87	0.23	0.17	Ovary width	Solanaceae
0.50	0.75	1.00	Self-incompatibility index	Solanaceae
0.61	0.63	0.50	Selfing rate	Brassicaceae

0.60	0.64	0.71	Pollen size	Brassicaceae
0.40	0.84	0.96	Pollen per anther	Brassicaceae
0.62	0.61	0.42	Number of ovules	Brassicaceae
0.49	0.76	0.96	Pollen-ovule ratio	Brassicaceae
0.55	0.70	0.58	Stigmatic area	Brassicaceae
0.92	0.15	0.08	Stigma length	Brassicaceae
0.63	0.60	0.38	Stigma width	Brassicaceae
0.83	0.31	0.08	Style length	Brassicaceae
0.79	0.37	0.33	Style width	Brassicaceae
0.59	0.65	0.50	Ovary length	Brassicaceae
0.54	0.70	1.00	Ovary width	Brassicaceae
0.50	0.75	0.83	Self-incompatibility index	Brassicaceae

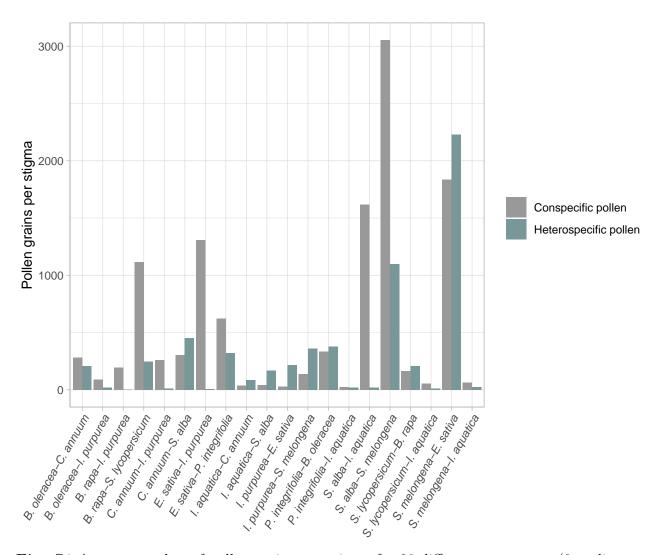


Fig. S1 Average number of pollen grains per stigma for 20 different treatments (3 replicates per tratment). For each treatment, we show the average number of conspecific pollen grains (grey) and heterospecific pollen grains (light blue) per stigma. For each pair of species on the x-axis, the first species is the recipient species, and the second, the donor species.

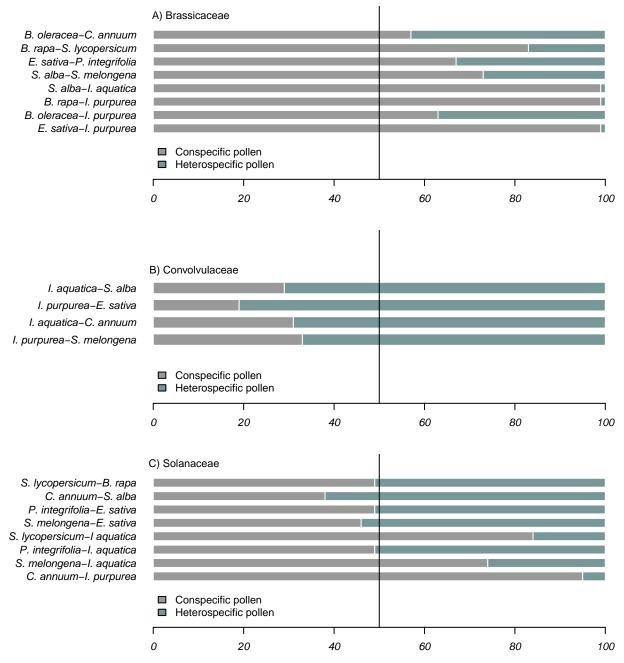


Fig. S2 Average proportion of conspecific and heterospecific pollen per stigma grouped by family, A) Brassicaceae, B) Solanaceae and C) Convolvulaceae. These proportions (%) are the number of conspecific pollen grains or heterospecific pollen grains divided by the total number of pollen grains per stigma for 20 different treatments. We conducted 3 count replicates per treatment and then we calculated the average number of pollen grains for these treatments. The proportion of conspecific and heterospecific pollen are shown in grey and light blue respectively. For each pair of species on the y-axis, the first species is the pollen recipient and the second the pollen donor. The vertical black line represents 50% pollen of both donor and recipient.

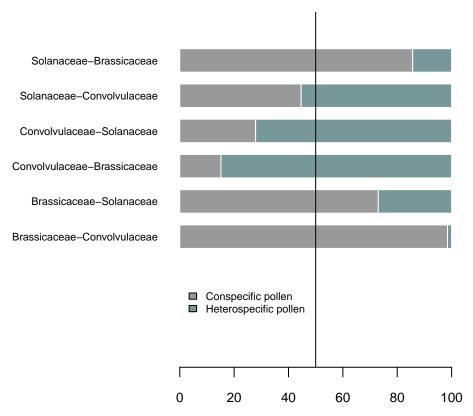


Fig. S3 Average proportion (%) of heterospecific and conspecific pollen per family for the different 20 treatments counted. We conducted 3 count replicates per treatment and then we calculated the average number of pollen grains for these different 20 treatments. Finally, we grouped by family these treatments in order to see general tendencies across families as pollen donor and as pollen recipient. Pollen ratios were considered as the number of conspecific or heterospecific pollen grains divided by the total number of pollen grains per stigma. On the y-axis, the first family on each pair of plant families is the recipient one, and the second, the family of the donor. The vertical bar on intercept 50, represents equal proportions of both recipient (grey) and donor (light blue) pollen.

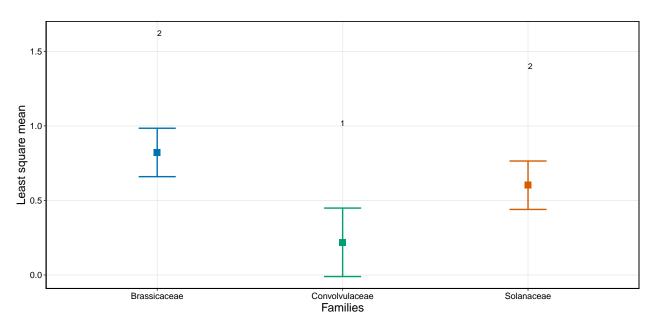


Fig. S4 Pollen ratios comparisons between the different pollen recipient families where the boxes represent least square means, the error bars, confidence intervals 95%, and sharing numbers indicate no significant differences between groups (Tukey adjusted comparisons). These pollen ratios (%) are the total number of heterospecific pollen grains divided by the total quantity of pollen (conspecific pollen + heterospecific pollen), and then compared by family (N=20). Brassicacea family is coloured in blue, Convolvulaceae in green and Solanaceae in orange.

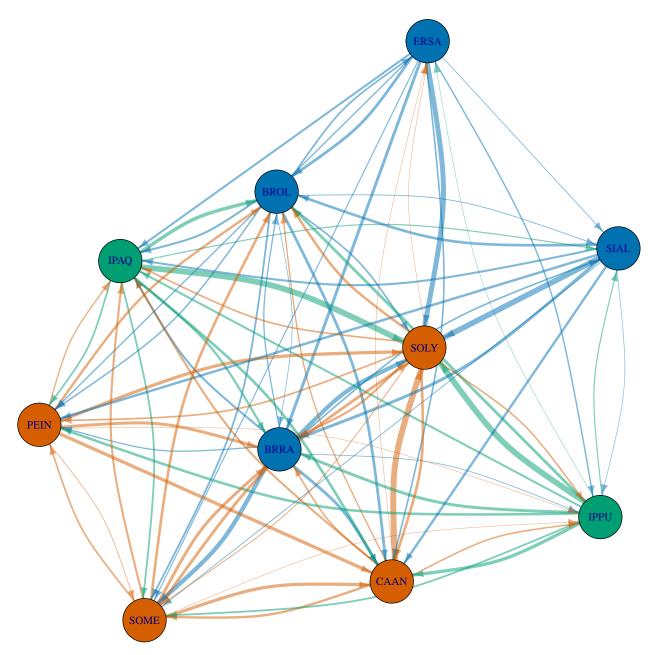


Fig. S5 Unipartite bidirectional network with asymmetrical effect. The lines with the arrow heads connect the impact of foreign pollen (effect size) of each pollen donor species on each recipient species. All the arrow heads point to the recipient species of the reciprocal interaction. Lines of species that did not have a negative impact are not represented. The different nodes and the effect of the donor species on the recipient species appear coloured by family: Solanaceae (orange), Brassicaceae (blue) and Convolvulaceae (green). The intensity of the effect is represented by the line 's size where a larger effect size corresponds to a thicker line and a thinner line to a smaller effect size. Species code: BROL: Brassica oleracea, BRRA: Brassica rapa, ERSA: Eruca sativa, SIAL: Sinapis alba, IPAQ: Ipomoea aquatica, IPPU: Ipomoea purpurea, CAAN: Capsicum annuum, PEIN: Petunia integrifolia, SOLY: Solanum lycopersicum, SOME: Solanum melongena.

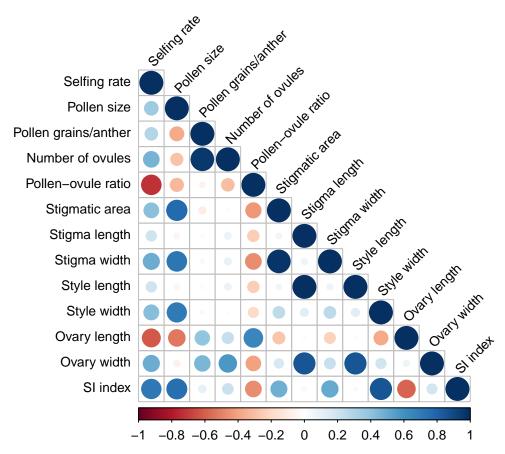


Fig. S6 Graphical representation of the correlation matrix of the different reproductive traits considered in the experiment. Positive correlations are displayed in blue and negative in red. The intensity, size and colour of the circles are proportional to the correlation coefficient from Pearson's r.

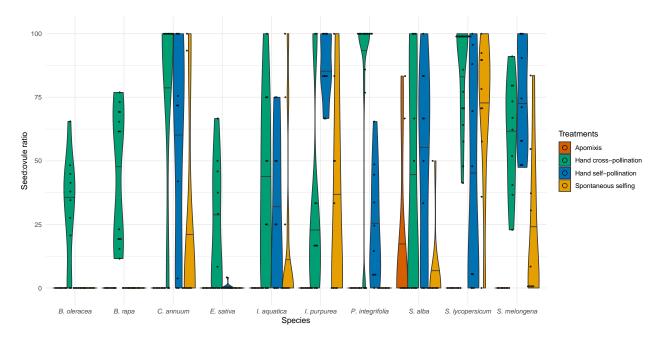


Fig. S7 Violin plot of the proportion of seeds coverted to ovule (%) for all species with four different hand-pollination treatments: apomixis (orange), hand cross pollination (green), hand self pollination (blue) and spontaneous selfing (yellow). The coloured dots, represent the different values of seed set for each treatment.

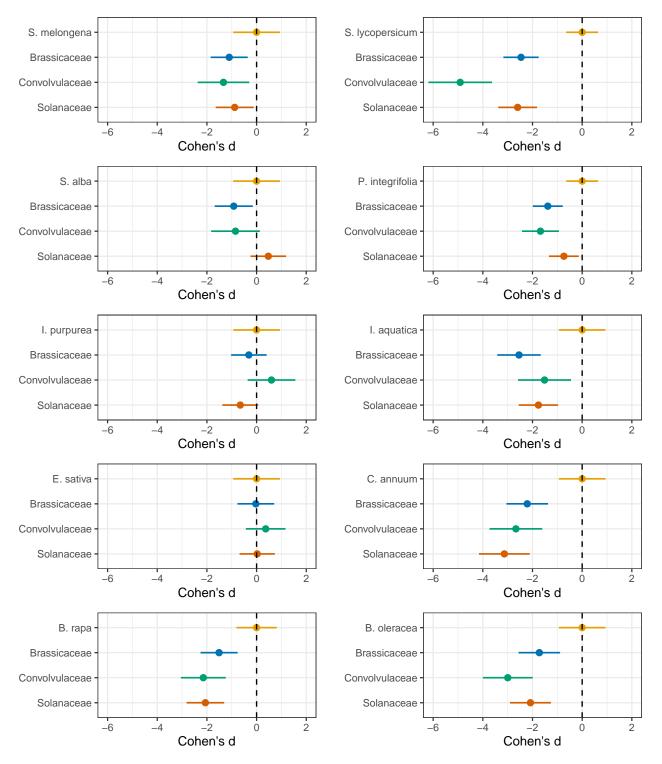


Fig. S8 Grouped effect sizes (95% confidence intervals) of the different families on each focal species. For each species, the grouped effect by family is compared with the control treatment of hand cross pollination with conspecific pollen. The control treatment is represented in yellow and vertically intersected with a dashed line through the mean effect size in order to help the visual interpretation of the effect sizes. Any value to the left of the vertical dashed line represents a negative impact of foreign pollen. The different effect sizes and confidence intervals are coloured by family: Solanaceae (orange), Brassicaceae (blue) and Convolvulaceae (green).