

Supplementary material for:

Intersexual conflict over seed size is stronger in more outcrossed populations of a mixed mating plant

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Table S1. Coordinates, average herkogamy measured by the anther-stigma distance (ASD) and average seed diameter of the nine populations of *Dalechampia scandens* included in the first data set.

Population	Coordinates	ASD \pm SE (mm)	Seed dia \pm SE (mm)
Bacalar (BA)	18°36'40" N, 88°26'32" W	3.01 \pm 0.31	4.01 \pm 0.027
Comalcalco (C)	18°21'26" N, 93°20'43" W	2.61 \pm 0.28	3.89 \pm 0.023
Cacalchén (CA)	20°58'01" N, 89°13'18" W	1.79 \pm 0.17	4.02 \pm 0.031
Ciudad del Carmen (CC)	18°56'29" N, 91°18'01" W	3.40 \pm 0.16	4.15 \pm 0.043
Graciano Sánchez (GS)	19°08'04" N, 88°30'18" W	2.68 \pm 0.23	4.23 \pm 0.022
La Mancha (LM)	19°37'15" N, 96°28'09" W	0.33 \pm 0.15	3.87 \pm 0.009
Martinez de la Torre (M)	20°05'09" N, 97°01'55" W	2.95 \pm 0.27	4.14 \pm 0.031
Puerto Morelos (PM)	20°51'11" N, 86°53'43" W	2.62 \pm 0.19	4.30 \pm 0.035
Tulum (T)	20°12'26" N, 87°27'04" W	3.34 \pm 0.18	4.22 \pm 0.018

Table S2. Number of seeds and (number of crosses) in crosses among the nine populations of *Dalechampia scandens* included in the first data set. Between-populations crosses: $n = 635$ seeds (86 crosses). Within-population crosses (on the diagonal): $n = 1427$ seeds (176 crosses).

	♂BA	♂C	♂CA	♂CC	♂GS	♂LM	♂M	♂PM	♂T
♀BA	68 (8)	26 (4)	82 (11)	20 (3)	5 (1)	15 (2)	25 (3)	5 (1)	12 (2)
♀C	9 (2)	88 (11)	-	-	8 (1)	-	4 (1)	-	4 (1)
♀CA	39 (5)	-	80 (9)	-	-	-	-	-	-
♀CC	5 (1)	17 (2)	9 (1)	328 (40)	5 (1)	-	6 (1)	9 (1)	23 (3)
♀GS	-	-	-	8 (1)	87 (10)	-	7 (1)	-	-
♀LM	-	7 (1)	7 (1)	24 (3)	9 (1)	408 (50)	37 (5)	-	31 (4)
♀M	15 (2)	4 (1)	22 (3)	30 (4)	8 (1)	37 (5)	86 (10)	6 (1)	15 (2)
♀PM	-	-	-	9 (1)	-	-	-	196 (28)	-
♀T	-	8 (1)	9 (1)	-	6 (1)	-	-	8 (1)	86 (10)

Table S3. Coordinates, average herkogamy measured as the anther-stigma distance (ASD) and average seed diameter of the four study populations of *D. scandens* used for the diallel crosses.

Population	Coordinates	ASD \pm SE (mm)	Seed dia \pm SE (mm)
Comalcalco (C)	18°21'26" N, 93°20'43" W	2.61 \pm 0.28	3.89 \pm 0.023
Puerto Morelos (PM)	20°51'11" N, 86°53'43" W	2.62 \pm 0.19	4.30 \pm 0.035
Martinez de la Torre (M)	20°05'09" N, 97°01'55" W	2.95 \pm 0.27	4.14 \pm 0.031
Ciudad del Carmen (CC)	18°56'29" N, 91°18'01" W	3.40 \pm 0.16	4.15 \pm 0.043

Table S4. Number of seeds and (number of crosses) in the 16 cross-combinations within and between the four populations of *D. scandens* (♀: maternal population, ♂: paternal population) used for the diallel crosses. On the diagonal (within-population crosses), the first set of numbers correspond to self-crosses, the second set corresponds to outcrosses.

	♂C	♂CC	♂M	♂PM
♀C	80 (11)/199 (15)	248 (29)	220 (28)	244 (30)
♀CC	230 (28)	82 (10)/174 (20)	255 (30)	258 (30)
♀M	232 (26)	234 (28)	88 (10)/180 (21)	261 (30)
♀PM	216 (28)	235 (29)	216 (27)	73 (9)/169 (21)

Table S5. Results of the statistical test of the tug-of-war hypothesis in the two datasets (crosses among nine populations and diallel among four population). The support for the tug-of-war hypothesis is given by the effect of the relative outcrossing rate [$\log_e(\text{paternal population herkogamy}/\text{maternal population herkogamy})$] on the hybrid relative seed size, that is, the hybrid seed diameter expressed as a percentage of the average seed diameter within the maternal population (see Table S1 and S8 for maternal averages). For both datasets, the mixed effect models include the random factors blossom identity nested within maternal plant identity, itself nested within maternal population identity, and paternal plant identity nested within paternal population identity. For the diallel data, the covariates included in the model, peduncle diameter (mm) and seed set (number of seeds per blossom), were centred on the mean of the maternal population. The units of the estimates are % for the intercept, and % change per unit change in the explanatory variables for the other estimates.

Model	Parameter	Estimate \pm SE	<i>T</i>	<i>P</i>
9 pops	Intercept	99.9 \pm 0.61	163.1	<0.001
	Relative outcrossing rate	1.34 \pm 0.46	2.91	<0.001
4 pops	Intercept	100 \pm 0.29	344.7	<0.001
	Relative outcrossing rate	3.28 \pm 1.193	2.75	<0.01
	Peduncle	3.142 \pm 2.447	1.28	0.20
	Seed set	0.509 \pm 0.350	1.46	0.15
	Peduncle \times matpop CC	5.155 \pm 3.186	1.62	0.11
	Peduncle \times matpop M	3.805 \pm 2.970	1.28	0.20
	Peduncle \times matpop PM	- 5.776 \pm 3.014	- 1.92	0.056
	Seed set \times matpop CC	- 0.733 \pm 0.363	- 2.02	0.044
	Seed set \times matpop M	0.155 \pm 0.416	0.37	0.71
	Seed set \times matpop PM	- 0.579 \pm 0.323	- 1.80	0.074
	Seed set \times patpop CC	- 0.466 \pm 0.323	- 1.44	0.15
	Seed set \times patpop M	0.141 \pm 0.296	0.48	0.64
Seed set \times patpop PM	- 0.298 \pm 0.295	- 0.76	0.45	

Table S6a. Results of the first statistical test for the recognition-avoidance hypothesis in the two datasets (crosses among nine populations and diallel among four populations). Support for the recognition-avoidance hypothesis is given by the effect of cross type (within- and between populations) on hybrid relative seed size, that is, the hybrid seed diameter expressed as a percentage of the average seed diameter within the maternal population (see Table S1 and S8 for maternal averages). For both datasets, the mixed effect models include the random factors blossom identity nested within maternal plant identity, itself nested within maternal population identity, and paternal plant identity nested within paternal population identity. For the diallel data, the covariates included in the model, peduncle diameter (mm) and seed set (number of seeds per blossom), were centred on the mean of the maternal population. The units of the estimates are % for the intercept, and % change per unit change in the explanatory variables for the other estimates.

Dataset	Parameter	Estimate \pm SE	<i>T</i>	<i>P</i>
9 pops	Within populations (intercept)	99.9 \pm 0.42	237.9	<0.001
	Between populations	0.45 \pm 0.57	0.79	0.43
4 pops	Within populations (intercept)	100 \pm 2.14	46.7	<0.001
	Between populations	0.11 \pm 0.30	0.37	0.71
	Peduncle	2.84 \pm 2.16	1.31	0.19
	Seed set	0.29 \pm 0.27	1.06	0.29
	Peduncle \times matpop CC	4.29 \pm 2.89	1.49	0.14
	Peduncle \times matpop M	3.94 \pm 2.65	1.48	0.14
	Peduncle \times matpop PM	- 5.16 \pm 2.71	- 1.91	0.057
	Seed set \times matpop CC	- 0.57 \pm 0.33	- 1.71	0.088
	Seed set \times matpop M	- 0.14 \pm 0.33	- 0.43	0.67
	Seed set \times matpop PM	- 0.49 \pm 0.26	- 1.88	0.06
	Seed set \times patpop CC	- 0.17 \pm 0.29	- 0.58	0.56
	Seed set \times patpop M	0.15 \pm 0.26	0.58	0.56
Seed set \times patpop PM	- 0.15 \pm 0.27	- 0.54	0.59	

Table S6b. Results of the second statistical test for the recognition-avoidance hypothesis in the two datasets (crosses among nine populations and diallel among four populations). Support for the recognition-avoidance hypothesis is given by the effect of the outcrossing rate of the paternal population (inferred from the anther-stigma distance (mm)) on hybrid relative seed size, that is, the hybrid seed diameter expressed as a percentage of the average seed diameter within the maternal population (see Table S1 and S8 for maternal averages). For both datasets, the mixed effect models include the random factors blossom identity nested within maternal plant identity, itself nested within maternal population identity, and paternal plant identity nested within paternal population identity. For the diallel data, the covariates included in the model, peduncle diameter (mm) and seed set (number of seeds per blossom), were centred on the mean of the maternal population. The units of the estimates are % for the intercept, and % change per unit change in the explanatory variables for the other estimates.

Dataset	Parameter	Estimate \pm SE	<i>T</i>	<i>P</i>
9 pops	Intercept	96.8 \pm 1.91	50.6	<0.001
	Paternal pop. herkogamy	1.29 \pm 0.66	1.94	0.09
4 pops	Intercept	97.9 \pm 1.48	66.3	<0.001
	Paternal pop. herkogamy	0.812 \pm 0.49	1.67	0.10
	Peduncle	3.05 \pm 2.45	1.24	0.21
	Seed set	0.526 \pm 0.351	1.50	0.14
	Peduncle \times matpop CC	5.25 \pm 3.19	1.65	0.10
	Peduncle \times matpop M	3.86 \pm 2.97	1.30	0.20
	Peduncle \times matpop PM	- 5.67 \pm 3.02	- 1.88	0.061
	Seed set \times matpop CC	- 0.744 \pm 0.363	- 2.05	0.041
	Seed set \times matpop M	0.136 \pm 0.417	0.33	0.74
	Seed set \times matpop PM	- 0.584 \pm 0.324	- 1.80	0.072
	Seed set \times patpop CC	- 0.476 \pm 0.324	- 1.47	0.14
	Seed set \times patpop M	0.124 \pm 0.297	0.419	0.68
	Seed set \times patpop PM	- 0.309 \pm 0.395	- 0.782	0.44

Table S7. Results for the statistical test of a simple additive genetic effect on seed diameter in the two datasets. The support for this hypothesis is given by the parameter estimate for the effect of the parental relative seed size [$\log_e(\text{average seed size of the paternal population}/\text{average seed size of the maternal population})$] on the hybrid relative seed size, that is, the hybrid seed diameter expressed as a percentage of the average seed diameter within the maternal population (see Table S1 and S8 for maternal averages). For both data sets, the mixed effect models included the random factors blossom identity nested within maternal plant identity, itself nested within maternal population identity, and paternal plant identity nested within paternal population identity. In the models analysing the data set from the diallel, the covariates, peduncle diameter (mm) and seed set (number of seeds per blossom), were centred on the mean of the maternal population. The units of the estimates are % for the intercept and % change per unit change in the explanatory variables for the other estimates.

Model	Parameter	Estimate \pm SE	<i>t</i>	<i>P</i>
Additive	Intercept	100 \pm 0.817	122.4	<0.001
9 pops	Parental relative seed size	10.37 \pm 13.38	0.775	0.454
Additive	Intercept	100 \pm 0.487	205	<0.001
4 pops	Parental relative seed size	3.34 \pm 4.12	0.81	0.42
	Peduncle	2.73 \pm 2.45	1.12	0.27
	Seed set	0.563 \pm 0.351	1.60	0.11
	Peduncle \times matpop CC	5.62 \pm 3.19	1.76	0.079
	Peduncle \times matpop M	3.87 \pm 2.98	1.30	0.20
	Peduncle \times matpop PM	- 5.30 \pm 3.02	- 1.76	0.080
	Seed set \times matpop CC	- 0.78 \pm 0.36	- 2.15	0.033
	Seed set \times matpop M	0.089 \pm 0.42	0.22	0.83
	Seed set \times matpop PM	- 0.60 \pm 0.32	- 1.86	0.064
	Seed set \times patpop CC	- 0.50 \pm 0.32	- 1.55	0.12
	Seed set \times patpop M	0.11 \pm 0.30	0.36	0.72
	Seed set \times patpop PM	- 0.33 \pm 0.40	- 0.82	0.41

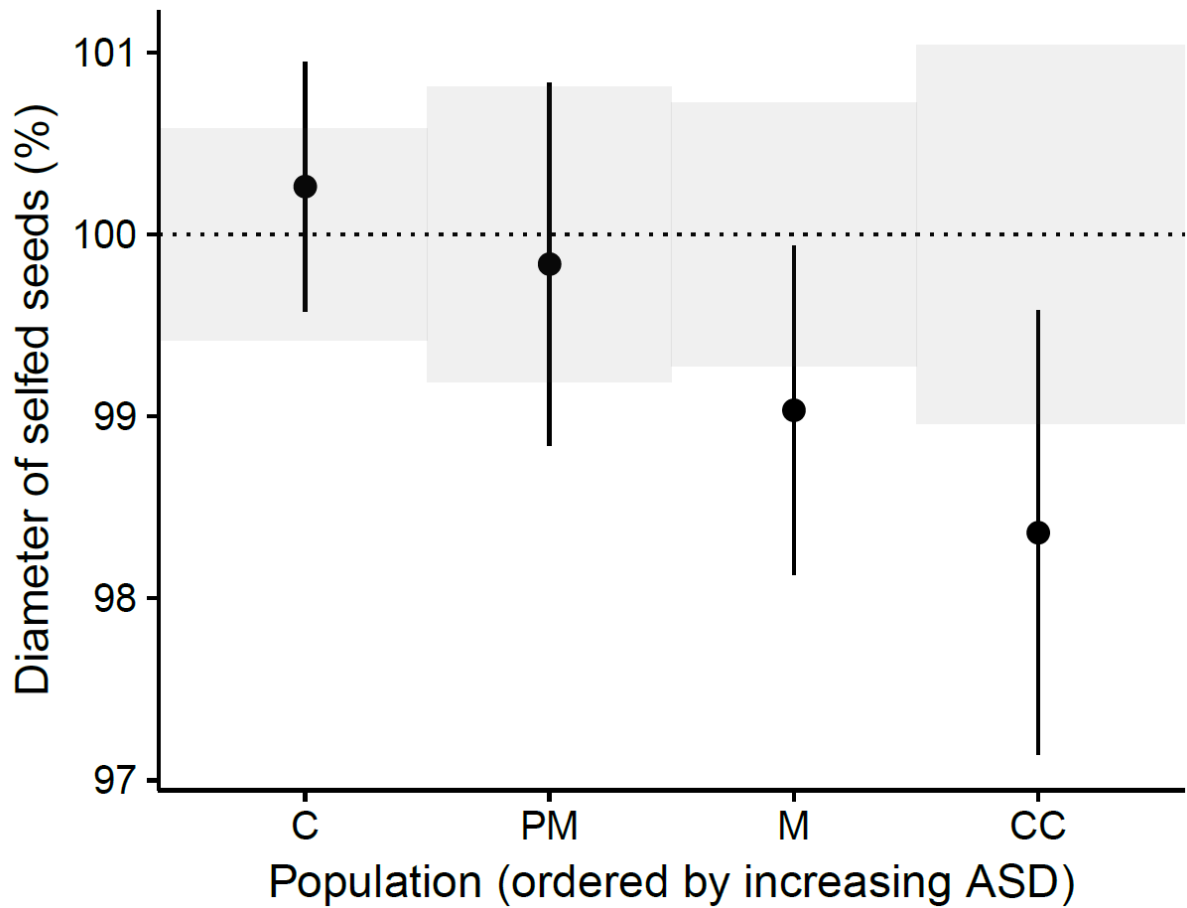


Figure S1. Effect of selfing on hybrid relative seed size (diameter of selfed seeds expressed as a percentage of the average seed diameter of outcrossed seeds within populations; dashed horizontal line) in the four populations of *D. scandens* used in the diallel crosses. Shaded areas represent the standard error in seed size for outcrossed seeds in each population. Means \pm SE for each cross combination were obtained from the linear mixed-effects models fitted for each maternal population (Table S8).

Table S8. Effect of paternal population (C, CC, M, PM) and selfing on seed diameter (mm) in four populations (Pop) of *D. scandens*. For each population, the average seed diameter of outcrossed seeds is given by the intercept of the model while the effects of selfing or paternal population in hybrid crosses are given as a contrast from the intercept. Covariates, peduncle diameter (mm) and seed set (number of seeds per blossom), were centred on the mean of the maternal population. In each model blossom identity nested within maternal plant identity, and paternal plant identity, were set as random factors. Note that although the effects of the covariates were not statistically significant in all populations, we included them in all the models in order to have similar models for all the populations.

Pop	Parameter	Estimate \pm SE	Estimate \pm SE	<i>t</i>	<i>P</i>
C	C outcross (intercept)	3.893 \pm 0.023	100 \pm 0.582	171	<0.001
	C self	0.010 \pm 0.031	0.263 \pm 0.799	0.33	0.74
	PM	0.030 \pm 0.027	0.766 \pm 0.690	1.11	0.27
	M	0.036 \pm 0.028	0.915 \pm 0.710	1.29	0.20
	CC	0.028 \pm 0.028	0.711 \pm 0.706	1.01	0.32
	Peduncle	0.112 \pm 0.063	2.867 \pm 1.612	1.78	0.08
	Seed set	-0.019 \pm 0.016	-0.488 \pm 0.410	-1.19	0.24
	Seed set \times C self	0.010 \pm 0.020	0.250 \pm 0.516	0.88	0.63
	Seed set \times PM	0.028 \pm 0.018	0.712 \pm 0.563	1.54	0.13
	Seed set \times M	0.052 \pm 0.018	1.328 \pm 0.466	2.85	0.01
	Seed set \times CC	0.021 \pm 0.024	0.549 \pm 0.626	0.88	0.38
PM	PM outcross (intercept)	4.305 \pm 0.035	100 \pm 0.810	123	<0.001
	PM self	-0.007 \pm 0.039	-0.163 \pm 0.913	-0.18	0.86
	C	0.035 \pm 0.032	0.823 \pm 0.731	1.13	0.27
	M	0.039 \pm 0.031	0.905 \pm 0.719	1.26	0.22
	CC	0.044 \pm 0.031	1.106 \pm 0.708	1.44	0.16
	Peduncle	-0.098 \pm 0.070	-2.273 \pm 1.626	-1.40	0.17
	Seed set	-0.016 \pm 0.013	-0.366 \pm 0.306	-1.97	0.23
	Seed set \times PM self	0.011 \pm 0.025	0.244 \pm 0.574	0.43	0.67
	Seed set \times C	0.013 \pm 0.016	0.295 \pm 0.366	0.81	0.423
	Seed set \times M	0.027 \pm 0.017	0.628 \pm 0.389	1.62	0.11
	Seed set \times CC	-0.009 \pm 0.017	-0.220 \pm 0.391	-0.56	0.58
M	M outcross (intercept)	4.168 \pm 0.030	100 \pm 0.723	138	<0.001
	M self	-0.040 \pm 0.037	-0.967 \pm 0.875	-1.11	0.27
	C	-0.010 \pm 0.035	-0.239 \pm 0.850	-0.28	0.78
	PM	0.010 \pm 0.033	0.233 \pm 0.790	0.30	0.77
	CC	0.072 \pm 0.034	1.726 \pm 0.810	2.13	0.04
	Peduncle	0.375 \pm 0.062	9.00 \pm 1.478	6.09	<0.001
	Seed set	-0.024 \pm 0.019	-0.578 \pm 0.463	-1.25	0.22
	Seed set \times M self	-0.077 \pm 0.106	-1.843 \pm 2.550	-0.72	0.47
	Seed set \times C	0.132 \pm 0.076	3.165 \pm 1.823	1.74	0.09
	Seed set \times PM	0.009 \pm 0.044	0.214 \pm 1.061	0.20	0.84
	Seed set \times CC	0.038 \pm 0.022	0.914 \pm 0.538	1.70	0.09
CC	CC outcross (intercept)	4.134 \pm 0.043	100 \pm 1.039	96.3	<0.001
	CC self	-0.068 \pm 0.044	-1.640 \pm 1.061	-1.55	0.13
	C	-0.059 \pm 0.037	-1.427 \pm 0.888	-1.61	0.12
	PM	-0.036 \pm 0.036	-0.863 \pm 0.871	-0.99	0.33
	M	-0.050 \pm 0.036	-1.210 \pm 0.871	-1.39	0.17
	Peduncle	0.345 \pm 0.091	8.336 \pm 2.207	3.78	<0.001
	Seed set	0.028 \pm 0.060	0.676 \pm 1.459	0.46	0.64
	Seed set \times CC self	-0.045 \pm 0.066	-1.097 \pm 1.583	-0.69	0.49
	Seed set \times C	-0.027 \pm 0.064	-0.654 \pm 1.554	-0.42	0.68
	Seed set \times M	-0.077 \pm 0.065	-1.830 \pm 1.579	-1.16	0.25
	Seed set \times PM	-0.022 \pm 0.067	-0.524 \pm 1.613	-0.33	0.75

Incompatibility analysis of diallel crosses

To assess possible incompatibilities between populations, we calculated the percentage of crosses that failed to produce seeds, and the percentage of aborted seeds for each cross combination (Table S9). Because each *D. scandens* blossom can produce a maximum of nine seeds, we subtracted the number of viable seeds from nine, to obtain the number of aborted seeds (non-developed and abnormally small seeds). Both the percentage of crosses that failed, and the percentage of aborted seeds were low for all cross combinations, and did not seem to differ between within-population and between-population crosses. Additionally, the average number of viable seeds produced per blossom was high for each cross combination, regardless of cross type (Table S9). These results suggest that there is no incompatibility issues between these populations. When we had replicated a cross between two individuals, we only included data from the first cross performed when assessing incompatibility. In the analyses on seed size, we included the crosses that produced the highest number of seeds.

Table S9. Percentage of crosses that failed to produce seeds, percentage of aborted/abnormal seeds and average number of viable seeds (\pm SE) produced per blossom in the first series of crosses performed among and within four populations of *D. scandens* ($n = 480$ crosses).

Maternal population	Paternal population	Failed crosses (%)	Aborted/abnormal seeds (%)	Avg. number of viable seeds per blossom \pm SE
C	C outcross	6	17.4	7.44 \pm 0.47
	C self	10	13.6	7.78 \pm 0.28
	PM	0	10.7	8.03 \pm 0.31
	M	3	12.7	7.86 \pm 0.32
	CC	1	8.6	8.22 \pm 0.37
PM	PM outcross	5	11.1	8.00 \pm 0.48
	PM self	0	8.9	8.20 \pm 0.51
	C	0	15.9	7.57 \pm 0.37
	M	10	13.2	7.82 \pm 0.43
	CC	3	11.1	8.00 \pm 0.31
M	M outcross	0	12.1	8.35 \pm 0.32
	M self	10	12.4	8.89 \pm 0.11
	C	7	1.33	8.88 \pm 0.07
	PM	3	3.1	8.72 \pm 0.10
	CC	0	8.8	8.21 \pm 0.35
CC	CC outcross	0	4.4	8.60 \pm 0.17
	CC self	0	8.9	8.20 \pm 0.44
	C	0	8.7	8.21 \pm 0.23
	PM	0	4.8	8.57 \pm 0.16
	M	0	5.6	8.50 \pm 0.18