

Supplementary material for

Balao *et al.*

Genetic differentiation and admixture between sibling allopolyploids in the *Dactylorhiza majalis* complex

Table S1. Table of sampling locations of the diploid individuals investigated in the present study. Tetraploid populations within 100m radius from the diploid individuals are given in column “Sympatric 4x”.

Species	Region	N	Latitude/longitude		Sympatric 4x
<i>D. incarnata</i>	Pyrenees	6	42.862N	1.9810E	PYR1
	Alps	2	47.282N	11.185E	ALP11
		2	47.529N	12.579E	ALP13
		2	55.931N	14.068E	SCA2
	Scandinavia	2	57.340N	18.321E	SCA3
		3	57.817N	18.895E	SCA4
		5	57.685N	7.206W	BRI5
	Britain	1	54.670N	2.241W	-
	<i>D. fuchsii</i>	Pyrenees	9	42.894N	1.929E
		1	42.923N	0.803E	-
		1	42.862N	1.981E	PYR1
Alps		2	46.304N	14.426E	-
Scandinavia		1	57.340N	18.321E	SCA3
Britain		1	54.253N	0.685W	BRI1
		1	54.282N	0.690E	BRI2
		3	57.685N	7.206W	BRI5

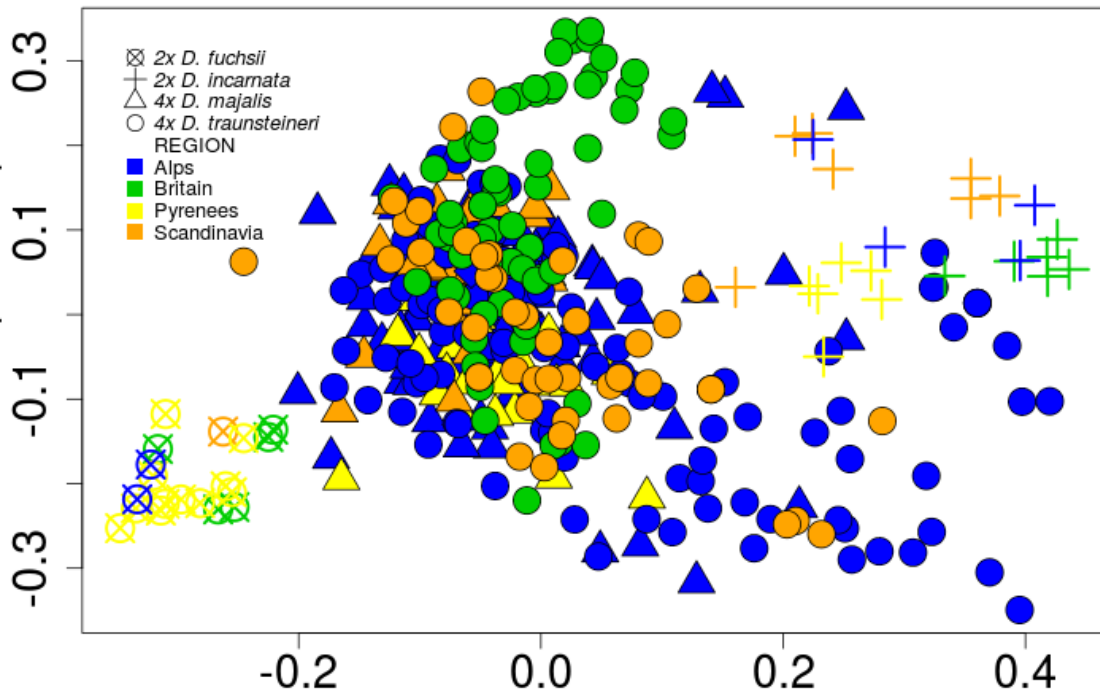
Table S2. Observed (Obs.) and expected (Exp.) frequencies of genotypes of the *Dactylorhiza* allotetraploids (FFFF, FFFI, FFII, FIII, IIII) for loci D2, D52 and D55, and G-tests for tetrasomic inheritance (N = 386). *f* is the frequency of *D. fuchsii* alleles and *i* is the frequency of *D. incarnata* alleles.

	Locus		FFFF	FFFI	FFII	FIII	IIII	<i>f</i>	<i>i</i>	G	<i>p</i>
All	D2	Obs.	0.008	0.003	0.842	0.018	0.130	0.43	0.57	513.87	<0.001
		Exp.	0.035	0.182	0.361	0.317	0.105				
<i>D. majalis</i>	D2	Obs.	0.012	0.006	0.899	0.030	0.054	0.46	0.54	392.75	<0.001
		Exp.	0.047	0.216	0.372	0.284	0.081				
<i>D. traunsteineri</i>	D2	Obs.	0.005	0.000	0.798	0.009	0.188	0.40	0.60	303.71	<0.001
		Exp.	0.027	0.157	0.348	0.342	0.126				
All	D52	Obs.	0.000	0.003	0.334	0.409	0.254	0.17	0.83	224.58	<0.001
		Exp.	0.001	0.016	0.119	0.388	0.476				
<i>D. majalis</i>	D52	Obs.	0.000	0.006	0.429	0.315	0.250	0.22	0.78	61.77	<0.001
		Exp.	0.002	0.033	0.176	0.417	0.372				
<i>D. traunsteineri</i>	D52	Obs.	0.000	0.000	0.261	0.482	0.257	0.13	0.87	121.00	<0.001
		Exp.	0.000	0.008	0.077	0.343	0.572				
All	D55	Obs.	0.018	0.109	0.707	0.109	0.057	0.45	0.55	498.53	<0.001
		Exp.	0.042	0.204	0.368	0.296	0.089				
<i>D. majalis</i>	D55	Obs.	0.006	0.042	0.845	0.071	0.036	0.46	0.54	334.96	<0.001
		Exp.	0.045	0.210	0.370	0.290	0.085				
<i>D. traunsteineri</i>	D55	Obs.	0.028	0.161	0.601	0.138	0.073	0.45	0.55	194.55	<0.001
		Exp.	0.041	0.200	0.367	0.300	0.092				

Table S3. Estimates (and confidence intervals) of effective population sizes (θ) and migration rates (M) for *Dactylorhiza* sibling allopolyploids for the five datasets with Migrate-n. The index $_m$ refers to *D. majalis*, the index $_t$ to *D. traunsteineri*.

	θ_m	θ_t	$M_{m \rightarrow t}$	$M_{t \rightarrow m}$	Bezier logLikelihood
Dataset1	6.8 (0.3-17.6)	2.1 (0.0-4)	2.3 (0.0-8.0)	2.5 (0.0-8.2)	-119203
Dataset2	2.1 (0.0-4.1)	1.8 (0.0-3.73)	3.4 (0.0-9.2)	3.5 (0.0-9.0)	-114331
Dataset3	3.1 (0.0-6.6)	1.8 (0.0-3.73)	6.6 (0.2-12.8)	1.3 (0.0-6.2)	-116191
Dataset4	2.2 (0.0-4.2)	2.40 (0.0-4.73)	4.6 (0.0-10.2)	3.8 (0.0-9.4)	-115098
Dataset5	2.1 (0.0-4.2)	1.9 (0.0-3.86)	3.9 (0.0-9.8)	3.5 (0.0-4.3)	-121336
Mean (SE)	3.29 (0.90)	2.02 (0.11)	4.20 (0.71)	2.93 (0.45)	

Figure S1. Analyses of a reduced dataset, including only the four least variable microsatellite loci (D2, D8, D51, and D81).
A). Principal Coordinates Analysis (PCO) based on the Bruvo distance matrix among 386 *Dactylorhiza* allopolyploids and 42 diploid progenitors. Individuals are labelled according to geographical provenance by different colours: blue, Alps; orange, Scandinavia; yellow, Pyrenees; and green; Britain. Species are indicated by shapes of the symbols: crosses, *D. incarnata*; crossed circles, *D. fuchsii*; triangles, *D. majalis*; and circles, *D. traunsteineri*. In the full dataset of eight SSR loci, the general patterns are retained, but the British *D. traunsteineri* tend to separate more than here from the rest of individuals.



B). Boxplot of population pairwise genetic divergence (pwG_{ST}) between sibling *Dactylorhiza* allopolyploid populations when found in allopatry, in regional sympatry (i.e., in the Alps) and in local sympatry (i.e., within max. 300m from one another). The first boxplot displays the overall variation in pwG_{ST} . Outliers are marked as circles. Medians not significantly different at $p < 0.05$ are indicated by the same letter (non-parametric multiple comparison test). In the full dataset analyses, the regional sympatric pairwise comparisons have been found to be significantly different from allopatric comparisons. This significant difference has been lost when analysing only the 4 least variable markers.

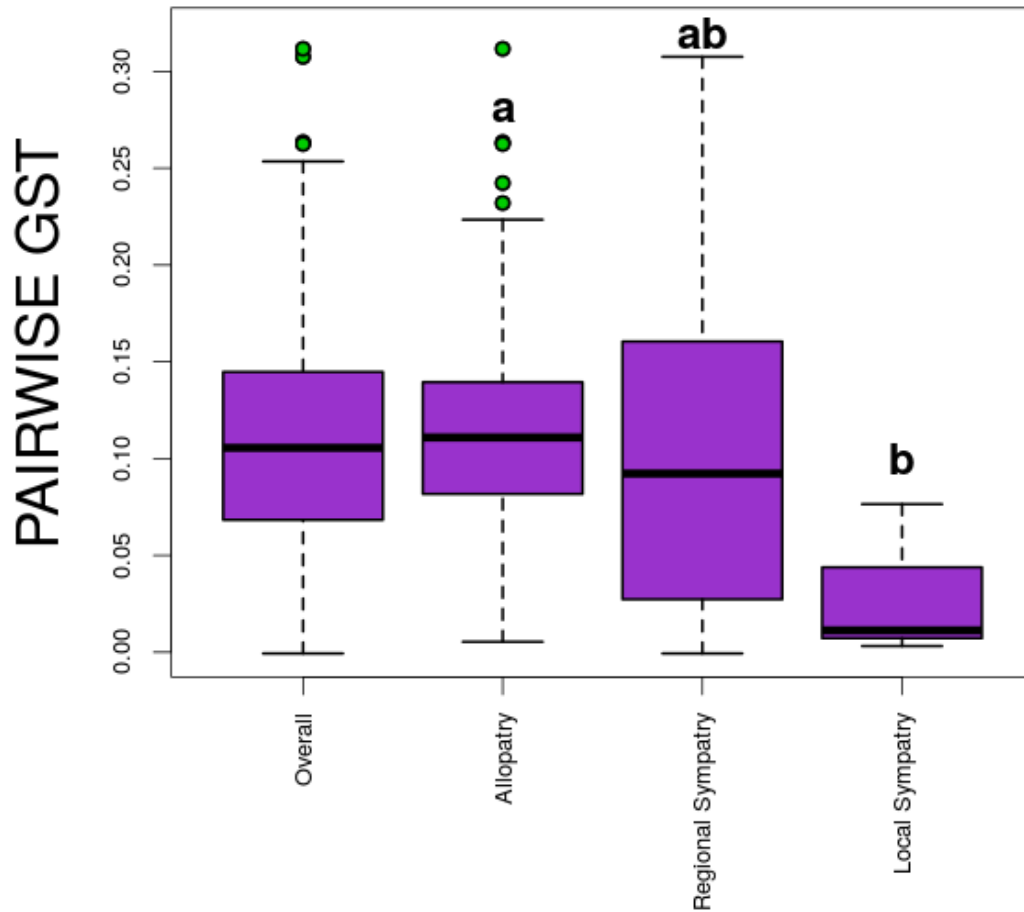


Figure S2. Allelic length distribution per locus for each of the eight loci investigated here across the *Dactylorhiza* allotetraploid and diploid individuals. Bubble size is proportional to the relative frequency of the respective allele.

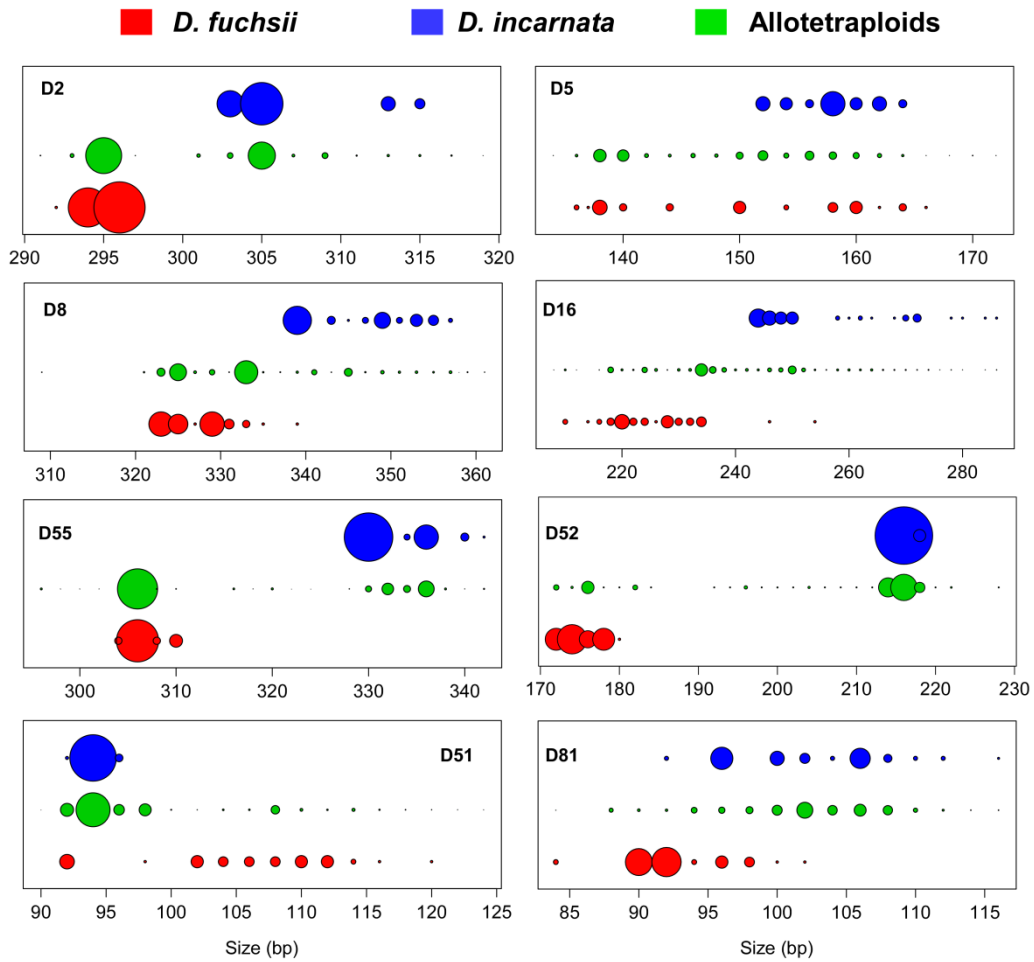
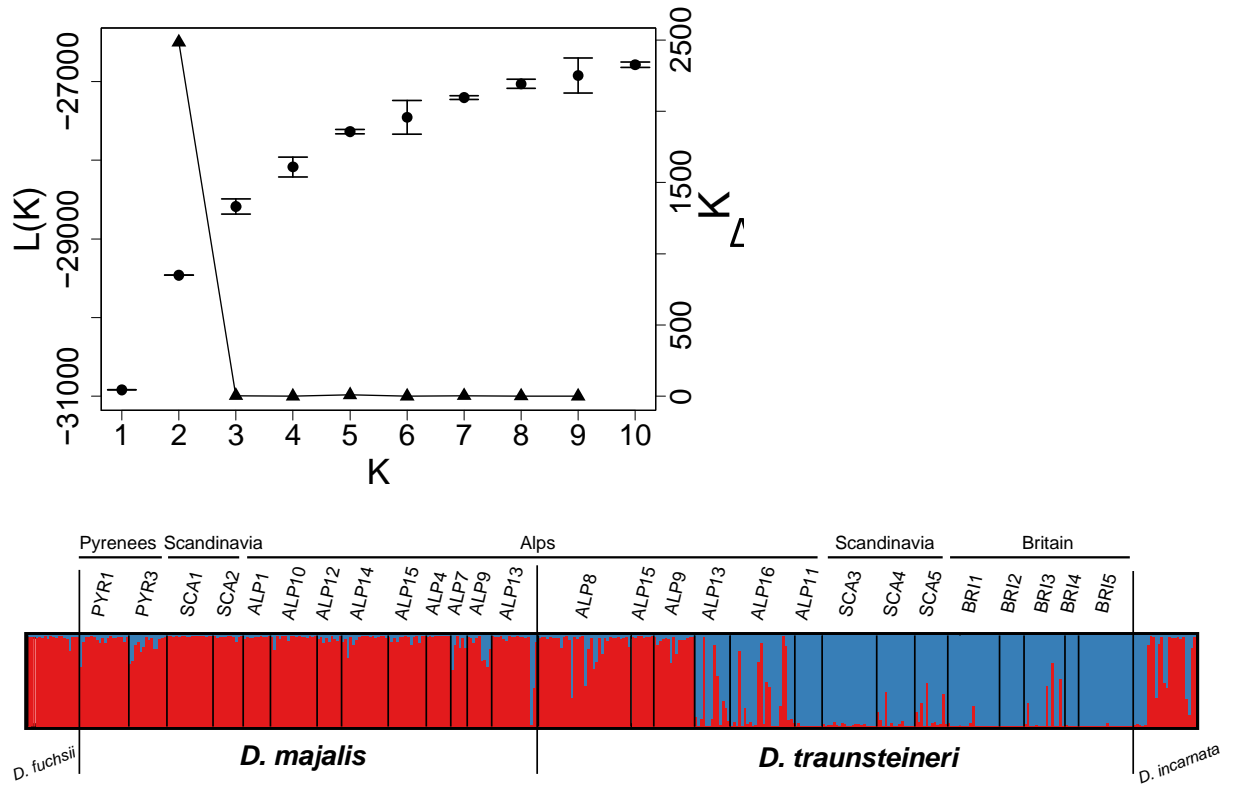


Figure S3. Likelihood (mean \pm SD over 10 replicates) and magnitude of ΔK from Structure analyses calculated following the method proposed by Evanno et al. (2005). **A).** The structure results of the analysis including all 428 individuals, polyploids and diploids.



B). The structure results of the analysis including the 386 polyploids only (for results see Fig. 3).

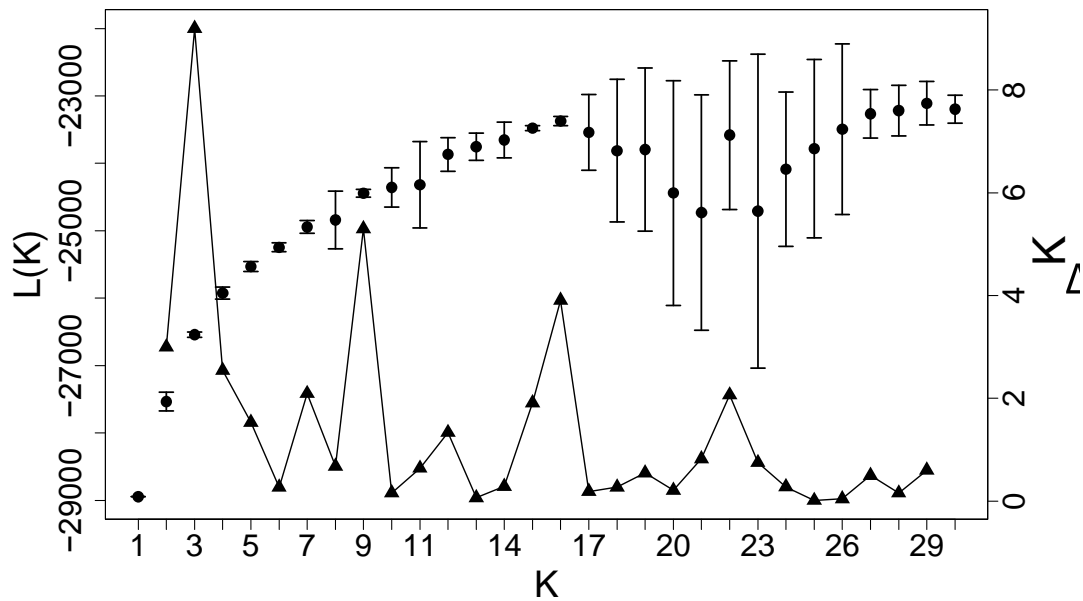
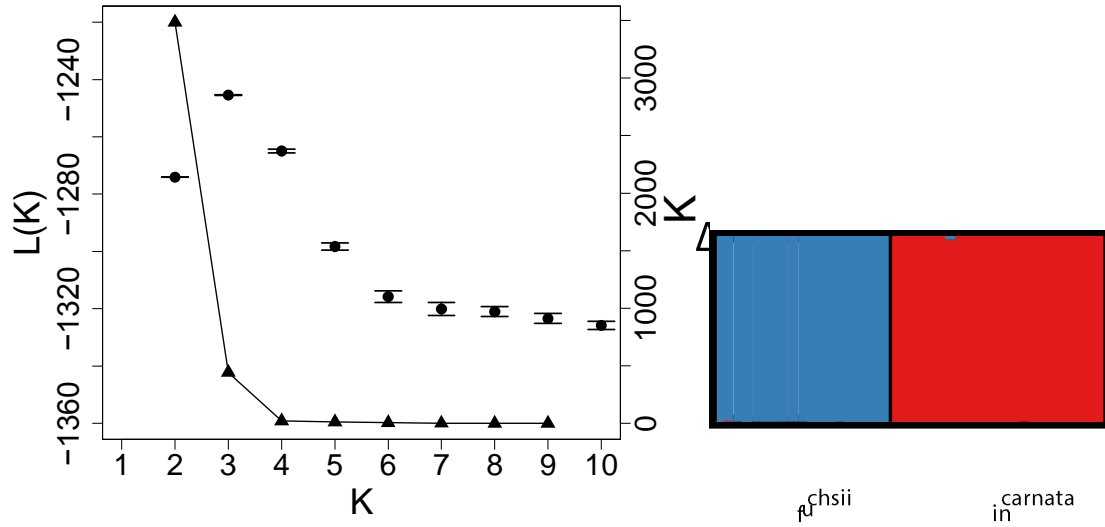


Figure S4. Results of Structure analyses considering only diploid individuals.
A). Structure results of analyses of all diploid individuals, including both *D. fuchsii* and *D. incarnata*.



B). Structure results of analyses of the *D. incarnata* individuals only.

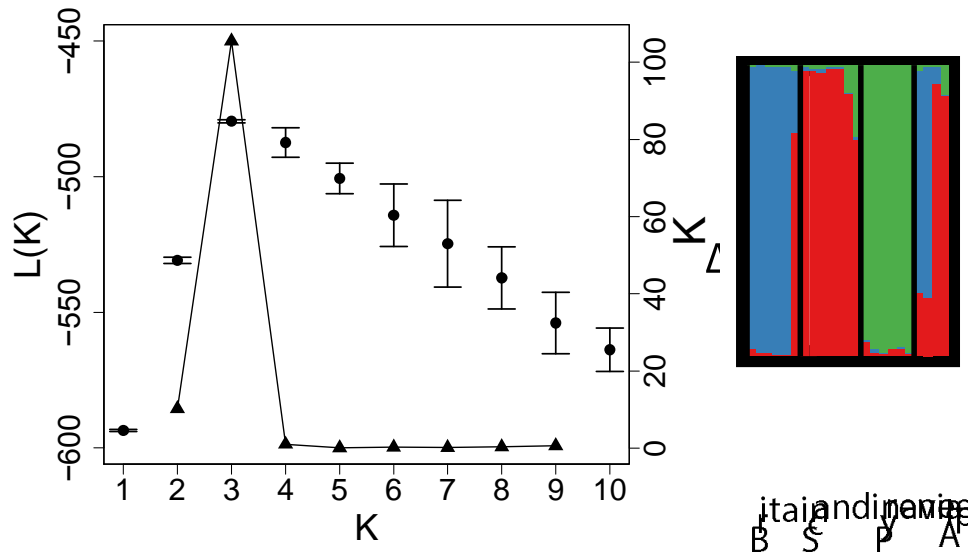


Figure S5. Structure results for each of the three Alpine sympatric populations of *D. majalis* and *D. traunsteineri* for a model bound to $K = 2$. Each bar represents a different individual, whereas each segment length is proportional to the estimated membership for each group. For Alp9 individuals 1429 to 1650 correspond to *D. traunsteineri*, individuals 1651 to 1659 to *D. majalis*. For Alp13 individuals 1619 to 1629, plus 1670 to 1672 correspond to *D. traunsteineri*, and individuals 1630 to 1638, 1660 to 1663 plus 1666 to 1669 correspond to *D. majalis*. Finally, for population Alp15 individuals 1690 to 1697 correspond to *D. traunsteineri*, and 1698 to 1705, plus 1773 to 1778 to *D. majalis*.

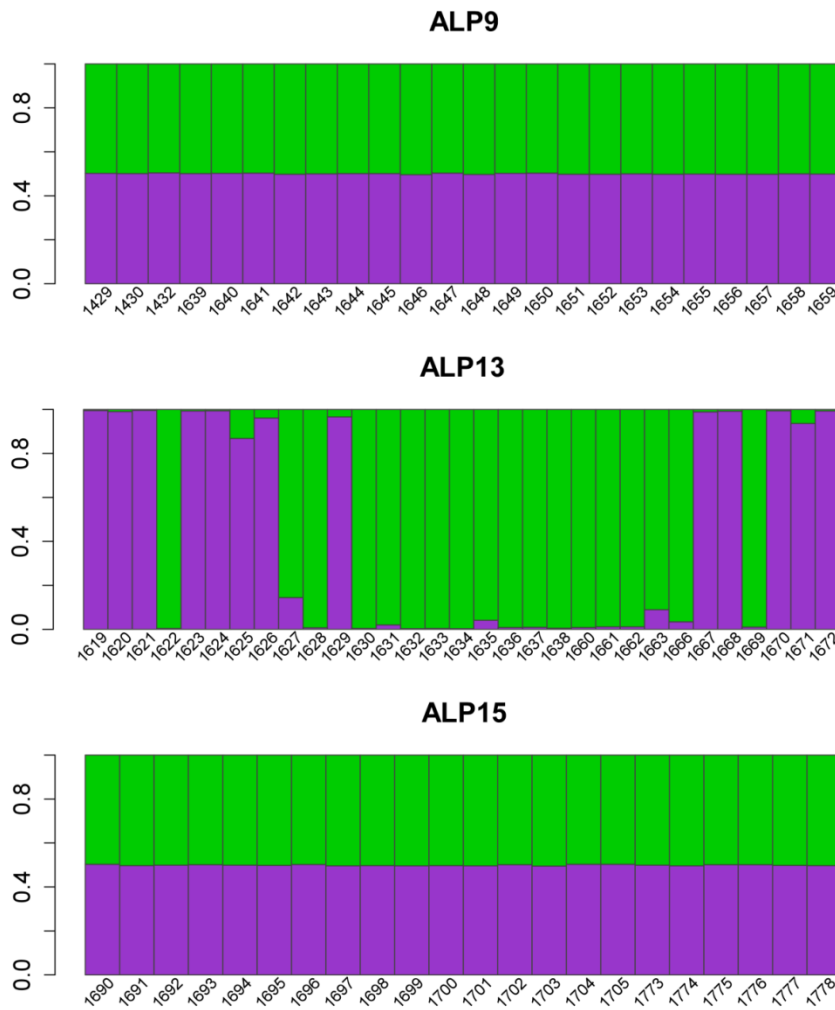


Figure S6. Results of the Reduced Major Axis (RMA) regression between genetic and geographic distances for the two *Dactylorhiza* allopolyploids studied here.

