

Moreira, B., Castellanos, M.C., Pausas, J.G. 2014. Genetic component of flammability variation in a Mediterranean shrub. *Molecular Ecology*, 23:1213-1223.

### Supporting information

**Table S1.** Geographical coordinates (Lat: latitude, Long.: longitude) and summary of the distances (in meters) between the individuals sampled for each site.

Location	Coordinates		Pairwise distances			Distance to the nearest neighbour	
	Lat.	Long.	Mean	Median	Maximum	Mean	Median
Ares del Maestrat	40.41	-0.08	37.39	35.27	130.38	6.52	5.66
Cheste	39.52	-0.62	65.94	64.43	149.95	8.48	7.61
Chiva	39.53	-0.80	217.3	173.27	538.29	16.42	14.31
Sot de Chera	39.60	-0.92	71.85	68.96	171.52	9.26	7.75

**Table S2.** Pairwise geographical distances between sites (in Km).

	Ares del Maestrat	Cheste	Sot de Chera
Cheste	109.8		
Sot de Chera	115.0	27.7	
Chiva	116.1	16.2	12.9

**Table S3.** Code, primer combination, number of markers, range sizes (bp) and scoring error rates for each of the nine combinations used. Primer combinations are indicated by the EcoRI-ANN and MseI-CNN selective nucleotides (where N represents any nucleotide) and dye used to label each EcoRI primer.

Code	Primer combination (Dye)	# markers	Range sizes	Scoring error rate (%)
P1	Eco-ACT/Mse-CAA (6FAM)	70	77-493	1.24
P2	Eco-AGG/Mse-CAA (NED)	36	95-426	3.89
P3	Eco-AAG/Mse-CAT (VIC)	30	94-353	7.56
P4	Eco-ACA/Mse-CTT (6FAM)	44	126-512	0.15
P5	Eco-AGG/Mse-CTA (NED)	31	105-525	0.43
P6	Eco-AAG/Mse-CTC (VIC)	42	116-497	2.06
P7	Eco-ACA/Mse-CTC (6FAM)	59	92-523	0.23
P8	Eco-AAC/Mse-CAC (NED)	34	120-458	0.59
P9	Eco-AAG/Mse-CTA (VIC)	30	119-505	2.22

**Table S4.** Number of individuals and AFLP loci included in each analysis

	Individuals	AFLP loci
Total sampled	169	376
Genetic variation (loci present in $\geq 1\%$ or $\leq 99\%$ of individuals)	169	329
Phenotypic flammability characterization	160	--
Phenotypic-genetic association ( $\geq 5\%$ - $\leq 95\%$ )	160	226
$F_{ST}$ -based analysis ( $\geq 5\%$ - $\leq 95\%$ )	169	220

**Table S5.** Loadings and importance of components for flammability.

		flamPC1	flamPC2
Loadings	Time to ignition	-0.592	0.236
	Mass loss rate	-0.518	~0
	Heat released	0.531	-0.210
	Bulk Density	0.315	0.945
Variance	Standard deviation	1.523	0.926
	Proportion of Variance	0.580	0.215
	Cumulative Proportion	0.580	0.795

**Table S6.** Pairwise  $F_{ST}$  between populations, estimated from 329 AFLP loci (loci present in  $\geq 1\%$  or  $\leq 99\%$  of individuals).

	Ares del Maestrat	Ceste	Sot de Chera
Ceste	0.063		
Sot de Chera	0.070	0.031	
Chiva	0.073	0.025	0.042

**Table S7.** Pairwise  $\Phi_{PT}$  between populations

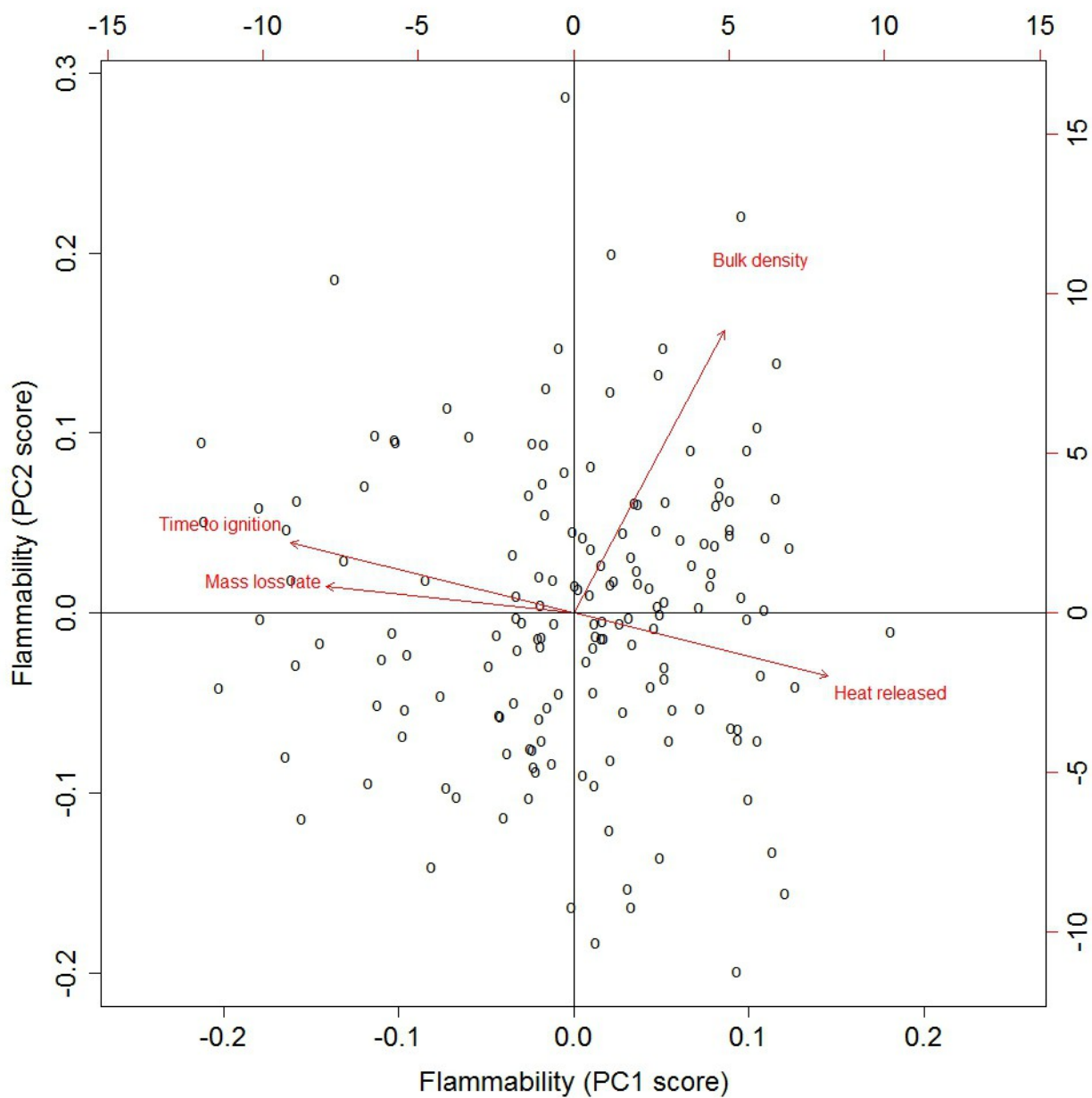
	Ares del Maestrat	Ceste	Sot de Chera
Ceste	0.118		
Sot de Chera	0.103	0.066	
Chiva	0.103	0.045	0.043

**Table S8.** Allelic frequency for each site and results of the independent logistic regressions across individuals of AFLP loci presence/absence against flammability (flamPC1). Only the results for the 16 loci with statistically significant relationships after correction for false discovery rates with the q-value method are shown. Sites are Ares del Maestrat and Cheste (NoFi), Chiva and Sot de Chera (HiFi).

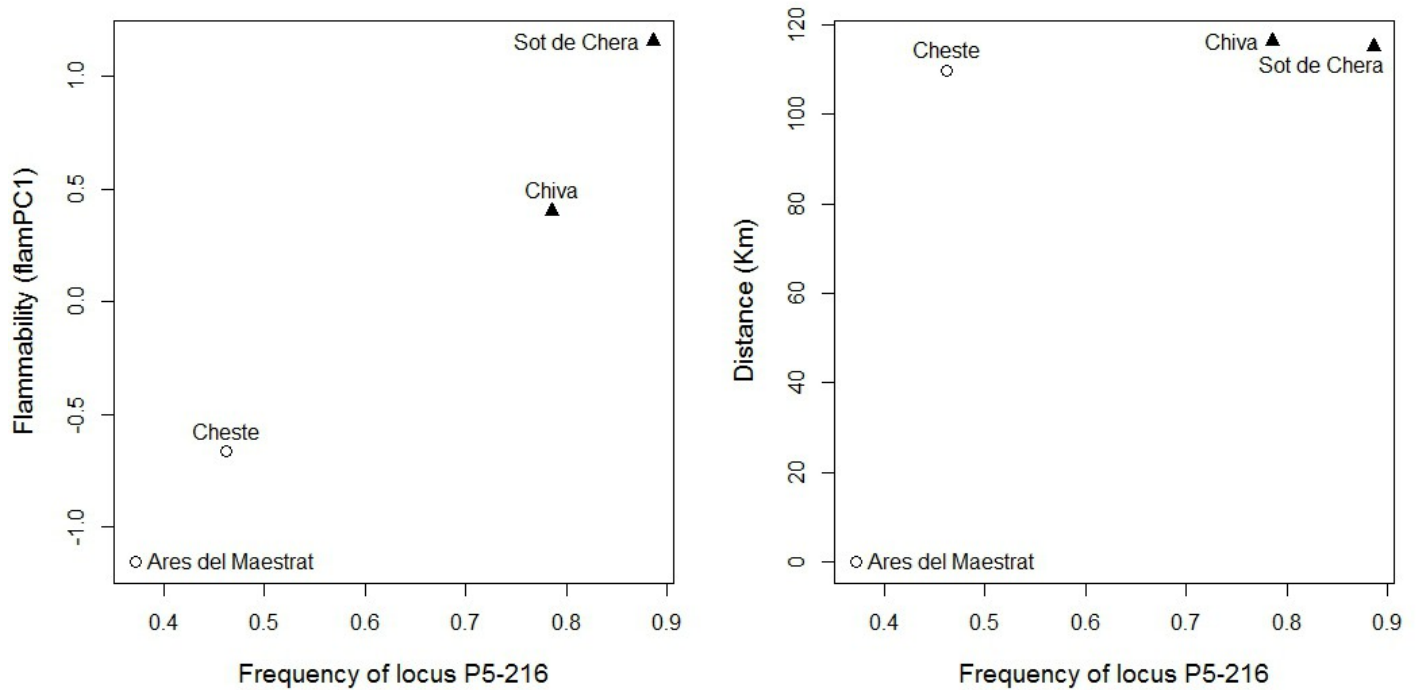
Locus	Allelic frequency				Regression parameters			Bayesian estimation		
	Ares del Maestrat	Cheste	Chiva	Sot Chera	Coef.	se	P	Coef.	95% credible interval	
P2 293	0.06	0.33	0.31	0.50	0.41	0.13	0.001	0.43	0.18	0.73
P2 395	0.37	0.56	0.64	0.77	0.32	0.11	0.003	0.33	0.11	0.57
P3 195	0.51	0.87	0.86	0.95	0.45	0.13	0.001	0.46	0.17	0.73
P3 314	0.11	0.33	0.02	0.05	-0.47	0.15	0.002	-0.48	-0.80	-0.18
P1 199	0.80	0.74	0.67	0.30	-0.33	0.12	0.004	-0.33	-0.59	-0.10
P5 208	0.31	0.08	0.00	0.02	-0.54	0.17	0.001	-0.56	-0.94	-0.24
P5 216	0.37	0.46	0.79	0.89	0.31	0.11	0.005	0.32	0.09	0.56
P5 425	0.26	0.18	0.02	0.07	-0.59	0.16	<0.001	-0.60	-0.94	-0.28
P6 161	0.51	1.00	1.00	1.00	0.64	0.17	<0.001	0.66	0.33	1.02
P4 189	0.43	0.10	0.10	0.14	-0.47	0.14	<0.001	-0.48	-0.76	-0.18
P4 344	0.51	1.00	0.93	1.00	0.56	0.16	<0.001	0.56	0.25	0.89
P8 179	0.09	0.15	0.29	0.45	0.60	0.16	<0.001	0.63	0.31	0.95
P8 213	0.86	0.87	0.98	0.86	0.46	0.16	0.004	0.47	0.14	0.80
P8 228	0.40	0.28	0.24	1.00	0.31	0.11	0.005	0.31	0.10	0.56
P9 151	0.83	0.23	0.29	0.34	-0.33	0.11	0.002	-0.34	-0.56	-0.10
P7 211	0.20	0.08	0.26	0.27	0.41	0.16	0.005	0.44	0.16	0.80

**Table S9.** Allelic frequency for each site and results of the independent logistic regressions across individuals of AFLP loci presence/absence against flammability (flamPC2). Only the results for the 13 loci with statistically significant relationships after correction for false discovery rates with the q-value method are shown. Sites are Ares del Maestrat and Cheste (NoFi), Chiva and Sot de Chera (HiFi).

Locus	Allelic frequency				Regression parameters			Bayesian estimation		
	Ares del Maestrat	Cheste	Chiva	Sot de Chera	Coef.	se	P	Coef.	95% credible interval	
P2 95	0.40	0.38	0.40	0.16	-0.60	0.20	0.002	-0.63	-1.07	-0.20
P2 222	0.09	0.28	0.07	0.02	-0.90	0.31	0.004	-0.94	-1.62	-0.30
P2 381	0.71	0.92	0.93	1.00	1.19	0.35	0.001	1.24	0.62	1.94
P3 289	0.74	0.59	0.55	0.41	-0.62	0.19	0.002	-0.63	-1.01	-0.20
P1 284	0.77	0.13	0.19	0.20	-0.55	0.21	0.005	-0.56	-1.01	-0.15
P1 289	0.63	0.00	0.00	0.00	-1.86	0.39	<0.001	-1.94	-2.78	-1.18
P6 161	0.51	1.00	1.00	1.00	1.15	0.34	<0.001	1.19	0.58	1.90
P6 239	0.37	0.15	0.14	0.05	-0.72	0.26	0.003	-0.74	-1.30	-0.23
P4 344	0.51	1.00	0.93	1.00	0.90	0.30	0.001	0.92	0.33	1.57
P8 137	0.34	0.00	0.02	0.00	-1.21	0.38	<0.001	-1.26	-2.05	-0.50
P8 300	0.54	0.00	0.00	0.00	-1.99	0.43	<0.001	-2.08	-3.06	-1.28
P9 151	0.83	0.23	0.29	0.34	-0.64	0.20	<0.001	-0.67	-1.07	-0.26
P7 365	0.29	0.69	0.48	0.66	0.61	0.19	<0.001	0.63	0.26	1.03



**Figure S1** - Flammability variables (time to ignition, mass loss rate, heat released and bulk density) summarized into two orthogonal axes of variation using a principal components analysis (PCA).



**Figure S2.** Relationship between allelic frequency of locus P5-216 and average flammability (flamPC1 score, left panel, as in Fig. 3 of the main text) and geographic distance (distance of each site to Ares del Maestrat; right panel). Sites are Ares del Maestrat and Cheste (NoFi; in open circles), Sot de Chera and Chiva (HiFi, in closed triangles).

**Protocol S1. AFLP protocol**

We used the AFLP technique to obtain markers across the genome using the technique described by (Vos *et al.*, 1995), with variations including the use of fluorescent primers. Restriction and ligation were performed in separate steps. Genomic DNA (5µl; 0.2-0.5 µg) was digested for 2h at 37°C in a total volume of 10 µl containing 1 µl of 10x Buffer A (Roche), 0.2 µl BSA (20mg/ml), 0.1 µl MseI (5U; New England Biolabs), and 0.25 µl EcoRI (10U; Roche). The restriction digest was then added to 10 µl of ligation mixture composed by 2 µl of 10x Buffer T4 ligase (Roche), 0.2 µl BSA (20mg/ml), 0.25 µl T4 DNA ligase (1.25U; Roche), 1 µl EcoRI-Adapter (5 pmol), 1 µl MseI-Adapter (50 pmol) and incubated for 3h at 37°C. The mix was then diluted with 180 µl double distilled water for a 1:10 final dilution. A preselective PCR was performed using 4 µl of the R-L dilution as template in a final volume of 20 µl containing 2µl of 10x Taq Buffer (Biotools), 2.5 mM MgCl<sub>2</sub>, 0.4 mM of each DNTP, 10 pmol of EcoRI+A primer, 10 pmol MseI+C primer and 1 U of Taq polymerase (Biotools). The thermocycler conditions were programmed as follows: a first step of 2 min at 72°C allowing polymerase to seal the nicks of the ligation step, 20 cycles at 94°C of denaturation for 30 sec, 56°C of annealing temperature for 1 min and a 72°C of extension step for 1 min followed by a final extension of 72°C for 10 min. Several preselective PCR dilutions were tested and finally fixed in a 1:40 dilution with double distilled water. Selective PCR was conducted in a 20 µl final volume containing 3µl of preselective dilution, 2µl of 10x Taq Buffer (Biotools), 2.5 mM MgCl<sub>2</sub>, 0.4 Mm of each DNTP, 10 pmol of MseI+ANN primer, 5 pmol of the fluorescent labelled EcoRI+CNN primer and 1 U of Taq polymerase (Biotools). Thermocycler conditions for this selective PCR step consisted of one step at 94°C for 4 min, 10 cycles at 94°C of denaturation for 30 sec, 66°C of annealing temperature 30 sec with the particularity to decrease one degree each cycle and continuing with 20 cycles at 56°C of annealing temperature. Both preselective and selective PCRs were performed in a Flexcycler 96 thermocycler, Control samples were included in each PCR reaction.