Spatial patterns of selectively neutral genetic structure and herbicide resistance in UK populations of *Alopecurus myosuroides*

Andrea Dixon, David Comont, Gancho T. Slavov & Paul Neve

Supporting Information

Table S1. Raw and recovered reads from 380 blackgrass individuals sequenced using RAD-seq with the *PstI* restriction enzyme across four plates pooled across four lanes of a NextSeq 500.

	Raw reads			Reads with good barcodes		
	No. of reads	SD	Range	No. of reads	SD	Range
Average number of reads per lane	256,208,097	66,367,779	144,145,990-314,373,265	226,470,278	58,491,274	128,212,242-279,773,706
Average number of reads per plate	256,208,097	27,123,761	227,300,692-283,631,306	226,470,278	23,729,673	200,570,031-257,241,003
Total number of reads across all plates and lanes	1,024,832,390			905,881,114		

Table S2. Tag and SNP generation from the UNEAK pipeline and filtering using VCFtools. The corresponding UNEAK plug-in is denoted by superscripts.

UNEAK pipeline results	
Merged tag counts[1]	5,606,854 tags covered by 857,464,002 matching reads
Tag count to tag pair[2]	391,667 reciprocal tag pairs
Tag pairs to TBT[3]	783,334 haplotypes
Final number of SNPs[4]	26,476 SNPs
VCFtools filtering	
Remove individuals with > 45% missing data across loci	369 individuals remain
Remove loci with MAF < 1% of > 20% missing data	22,862 loci remain
Remove loci in linkage disequilibrium ($r^2 > 0.8$)	22,849 loci remain
Remove loci that deviate from HWE ($P < 0.01$)	20,426 loci for final analysis

[1] UMergeTaxaTagCountPlugin
[2] UTagCountToTagPairPlugin
[3] UTagPairToTBTPlugin
[4] UMapInfoToHapMapPlugin

Table S3. Phenotypic variation in dry weight (natural log transformed) of herbicide treated plants, analysed using a linear mixed modelling approach. Results show the estimated phenotypic variance associated with each factor (entered as random effects within the model). Estimated 95% confidence limits are given, with significance attributed to intervals which do not include zero.

	Mesosulfuron + iodosulfuron			Cycloxydim			Fenoxaprop		
Source	Variance	Lower 95% CI	Upper 95% CI	Variance	Lower 95% CI	Upper 95% CI	Variance	Lower 95% CI	Upper 95% CI
Pop*Dose	0.22	0.14	0.30	0.34	0.21	0.47	0.06	0.03	0.09
Population	0.00	0.00	0.08	0.00	0.00	0.13	0.00	0.00	0.03
Position	0.00	0.00	0.00	0.02	0.00	0.05	0.00	0.00	0.00
Tray	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00
Glasshouse	0.00	0.00	0.04	0.03	0.00	0.21	0.00	0.00	0.04
Residual	0.08	0.07	0.10	0.15	0.12	0.19	0.06	0.05	0.08

Table S4. Phenotypic variation in mortality of herbicide treated plants, analysed using a linear mixed modelling approach. Results show the estimated phenotypic variance associated with each factor (entered as random effects within the model). Estimated 95% confidence limits are given, with significance attributed to intervals which do not include zero.

	Mesosulfuron + iodosulfuron			Cycloxydim			Fenoxaprop		
Source	Variance	Lower 95% CI	Upper 95% CI	Variance	Lower 95% CI	Upper 95% CI	Variance	Lower 95% CI	Upper 95% CI
Pop*Dose	815.80	534.04	1090.7	545.50	350.91	736.46	303.52	190.18	414.61
Population	0.00	0.00	265.76	0.00	0.00	157.84	0.33	0.00	106.58
Position	0.00	0.00	7.98	1.30	0.00	14.95	1.23	0.00	14.21
Tray	6.88	0.00	26.19	0.00	0.00	8.80	0.00	0.00	8.80
Glasshouse	0.00	0.00	6.61	0.37	0.00	18.99	0.00	0.00	7.00
Residual	152.30	130.25	179.54	118.90	96.73	148.13	106.55	86.61	132.75

Table S5. Genetic correlation between BLUPs, computed from linear mixed model analysis of herbicide phenotyping data. Correlation shown are for two phenotypic measures; dry weight and mortality, in either herbicide treated or untreated (Control) treatments, for the herbicides Mesosulfuron + iodosulfuron (M+I), Fenoxaprop (Fen), and Cycloxydim (Cyc).

		Herbicide treated biomass			Herbicide treated mortality			Control biomass			Control mortality		
		M+I	Cyc	Fen	M+I	Cyc	Fen	M+I	Cyc	Fen	M+I	Cyc	Fen
Herbicide treated biomass	M+I	1.00											
	Cyc	0.50	1.00										
	Fen	0.41	0.61	1.00									
Herbicide treated mortality	M+I	-0.94	-0.52	-0.49	1.00								
	Cyc	-0.42	-0.90	-0.56	0.44	1.00							
	Fen	-0.36	-0.67	-0.89	0.46	0.59	1.00						
Control biomass	M+I	-0.03	-0.01	0.06	0.06	-0.05	-0.07	1.00					
	Cyc	0.09	-0.03	-0.17	-0.04	0.04	0.05	0.14	1.00				
	Fen	0.09	-0.14	-0.14	-0.01	0.15	0.25	0.31	0.03	1.00			
Control mortality	M+I	0.14	0.08	0.10	-0.12	-0.10	-0.09	0.13	0.02	0.16	1.00		
	Cyc	-0.08	0.09	0.17	0.07	-0.11	-0.10	-0.06	-0.55	0.06	-0.19	1.00	
	Fen	-0.03	0.19	0.20	-0.01	-0.17	-0.07	0.24	-0.18	0.28	0.03	0.11	1.00

Table S6. Summary of the genetic analyses performed in the study and the populations that were excluded from each analysis, if applicable.

Analysis	Populations excluded
smartPCA	128 and 163*
AMOVA	N/A
RDA	128, 163, and 200
Population level GWAS	128, 163, and 200

*A smartPCA with all 47 populations was performed, but the smartPCA excluding population 128 and 163 was used for all downstream analysis

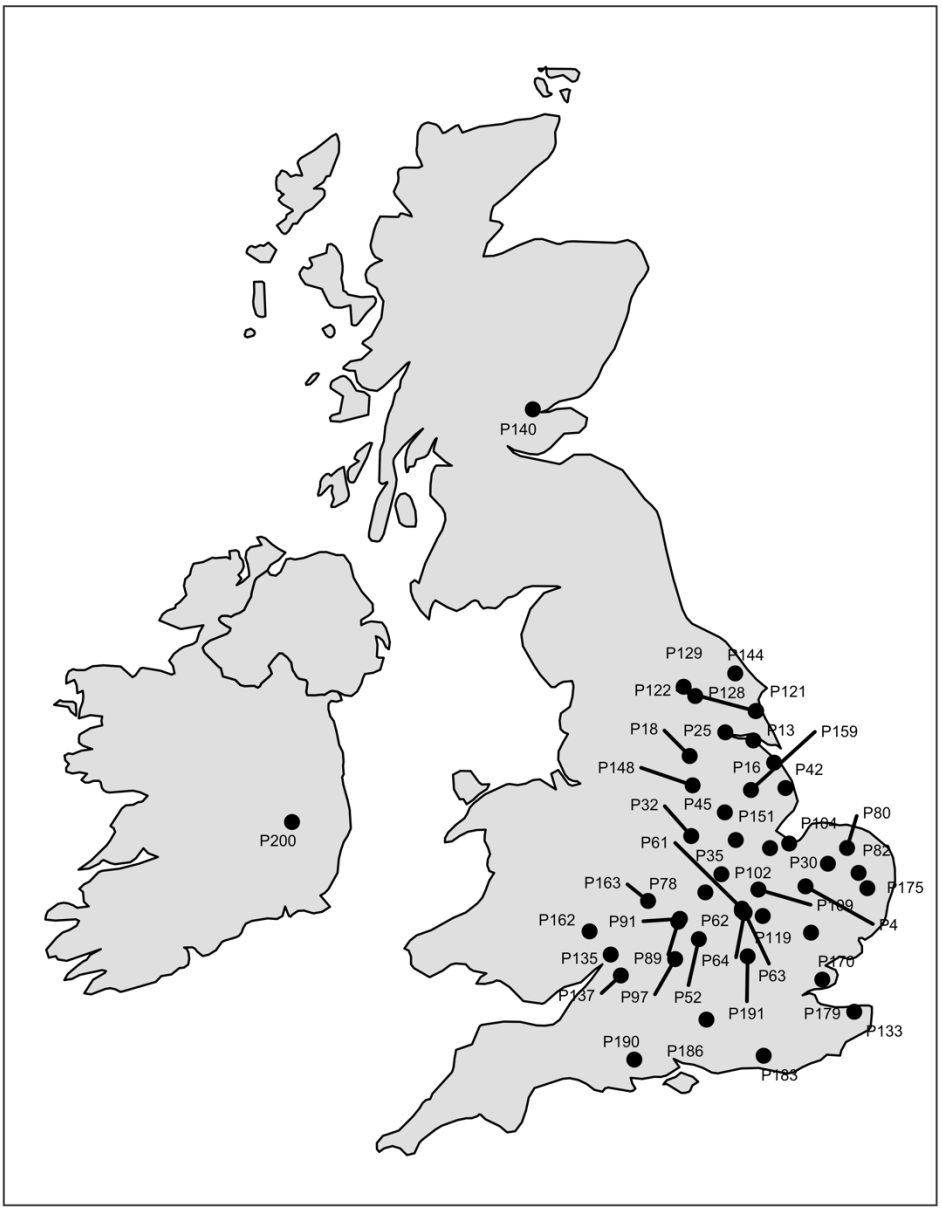

Figure S1. Map illustrating the sampling locations of the 47 *Alopecurus myosuroides* populations used in this study.

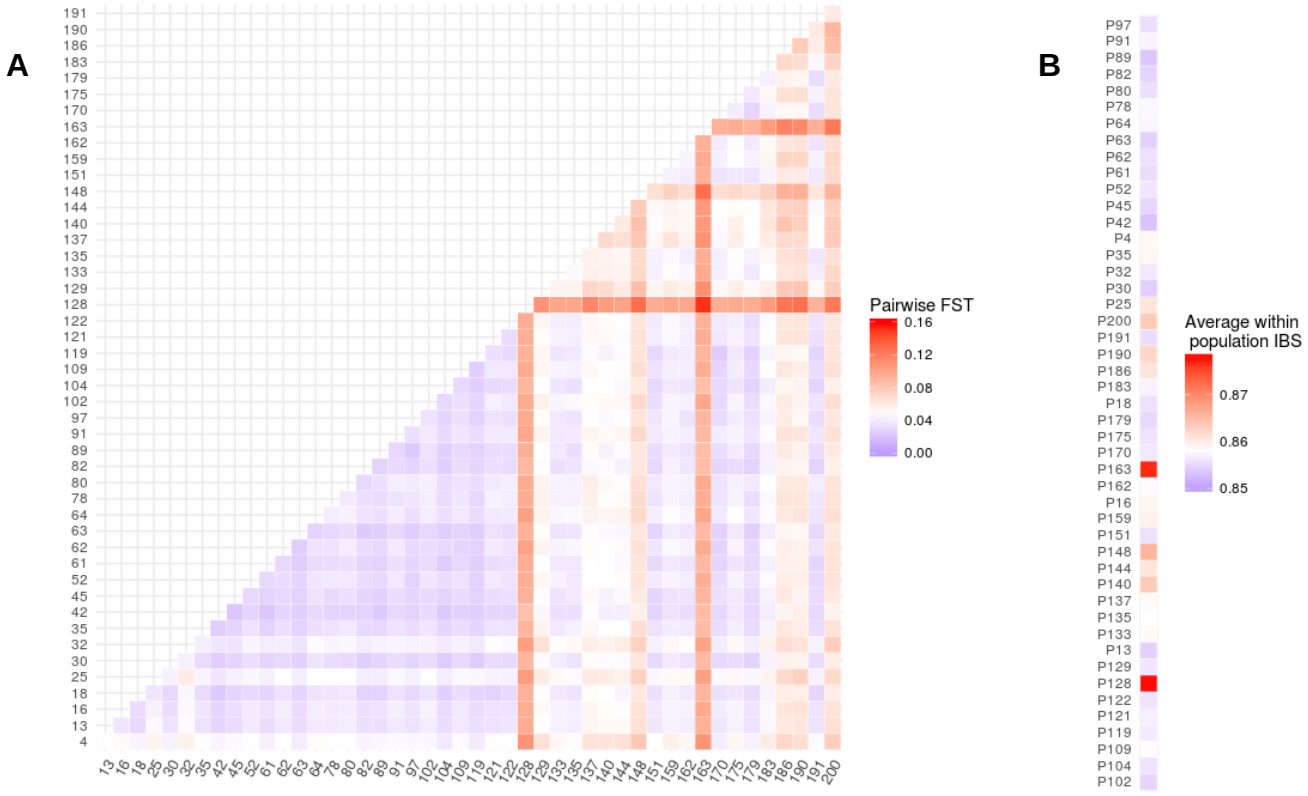

Figure S2. (A) The pair-wise F_{ST} for all 47 populations from Eigenstrat. The pair-wise F_{ST} value is reported as a color with increasing differentiation from blue to red. (B) The proportion of relatedness of each population as determined by an allele-sharing kinship matrix. The proportion is reported in the cells, and the color indicates increasing relatedness from blue to red.

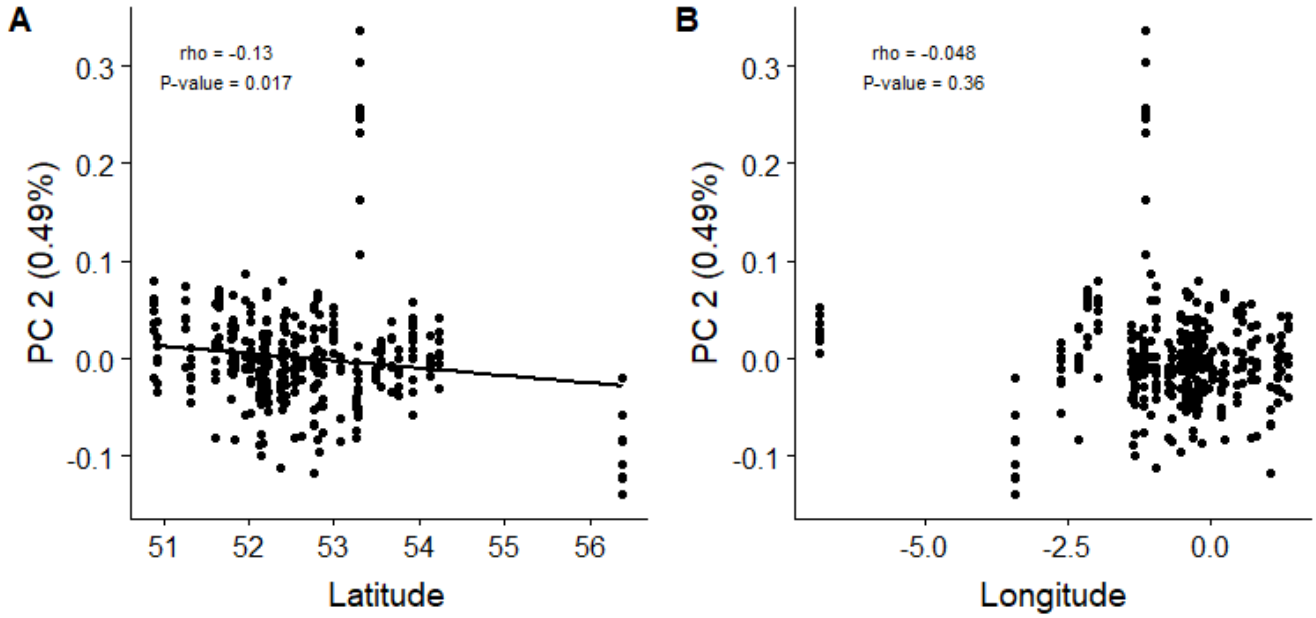

Figure S3. Spearman rank correlations between PC2 of the smartPCA excluding populations P128 and P163 (n = 45) and latitude (A) and longitude (B).

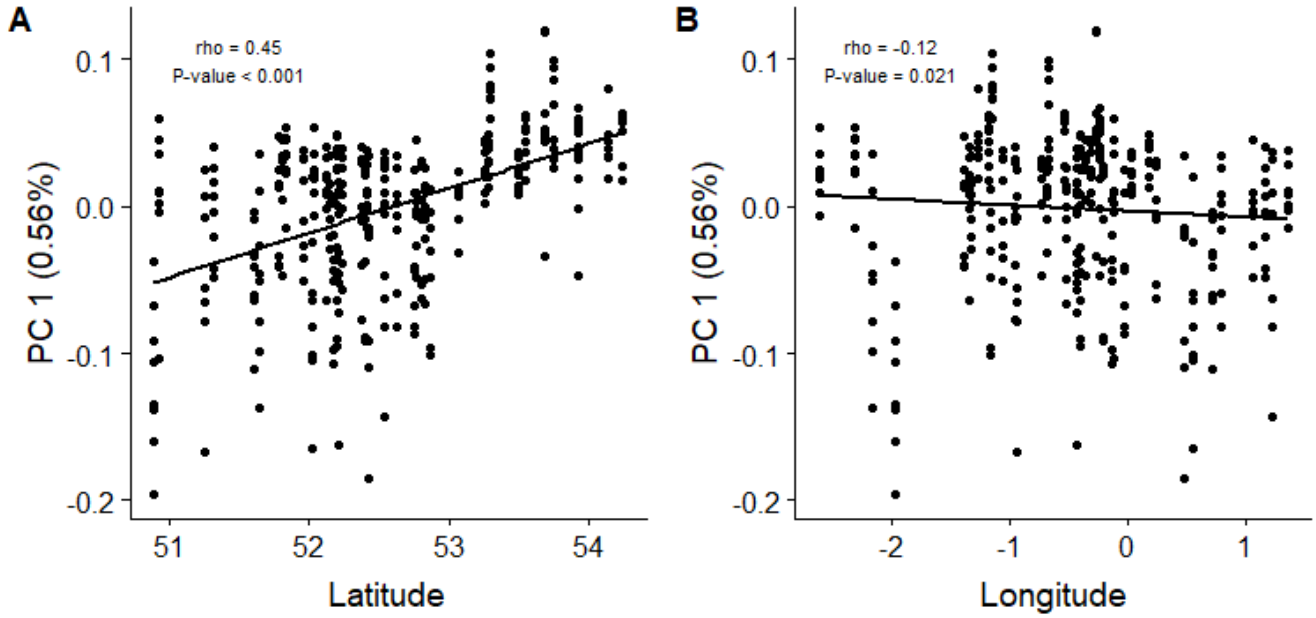

Figure S4. Spearman rank correlations between PC1 of the smartPCA for the English populations, excluding the Irish and Scottish populations and populations P128 and P163 (n = 43) and latitude (A) and longitude (B).

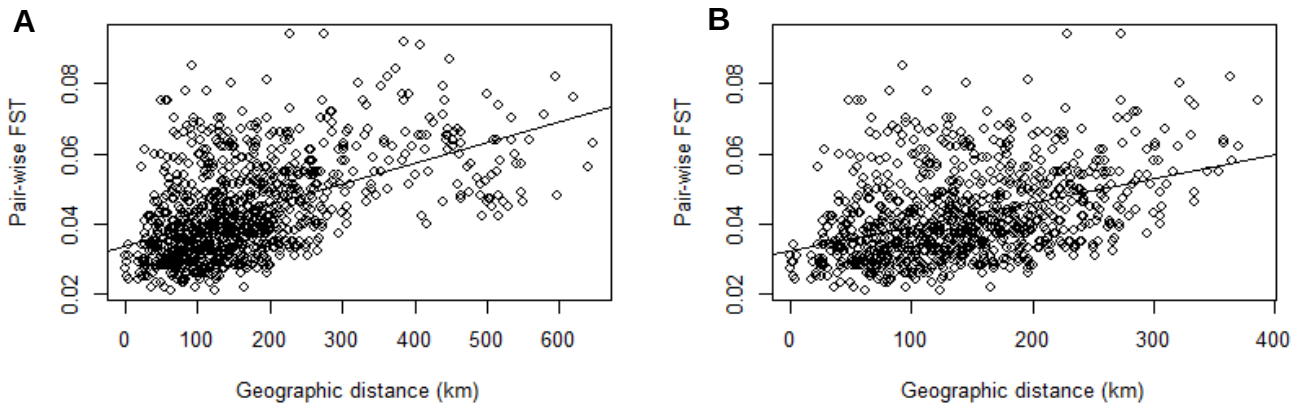

Figure S5. The pair-wise F_{ST} regressed against pair-wise geographic distance in kilometers for 45 populations A) after removing population P128 and P163 (Mantel r = 0.50, $P < 0.001$ and B) for all English populations (n = 43) (Mantel r = 0.41, $P < 0.001$; B).

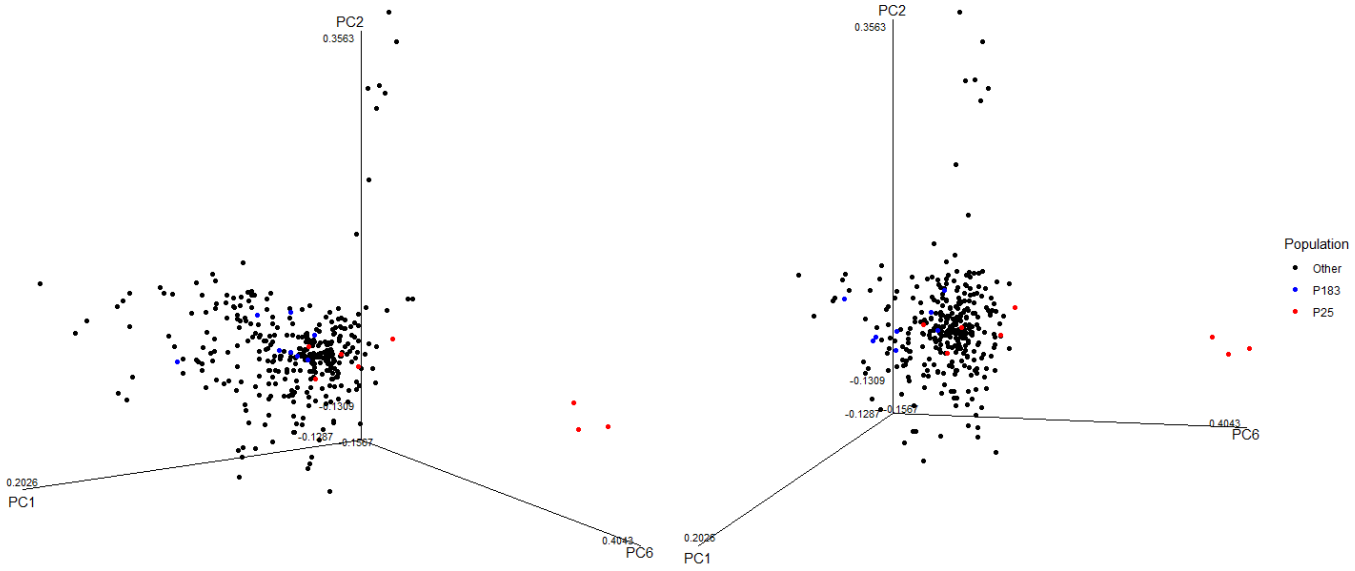

Figure S6. 3-D scatter plot of PCs 1, 2, and 6 from the smartPCA including 44 populations (excluding P128, P163, and P200 [for which no resistance phenotype data was available]). PC6 was found to significantly explain population-level phenotypic BLUPs for mortality and biomass in all three RDA models assessing resistance to fenoxaprop, mesosulfuron + iodosulfuron, and cycloxydim.