

Supplementary material

Distinct spatial patterns of genetic structure and diversity in the butterfly Marbled White (*Melanargia galathea*) inhabiting fragmented grasslands

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Table S1 Most recent PCR protocol for nuclear microsatellite markers of *Melanargia galathea*, following in Schmid et al. (2016).

Step	Temperature	Duration	Repetitions
Polymerase activation	95 °C	5 min	1 x
Denaturation	95 °C	30 s	
Annealing	60 °C	90 s	32 x
Elongation	72 °C	30 s	
Final elongation	60 °C	60 min	1 x

Table S2 Most recent PCR mix for Multiplex 1 and 2 used for primer application in a 10-µl PCR approach (Schmid et al. 2016).

Ingredient	Concentration of working solution [µM]	Amount of working solution [µl]	End concentration [µM]
<i>Multiplex 1</i>			
H ₂ O		2.02	
Qiagen Type-it	2x	5.00	1x
C2S5_F_FAM (MeGa_1_FAM)	10	0.04	0.04
C2S5_R (MeGa_1_F)	10	0.04	0.04
952H_F_ATTO532 (MeGa_2_ATTO532)	10	0.40	0.40
952H_R (MeGa_2_R)	10	0.40	0.40
BBJK_F_ATTO565 (MeGa_3_ATTO565)	10	0.05	0.05
BBJK_R (MeGa_3_R)	10	0.05	0.05
DNA	(~2ng/µl)	2.00	(~2ng/µl)

Multiplex 2

H ₂ O		1.84	
Qiagen Type-it	2x	5.00	1x
7QTP_F (MeGa_5_F)	10	0.12	0.12
7QTP_R_FAM (MeGa_5_R_FAM)	10	0.12	0.12
522M_F (MeGa_4_F)	10	0.36	0.36
522M_R_FAM (MeGa_4_R_FAM)	10	0.36	0.36
7QTP_F (MeGa_5_F)	10	0.10	0.10
48TK_R_ATTO532 (MeGa_6_R_ATTO532)	10	0.10	0.10
DNA	(~2ng/μl)	2.00	(~2ng/μl)

Table S3 General setting of empirical kriging in ArcMap 10.7 applied to infer rarefied allelic richness in *Melanargia galathea* across Switzerland.

General properties	
Subset size	100
Overlap factor	2
Number of simulations	100
Transformation	Empirical
Semivariogram type	K-Bassel
Search Neighbourhood	
Neighbourhood type	Standard circus
Maximum neighbours	15
Minimum neighbours	10
Sector type	1 sector

Table S4 Prediction errors of the empirical kriging in ArcMap 10.7 for the genetic diversity (rarefied allelic richness), average change rate per year and standard deviation of genetic diversity models

Model predictor errors						
Model	Samples	Mean	Root-mean-square	Mean standardised	Root-mean-square standardized	Average standard error
Genetic diversity	303	0.002	0.341	-0.010	0.987	0.341
Average change rate per year	232	-0.007	0.120	-0.001	0.990	0.126
Standard deviation	232	-0.001	0.119	0.003	0.981	0.113

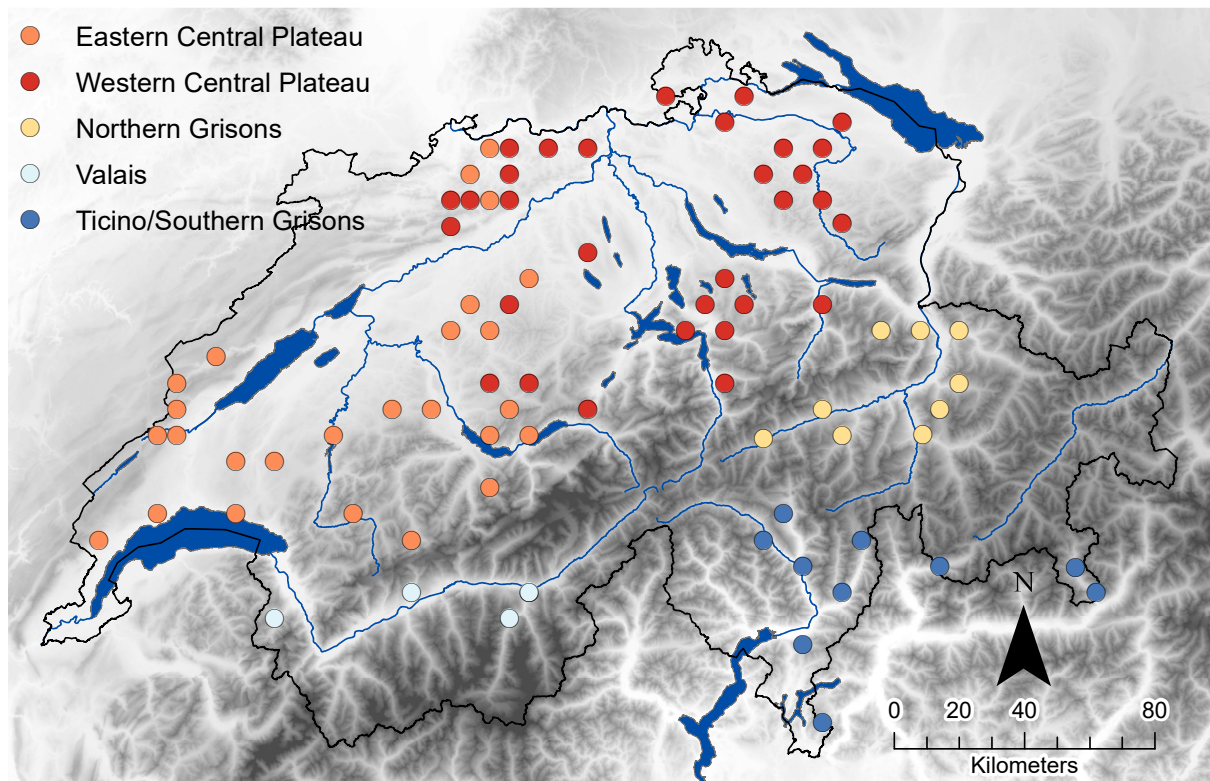


Figure S1 81 sampling locations, in which more than 10 individuals were sampled, were assigned to a cluster obtained by the K=5 STRUCTURE result according to its highest assignment probability.

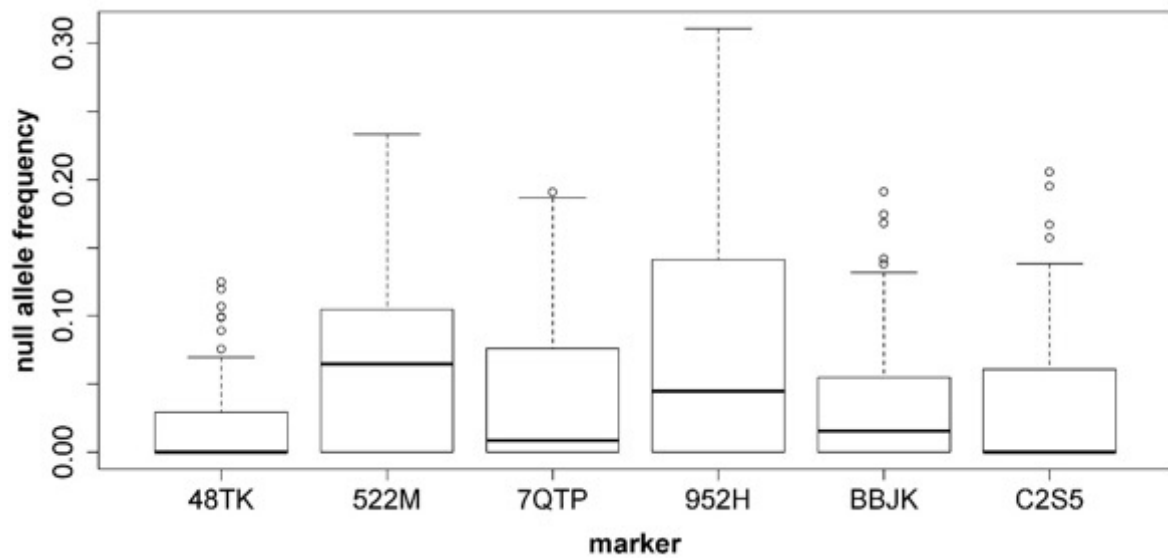


Figure S2 Boxplots of estimated null allele frequencies for the six nuclear microsatellite markers in each of the 81 sampling locations where 10 or more individuals of *Melanargia galathea* were sampled.

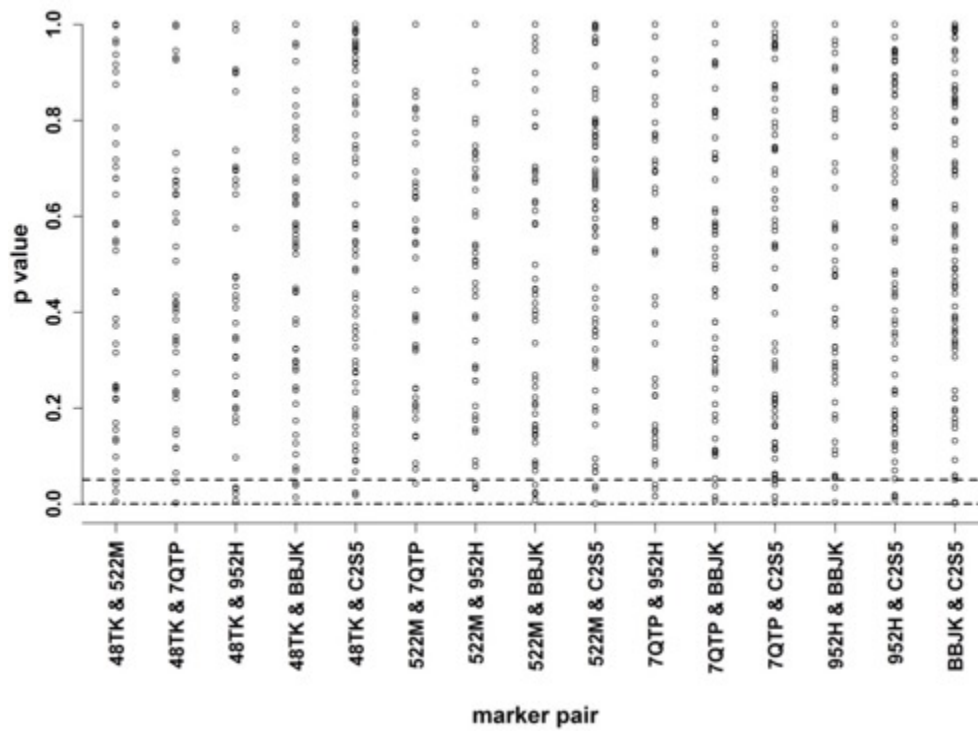


Figure S3 P values of the tests for linkage disequilibrium between marker pairs (H_0 : independence of markers) in each of the 81 sampling locations where 10 or more individuals of *Melanargia galathea* were sampled. The upper dashed line indicates the 5%-significance level and the lower dashed line the respective significance level after Bonferroni correction.

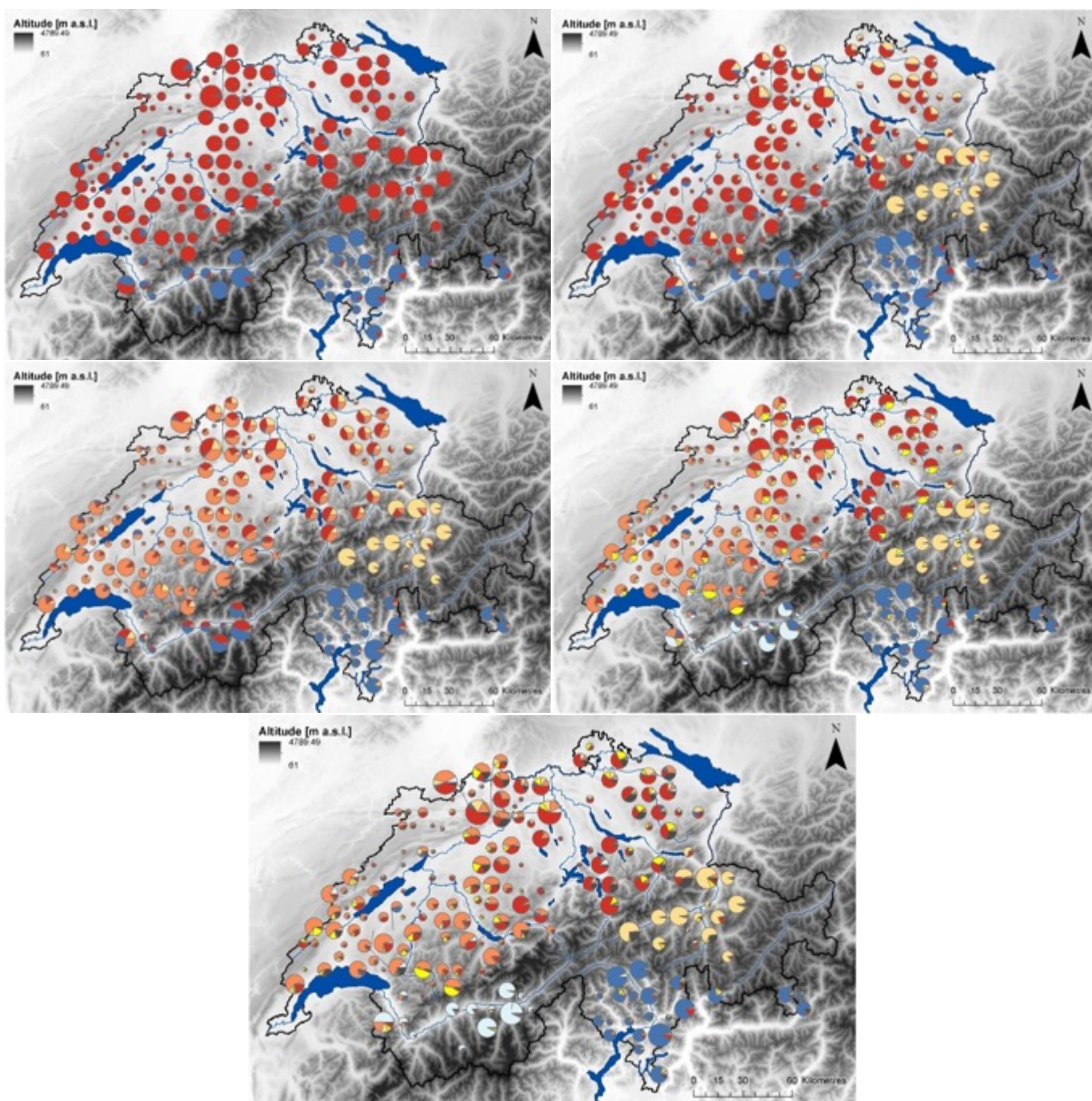


Figure S4 STRUCTURE result of 189 sampling locations of *Melanargia galathea* for $K=2$ (top left), $K=3$ (top right), $K=4$ (middle left), $K=6$ (middle right) and $K=7$ (bottom). The proportion in a pie chart indicates the assignment probability for each sampling location belonging to a specific cluster. Pie charts are scaled according to the sample size.

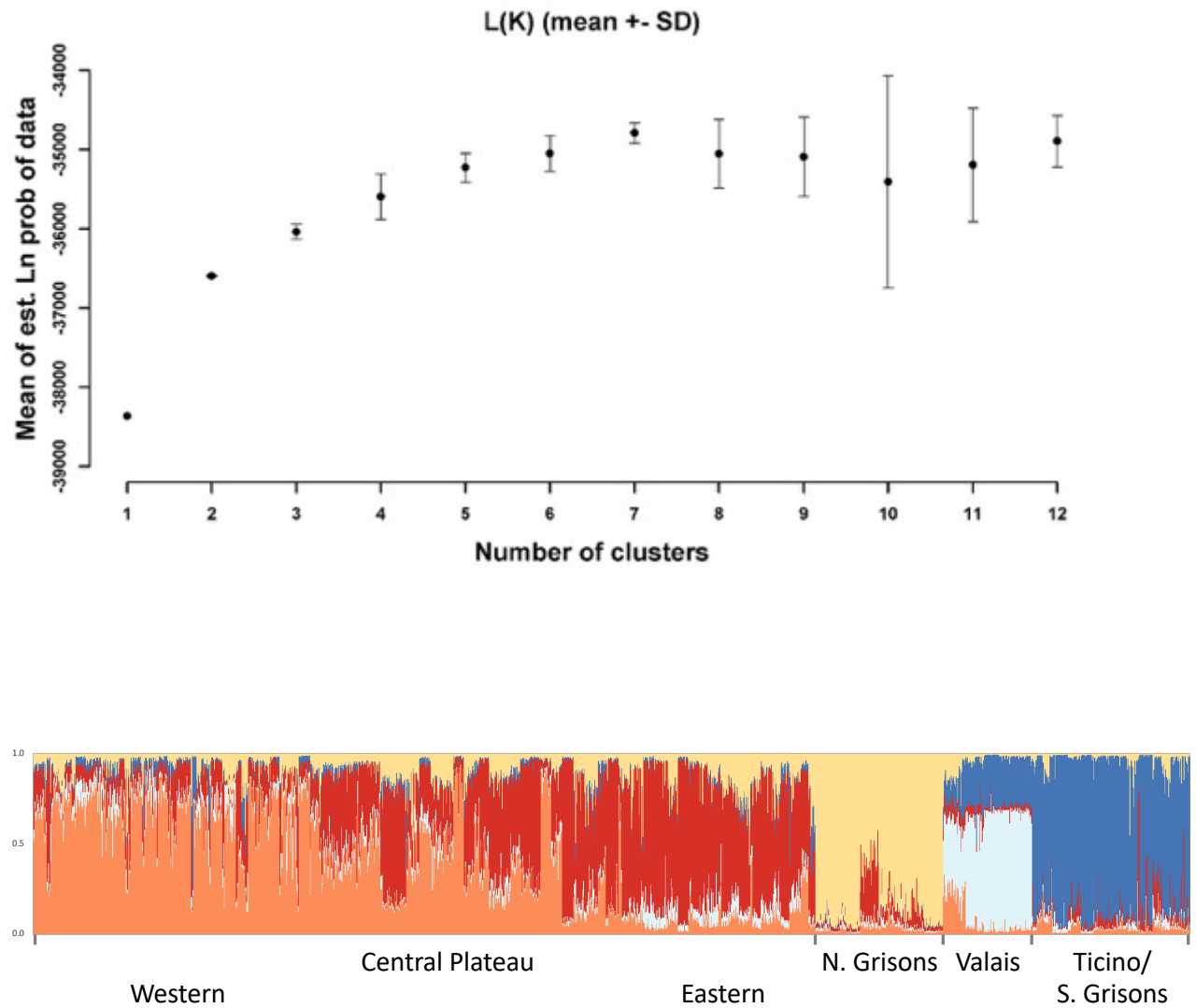


Figure S5 STRUCTURE analysis of *Melanargia galathea* based on 189 sampling locations in Switzerland Top: The mean likelihood estimates (\pm standard deviation) for each K ranging from 1 to 12 with 10 repetitions each. Bottom: Barplot of individual assignment probabilities for K=5, complementing population-wise assignment shown in Fig. 2.

Reference

Schmid, M., D. Csencsics, and F. Gugerli. 2016. Repetitive flanking sequences challenge microsatellite marker development: a case study in the lepidopteran *Melanargia galathea*. *Molecular Ecology Resources* **16**:1499–1507.