

**Lack of population-specific patterns of major histocompatibility complex (MHC) diversity  
in roe deer from lowland and mountain habitats in Croatia**

**SUPPLEMENT DOCUMENTATION**

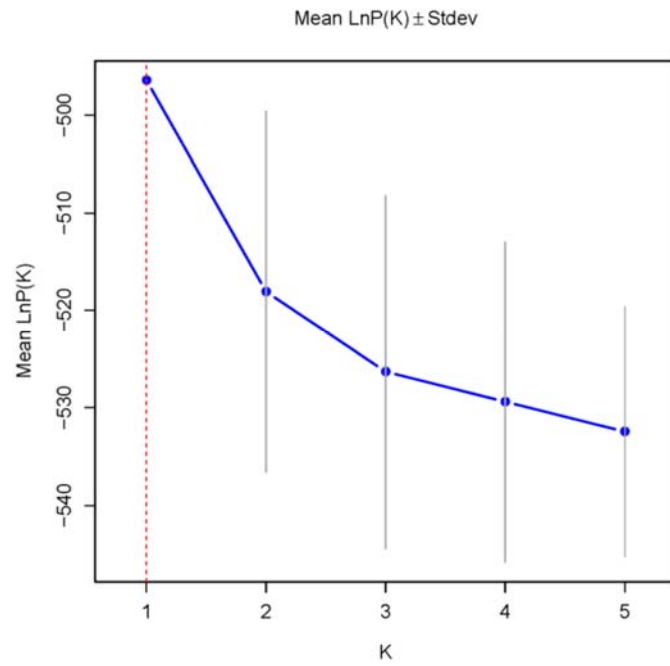
**Table S1.** Genotypes of 133 roe deer individuals sampled in four Croatian counties. Samples were categorised into two distinct populations: lowland (Bjelovar-Bilogora and Zagreb County, N=93) and mountain population (Lika-Senj and Primorje-Gorski Kotar County, N=40).

Sample name	County	Genotype	
S1BJ	Bjelovar-Bilogora	Caca-DRB*0201	Caca-DRB*0301
1	Bjelovar-Bilogora	Caca-DRB*0201	Caca-DRB*0304
3	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0304
4	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0102
8	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0401
9	Bjelovar-Bilogora	Caca-DRB*0302	Caca-DRB*0301
11	Bjelovar-Bilogora	Caca-DRB*0302	Caca-DRB*0301
12	Bjelovar-Bilogora	Caca-DRB*0303	Caca-DRB*0301
13	Bjelovar-Bilogora	Caca-DRB*0302	Caca-DRB*0401
14	Bjelovar-Bilogora	Caca-DRB*0302	Caca-DRB*0402
16	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0302
17	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0302
18	Bjelovar-Bilogora	Caca-DRB*0302	Caca-DRB*0402
20	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0302
22	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0301
23	Bjelovar-Bilogora	Caca-DRB*0302	Caca-DRB*0303
24	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0401
25	Bjelovar-Bilogora	Caca-DRB*0302	Caca-DRB*0402
28	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0304
29	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0303
30	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0303
33	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0303
35	Bjelovar-Bilogora	Caca-DRB*0401	Caca-DRB*0304
36	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0304
37	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0201
40	Bjelovar-Bilogora	Caca-DRB*0303	Caca-DRB*0304
41	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0303
42	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0201
47	Bjelovar-Bilogora	Caca-DRB*0102	Caca-DRB*0201

15	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0102
34	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0301
67	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0303
93	Bjelovar-Bilogora	Caca-DRB*0401	Caca-DRB*0303
95	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0303
98	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0301
99	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0301
106	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0401
107	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0303
108	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0102
109	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0401
SO-11	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0302
SO-12	Bjelovar-Bilogora	Caca-DRB*0303	Caca-DRB*0401
SO-13	Bjelovar-Bilogora	Caca-DRB*0302	Caca-DRB*0201
SO-14	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0302
SO-15	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0301
SO-16	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0102
SO-19	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0102
SO-20	Bjelovar-Bilogora	Caca-DRB*0303	Caca-DRB*0201
SO-21	Bjelovar-Bilogora	Caca-DRB*0303	Caca-DRB*0201
S2	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0301
S5	Bjelovar-Bilogora	Caca-DRB*0303	Caca-DRB*0301
S6	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0301
S7	Bjelovar-Bilogora	Caca-DRB*0301	Caca-DRB*0401
S9	Bjelovar-Bilogora	Caca-DRB*0302	Caca-DRB*0401
15SC	Zagreb	Caca-DRB*0302	Caca-DRB*0302
16SC	Zagreb	Caca-DRB*0301	Caca-DRB*0301
17SC	Zagreb	Caca-DRB*0301	Caca-DRB*0301
18SC	Zagreb	Caca-DRB*0301	Caca-DRB*0301
19SC	Zagreb	Caca-DRB*0301	Caca-DRB*0302
1SC	Zagreb	Caca-DRB*0301	Caca-DRB*0301
21SC	Zagreb	Caca-DRB*0301	Caca-DRB*0301
22SC	Zagreb	Caca-DRB*0302	Caca-DRB*0302
3SC	Zagreb	Caca-DRB*0301	Caca-DRB*0301
6SC	Zagreb	Caca-DRB*0302	Caca-DRB*0302
7SC	Zagreb	Caca-DRB*0301	Caca-DRB*0301
8SC	Zagreb	Caca-DRB*0301	Caca-DRB*0302
9SC	Zagreb	Caca-DRB*0301	Caca-DRB*0302
M11-35	Zagreb	Caca-DRB*0301	Caca-DRB*0303
M12-35	Zagreb	Caca-DRB*0201	Caca-DRB*0201
M4-65	Zagreb	Caca-DRB*0301	Caca-DRB*0302
M6-35	Zagreb	Caca-DRB*0302	Caca-DRB*0302
M7-35	Zagreb	Caca-DRB*0301	Caca-DRB*0301
M7-75	Zagreb	Caca-DRB*0302	Caca-DRB*0302
M8-25	Zagreb	Caca-DRB*0301	Caca-DRB*0301
M8-55	Zagreb	Caca-DRB*0301	Caca-DRB*0301

M8-75	Zagreb	Caca-DRB*0302	Caca-DRB*0303
M9-55	Zagreb	Caca-DRB*0201	Caca-DRB*0201
M9-75	Zagreb	Caca-DRB*0302	Caca-DRB*0302
12SC	Zagreb	Caca-DRB*0304	Caca-DRB*0301
13SC	Zagreb	Caca-DRB*0401	Caca-DRB*0301
14SC	Zagreb	Caca-DRB*0102	Caca-DRB*0301
1SL	Zagreb	Caca-DRB*0403	Caca-DRB*0102
20SC	Zagreb	Caca-DRB*0304	Caca-DRB*0302
2SL	Zagreb	Caca-DRB*0402	Caca-DRB*0102
3SL	Zagreb	Caca-DRB*0403	Caca-DRB*0102
4SC	Zagreb	Caca-DRB*0302	Caca-DRB*0102
4SL	Zagreb	Caca-DRB*0304	Caca-DRB*0301
5SC	Zagreb	Caca-DRB*0304	Caca-DRB*0301
M6-55	Zagreb	Caca-DRB*0301	Caca-DRB*0201
M6-65	Zagreb	Caca-DRB*0302	Caca-DRB*0304
M6-75	Zagreb	Caca-DRB*0102	Caca-DRB*0301
M9-25	Zagreb	Caca-DRB*0301	Caca-DRB*0201
M7-15	Zagreb	Caca-DRB*0303	Caca-DRB*0402
L1	Lika-Senj	Caca-DRB*0301	Caca-DRB*0302
L10	Lika-Senj	Caca-DRB*0301	Caca-DRB*0302
L12	Lika-Senj	Caca-DRB*0302	Caca-DRB*0302
L13	Lika-Senj	Caca-DRB*0301	Caca-DRB*0301
L15	Lika-Senj	Caca-DRB*0301	Caca-DRB*0302
L17	Lika-Senj	Caca-DRB*0102	Caca-DRB*0102
L20	Lika-Senj	Caca-DRB*0301	Caca-DRB*0302
L3	Lika-Senj	Caca-DRB*0301	Caca-DRB*0301
L8	Lika-Senj	Caca-DRB*0201	Caca-DRB*0401
L11	Lika-Senj	Caca-DRB*0304	Caca-DRB*0405
L14	Lika-Senj	Caca-DRB*0102	Caca-DRB*0405
L18	Lika-Senj	Caca-DRB*0301	Caca-DRB*0405
L19	Lika-Senj	Caca-DRB*0401	Caca-DRB*0301
L2	Lika-Senj	Caca-DRB*0402	Caca-DRB*0405
L5	Lika-Senj	Caca-DRB*0301	Caca-DRB*0401
L6	Lika-Senj	Caca-DRB*0304	Caca-DRB*0301
L7	Lika-Senj	Caca-DRB*0301	Caca-DRB*0405
L16	Lika-Senj	Caca-DRB*0302	Caca-DRB*0402
L4	Lika-Senj	Caca-DRB*0301	Caca-DRB*0402
L9	Lika-Senj	Caca-DRB*0302	Caca-DRB*0402
K1	Primorje-Gorski kotar	Caca-DRB*0302	Caca-DRB*0302
K11	Primorje-Gorski kotar	Caca-DRB*0301	Caca-DRB*0302
K15	Primorje-Gorski kotar	Caca-DRB*0301	Caca-DRB*0302
K17	Primorje-Gorski kotar	Caca-DRB*0301	Caca-DRB*0301
K18	Primorje-Gorski kotar	Caca-DRB*0301	Caca-DRB*0302
K20	Primorje-Gorski kotar	Caca-DRB*0301	Caca-DRB*0303
K5	Primorje-Gorski kotar	Caca-DRB*0301	Caca-DRB*0301
K10	Primorje-Gorski kotar	Caca-DRB*0302	Caca-DRB*0102

<b>K13</b>	<b>Primorje-Gorski kotar</b>	<b>Caca-DRB*0304</b>	<b>Caca-DRB*0301</b>
<b>K14</b>	<b>Primorje-Gorski kotar</b>	<b>Caca-DRB*0304</b>	<b>Caca-DRB*0301</b>
<b>K19</b>	<b>Primorje-Gorski kotar</b>	<b>Caca-DRB*0301</b>	<b>Caca-DRB*0201</b>
<b>K3</b>	<b>Primorje-Gorski kotar</b>	<b>Caca-DRB*0102</b>	<b>Caca-DRB*0301</b>
<b>K4</b>	<b>Primorje-Gorski kotar</b>	<b>Caca-DRB*0302</b>	<b>Caca-DRB*0401</b>
<b>K6</b>	<b>Primorje-Gorski kotar</b>	<b>Caca-DRB*0304</b>	<b>Caca-DRB*0303</b>
<b>K7</b>	<b>Primorje-Gorski kotar</b>	<b>Caca-DRB*0402</b>	<b>Caca-DRB*0304</b>
<b>K8</b>	<b>Primorje-Gorski kotar</b>	<b>Caca-DRB*0401</b>	<b>Caca-DRB*0303</b>
<b>K9</b>	<b>Primorje-Gorski kotar</b>	<b>Caca-DRB*0302</b>	<b>Caca-DRB*0102</b>
<b>K12</b>	<b>Primorje-Gorski kotar</b>	<b>Caca-DRB*0301</b>	<b>Caca-DRB*0402</b>
<b>K16</b>	<b>Primorje-Gorski kotar</b>	<b>Caca-DRB*0302</b>	<b>Caca-DRB*0402</b>
<b>K2</b>	<b>Primorje-Gorski kotar</b>	<b>Caca-DRB*0302</b>	<b>Caca-DRB*0402</b>



**Figure S1.** Mean log likelihood values for K=1 to K=5, indicating that the highest likelihood is observed at K=1.

**Table S2.** Results of dN/dS test conducted in MEGA 11 using Nei-Gojobori (Jukes-Cantor) method on 249 bp long roe deer DRB sequences. Statistical significance (p) was tested with 1000 bootstrap replicates.

<b>Population</b>	<b>dN</b>	<b>dS</b>	<b>dN/dS</b>	<b>p</b>
Lowland	0.05 (0.01)	0.04 (0.02)	1.25	0.24
Mountain	0.05 (0.01)	0.04 (0.02)	1.25	0.24
Overall	0.04 (0.01)	0.04 (0.02)	1.00	0.27

**Table S3.** Results of dN/dS test conducted in MEGA 11 using Nei-Gojobori (Jukes-Cantor) method on antigen binding sites inferred from human MHC molecular structure (Brown et al. 1993). Statistical significance (p) was tested with 1000 bootstrap replicates.

<b>Population</b>	<b>dN</b>	<b>dS</b>	<b>dN/dS</b>	<b>p</b>
Lowland	0.10 (0.04)	0.05 (0.05)	2	0.14
Mountain	0.10 (0.04)	0.05 (0.05)	2	0.13
Overall	0.10 (0.04)	0.05 (0.05)	2	0.15

**Table S4.** Results of codon-specific selection tests conducted on 10 MHC-DRB alleles detected in this study with posterior probability >99%. Underlined codons correspond to antigen binding sites (ABS) inferred from the human MHC molecule structure (Brown et al., 1993).

Codon	MODEL			
	M8	FEL	FUBAR	MEME
<u>13</u>	X		X	X
57	X			X
<u>86</u>	X	X	X	X