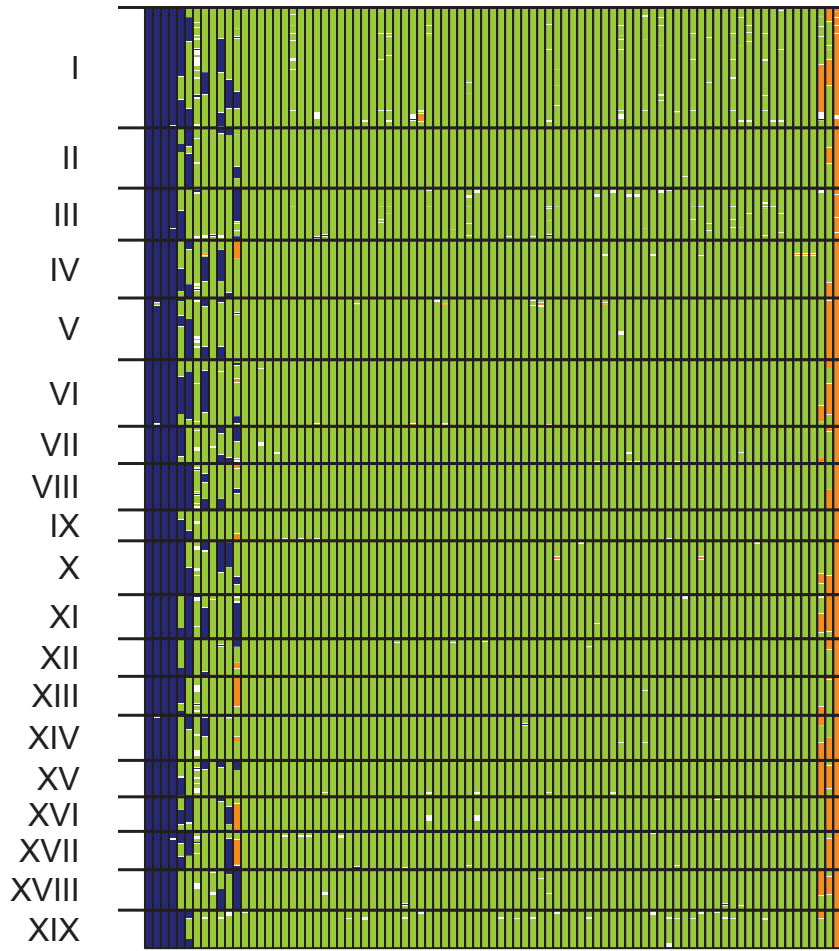
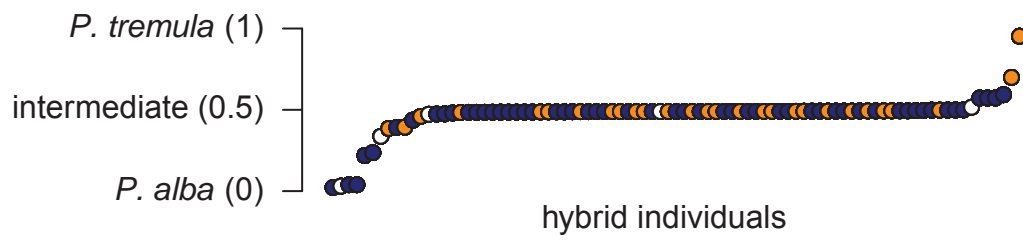


Italy

(A)

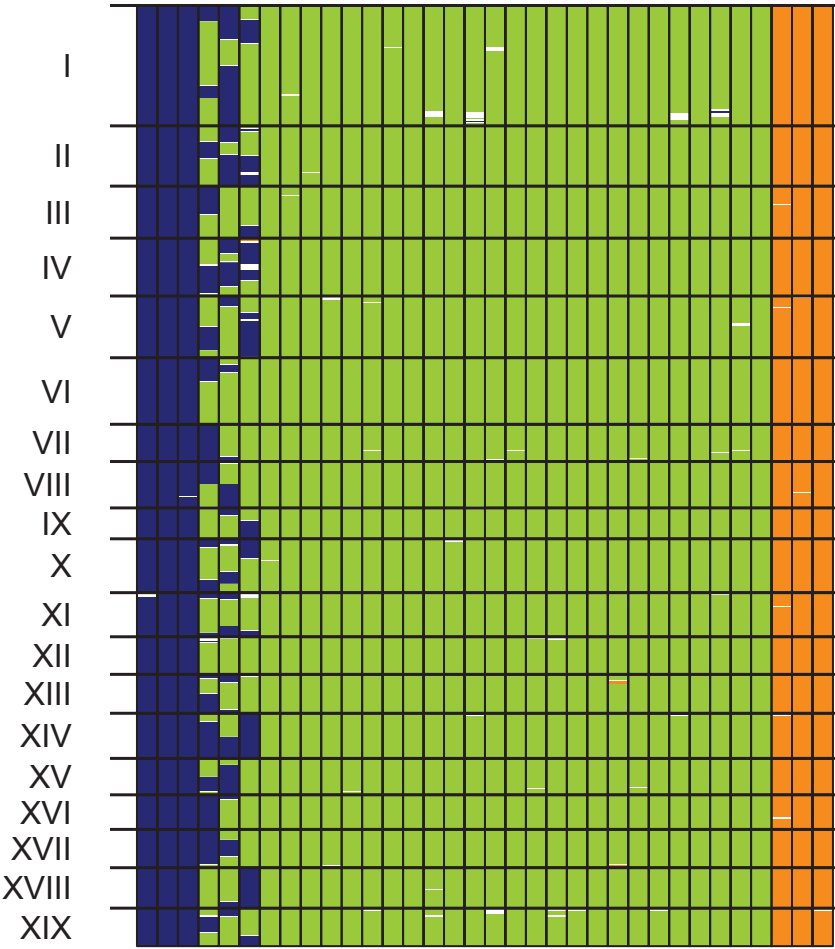


(B)

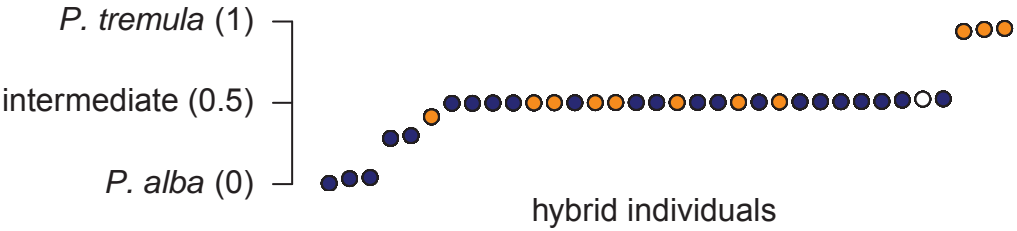


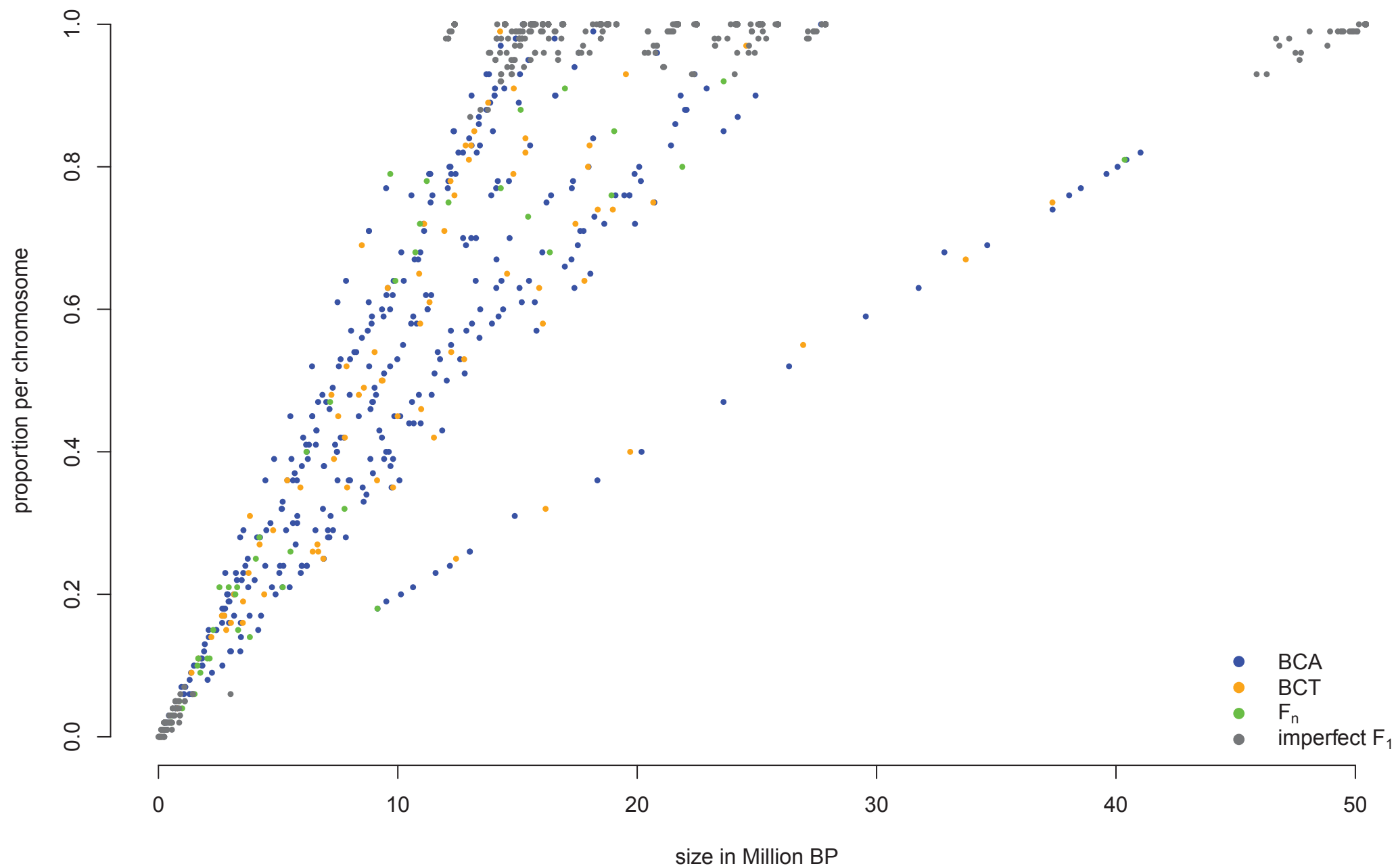
Hungary

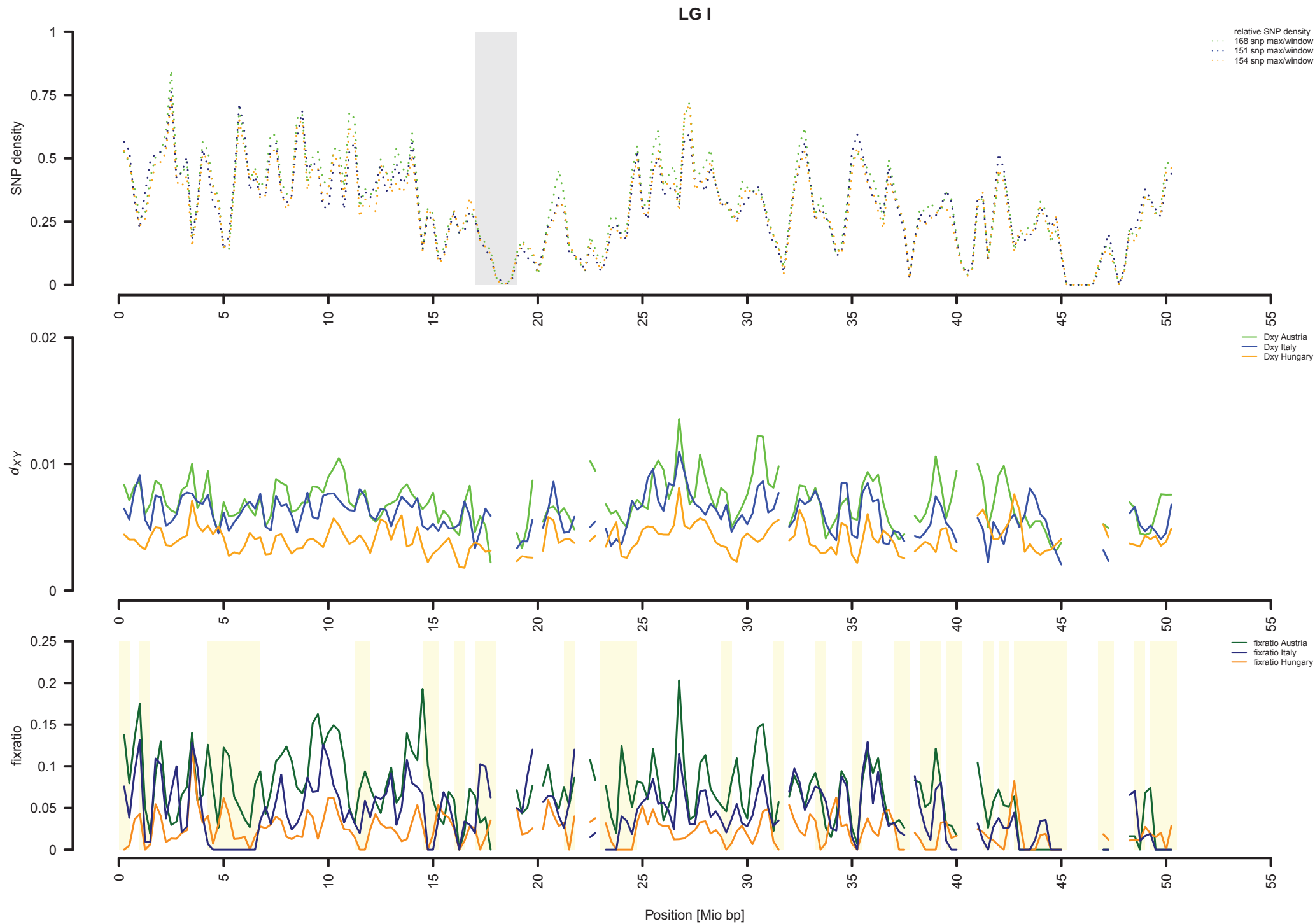
(A)

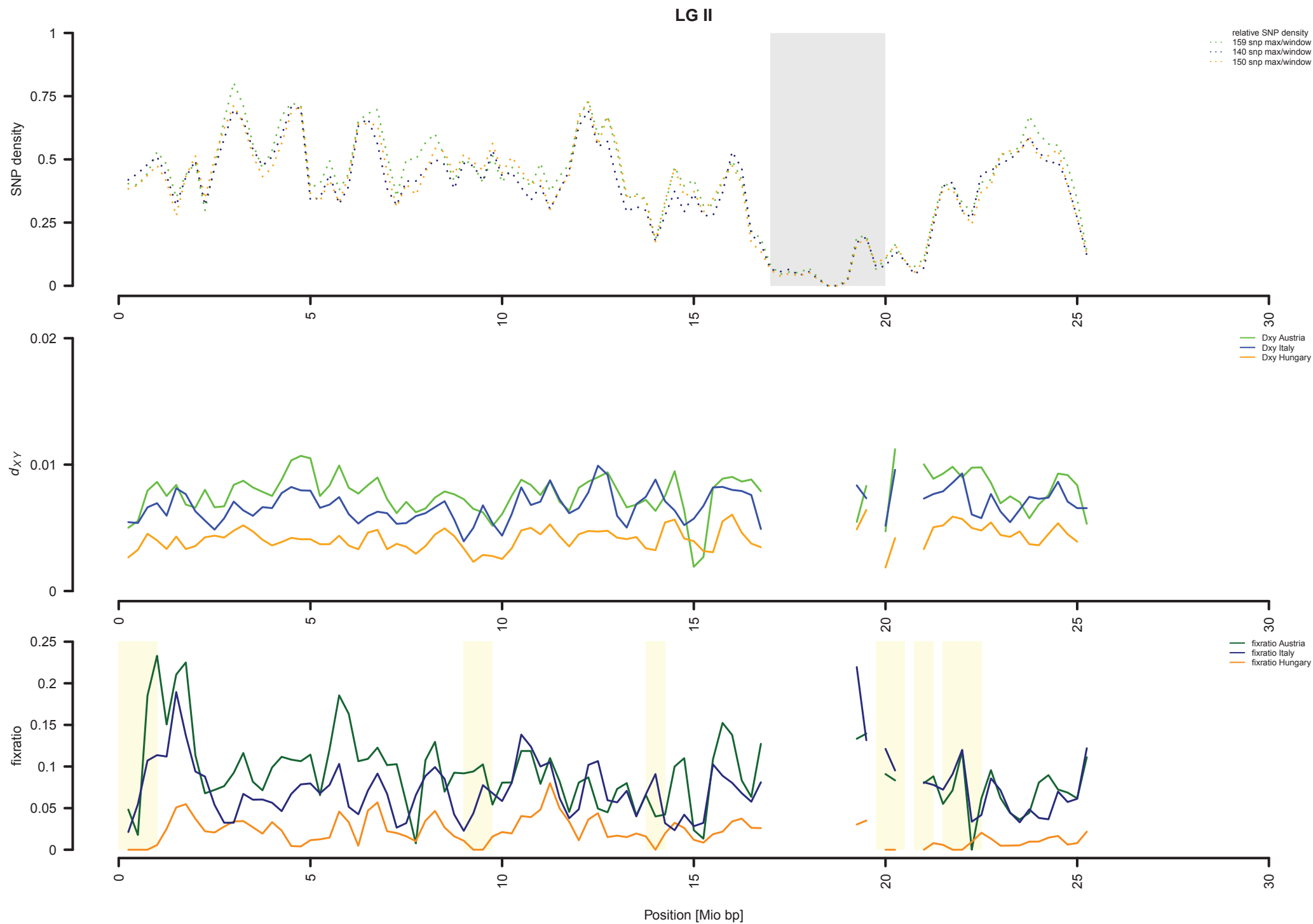


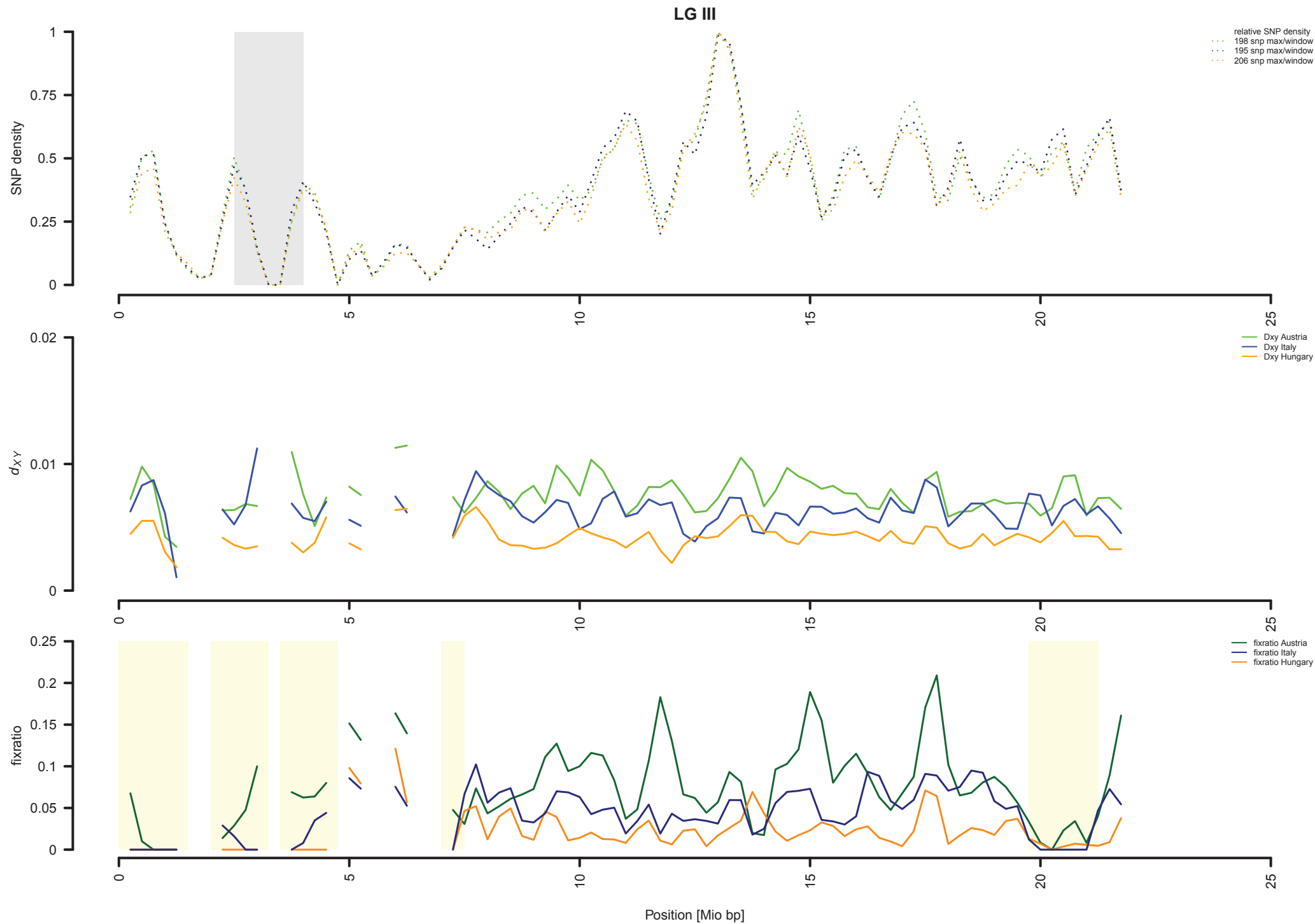
(B)

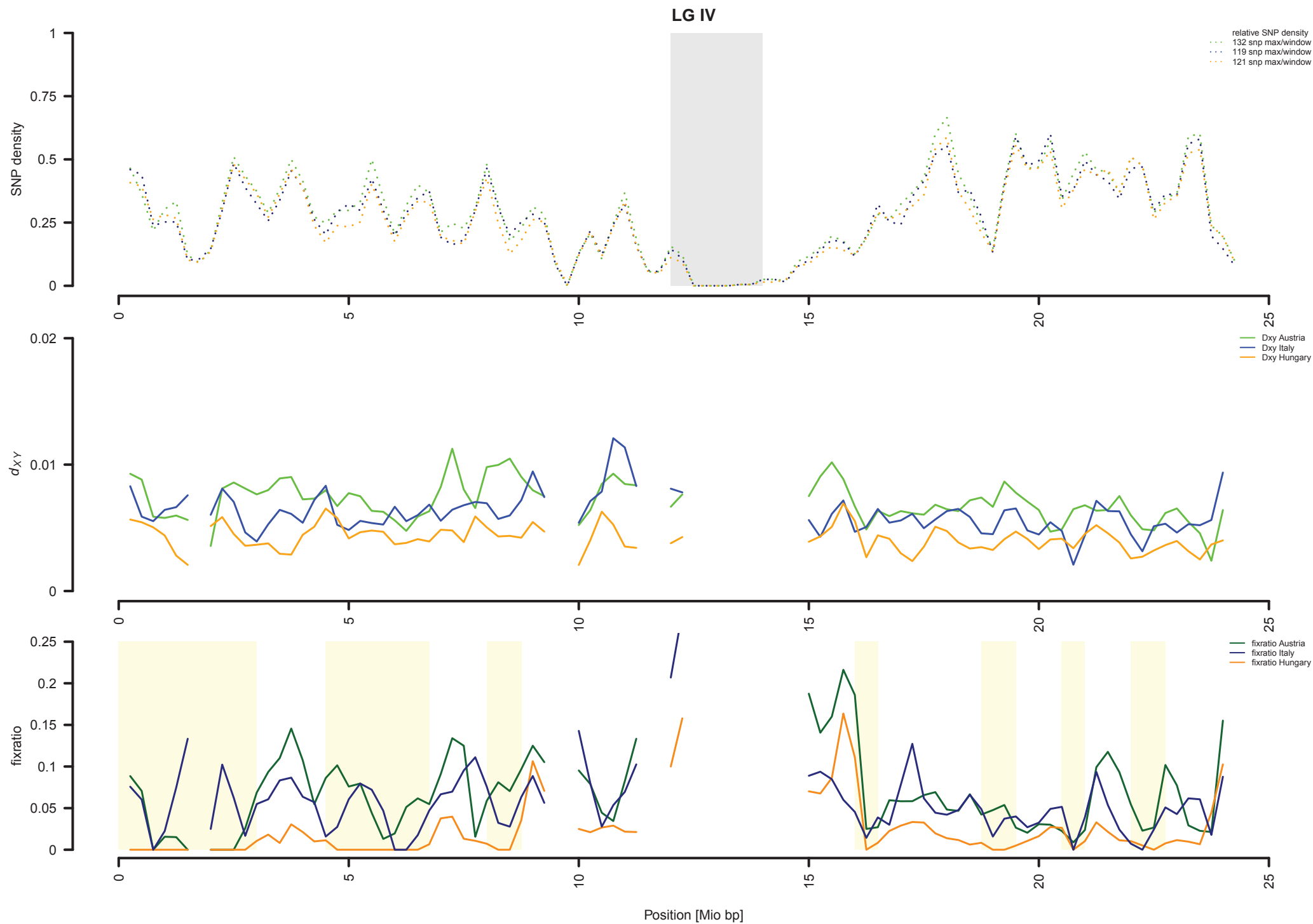


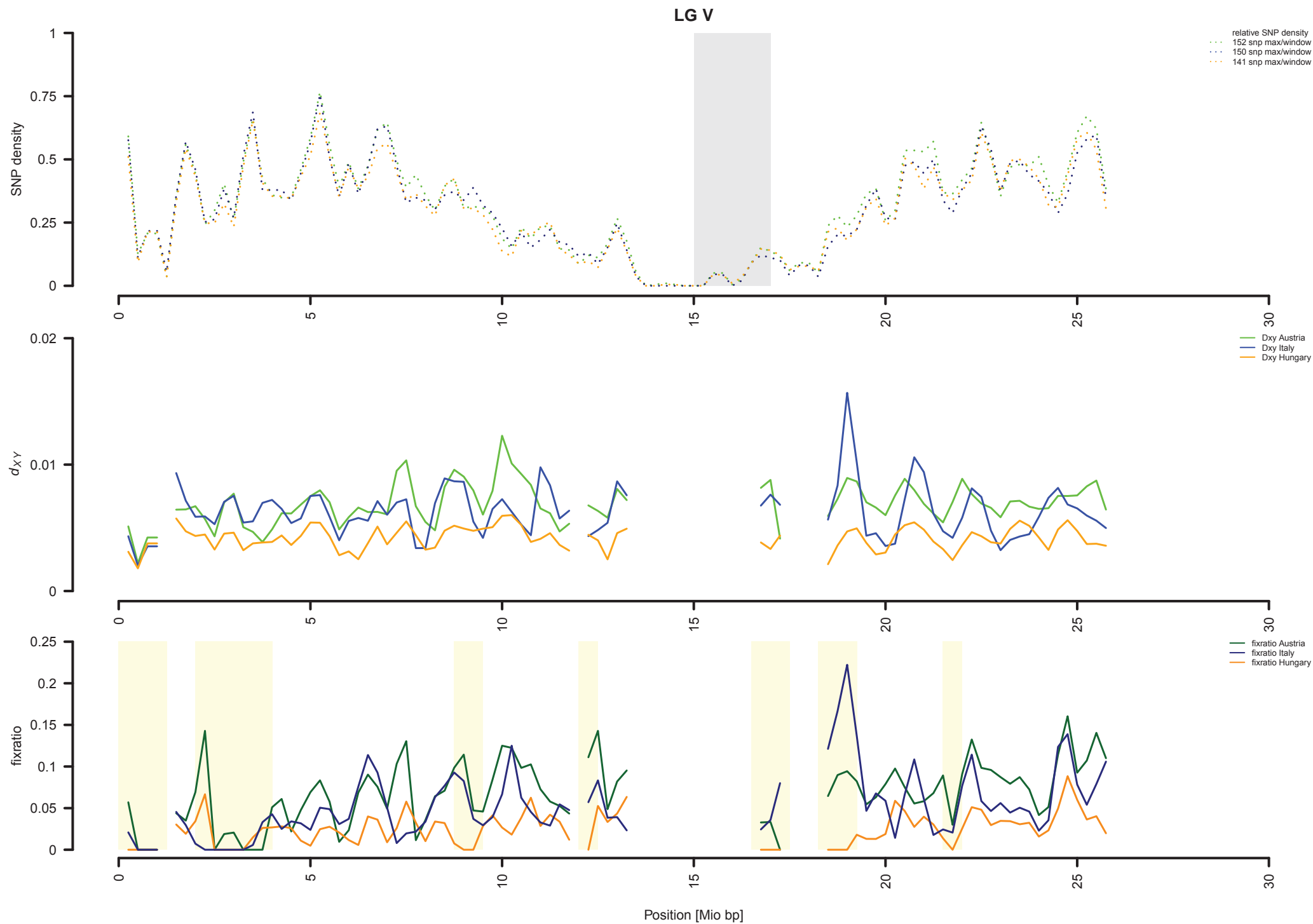


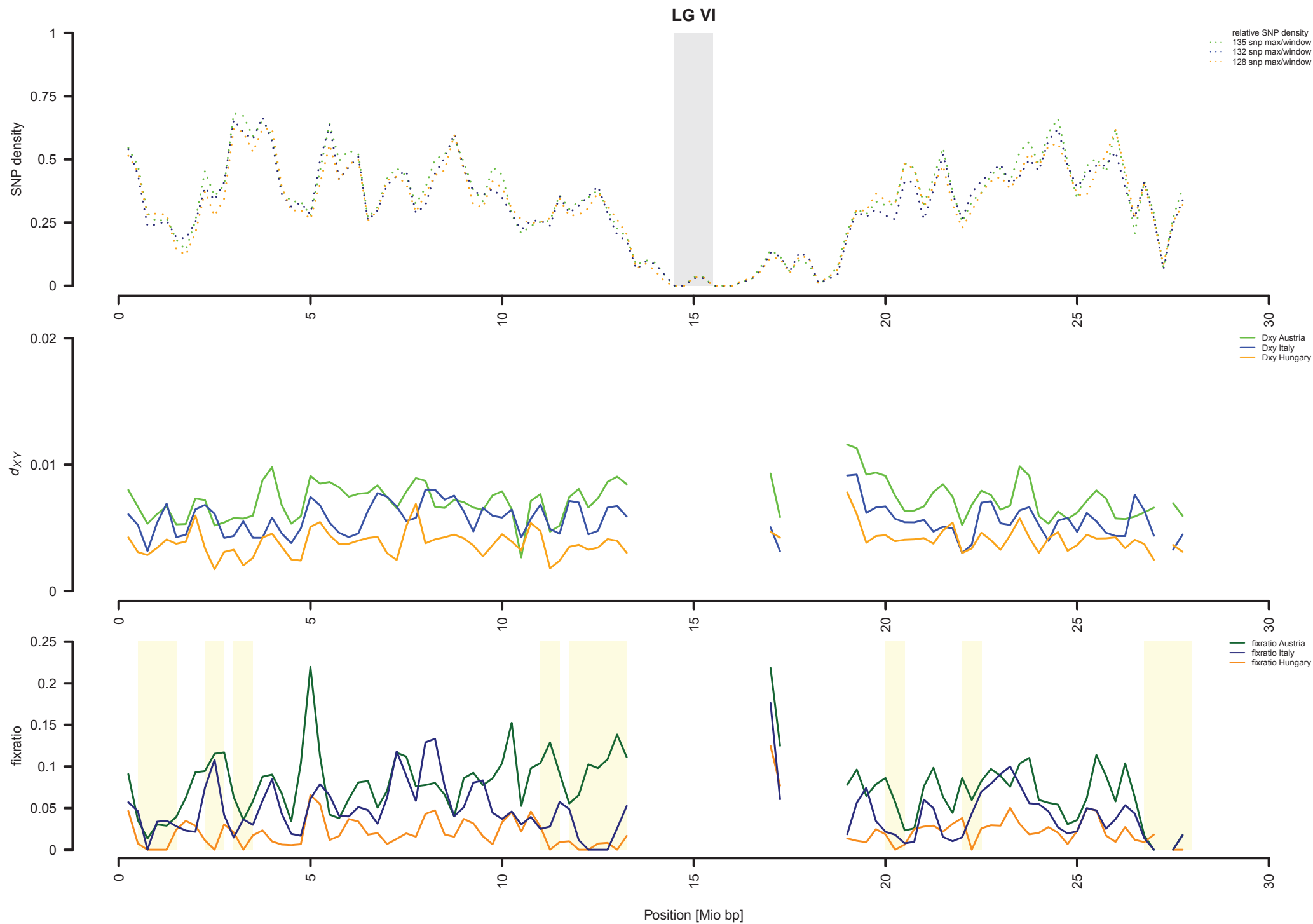




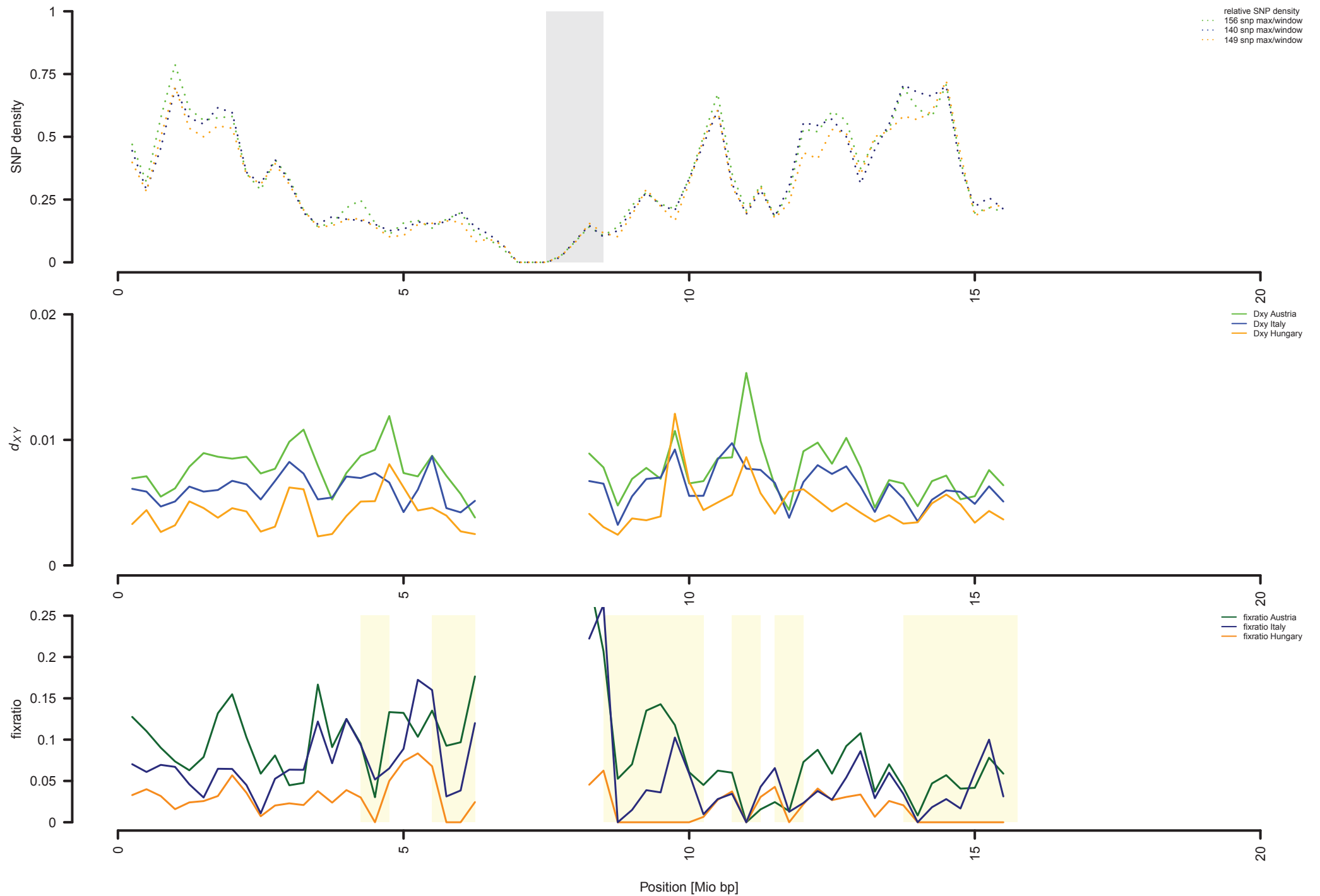




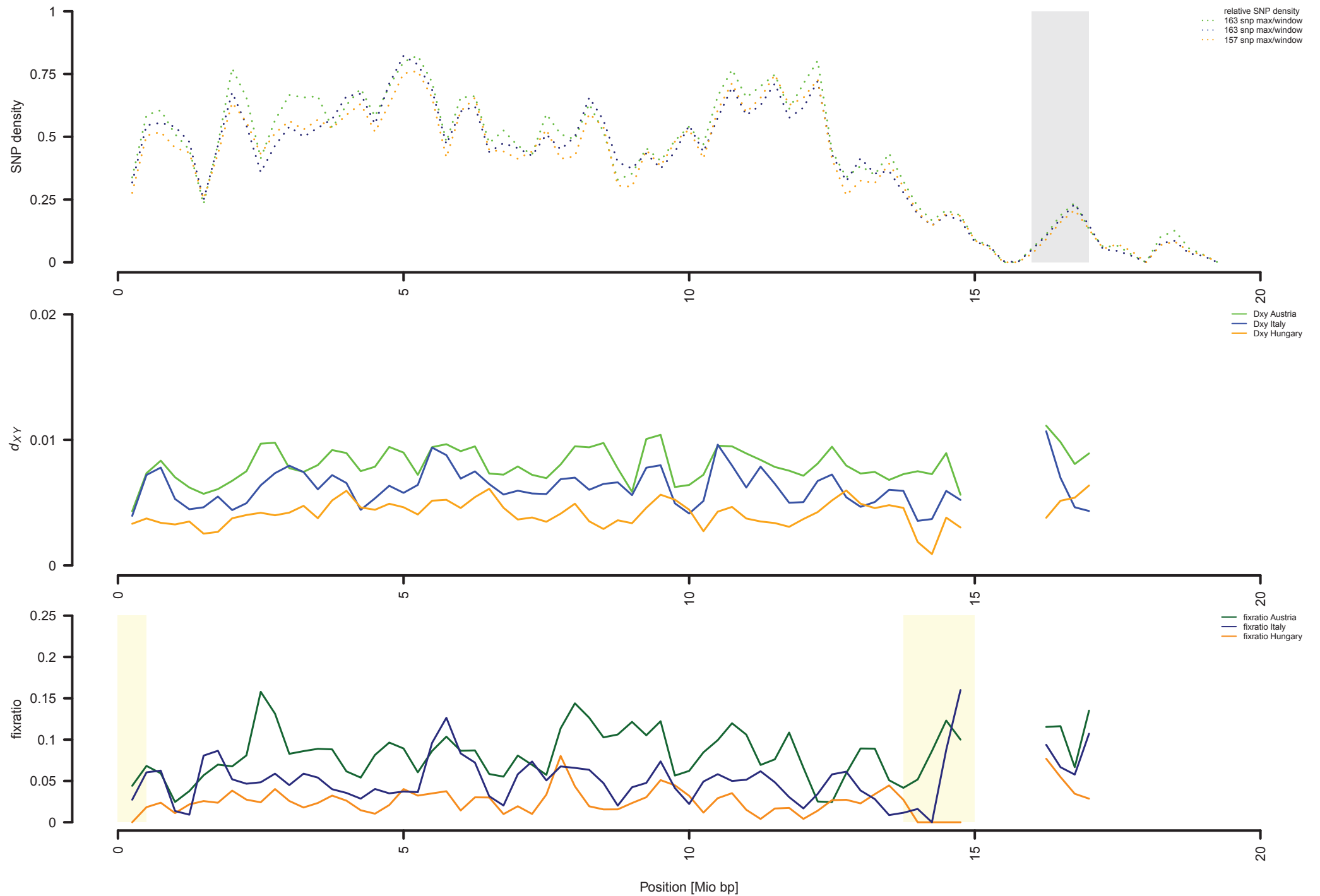




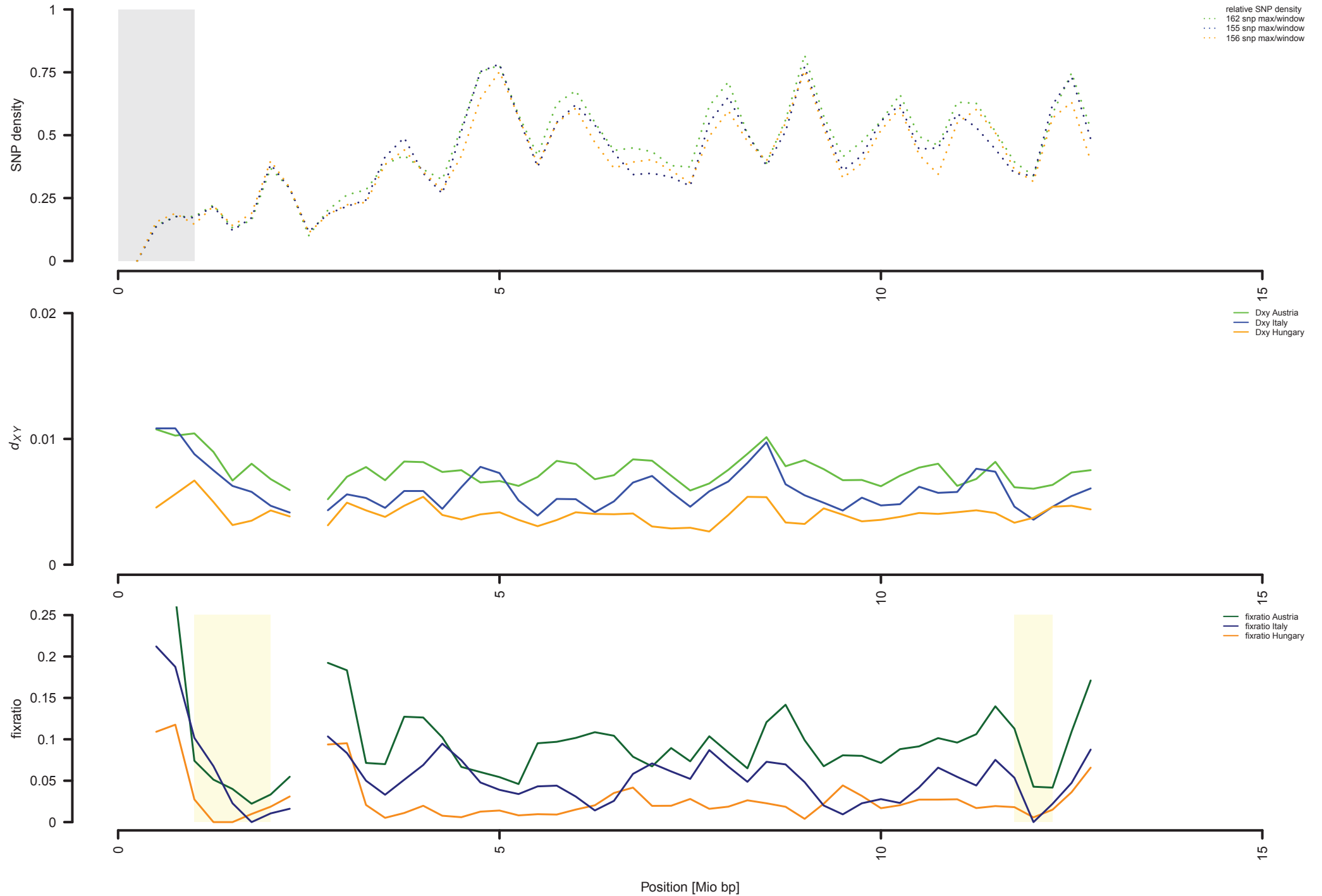
LG VII

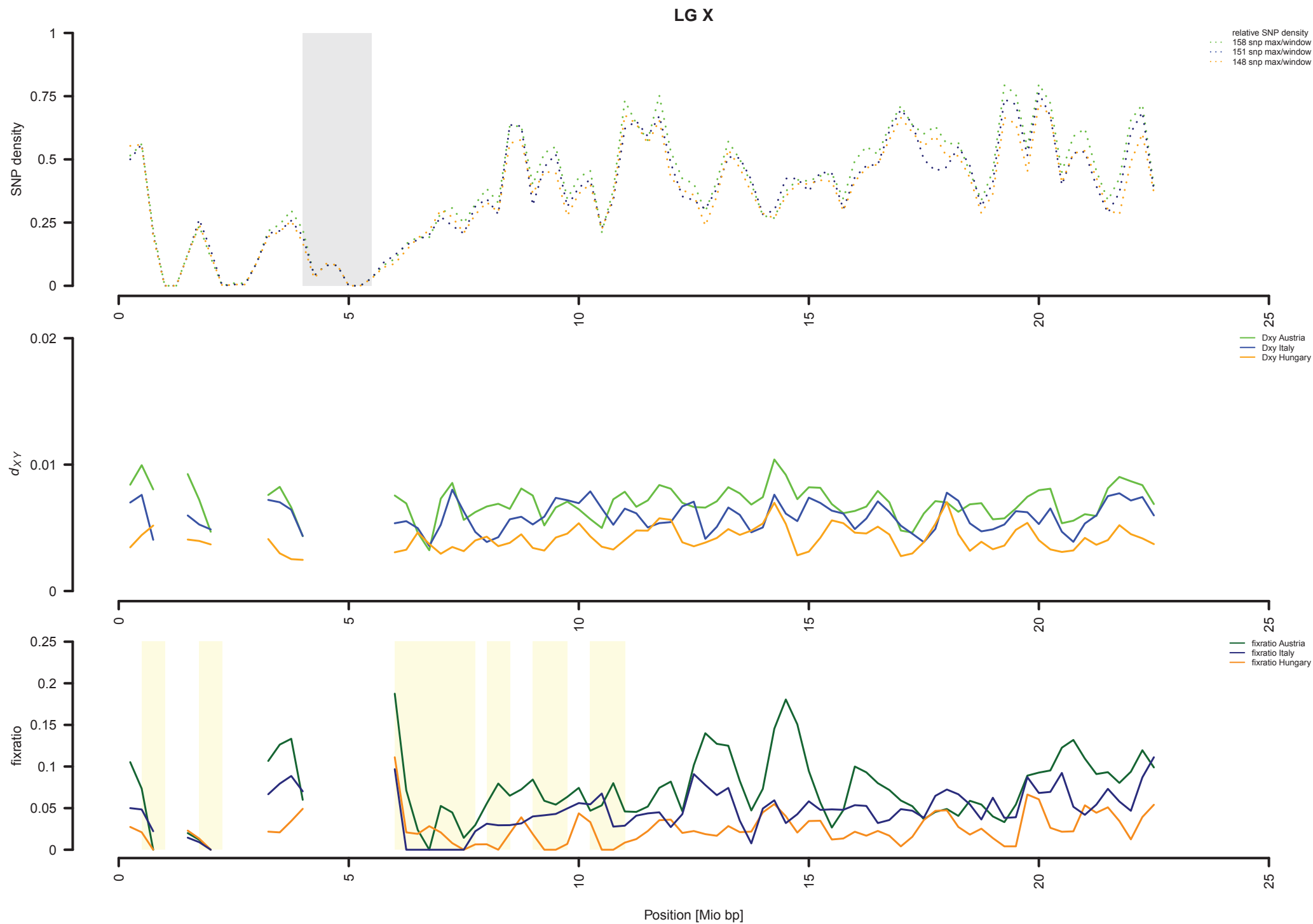


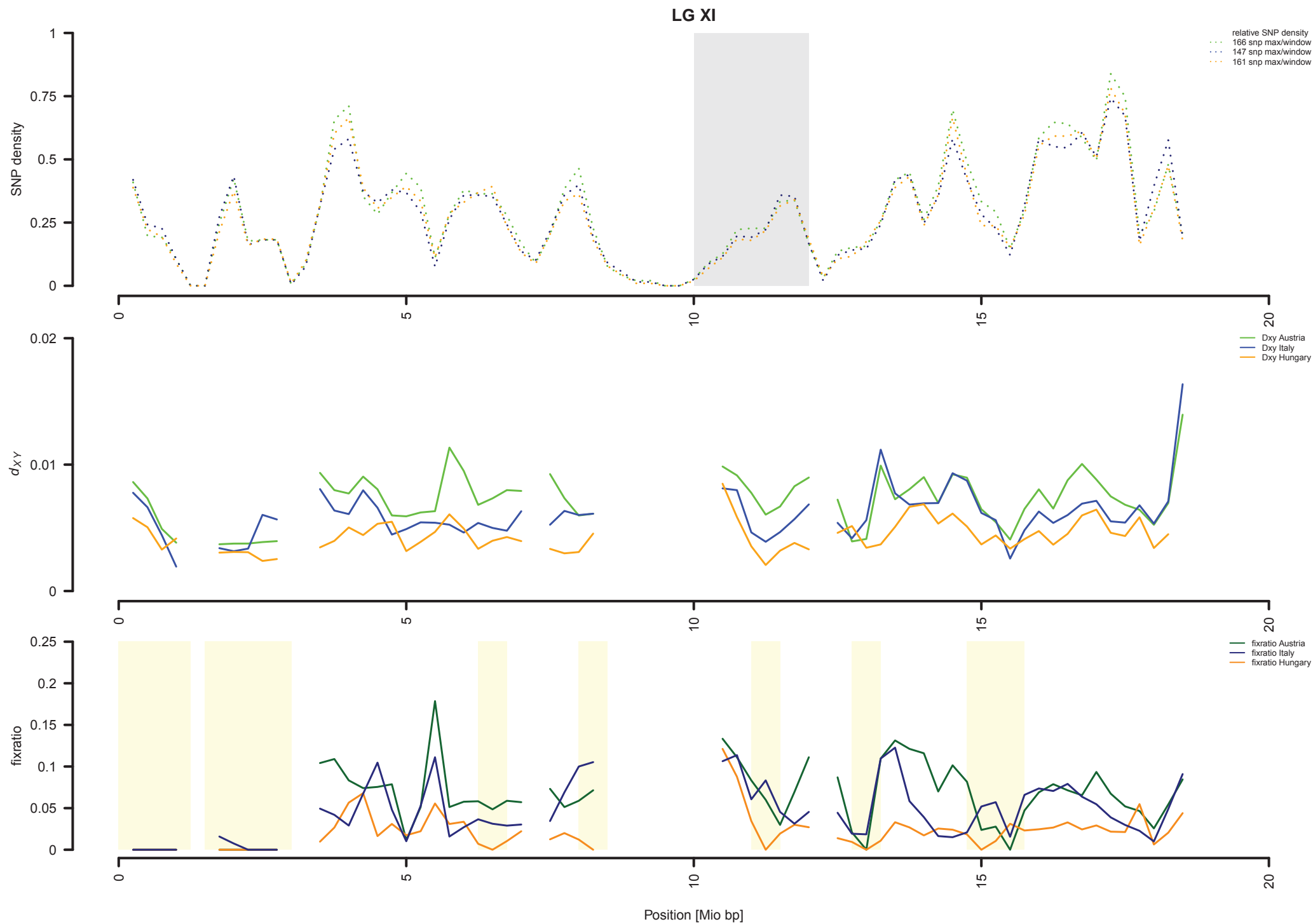
LG VIII



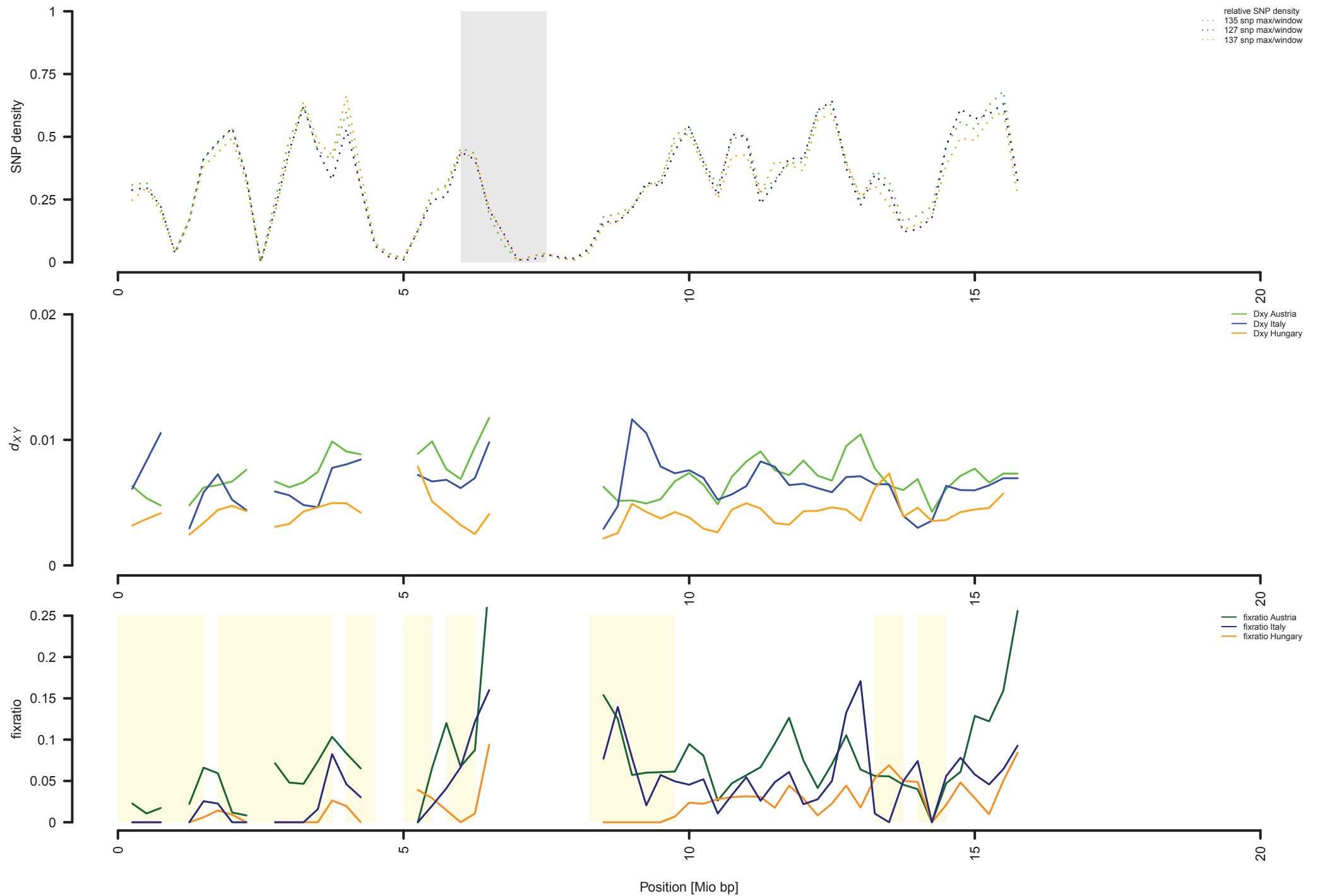
LG IX

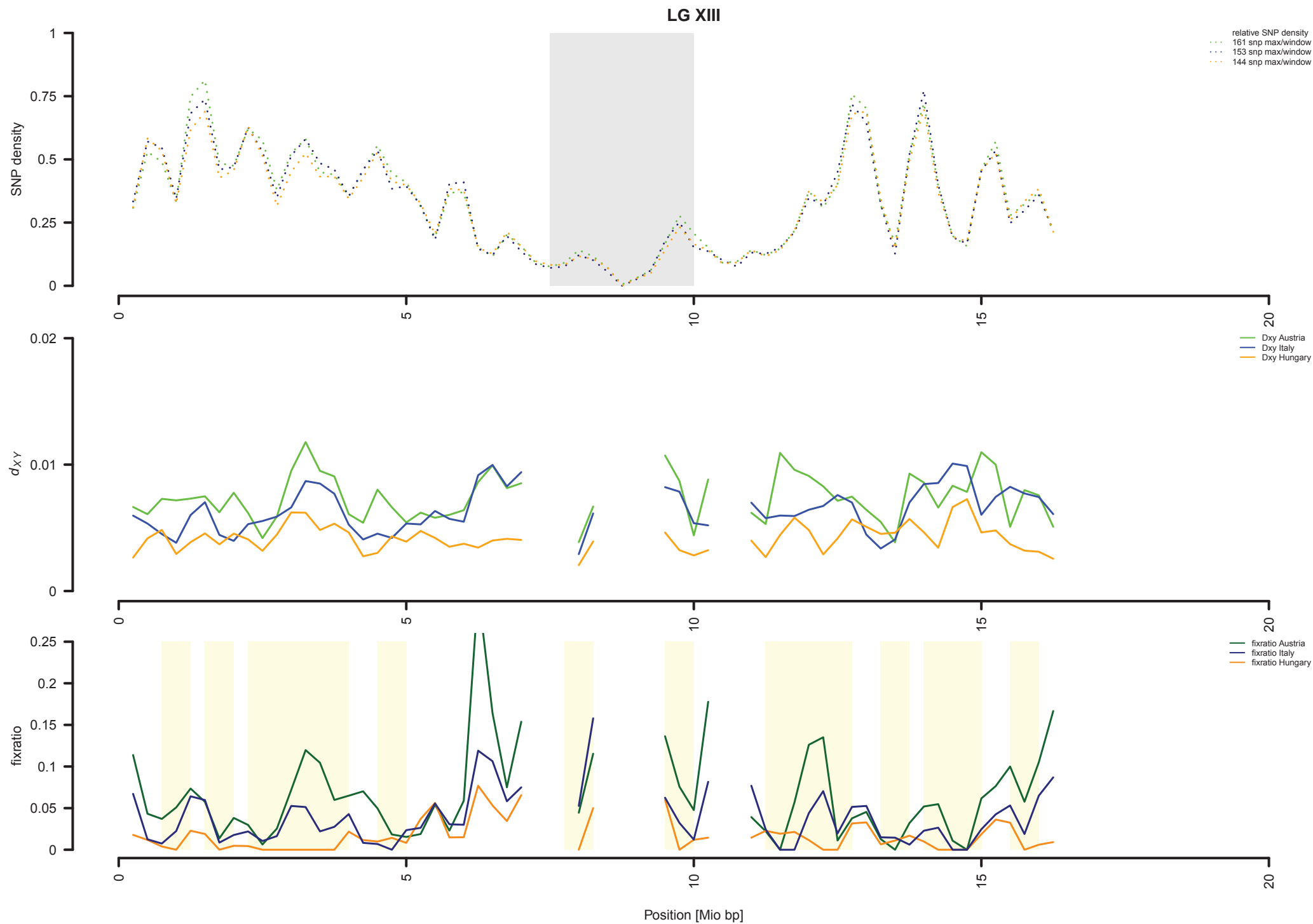




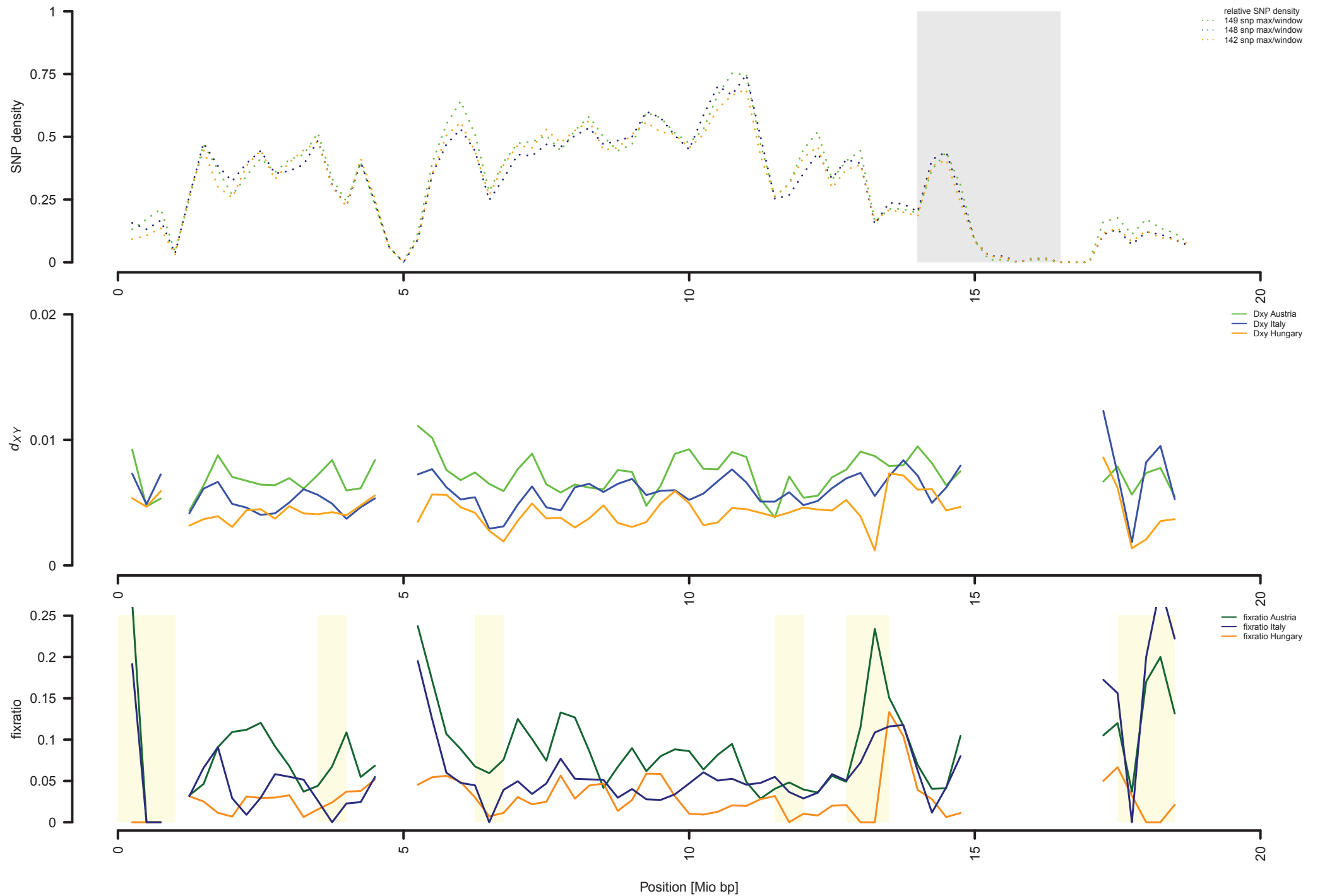


LG XII

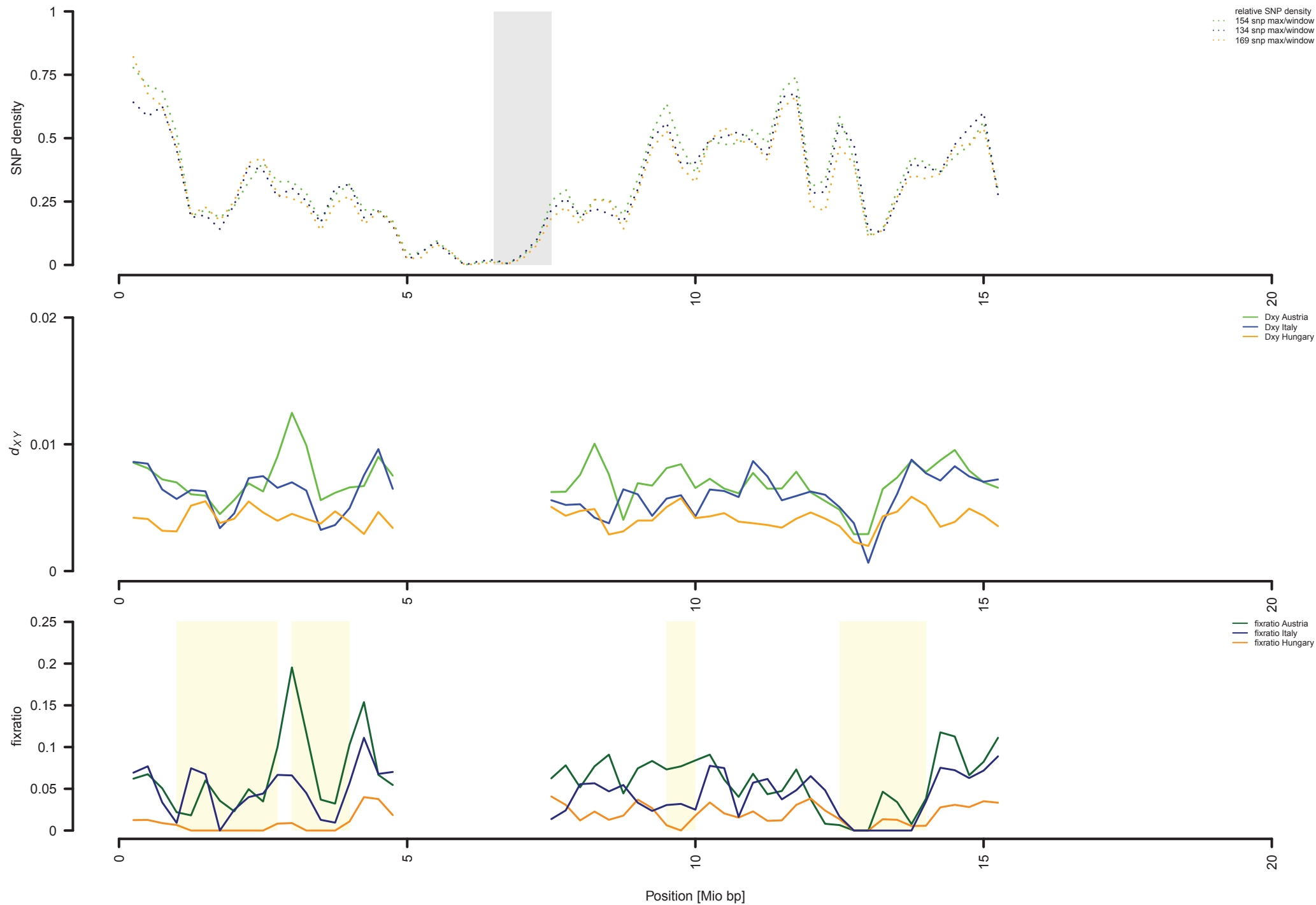




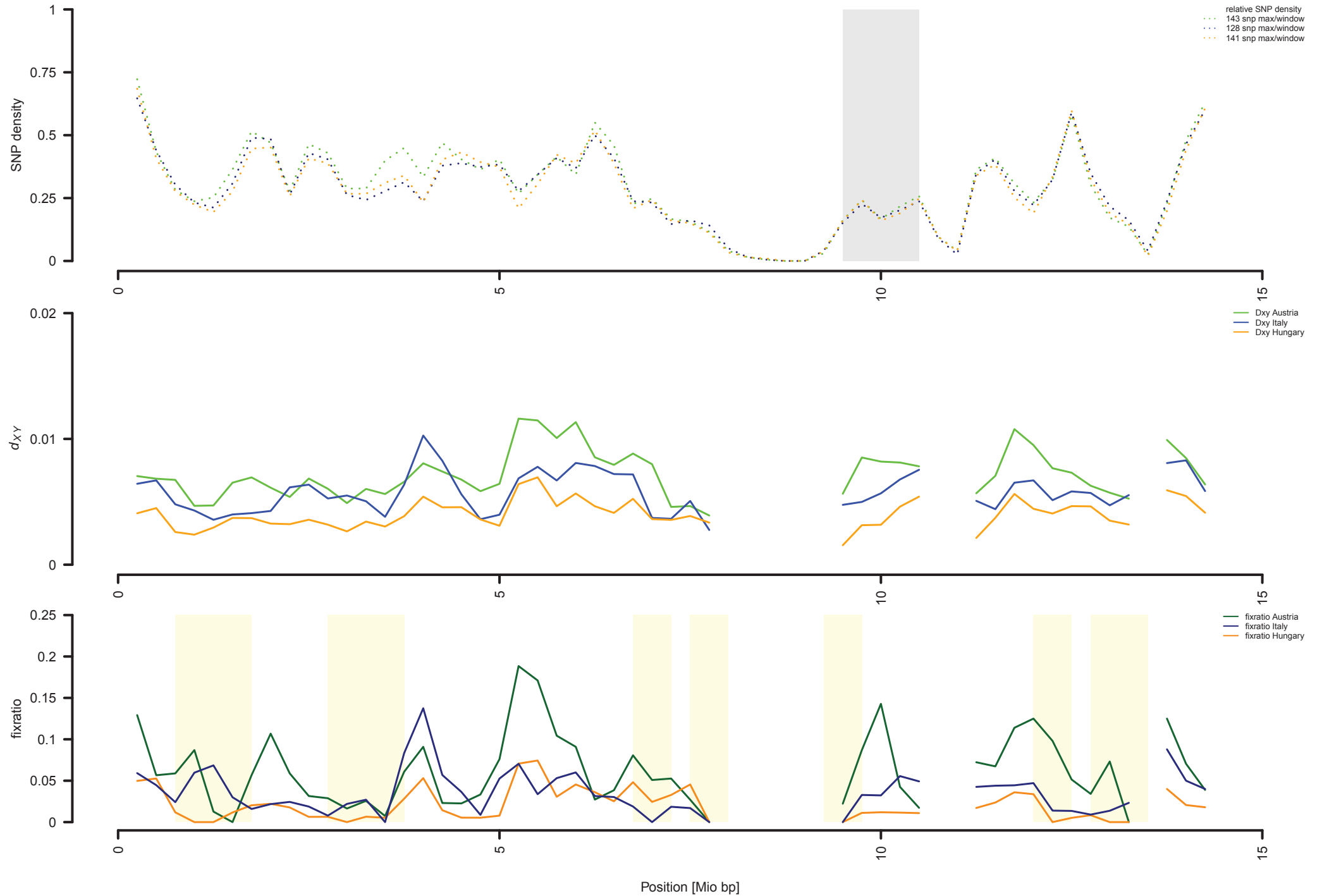
LG XIV

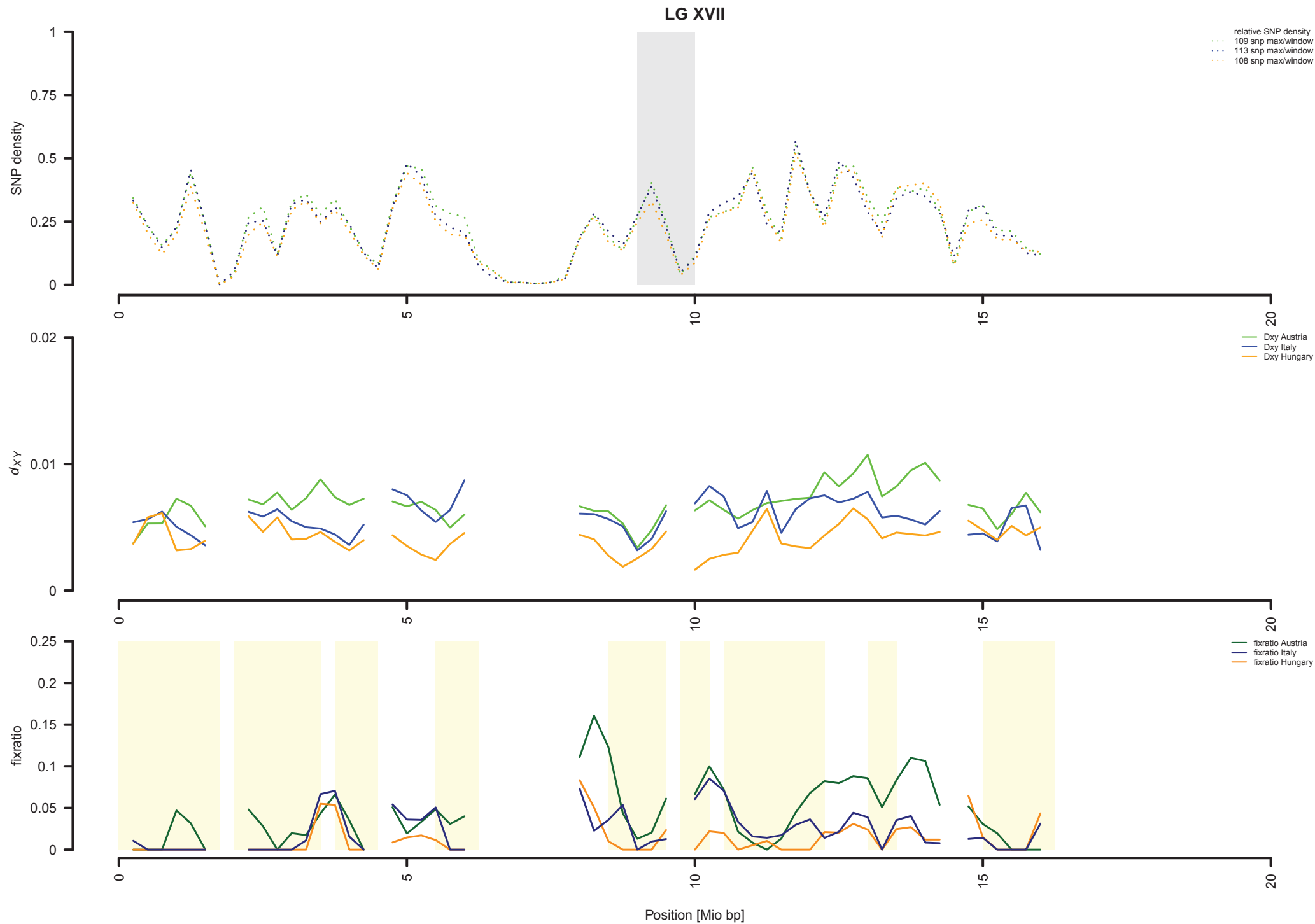


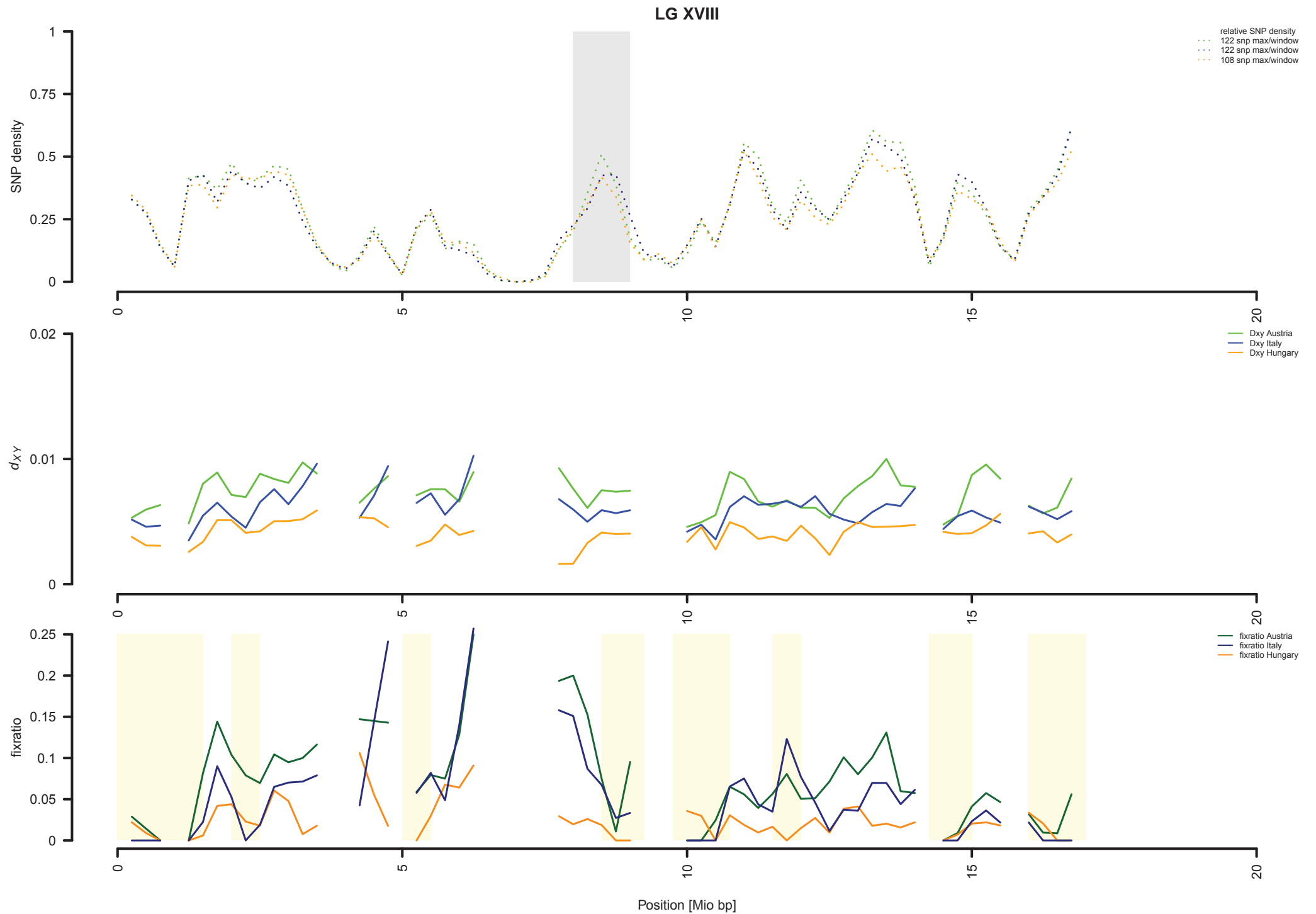
LG XV

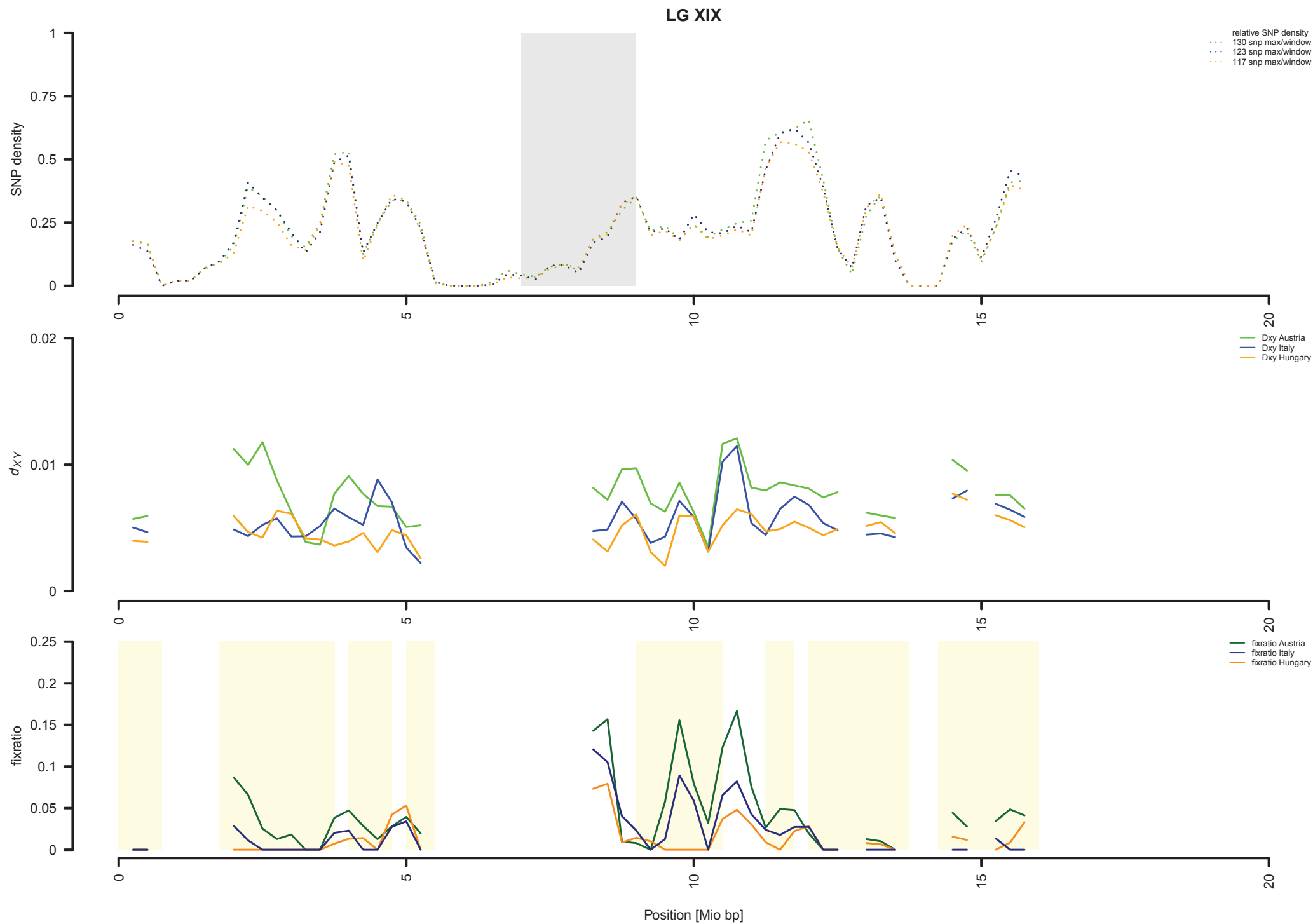


LG XVI

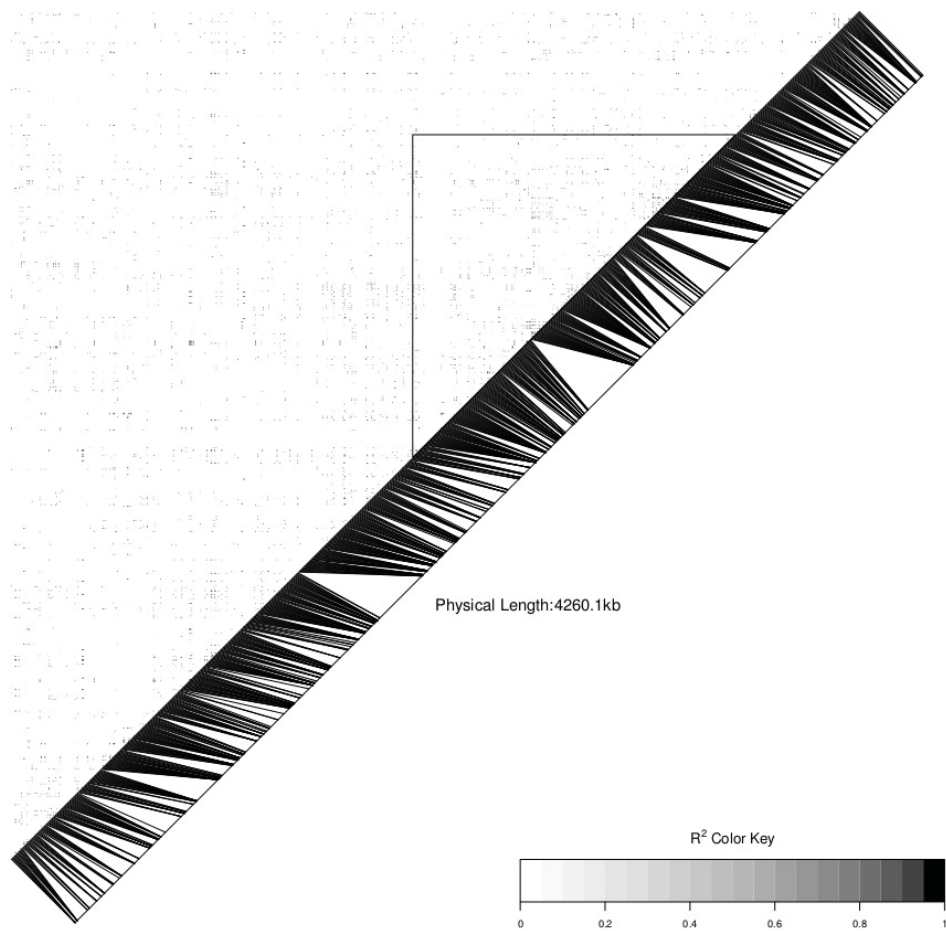




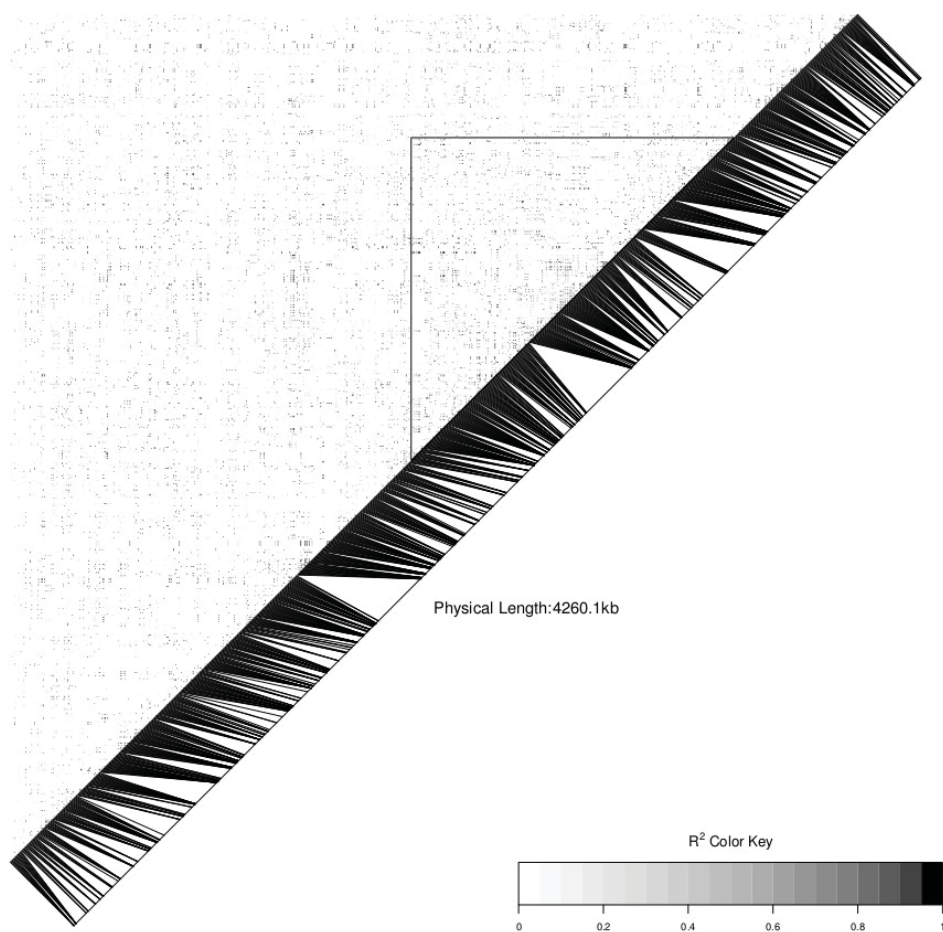




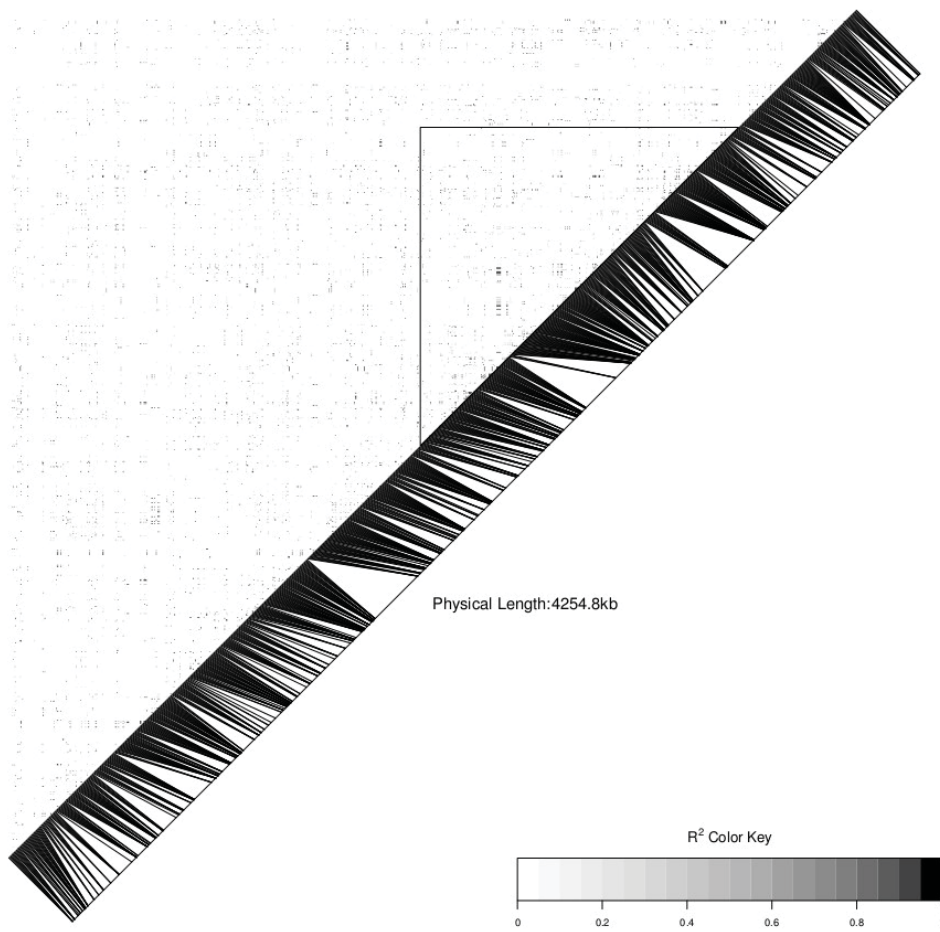
D' in Italy alba chr03 17.5–21.7 mio bp



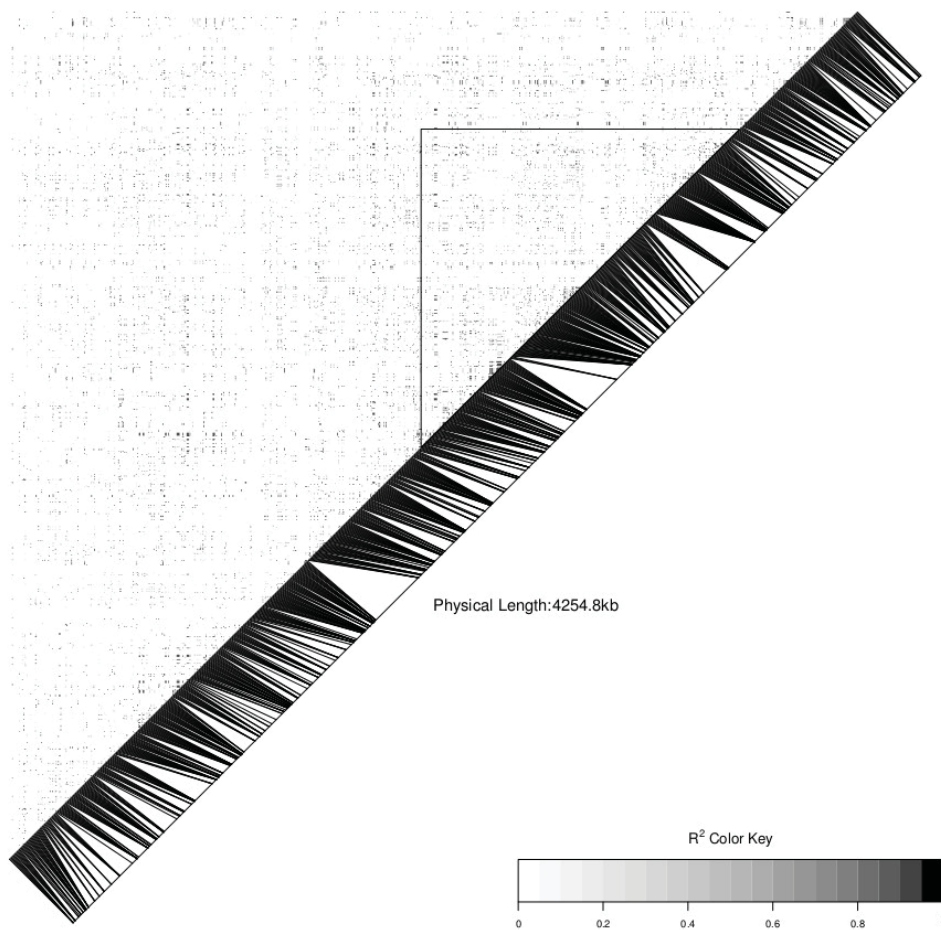
D' in Italy tremula chr03 17.5–21.7 mio bp



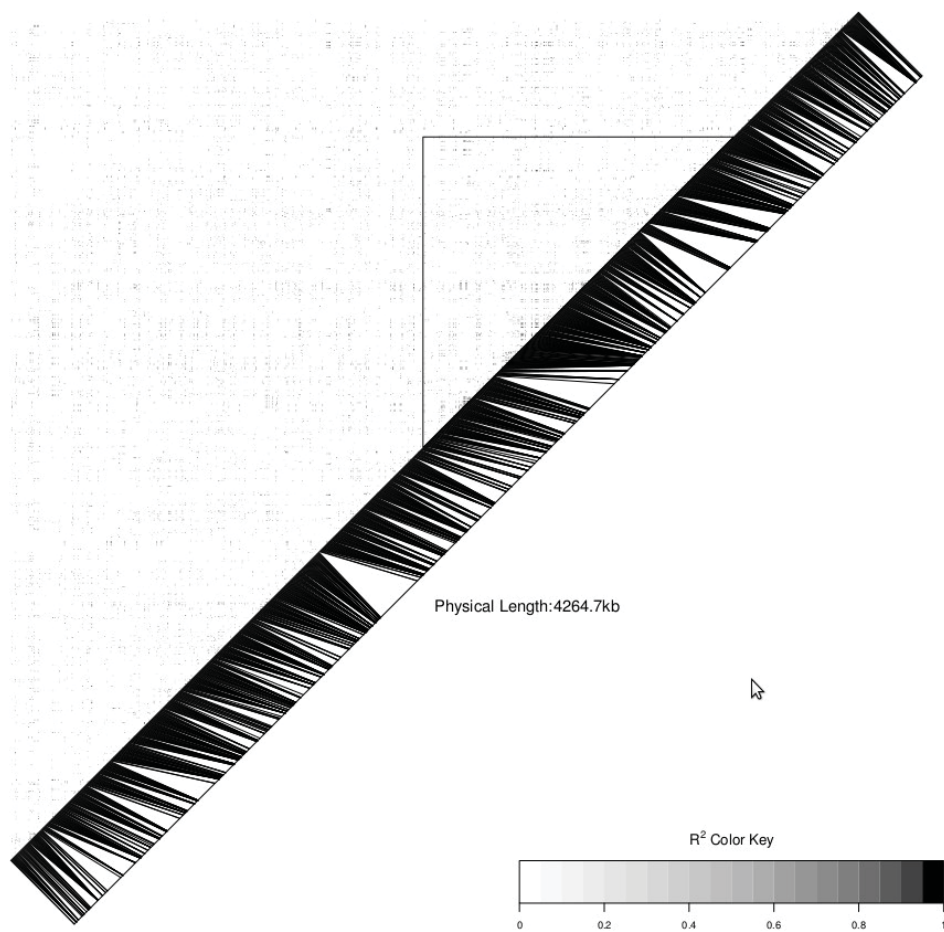
D' in *Austria alba* chr03 17.5–21.7 mio bp



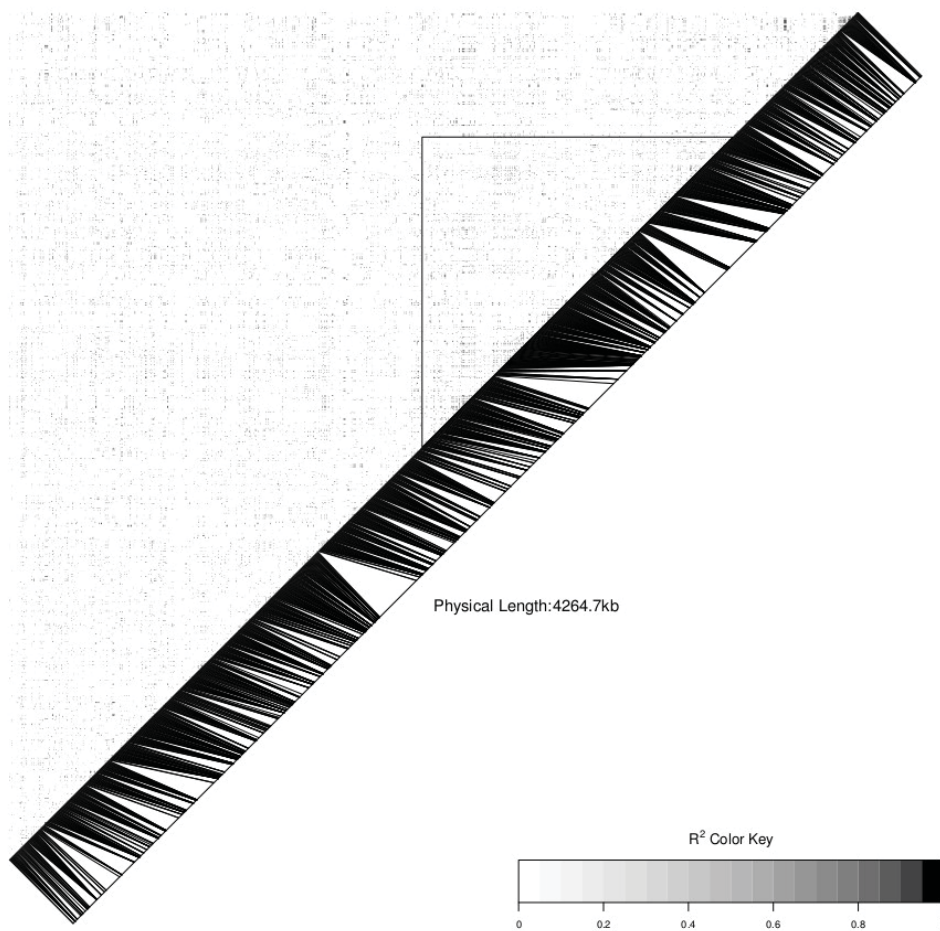
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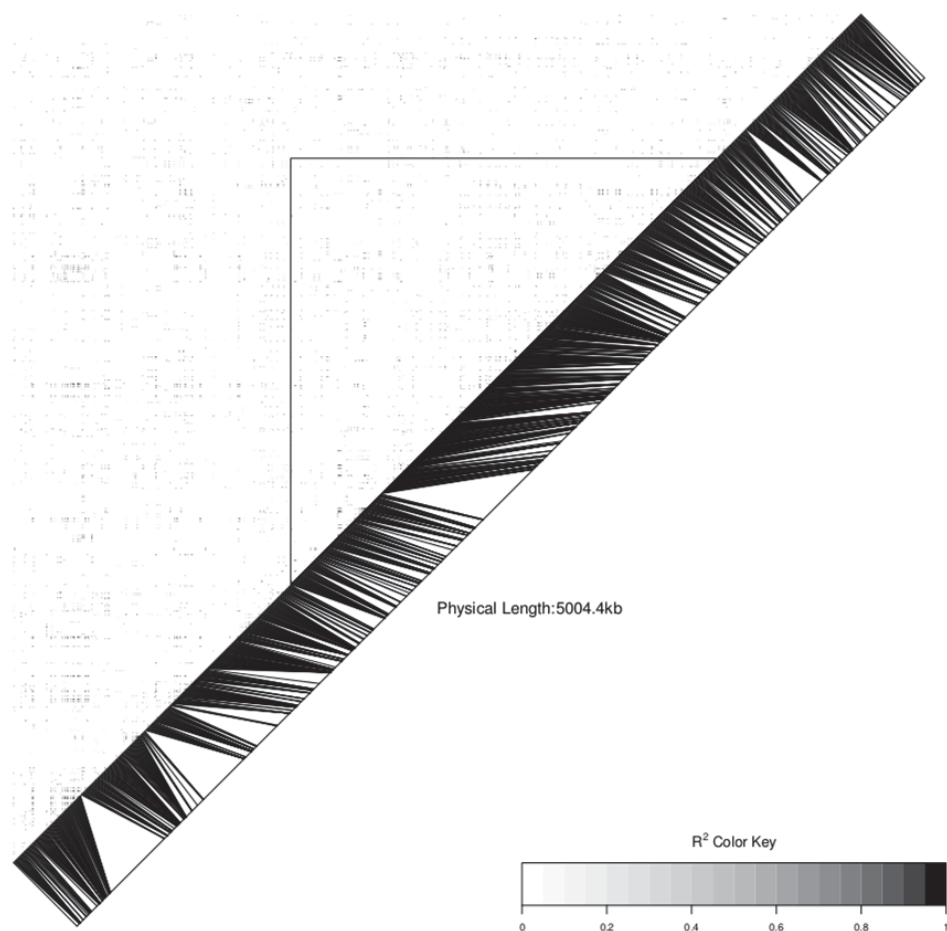
D' in Hungary alba chr03 17.5–21.7 mio bp



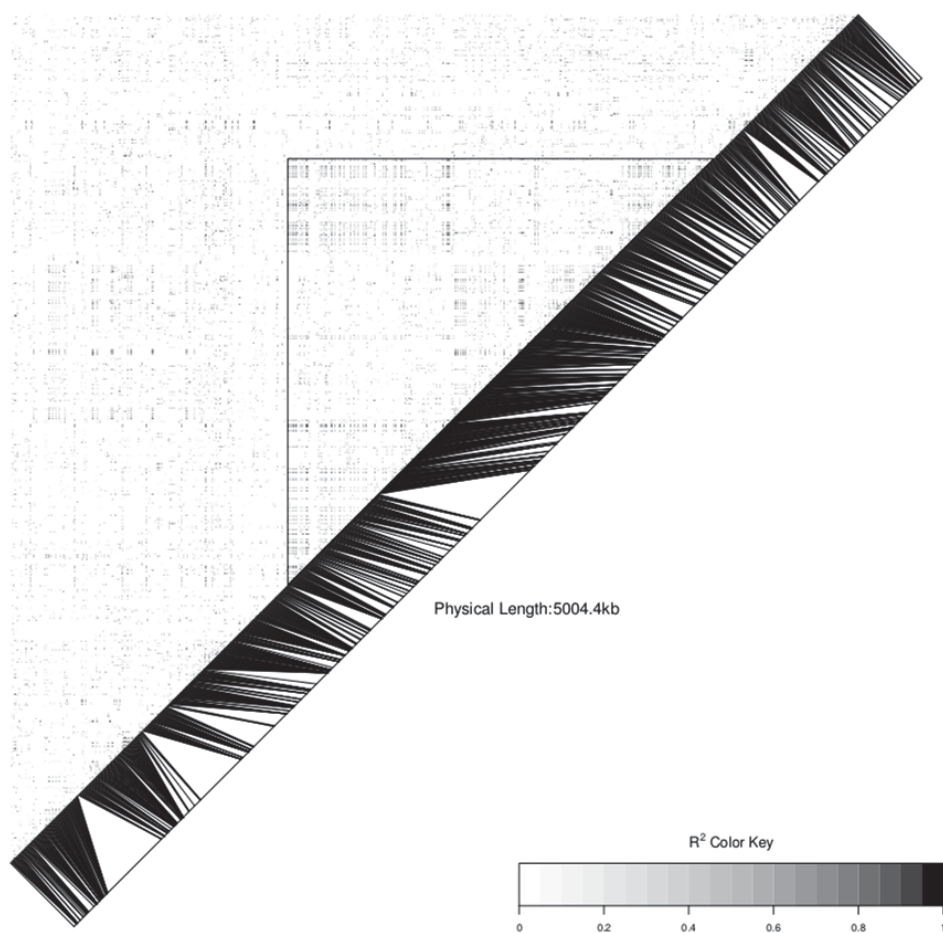
D' in Hungary tremula chr03 17.5–21.7 mio bp



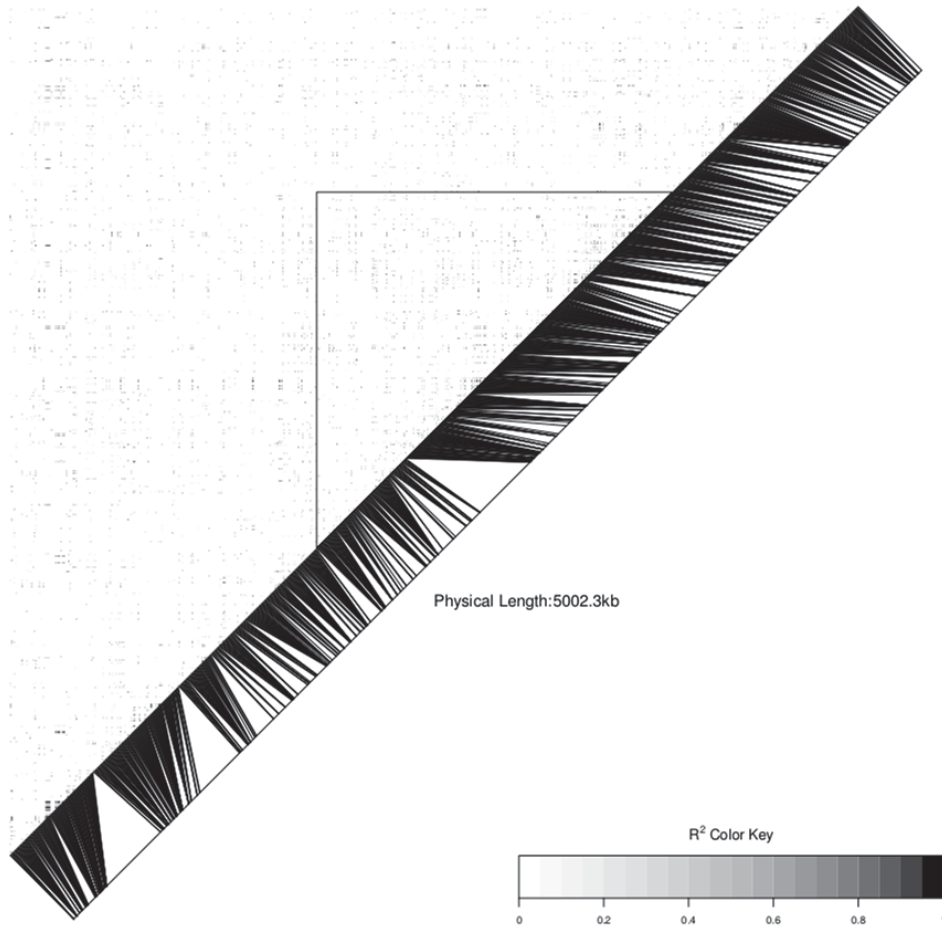
D' in Italy alba chr01 2.5–7.5 mio bp



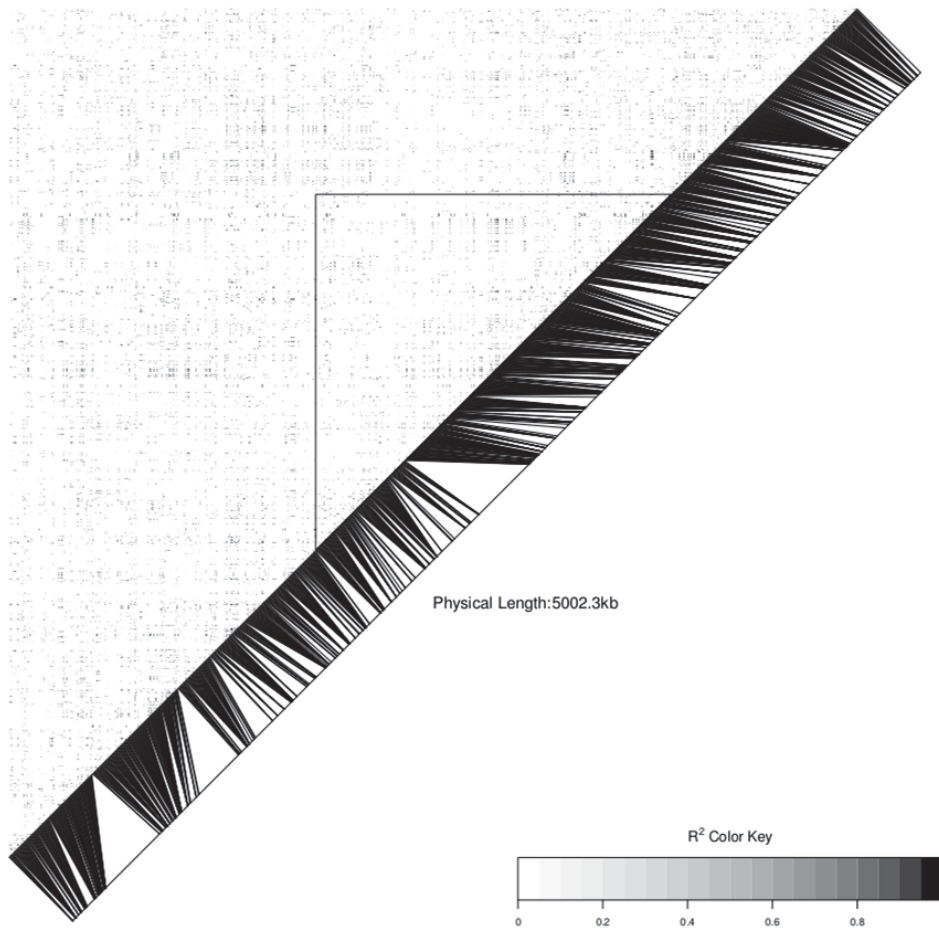
D' in Italy tremula chr01 2.5–7.5 mio bp



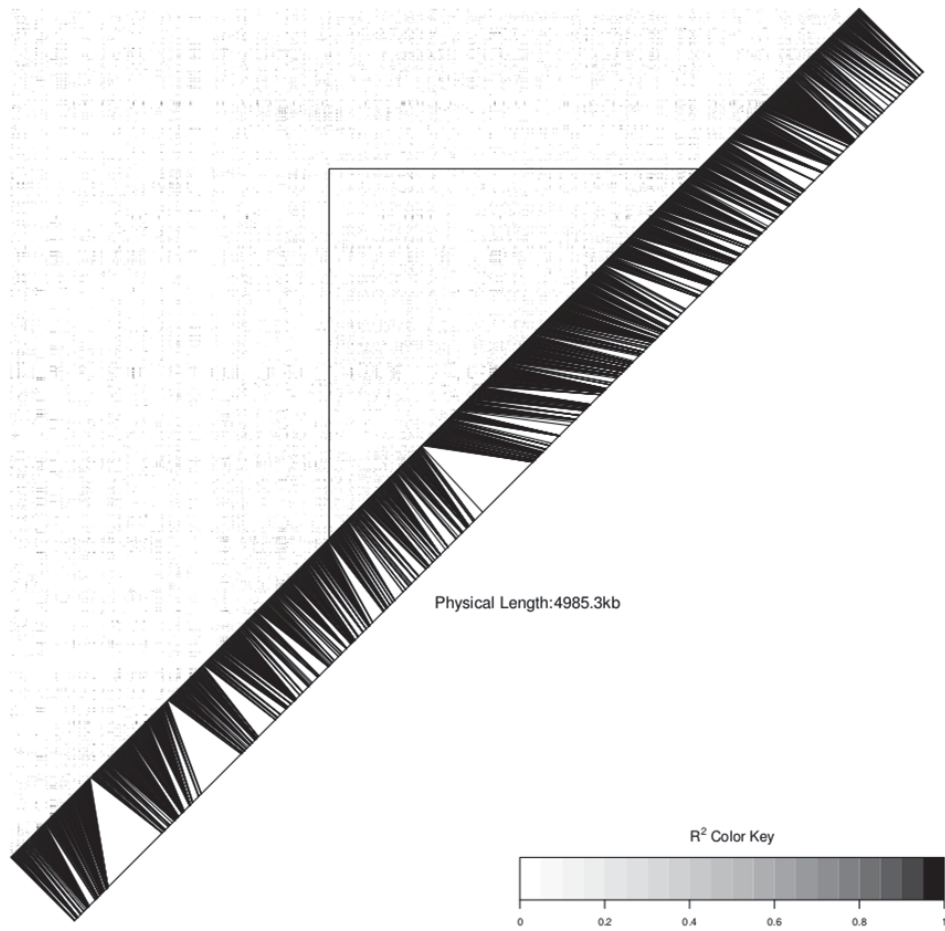
D' in *Austria alba* 2.5–7.5 mio bp



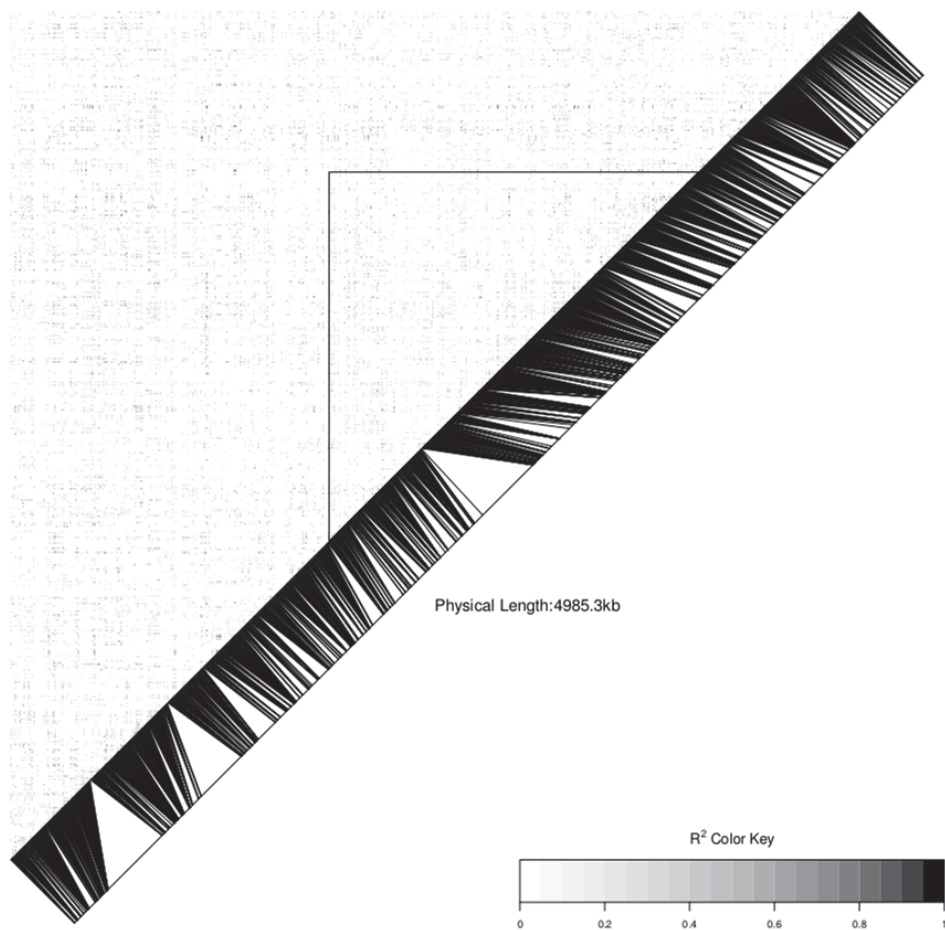
D' in *Austria tremula* 2.5–7.5 mio bp

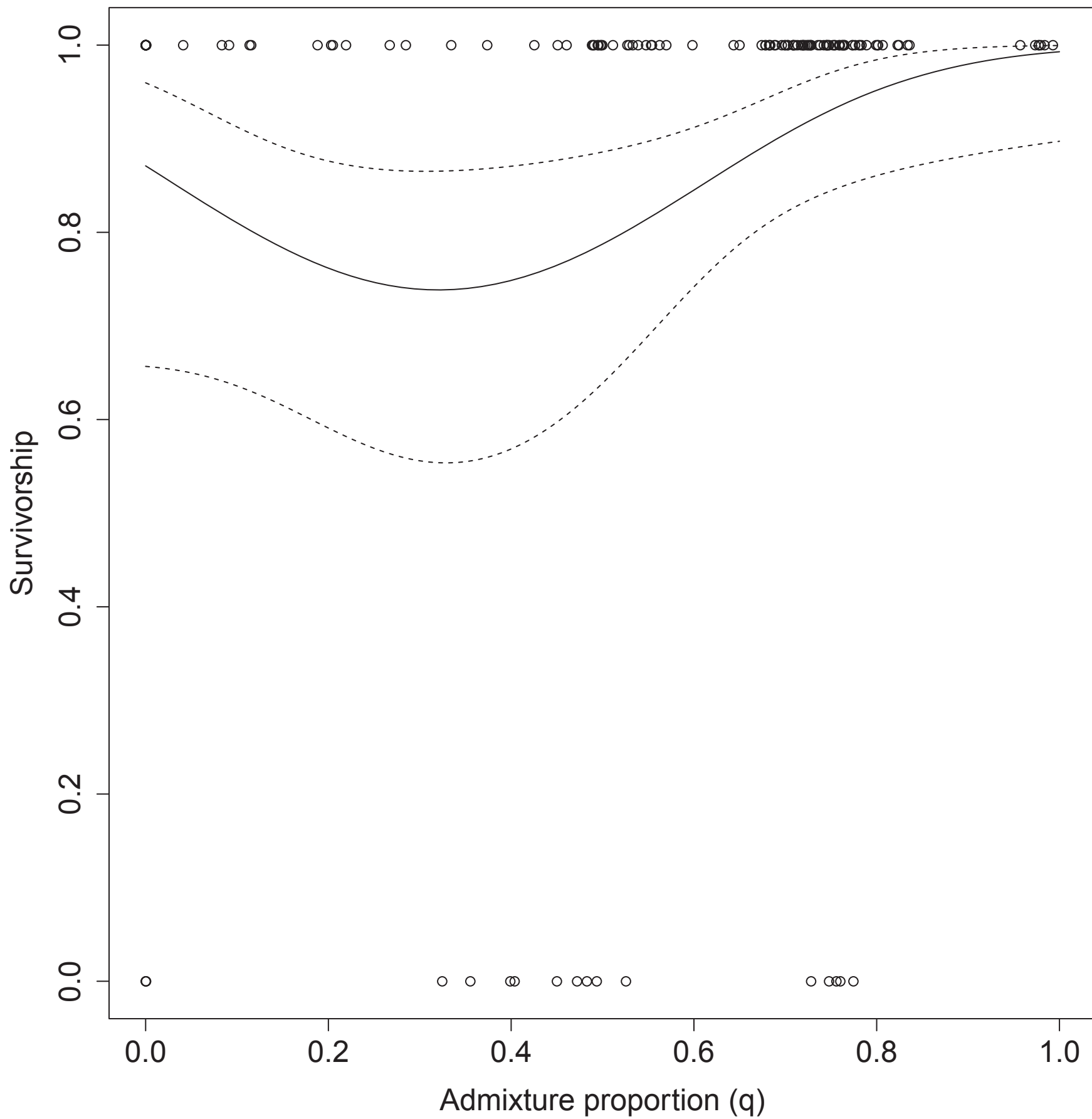


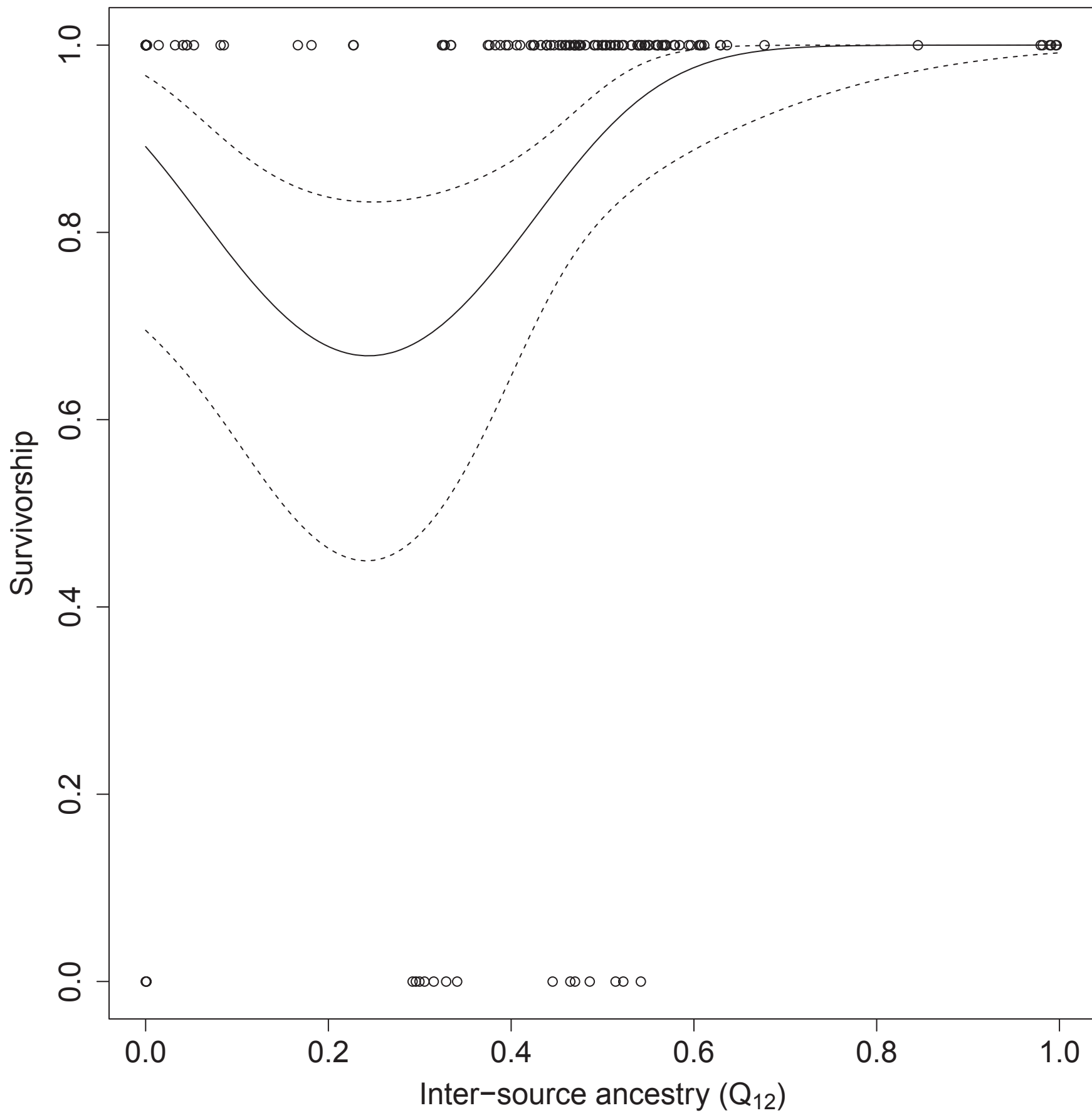
D' in Hungary alba chr01 2.5–7.5 mio bp



D' in Hungary tremula chr01 2.5–7.5 mio bp







Supporting Information

Selection against recombinant hybrids maintains reproductive isolation in hybridizing *Populus* species despite F₁ fertility and recurrent gene flow

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Validation of RAD-based genotyping of plastid DNA polymorphisms with the PCR-RFLP method

To validate RAD-based genotyping of plastid DNA polymorphisms (see main paper), haplotypes from six PCR-amplified plastid DNA fragments were screened for restriction site polymorphisms following Lexer *et al.* (2005) and Fussi *et al.* (2010) for a subset of the samples (total, 83; Italy, 48; Hungary, 35). Polymorphism data from the six fragments were combined into haplotypes and compared with haplotypes previously described by Fussi *et al.* (2010). A median joining network was built with the software Network (Bandelt *et al.* 1999) to be able to discriminate diagnostic haplotypes for *P. alba*, *P. tremula*, and haplotypes shared between the two species.

Twenty two plastid DNA haplotypes were found using PCR-RFLP, twelve of which had already been described by Fussi *et al.* (2010) (**Table S1**). Except for one single haplotype (H45), the median joining network allowed us to distinguish between haplotypes that were restricted or almost restricted (H21) to either *P. alba* or *P. tremula* (**Fig. S1**).

31 **Table S1.** Background information on individuals genotyped for the present study, including individual ID, hybrid zone
32 locality (A, Austria; H, Hungary; I, Italy), number of raw reads and quality reads in Mio, mean coverage, percentage of
33 missing data, admixture proportions q based on 70 microsatellites (Lindtke *et al.* 2012), q based on RAD-seq data
34 gathered in this study, plastid haplotypes identified by PCR-RFLP (NA, not analyzed), plastid haplotypes identified by
35 RAD-seq, and genotype class determined with RASPBerry for hybrids and with ADMIXTURE for the parental species.
36 Genotype classes Alba and Tremula correspond to the parental reference categories described in the main paper, classes
37 Alba-like, BCA, F1, imperfect F1, Fn, BCT, and Trem-like correspond to the genotypic classes reported in Table 2 and the
38 hybrid category in Table 1 of the main paper.

ID	Hybrid zone	Raw reads	Quality reads	Coverage	% missing data	q / micro-satellites	q / RAD	PCR-RFLP haplotype	RAD plastid haplotype	Genotype class
IT_006	I	5.22	4.86	23.13	0.29	0.002	0.00001	NA	alba1	Alba
IT_007	I	3.08	2.31	20.63	0.08	0.003	0.00001	NA	alba1	Alba
IT_012	I	7.67	5.9	49.97	0.01	0.004	0.00001	NA	alba1	Alba
IT_033	I	8.81	7.55	41.1	0.04	0.13	0.00001	NA	alba1	Alba
IT_087	I	4.91	4.31	30.34	0.05	0.672	0.00001	NA	alba1	Alba
IT_127	I	9.13	7.97	73.4	0.02	0.498	0.00001	NA	alba1	Alba
IT_129	I	6.06	3.43	20.04	0.25	0.002	0.00001	NA	alba1	Alba
IT_130	I	6.42	5.68	47.96	0.02	0.011	0.00001	H5	alba1	Alba
IT_131	I	5.92	5.09	42.51	0.02	0.011	0.00001	NA	alba1	Alba
IT_133	I	6.22	5.49	46.37	0.02	0.013	0.00001	NA	alba1	Alba
IT_138	I	8.14	7.06	46.47	0.02	0.013	0.00001	H9	alba1	Alba
IT_140	I	4.85	4.22	28.22	0.19	0.002	0.00001	NA	alba1	Alba
IT_152	I	8.13	6.09	46.33	0.01	0.066	0.00001	H5	alba1	Alba
IT_159	I	4.88	4.26	29.32	0.05	0.465	0.00001	NA	alba1	Alba
IT_178	I	5.19	4.36	33.03	0.03	0.062	0.00001	H5	alba1	Alba
IT_182	I	7.27	6.06	33.48	0.11	0.002	0.00001	H58	alba1	Alba

IT_039	I	5.48	4.79	15.16	0.37	0.003	0.02276	H58	alba1	Alba-like
IT_089	I	3.02	2.44	15.64	0.35	0.002	0.03138	NA	–	Alba-like
IT_040	I	5.09	4.34	24.25	0.22	0.002	0.03991	NA	alba1	Alba-like
IT_093	I	3.49	2.68	15.47	0.36	0.002	0.03992	NA	alba1	Alba-like
IT_156	I	4.76	4.16	34.64	0.03	0.276	0.21956	NA	alba1	BCA
IT_151	I	5.39	4.52	39.94	0.01	0.199	0.23745	H5	alba1	BCA
IT_142	I	5.63	4.29	30.77	0.02	0.363	0.38617	H45	trem2	BCA
IT_143	I	5.84	4.94	42.91	0.01	0.382	0.39434	H66	trem2	BCA
IT_139	I	4.8	4.22	36.71	0.01	0.46	0.43693	NA	alba1	BCA
IT_061	I	4.86	4.27	23.49	0.23	0.464	0.47459	NA	alba1	F ₁
IT_019	I	3.57	3.24	20.57	0.31	0.533	0.47621	H5	alba1	F ₁
IT_046	I	6.16	5.23	50.48	0.01	0.492	0.48141	H10	alba1	F ₁
IT_001	I	5.12	4.3	29.09	0.15	0.555	0.4847	H45	trem1	F ₁
IT_057	I	3.13	2.8	19.12	0.3	0.437	0.48557	NA	alba1	F ₁
IT_020	I	6.77	5.1	44.03	0.01	0.492	0.4865	H5	alba1	F ₁
IT_124	I	5.24	4.53	40.62	0.01	0.512	0.48685	NA	alba1	F ₁
IT_097	I	6.49	5.63	49.88	0.01	0.488	0.48717	NA	alba1	F ₁
IT_106	I	4.31	3.67	26.02	0.08	0.47	0.48838	NA	trem2	F ₁
IT_113	I	5.08	4.27	38.21	0.01	0.497	0.48857	NA	alba1	F ₁
IT_058	I	5.71	4.89	37.83	0.06	0.443	0.48861	NA	alba1	F ₁
IT_038	I	5.03	4.01	25.51	0.11	0.503	0.48917	H58	alba1	F ₁
IT_073	I	7.42	6.5	50.85	0	0.545	0.48963	NA	trem1	F ₁
IT_163	I	6.72	5.97	54.52	0	0.425	0.48971	H45	trem2	F ₁
IT_079	I	6.34	5.61	47.27	0.01	0.458	0.48996	NA	alba1	F ₁
IT_072	I	6.31	4.74	42.66	0.01	0.498	0.49005	NA	–	F ₁
IT_055	I	5.13	4.49	39.48	0.01	0.451	0.49032	NA	alba1	F ₁

IT_108	I	4.94	4.35	34.79	0.04	0.524	0.49038	NA	alba1	F ₁
IT_054	I	5.87	5.14	42.4	0.01	0.447	0.49054	NA	alba1	F ₁
IT_059	I	4.49	3.82	23.92	0.15	0.472	0.49103	H50	trem1	F ₁
IT_103	I	6.75	5.81	52.53	0.01	0.513	0.49112	NA	trem1	F ₁
IT_074	I	3.33	2.76	23.4	0.04	0.547	0.49165	NA	trem1	F ₁
IT_037	I	6.96	6.13	22.38	0.18	0.483	0.49208	H45	trem1	F ₁
IT_036	I	3.85	3.37	20.8	0.21	0.473	0.49268	NA	trem1	F ₁
IT_053	I	7.37	6.33	40.98	0.04	0.473	0.49282	NA	alba1	F ₁
IT_049	I	7.95	6.72	45.6	0.03	0.43	0.49408	NA	alba1	F ₁
IT_078	I	4.96	4.42	29.89	0.07	0.475	0.49473	NA	trem1	F ₁
IT_141	I	2.78	2.09	18.17	0.11	0.389	0.49486	H9	alba1	F ₁
IT_048	I	3.52	3.08	20.65	0.21	0.424	0.49565	NA	alba1	F ₁
IT_052	I	3.78	3.14	21.37	0.19	0.469	0.49732	NA	alba1	F ₁
IT_050	I	3.56	3.08	19.73	0.23	0.522	0.49851	NA	alba1	F ₁
IT_056	I	2.19	1.93	13.56	0.42	0.426	0.4989	H10	alba1	F ₁
IT_063	I	4.53	3.91	25.31	0.15	0.495	0.49971	NA	alba1	F ₁
IT_051	I	3.78	3.22	17.74	0.27	0.553	0.50089	H10	alba1	F ₁
IT_145	I	2.33	2.05	18.25	0.26	0.485	0.51685	H9	–	F ₁
IT_077	I	4.33	3.8	33.66	0.02	0.423	0.46033	NA	trem1	F _n
IT_064	I	3.58	3.18	26.55	0.11	0.503	0.39243	NA	alba1	Imp F ₁
IT_015	I	4.02	3.16	22.47	0.18	0.51	0.4736	NA	–	Imp F ₁
IT_099	I	7.32	5.3	48.26	0	0.441	0.48556	NA	alba1	Imp F ₁
IT_100	I	8	6.88	60.52	0.01	0.458	0.48569	NA	alba1	Imp F ₁
IT_098	I	8.08	6.04	52.98	0.01	0.458	0.48604	NA	alba1	Imp F ₁

IT_150	I	5.7	4.99	44.13	0.01	0.488	0.48629	H9	alba1	Imp F ₁
IT_065	I	4.36	3.8	32.35	0.02	0.492	0.48636	H5	alba1	Imp F ₁
IT_085	I	7.25	6.44	58.87	0.01	0.52	0.48813	NA	trem1	Imp F ₁
IT_095	I	7.77	5.73	50.55	0	0.511	0.48932	NA	trem1	Imp F ₁
IT_119	I	8	6.65	58.98	0.01	0.572	0.48935	H5	alba1	Imp F ₁
IT_092	I	5.08	4.45	39.29	0.01	0.46	0.48954	NA	alba1	Imp F ₁
IT_104	I	6.75	5.92	51.33	0.01	0.492	0.4896	H9	alba1	Imp F ₁
IT_090	I	5.26	4.5	35.61	0.01	0.483	0.48976	NA	alba1	Imp F ₁
IT_080	I	6.29	5.47	41.65	0.01	0.527	0.48987	NA	trem1	Imp F ₁
IT_107	I	5.59	4.69	36.8	0.01	0.438	0.48992	H50	trem1	Imp F ₁
IT_084	I	4.72	4.07	35.51	0.01	0.512	0.49015	NA	trem1	Imp F ₁
IT_164	I	6.74	5.64	51.98	0.01	0.47	0.49054	H45	trem2	Imp F ₁
IT_096	I	5.12	3.81	30.4	0.02	0.548	0.49055	NA	trem1	Imp F ₁
IT_165	I	4.9	4.37	35.46	0.01	0.489	0.49083	H45	trem2	Imp F ₁
IT_076	I	2.77	2.36	17.49	0.26	0.483	0.49089	NA	alba1	Imp F ₁
IT_062	I	3.01	2.53	18.61	0.26	0.493	0.49101	NA	alba1	Imp F ₁
IT_121	I	6.88	5.95	51.78	0.01	0.502	0.4911	H5	alba1	Imp F ₁
IT_120	I	7.39	6.41	53.52	0.01	0.491	0.49112	NA	alba1	Imp F ₁
IT_160	I	5.49	4.83	42.36	0.01	0.495	0.49118	H63	trem3	Imp F ₁
IT_027	I	3.9	3.44	26.95	0.04	0.579	0.49139	NA	alba1	Imp F ₁
IT_091	I	5.19	4.49	35.17	0.01	0.457	0.49161	H10	alba1	Imp F ₁
IT_025	I	4.2	3.52	19.53	0.27	0.607	0.49232	NA	alba1	Imp F ₁
IT_026	I	6.25	4.5	26	0.16	0.577	0.4925	NA	alba1	Imp F ₁
IT_102	I	3.7	3.08	25.94	0.03	0.541	0.4927	H9	alba1	Imp F ₁

IT_136	I	3.3	2.84	20.77	0.12	0.467	0.49286	H45	trem1	Imp F ₁
IT_083	I	2.79	2.33	20.47	0.07	0.47	0.49377	H9	alba1	Imp F ₁
IT_122	I	5.02	4.37	40.05	0.01	0.443	0.49404	H50	trem1	Imp F ₁
IT_021	I	4.99	4.29	30.42	0.16	0.516	0.49416	H5	trem1	Imp F ₁
IT_118	I	6.02	5.3	42.28	0.01	0.562	0.49489	NA	alba1	Imp F ₁
IT_162	I	4.74	4.19	33.01	0.02	0.494	0.49872	H50	trem2	Imp F ₁
IT_153	I	7.25	6.15	54.77	0	0.638	0.57313	NA	alba1	Imp F ₁
IT_155	I	6.98	5.89	55.2	0.01	0.643	0.57329	H59	alba1	Imp F ₁
IT_154	I	6.49	5.58	51.42	0.01	0.648	0.57363	H5	alba1	Imp F ₁
IT_088	I	4.73	4.12	36.52	0.02	0.576	0.59372	NA	alba1	BCT
IT_086	I	5.45	4.76	40.37	0.02	0.196	0.70044	NA	trem1	BCT
IT_043	I	4.04	3.29	18.49	0.26	0.981	0.95361	H50	trem1	Trem-like
IT_041	I	5.23	4.55	39.5	0.03	0.998	0.99999	H45	trem1	Tremula
IT_070	I	6.26	4.43	41.09	0.03	0.998	0.99999	NA	trem2	Tremula
IT_081	I	7.95	6.79	59.21	0.02	0.971	0.99999	NA	trem1	Tremula
IT_110	I	7.91	5.43	49.42	0.02	0.998	0.99999	NA	trem1	Tremula
IT_114	I	6.04	5.07	45.48	0.02	0.988	0.99999	H36	trem1	Tremula
IT_115	I	5.14	4.52	40.69	0.03	0.987	0.99999	NA	trem1	Tremula
IT_135	I	4.96	4.23	38.08	0.02	0.972	0.99999	H64	trem1	Tremula
IT_137	I	7.31	5.71	32.92	0.03	0.991	0.99999	H63	trem3	Tremula
IT_166	I	7.57	6.35	53.6	0.02	0.989	0.99999	NA	trem1	Tremula
IT_171	I	8.22	6.13	56.45	0.02	0.997	0.99999	NA	trem2	Tremula
IT_176	I	7.43	6.21	53.33	0.02	0.989	0.99999	NA	trem2	Tremula
IT_180	I	12.41	10.91	48.11	0.03	0.996	0.99999	NA	trem1	Tremula
IT_184	I	4.08	3.07	27.69	0.08	0.998	0.99999	NA	trem1	Tremula
IT_186	I	7.7	6.42	48.68	0.02	0.989	0.99999	NA	trem1	Tremula
IT_187	I	5.32	4.67	37.8	0.05	0.97	0.99999	H45	trem2	Tremula

IT_190	I	7.11	5.95	54.66	0.02	0.989	0.99999	NA	trem2	Tremula
IT_193	I	6.55	4.99	43.3	0.03	0.998	0.99999	NA	trem1	Tremula
IT_196	I	6.18	4.6	41.6	0.03	0.998	0.99999	H50	trem1	Tremula
IT_200	I	7.21	5.02	44.83	0.02	0.997	0.99999	NA	trem1	Tremula
IT_210	I	4.55	3.51	31.08	0.04	0.997	0.99999	NA	trem1	Tremula
IT_214	I	7.68	5.74	50.34	0.02	0.996	0.99999	NA	trem1	Tremula
IT_217	I	6.2	4.39	38.34	0.04	0.998	0.99999	H51	trem1	Tremula
IT_222	I	7.58	5.43	50.31	0.02	0.997	0.99999	H62	trem1	Tremula
IT_008	I	4.02	3.64	—	0.34	0.002	0.00001	—	—	—
IT_030	I	2.96	2.5	—	0.3	0.002	0.00001	—	—	—
IT_031	I	8.32	3.14	—	0.35	0.002	0.00001	—	—	—
IT_034	I	3.25	2.67	—	0.48	0.002	0.00001	—	—	—
IT_148	I	2.3	1.98	—	0.54	1	0.02614	—	—	—
IT_022	I	2.58	2.36	—	0.62	0.552	0.49486	—	—	—
IT_016	I	0.61	0.45	—	—	0.527	—	—	—	—
IT_060	I	1.53	1.09	—	—	0.472	—	—	—	—
IT_067	I	1.48	1.31	—	—	0.012	—	—	—	—
IT_146	I	1.28	1.14	—	—	0.43	—	—	—	—
IT_157	I	1.6	1.41	—	—	0.45	—	—	—	—
12A4	A	2.01	1.89	18.93	0.15	0.001	0.00001	NA	alba1	Alba
14A4	A	2.3	2.17	19.63	0.2	0.001	0.00001	NA	alba1	Alba
28A4	A	3.44	3.21	30.61	0.06	0.001	0.00001	NA	alba1	Alba
29A4	A	2.34	2.12	29.15	0.09	0.001	0.00001	NA	alba1	Alba
31A4	A	3.21	2.69	21.9	0.1	0.029	0.00001	NA	alba1	Alba
35A4	A	6.55	5.92	83.54	0.01	0.005	0.00001	NA	alba1	Alba
44A4	A	2.39	2.2	32.37	0.14	0.001	0.00001	NA	alba1	Alba
4M19	A	4.62	3.9	36.84	0.04	0.001	0.00001	NA	alba1	Alba
4M34	A	10.16	8.62	83	0.01	0.001	0.00001	NA	alba1	Alba
4M38	A	7.71	6.63	65.01	0.01	0.001	0.00001	NA	alba1	Alba
53A4	A	2.88	2.63	38.42	0.02	0	0.00001	NA	alba1	Alba

60A4	A	5.6	4.62	42.93	0.03	0.001	0.00001	NA	alba1	Alba
61A4	A	3.55	3.17	39.21	0.03	0.001	0.00001	NA	alba1	Alba
71A4	A	2.76	2.54	38.07	0.02	0.001	0.00001	NA	alba1	Alba
73A4	A	7.34	6.8	96.82	0.02	0.001	0.00001	NA	alba1	Alba
83A4	A	4.15	3.8	53.23	0.03	0.001	0.00001	NA	alba1	Alba
84A4	A	5.41	5	71.99	0.01	0.001	0.00001	NA	alba1	Alba
J121	A	5.33	4.83	72.59	0.01	0.001	0.00001	NA	alba1	Alba
J13	A	6.36	5.77	83.24	0.01	0.001	0.00001	NA	alba1	Alba
J235	A	4.06	3.83	36.76	0.02	0.041	0.00001	NA	alba1	Alba
J218	A	3.84	3.44	32.11	0.02	0.372	0.19478	NA	trem1	BCA
J237	A	2.23	2.11	19.33	0.22	0.022	0.20625	NA	—	BCA
J238	A	5.86	5.09	56.87	0.01	0.325	0.25754	NA	trem1	BCA
J209	A	6.76	5.33	54.74	0.02	0.337	0.27649	NA	alba1	BCA
18A4	A	4.14	3.5	30.89	0.03	0.256	0.29144	NA	alba1	BCA
J8	A	6.47	6.08	59.87	0.01	0.511	0.49046	NA	alba1	F ₁
J257	A	3.54	3.27	31.16	0.02	0.517	0.49136	NA	alba1	F ₁
J188	A	3.75	3.05	27.83	0.04	0.486	0.49009	NA	alba1	Imp F ₁
4M23	A	3.17	2.82	26.67	0.04	0.508	0.49235	NA	alba1	Imp F ₁
25A4	A	2.82	2.62	23.78	0.07	0.758	0.49256	NA	alba1	Imp F ₁
11A4	A	2.85	2.68	26	0.04	0.558	0.49308	NA	alba1	Imp F ₁
J185	A	3.05	2.84	25.57	0.05	0.507	0.50165	NA	alba1	Imp F ₁
J104	A	5.16	4.48	42.85	0.02	0.557	0.5044	NA	alba1	Imp F ₁
J184	A	5.18	4.47	41.81	0.02	0.494	0.51152	NA	alba1	Imp F ₁
4M25	A	3.95	3.54	32.84	0.04	0.498	0.7249	NA	trem1	BCT
95A4	A	5.83	4.78	44.55	0.06	0.976	0.90759	NA	trem2	Trem-like
J220	A	3.23	3.01	28.7	0.05	1	0.99999	NA	trem1	Tremula
J226	A	3.82	3.5	32.39	0.04	0.948	0.99999	NA	trem1	Tremula

J231	A	4.16	3.79	50.95	0.02	0.994	0.99999	NA	trem1	Tremula
J256	A	7.51	6.81	94.89	0.02	0.997	0.99999	NA	trem1	Tremula
TM1M4	A	3.64	3.33	46.03	0.04	0.999	0.99999	NA	trem1	Tremula
TM2M4	A	2.99	2.7	37.12	0.05	0.999	0.99999	NA	trem1	Tremula
TO1M4	A	3.54	3.16	44.82	0.02	0.999	0.99999	NA	trem1	Tremula
TO2M4	A	5.64	5.06	73.84	0.02	1	0.99999	NA	trem1	Tremula
TO4M4	A	5.82	4.56	26.53	0.14	1	0.99999	NA	trem1	Tremula
TO5M4	A	4.64	4.18	58.04	0.02	0.999	0.99999	NA	trem1	Tremula
TO6M4	A	5.09	4.57	63.63	0.02	1	0.99999	NA	trem1	Tremula
TO8M4	A	4.78	4.07	25.02	0.21	0.999	0.99999	NA	trem1	Tremula
TR1M4	A	2.11	1.91	24.14	0.1	0.999	0.99999	NA	trem1	Tremula
TR2M4	A	3.49	3.1	42.95	0.02	0.999	0.99999	NA	trem1	Tremula
TR3M4	A	2.66	2.43	32.78	0.04	0.999	0.99999	NA	trem2	Tremula
J168	A	3.41	2.76	—	0.32	0	0.99999	—	—	—
8A4	A	1.2	1.09	—	—	0.001	—	—	—	—
93A4	A	0.47	0.44	—	—	0.001	—	—	—	—
TO3M4	A	0.73	0.58	—	—	0.999	—	—	—	—
TO7M4	A	1.3	1.09	—	—	1	—	—	—	—
HU_022	H	8.29	6.19	60.36	0.02	0.001	0.00001	NA	alba1	Alba
HU_070	H	8.01	5.94	55.23	0.03	0.002	0.00001	NA	alba2	Alba
HU_076	H	5.98	4.5	40.19	0.02	0.001	0.00001	NA	alba1	Alba
HU_080	H	6.87	5.05	46.2	0.02	0.011	0.00001	NA	alba1	Alba
HU_093	H	5.34	4.11	39.11	0.02	0.017	0.00001	NA	alba1	Alba
HU_102	H	7.43	5.75	53.34	0.02	0.001	0.00001	NA	alba1	Alba
HU_113	H	4.05	3.13	29.23	0.1	0.001	0.00001	NA	alba1	Alba
HU_130	H	6.52	4.75	46.83	0.04	0.001	0.00001	NA	alba1	Alba
HU_143	H	3.34	2.29	19.48	0.13	0.001	0.00001	NA	alba1	Alba
HU_149	H	6.71	5.11	48.95	0.02	0.001	0.00001	H21	alba1	Alba
HU_157	H	6.13	4.2	37.77	0.06	0.001	0.00001	NA	alba1	Alba
HU_164	H	6.63	4.97	44.69	0.02	0.001	0.00001	NA	alba1	Alba
HU_172	H	4.06	2.84	23.25	0.11	0.001	0.00001	NA	alba1	Alba

HU_176	H	8.43	5.74	47.16	0.02	0.001	0.00001	NA	alba1	Alba
HU_184	H	7.88	5.48	48.24	0.03	0.001	0.00001	NA	alba1	Alba
HU_007	H	6.17	4.63	40.58	0.06	0.076	0.00524	NA	alba1	Alba-like
HU_053	H	5.49	4.21	38.93	0.07	0.001	0.03413	NA	alba1	Alba-like
HU_057	H	3.48	2.71	24.14	0.18	0.001	0.04145	NA	alba1	Alba-like
HU_092	H	5.33	3.64	33.14	0.04	0.363	0.28245	H21	alba1	BCA
HU_074	H	3.87	2.5	23.04	0.1	0.323	0.29902	H21	alba1	BCA
HU_106	H	2.73	2.1	18.28	0.2	0.309	0.41455	H45	trem2	BCA
HU_086	H	6.95	4.64	41.63	0.02	0.458	0.49748	H21	alba1	F ₁
HU_097	H	8.27	5.69	51.47	0.01	0.552	0.49777	H21	alba1	F ₁
HU_060	H	4.53	3.38	27.25	0.04	0.507	0.50039	H53	trem1	F ₁
HU_121	H	3.16	2.19	21.02	0.25	0.481	0.50131	H45	trem2	F ₁
HU_058	H	8.52	6.03	58.38	0.01	0.489	0.50149	H21	alba1	F ₁
HU_163	H	5.5	3.79	34.89	0.03	0.501	0.50228	H21	trem2	F ₁
HU_098	H	6.87	4.75	41.46	0.02	0.412	0.50271	H21	alba1	F ₁
HU_116	H	7.15	5.01	47.31	0.01	0.581	0.50342	H21	alba1	F ₁
HU_099	H	6.85	4.6	42.95	0.01	0.514	0.50353	H61	alba1	F ₁
HU_111	H	7.67	5.41	47.69	0.01	0.51	0.5053	H21	alba1	F ₁
HU_069	H	4.75	3.25	28.43	0.04	0.521	0.50637	H36	trem1	F ₁
HU_117	H	5.83	3.96	36.48	0.02	0.536	0.50746	H21	alba1	F ₁
HU_103	H	2.45	1.69	15.63	0.27	0.47	0.5076	H21	alba1	F ₁
HU_125	H	2.22	1.7	15.76	0.32	0.499	0.50953	H21	alba1	F ₁
HU_062	H	1.59	1.24	11.92	0.5	0.44	0.52353	H45	–	F ₁
HU_096	H	4.36	3.22	28	0.12	0.614	0.52522	H21	alba1	F ₁
HU_073	H	8.25	5.8	54.36	0.01	0.461	0.49983	H21	alba1	Imp F ₁

HU_081	H	5.47	4.1	36.06	0.02	0.559	0.50008	H15	alba1	Imp F ₁
HU_055	H	7.65	5.71	47.84	0.01	0.534	0.50159	H50	trem1	Imp F ₁
HU_090	H	5.31	3.75	35.66	0.03	0.492	0.50274	H21	alba1	Imp F ₁
HU_167	H	6.32	4.85	45.92	0.01	0.454	0.50288	NA	trem3	Imp F ₁
HU_141	H	5.07	3.61	29.34	0.04	0.525	0.50402	H45	trem2	Imp F ₁
HU_079	H	4.37	3.33	30.34	0.03	0.484	0.50695	H15	alba1	Imp F ₁
HU_170	H	5.42	4.57	42.43	0.03	0.434	0.50908	H21	alba1	Imp F ₁
HU_082	H	5.56	4.11	35.69	0.03	0.497	0.51757	H15	alba1	Imp F ₁
HU_029	H	6.08	5.17	49.55	0.07	0.999	0.94015	NA	trem1	Trem-like
HU_018	H	6.19	5.34	52.8	0.06	0.999	0.95262	NA	trem2	Trem-like
HU_005	H	7.59	6.43	55.58	0.05	0.999	0.95864	NA	trem1	Trem-like
HU_001	H	4.49	3.87	36.29	0.07	0.998	0.99999	H36	trem1	Tremula
HU_012	H	5.69	4.9	47.72	0.04	0.999	0.99999	NA	trem1	Tremula
HU_013	H	4.87	4.15	36.84	0.08	0.997	0.99999	H50	trem1	Tremula
HU_023	H	8.59	7.36	69.44	0.04	0.998	0.99999	NA	trem1	Tremula
HU_034	H	6.7	5.93	58.33	0.02	0.997	0.99999	NA	trem1	Tremula
HU_044	H	6.82	5.88	57.09	0.04	0.999	0.99999	NA	trem1	Tremula
HU_046	H	8.75	7.52	70.84	0.04	0.999	0.99999	NA	trem1	Tremula
HU_059	H	6.7	5.76	53.84	0.05	0.999	0.99999	H67	trem1	Tremula
HU_065	H	7.61	6.59	63.77	0.03	0.999	0.99999	NA	trem1	Tremula
HU_078	H	7.5	6.52	61.3	0.03	0.998	0.99999	H53	trem1	Tremula
HU_101	H	8.13	7.07	66.85	0.04	0.999	0.99999	H68	trem1	Tremula
HU_120	H	8.75	7.62	67.24	0.03	0.998	0.99999	H67	trem1	Tremula
HU_122	H	3.49	2.72	26	0.16	0.981	0.99999	H50	trem1	Tremula
HU_054	H	0.95	0.73	—	—	0.534	—	—	—	—
HU_088	H	1.8	1.3	—	—	0.99	—	—	—	—

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40 **Table S2.** F_{ST} between pairs of populations and localities, estimated from RAD-seq data.
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		Italy			Austria			Hungary		
		<i>P. tremula</i>	<i>P. alba</i>	Hybrids	<i>P. tremula</i>	<i>P. alba</i>	Hybrids	<i>P. tremula</i>	<i>P. alba</i>	Hybrids
Italy	<i>P. tremula</i>	0								
	<i>P. alba</i>	0.526	0							
	Hybrids	0.175	0.151	0						
Austria	<i>P. tremula</i>	0.012	0.538	0.176	0					
	<i>P. alba</i>	0.520	0.026	0.156	0.529	0				
	Hybrids	0.195	0.175	0.005	0.188	0.167	0			
Hungary	<i>P. tremula</i>	0.013	0.519	0.154	0.008	0.509	0.162	0		
	<i>P. alba</i>	0.506	0.063	0.156	0.514	0.022	0.158	0.494	0	
	Hybrids	0.175	0.180	0.011	0.170	0.170	-0.001	0.143	0.151	0

42 **Table S3.** Plastid DNA haplotypes identified by RAD-seq using the *P. trichocarpa* reference plastome, including
 43 SNPs named by their positions (mean coverage in brackets), and nucleotide state for each haplotype found in
 44 *P. alba* and *P. tremula*.

Haplotype name	SNP 81893 (3338)	SNP 81901 (3338)	SNP 81996 (6666)	SNP 82711 (5404)	SNP 82819 (2955)	SNP 118145 (4053)	SNP 118190 (3476)
alba1	G	T	A	G	G	A	A
alba2	G	C	A	G	G	A	A
trem1	T	T	G	T	G	G	A
trem2	T	T	G	T	G	G	C
trem3	T	T	G	T	C	G	A

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48 **Table S4.** Linear and quadratic effects of the two genetic ancestry parameters Q12 (interspecific ancestry) and q
 49 (admixture proportion) on seedling survivorship in a common garden trial of *Populus alba* and *P. tremula*, estimated using
 50 mixed effect models including family membership of each seedling as a random factor. Shown are the three top-ranking
 51 models based on AIC_c, the Akaike information criterion corrected for finite sample sizes. Only models with a difference in
 52 AIC_c (delta AIC_c) of <7 to the best supported model are shown.

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Intercept	Q12 linear	Q12 quad	q linear	q quad	df	Log likelihood	AIC _c	delta AIC _c	AIC _c weight
2.63	2.96		-8.23	7.69	5.00	-39.39	89.2	0.00	0.51
2.92		3.08	-5.57	5.45	5.00	-39.94	90.3	1.09	0.29
2.65	2.78	0.21	-8.08	7.56	6.00	-39.39	91.4	2.18	0.17

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56 **Table S5.** Effect estimates, standard errors (SE), and probabilities (p-values) for
 57 the linear and quadratic effects of Q12 and q from the top-ranking model shown in
 58 Table S4, receiving >50% of the AIC weights.

	Estimate	SE	p-value
Q12 linear	2.9631	0.9689	0.002226
q linear	-8.2286	2.4644	0.000841
q quad	7.6852	2.1743	0.000408

Legends to Supporting information Figures S1 – S7

Figure S1 Median joining network for plastid DNA haplotypes visualised by the PCR-RFLP method. Haplotype names are congruent with the terminology used by Fussi *et al.* (2010). New haplotypes (newly found in the PCR-RFLP validation dataset) are labeled with an asterisk. Missing haplotypes are represented as black dots along lines connecting haplotypes. Pie sizes are proportional to haplotype frequencies. Haplotypes are colored according to their population origin: *P. alba* Italy, dark blue; *P. alba* Hungary, light blue; *P. tremula* Italy, dark green; *P. tremula* Hungary, light green; *P. x canescens* hybrids Italy, dark purple; *P. x canescens* hybrids Hungary, light purple.

Figure S2 (A) Local genetic ancestry along the 19 chromosomes of hybrids in the Italian hybrid zone, representing homozygous segments for *P. alba* (blue), homozygous segments for *P. tremula* (orange), heterospecific ancestry segments (green) and undefined segments (white). (B) Genomic admixture proportions and species origin of plastid DNA for each individual (*P. alba*, blue; *P. tremula*, orange; no data, white).

Figure S3 (A) Local genetic ancestry along the 19 chromosomes of hybrids in the Hungarian hybrid zone, representing homozygous segments for *P. alba* (blue), homozygous segments for *P. tremula* (orange), heterospecific ancestry segments (green) and undefined segments (white). (B) Genomic admixture proportions and species origin of plastid DNA for each individual (*P. alba*, blue; *P. tremula*, orange; no data, white).

Figure S4 Sizes of genomic segments (x-axis) found in backcrosses to *P. alba* (BCA, blue), backcrosses to *P. tremula* (BCT, orange), a single advanced recombinant hybrid (F_n , green), and ‘imperfect F_1 ’ hybrids (gray), plotted against the percentage they cover on each chromosome (y-axis). Segment sizes and percentages were extracted from RASPBerry ancestry results.

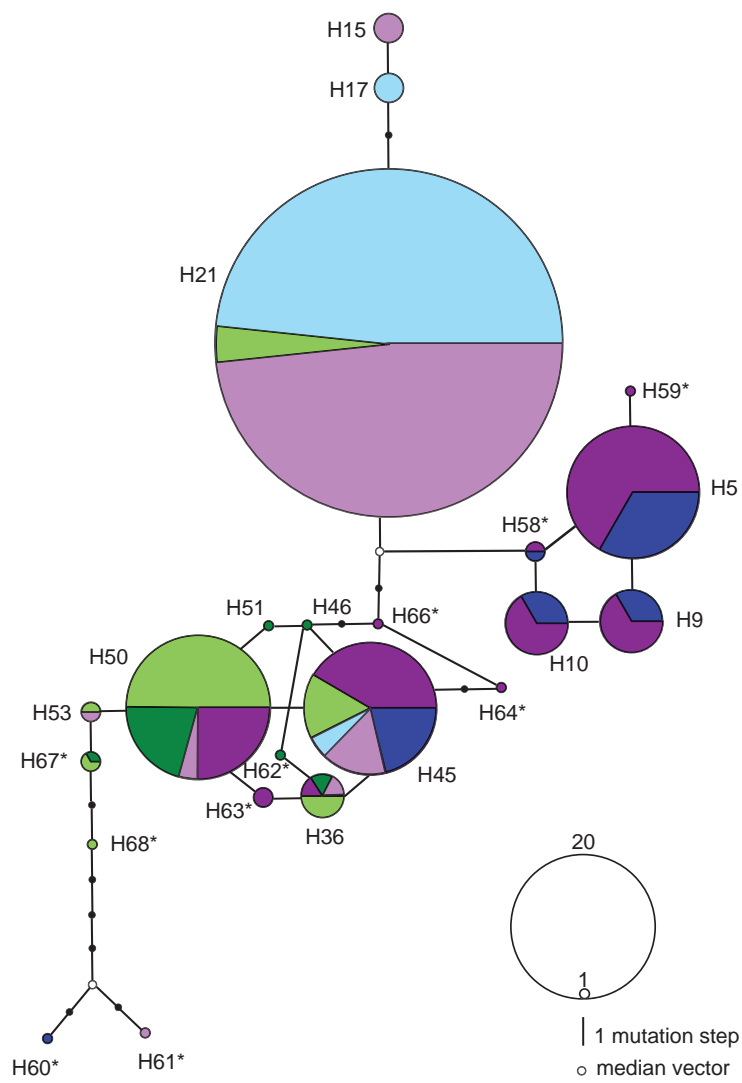
Figure S5 Patterns of SNP density, interspecific genomic divergence (d_{XY}) and differentiation (fixratio) between *Populus alba* and *P. tremula* for Italy (blue), Austria (green) and Hungary (orange). Shown are sliding window analyses (window size, 500 kb; step size, 250 kb) for relative SNP density (top), D_{XY} (middle) and proportion of fixed SNPs among all variable SNPs (fixratio, bottom). Results of all windowed analyses are plotted against window midpoints in million bp for windows with >20 SNPs. Zero fixation windows are highlighted in light yellow, thus facilitating inspection of low-differentiation regions with spatially variable and spatially uniform patterns. Approximate *P. trichocarpa* centromere positions are highlighted in gray.

Figure S6 Heat map showing linkage disequilibrium (D') from 17.5 to 21.7 million base pairs (bp) of chromosome III for the parental populations of *P. alba* and *P. tremula* in the Italian, Austrian, and Hungarian hybrid zone, respectively. D' is depicted on a gray scale as indicated by the chart below the heat map. Positions of markers along the reference genome are indicated by black bars along the diagonale. The genome region of interest is framed by a bold black line.

Figure S7 Heat map showing linkage disequilibrium (D') from 2.5 to 7.5 million base pairs (bp) of chromosome I for the parental populations of *P. alba* and *P. tremula* in the Italian, Austrian, and Hungarian hybrid zone, respectively. D' is depicted on a gray scale as indicated by the chart below the heat map. Positions of markers along the reference genome are indicated by black bars along the diagonale. The genome region of interest is framed by a bold black line.

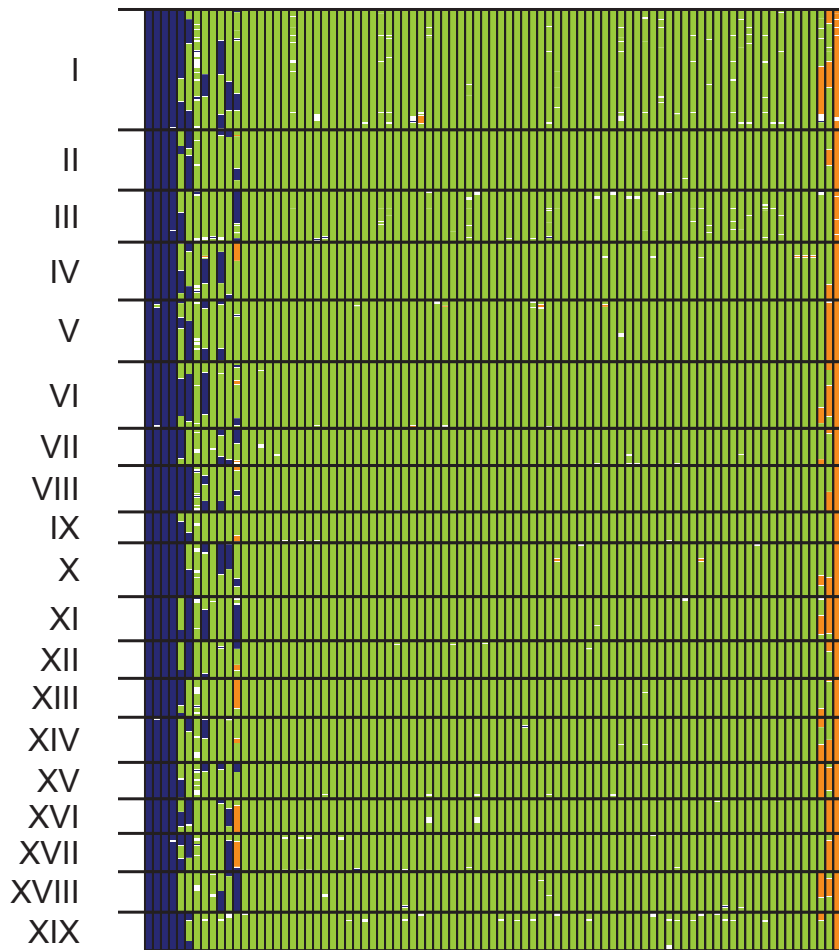
Figure S8 Graphical representation of the linear and quadratic effects of admixture proportions q on seedling survivorship in a common garden trial. Reduced survivorship of individuals with intermediate q (early generation hybrids) and increased survivorship of individuals with large q (*P. alba*-like plants including backcrosses) are visible from the plotted regression line (dashed lines are 95% confidence intervals).

Figure S9 Graphical representation of the linear and quadratic effects of intersource ancestry Q_{12} on seedling survivorship in a common garden trial. Reduced survivorship of individuals with intermediate Q_{12} (recombinant early generation hybrids) and increased survivorship of individuals with maximum Q_{12} (F_1 hybrids) is visible from the plotted regression line (dashed lines are 95% confidence intervals). See Milne & Abbott (2008) and Lindtke *et al.* (2012) for the expected biologically relevant parameter space of Q_{12} and related measures of interspecific heterozygosity.

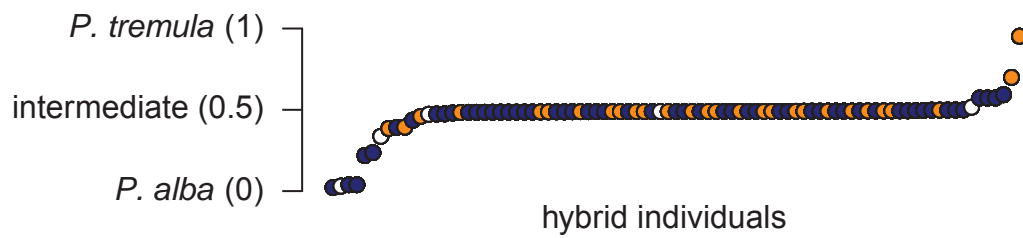


Italy

(A)

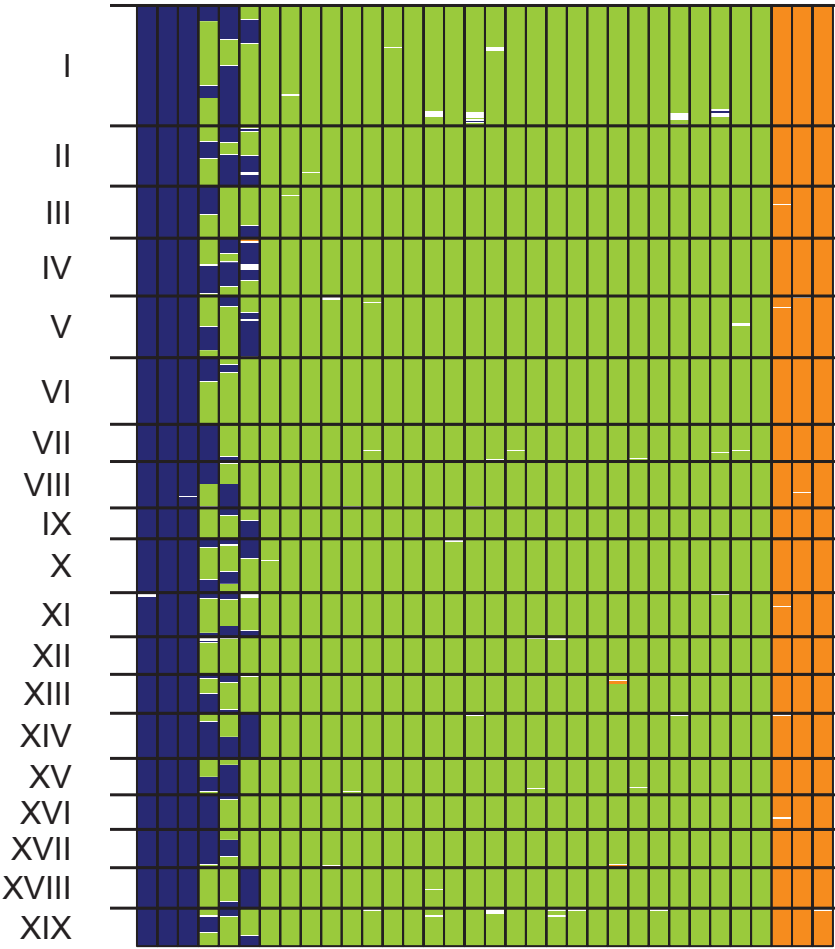


(B)

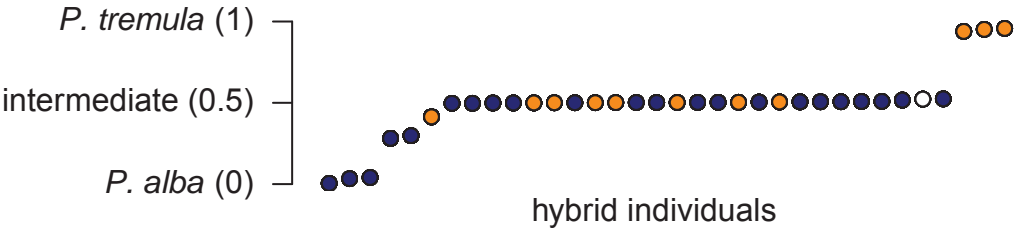


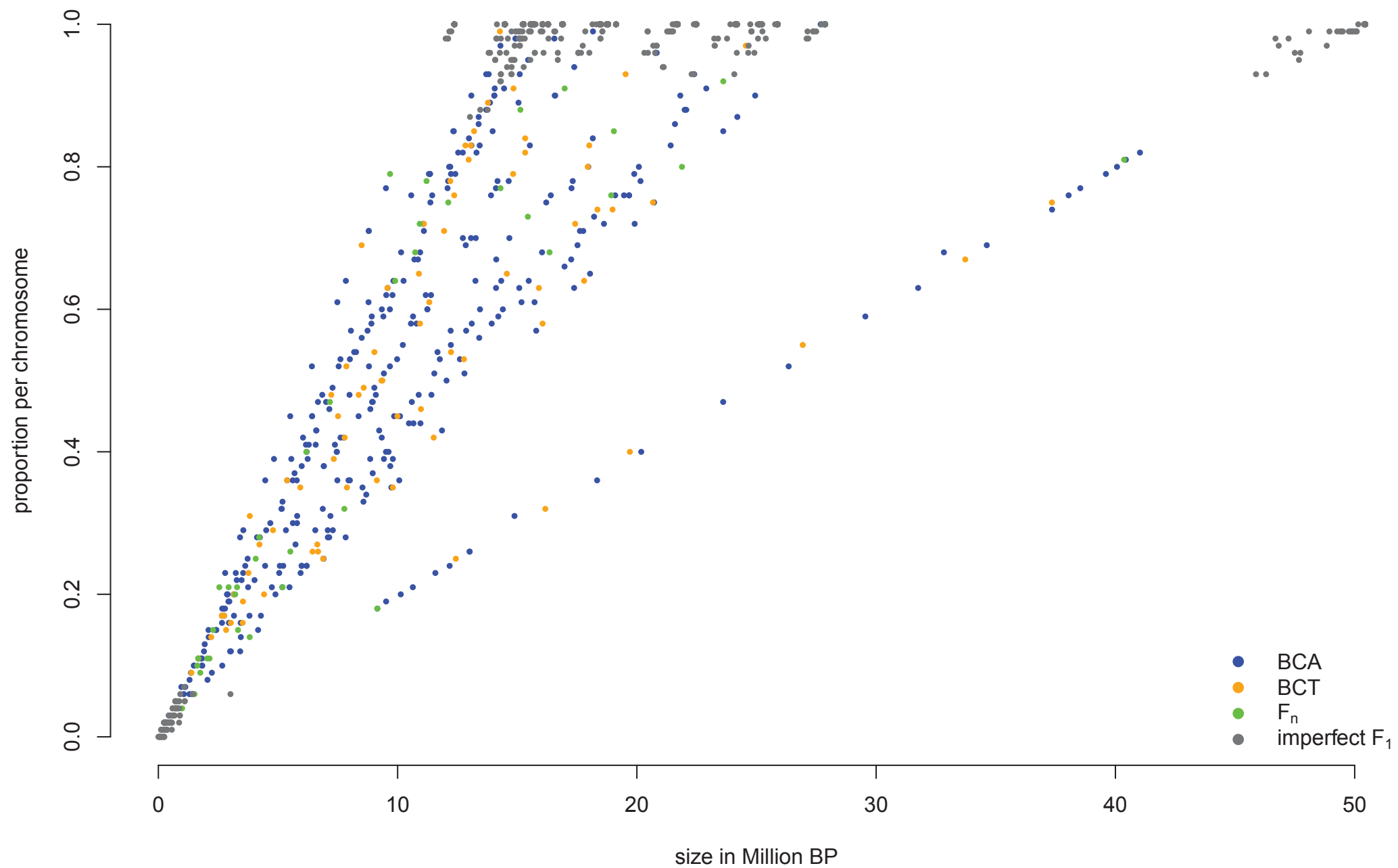
Hungary

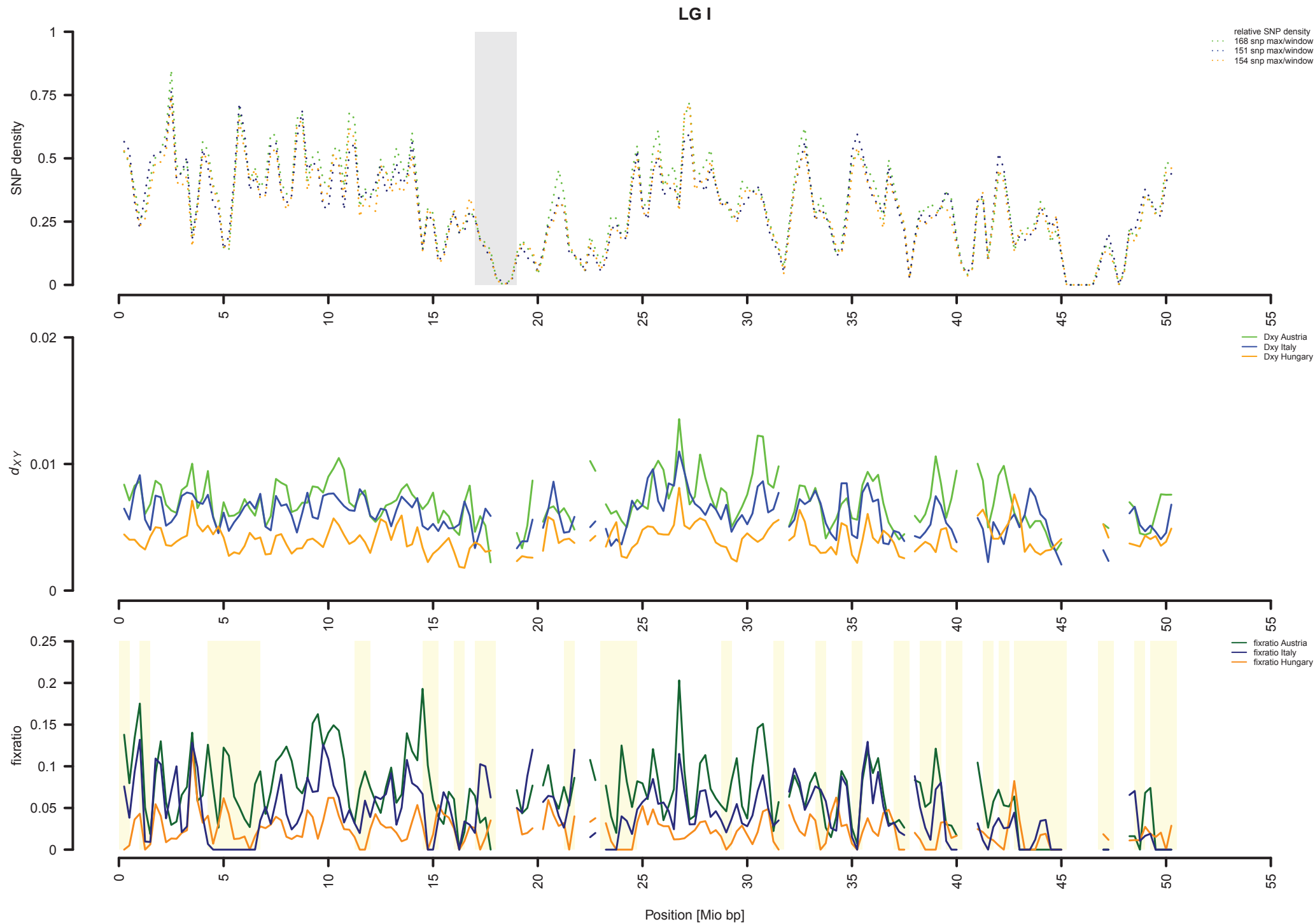
(A)

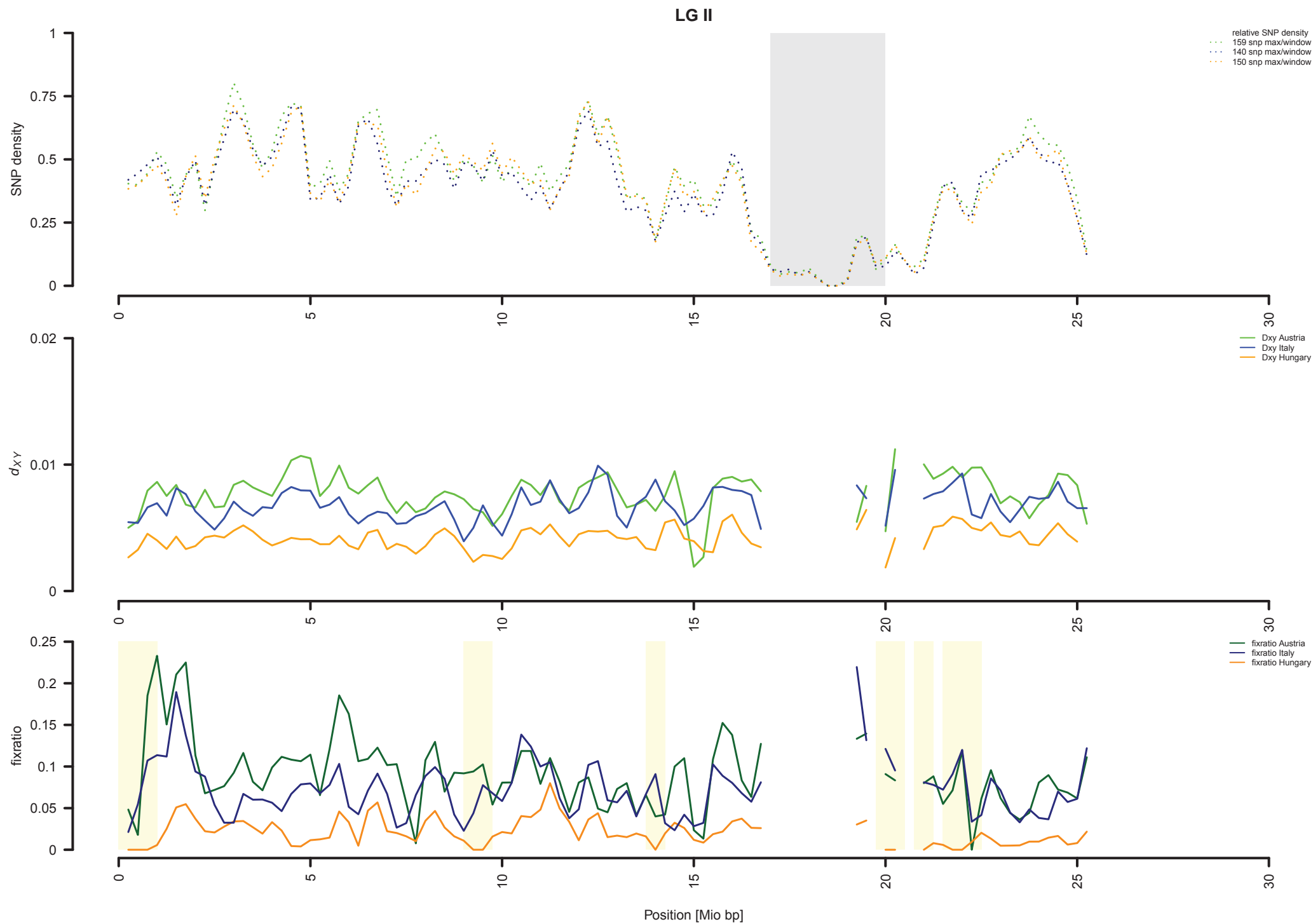


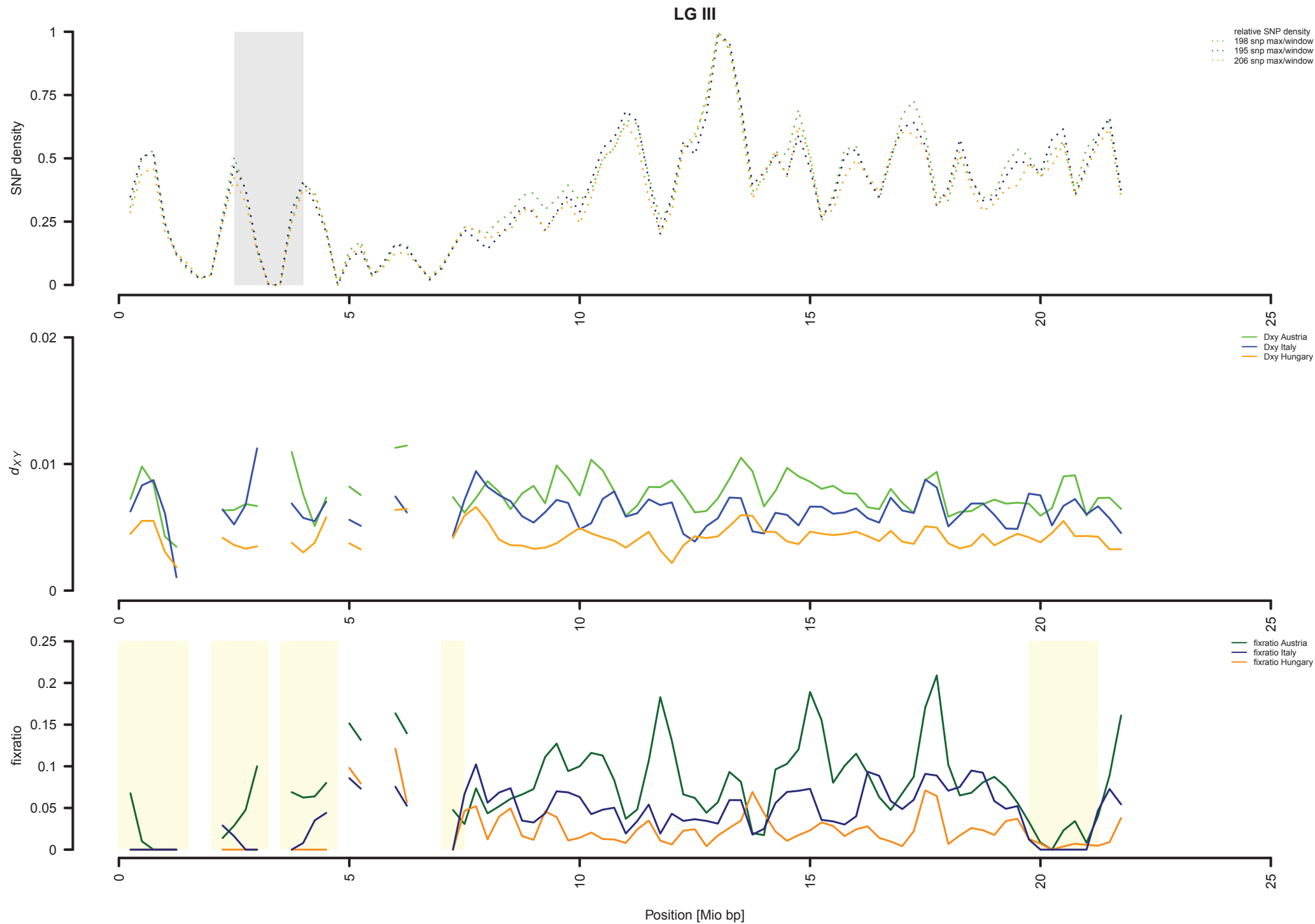
(B)

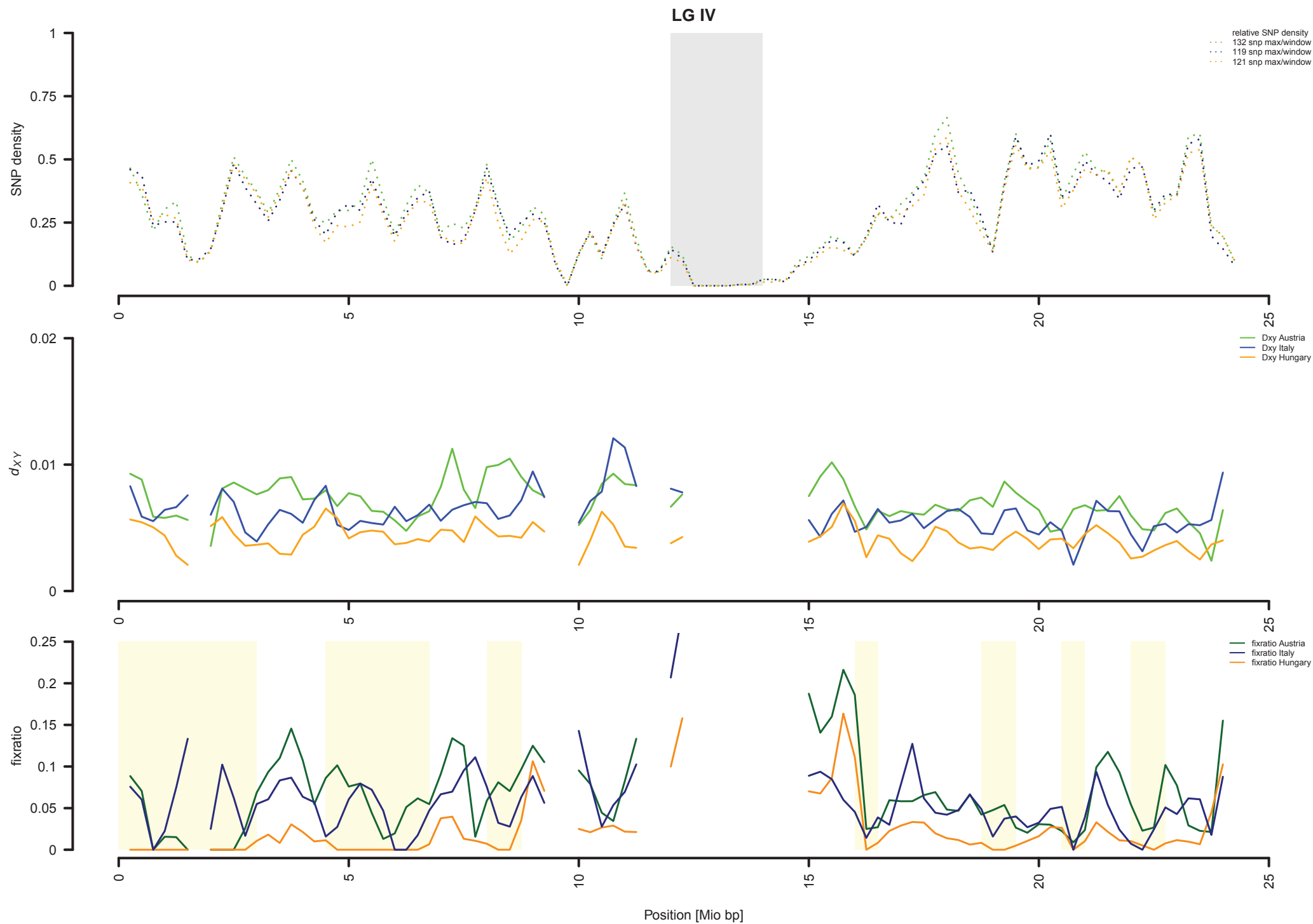


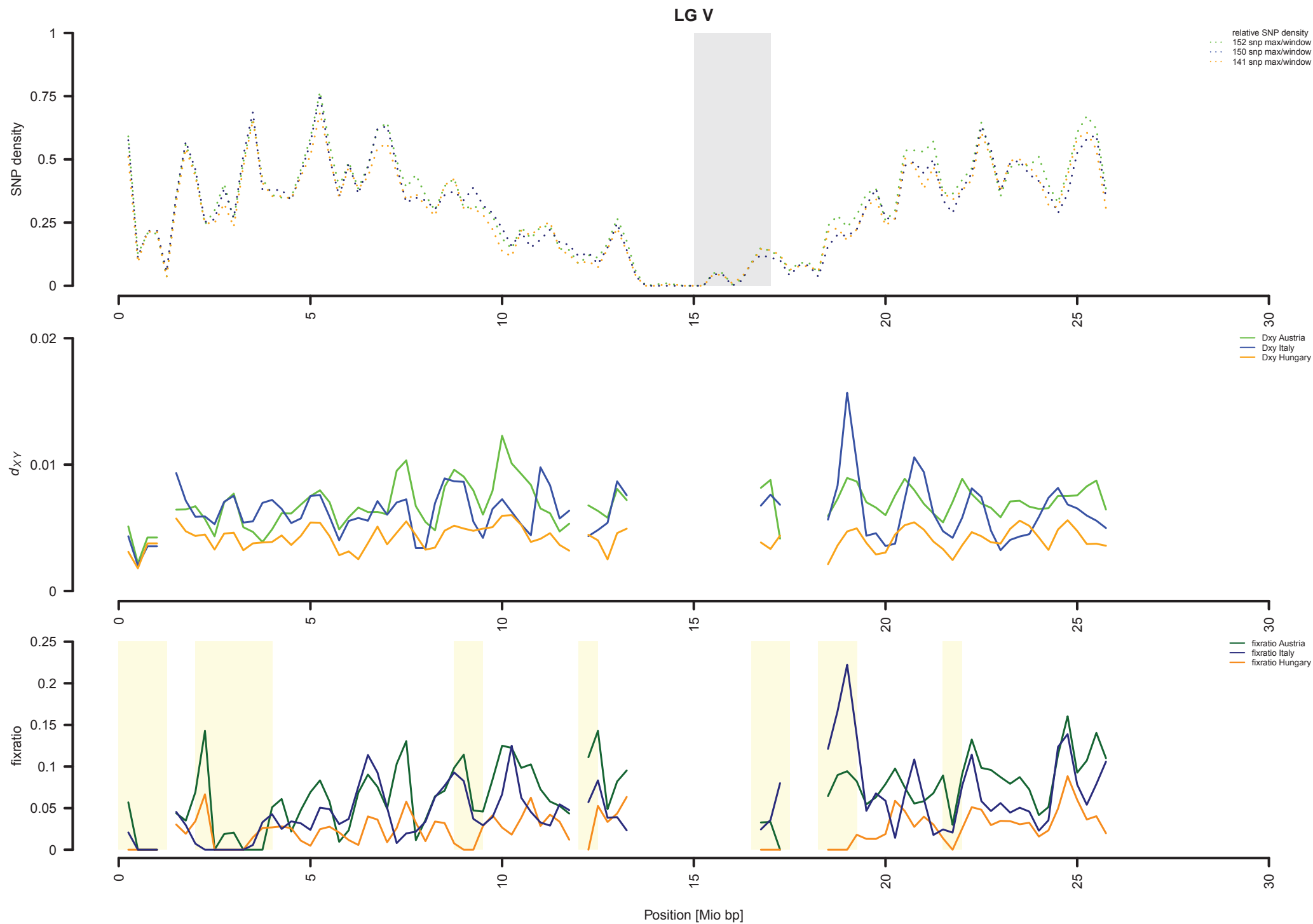


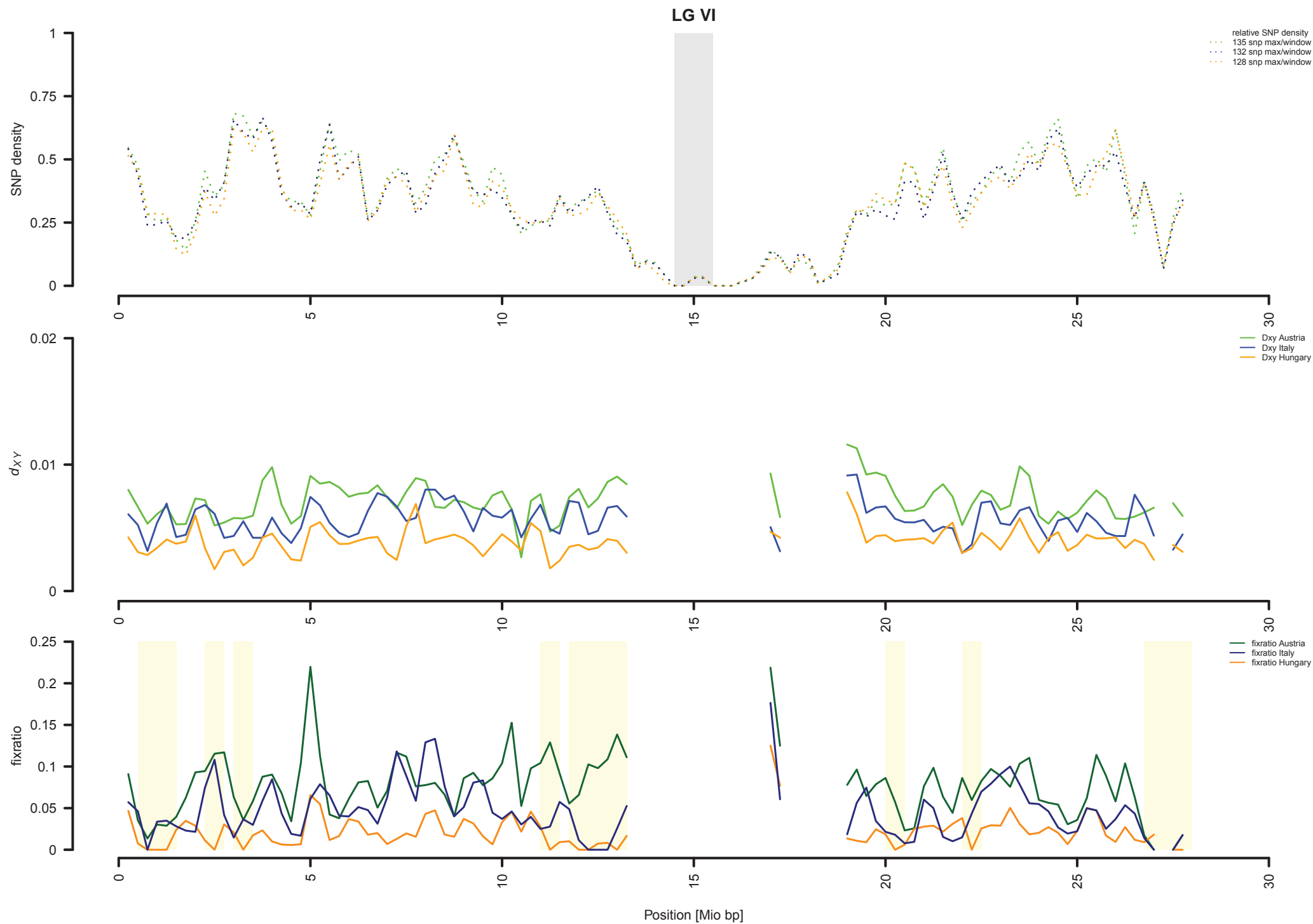




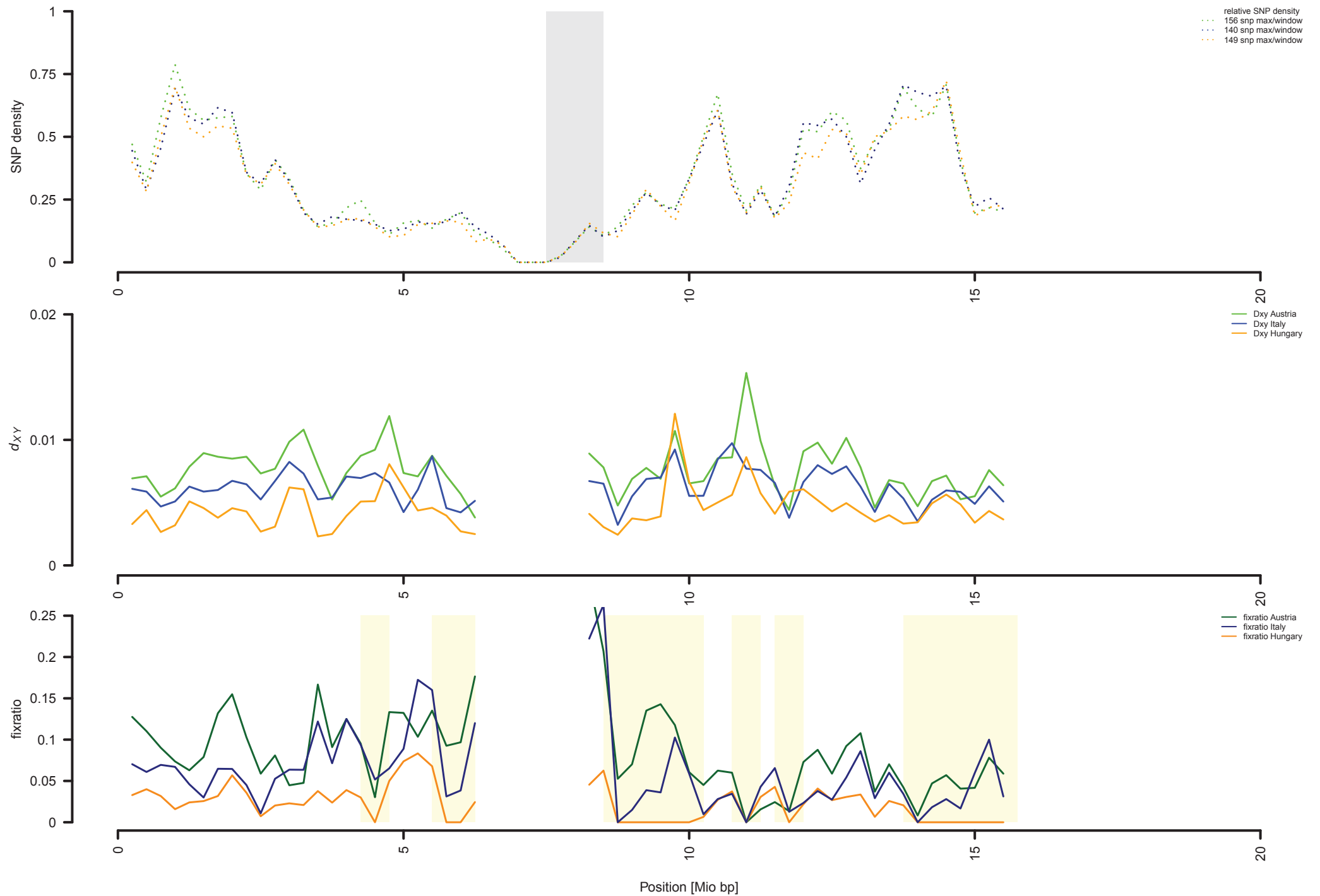




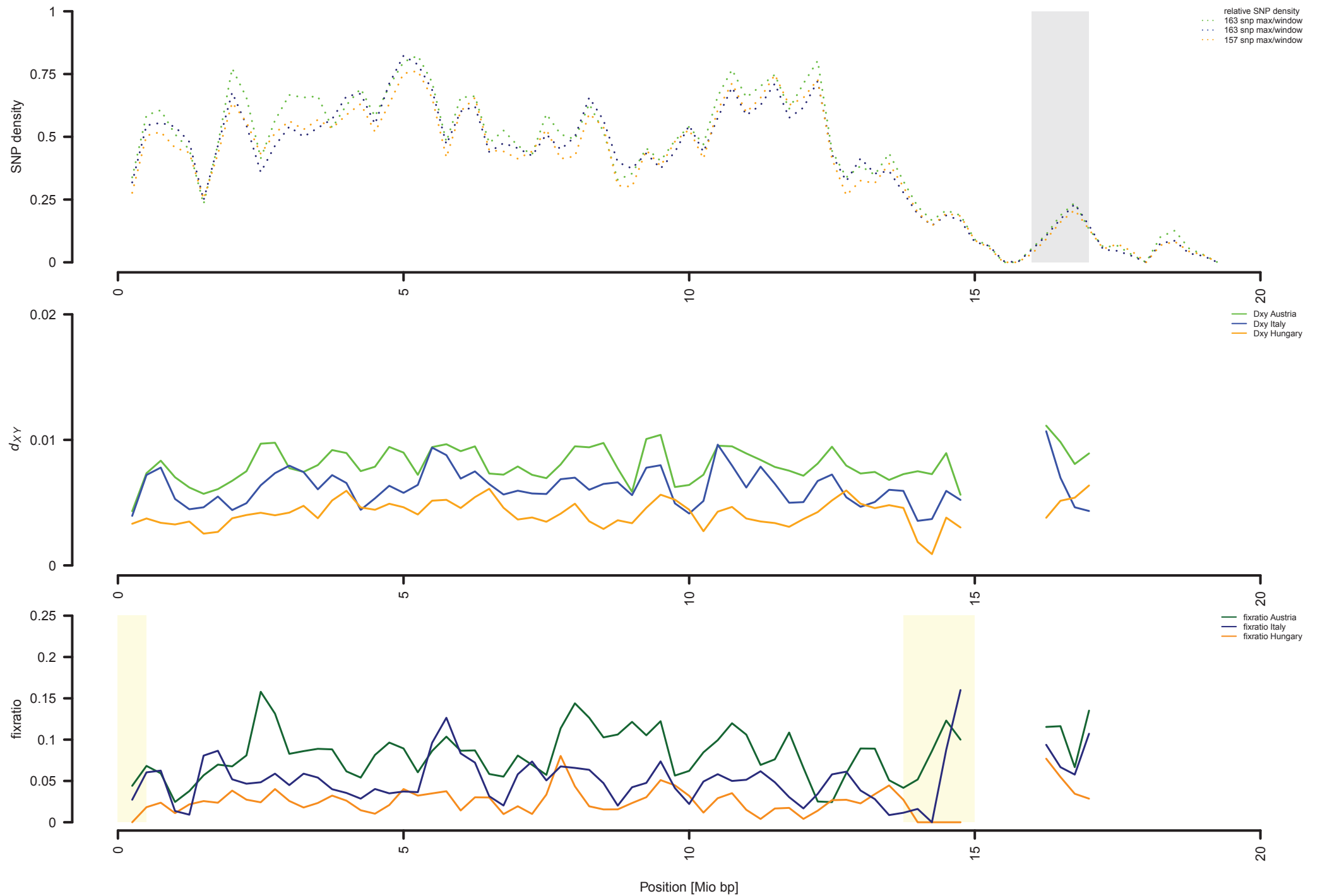




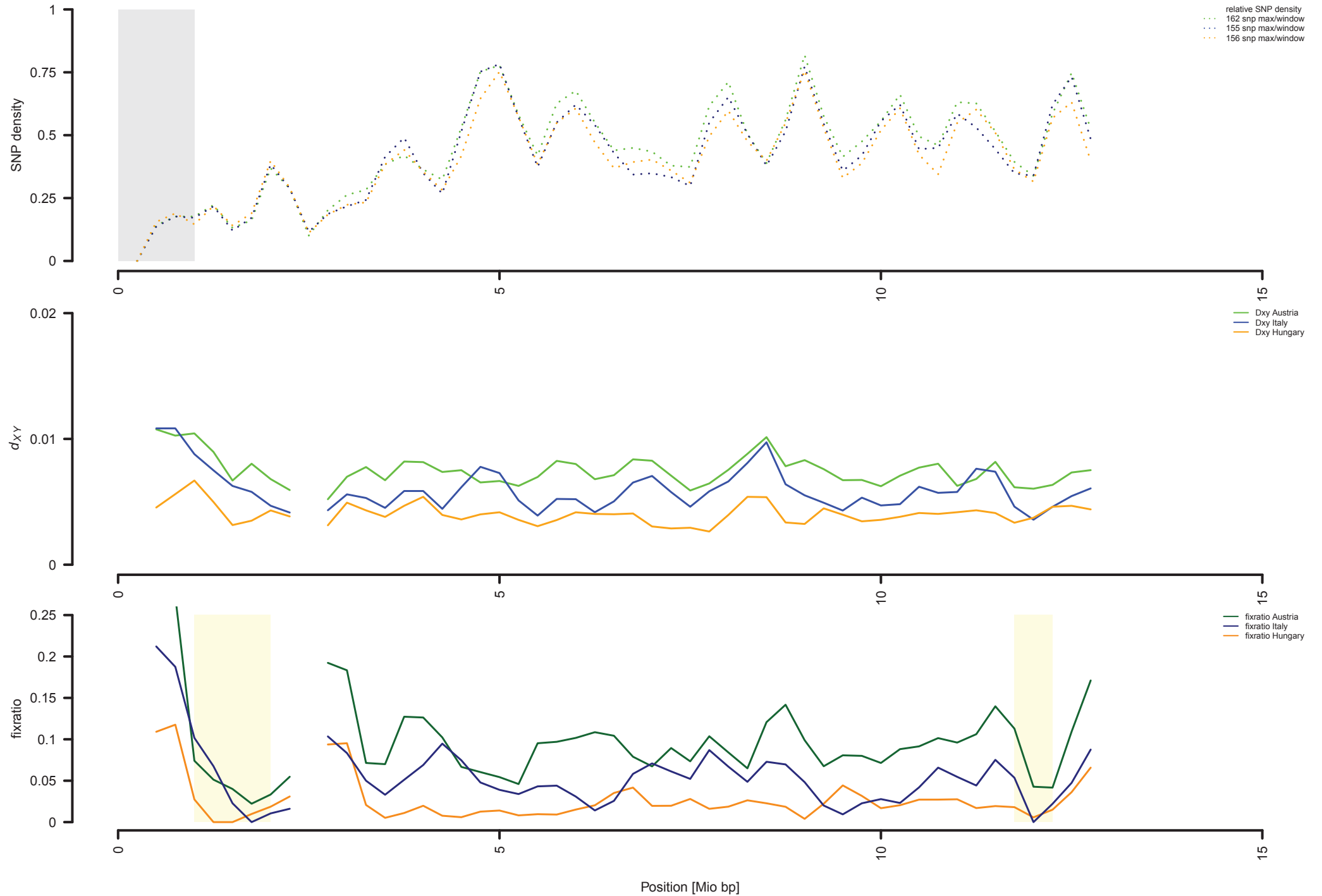
LG VII

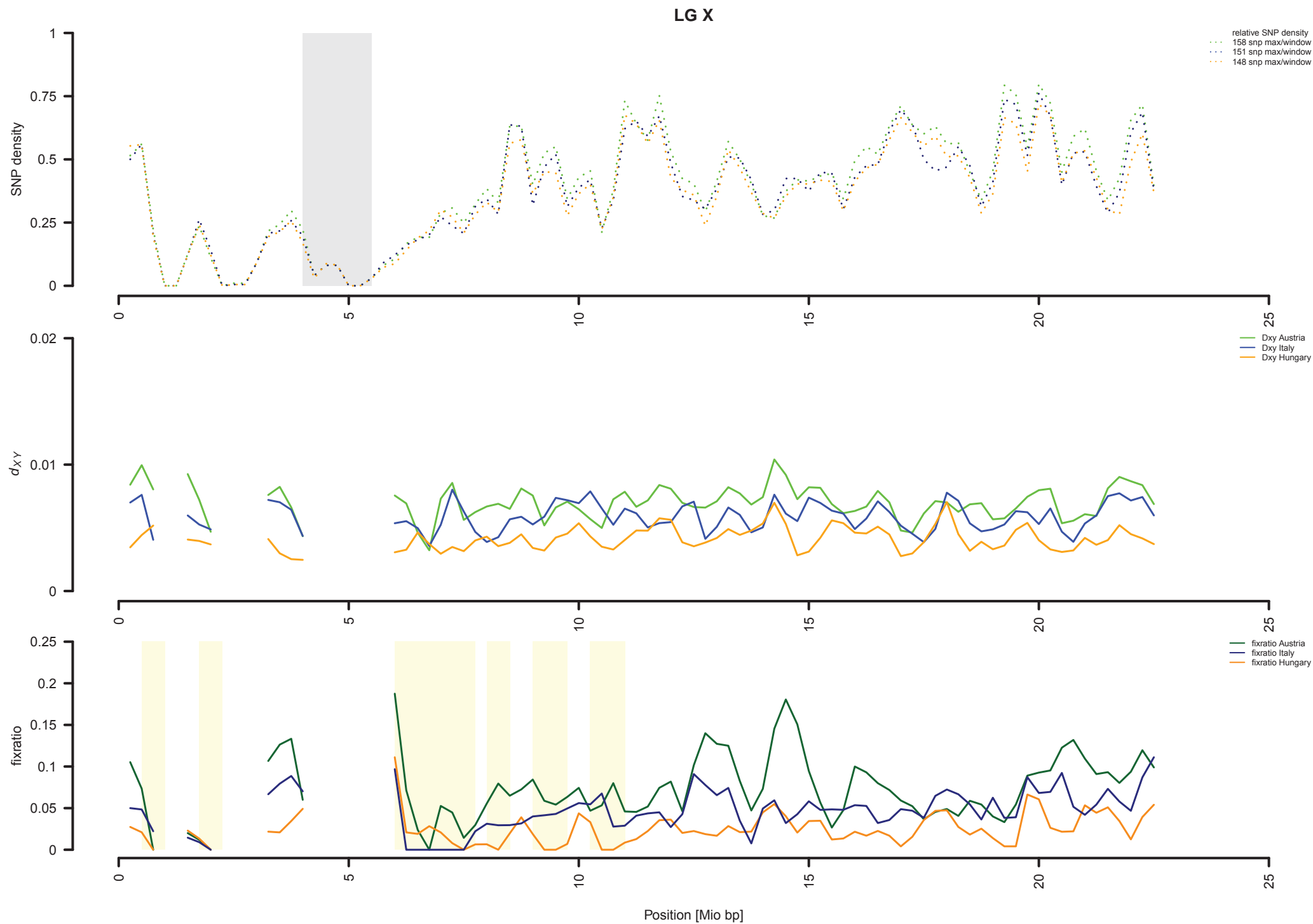


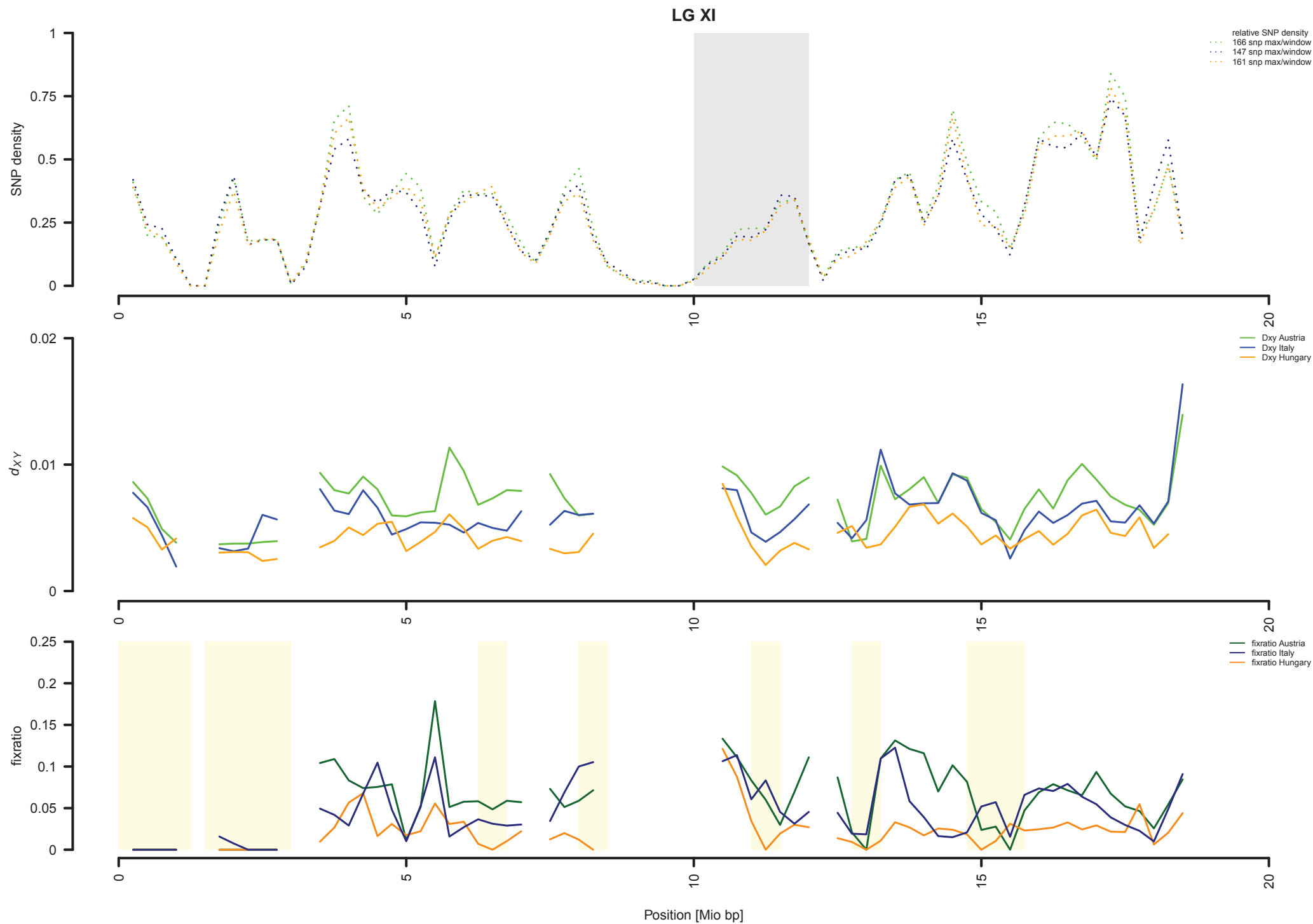
LG VIII



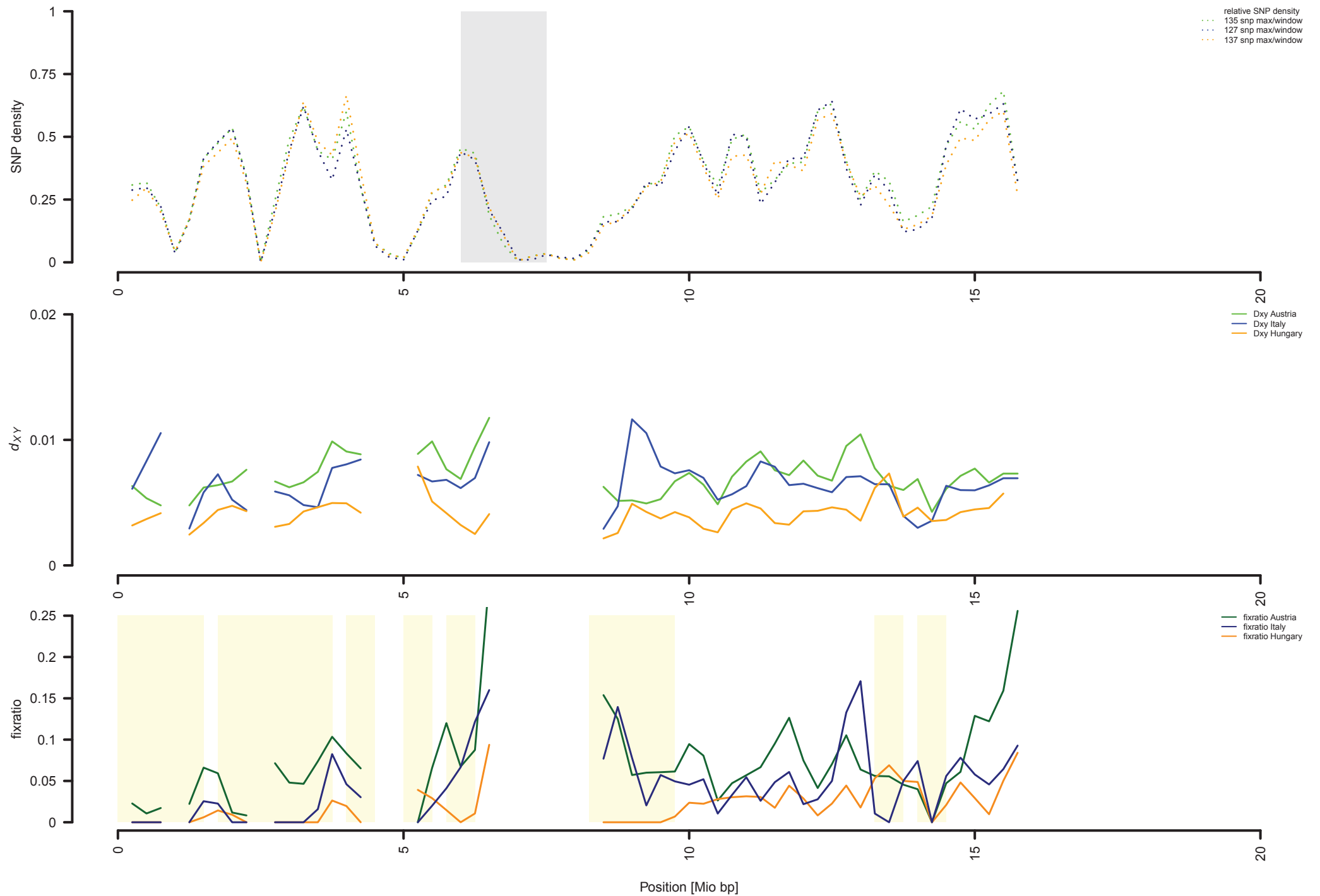
LG IX

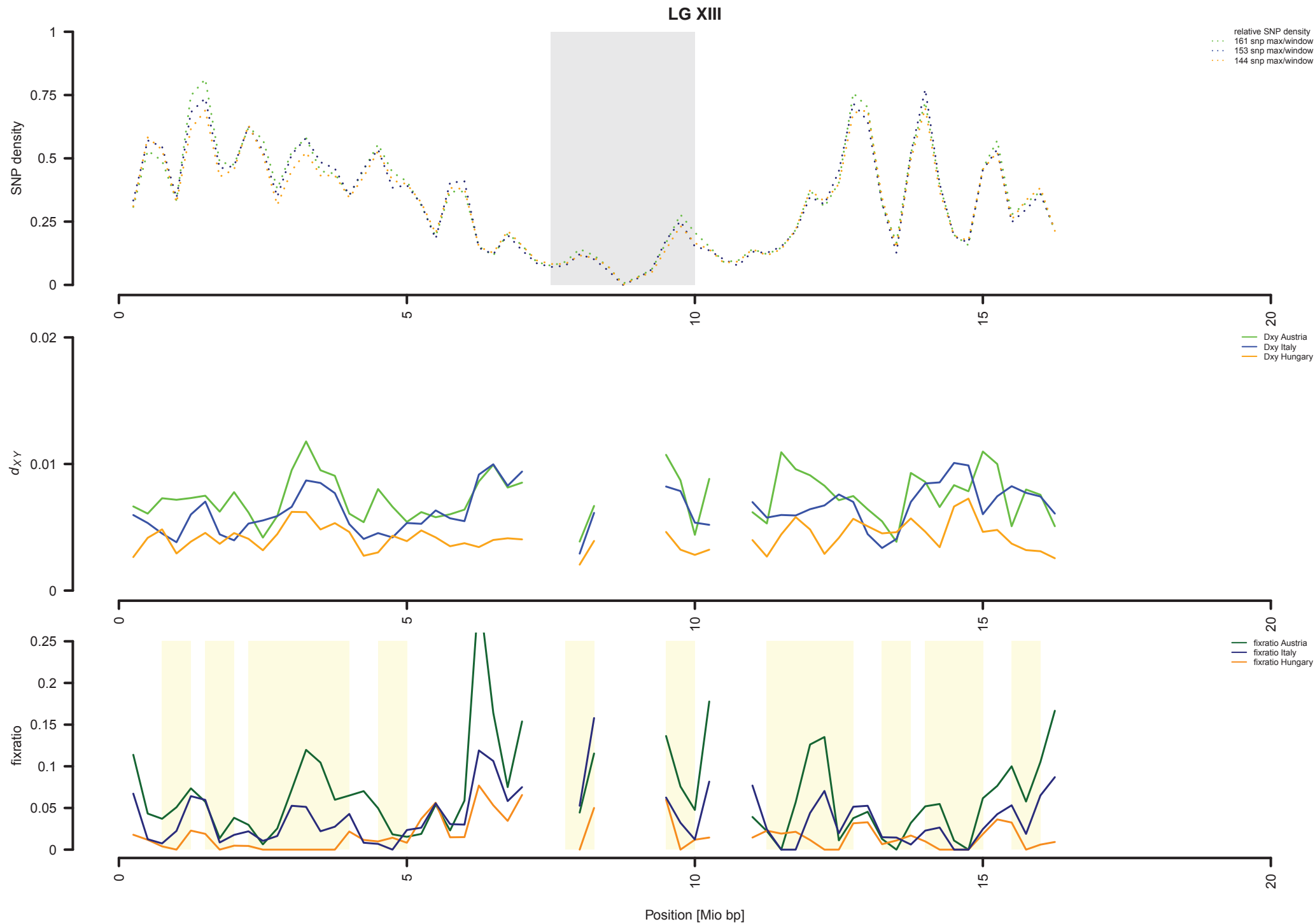




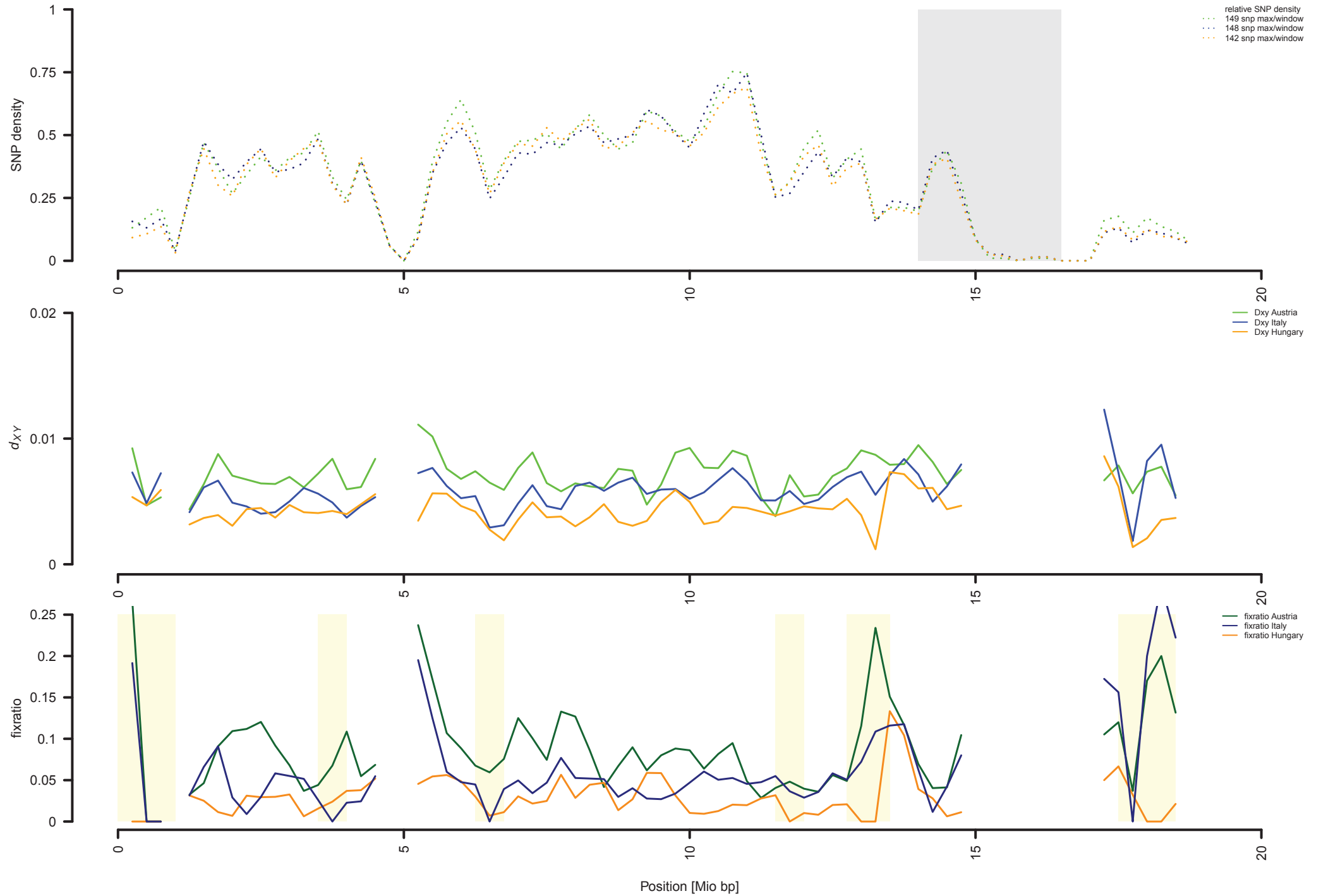


LG XII

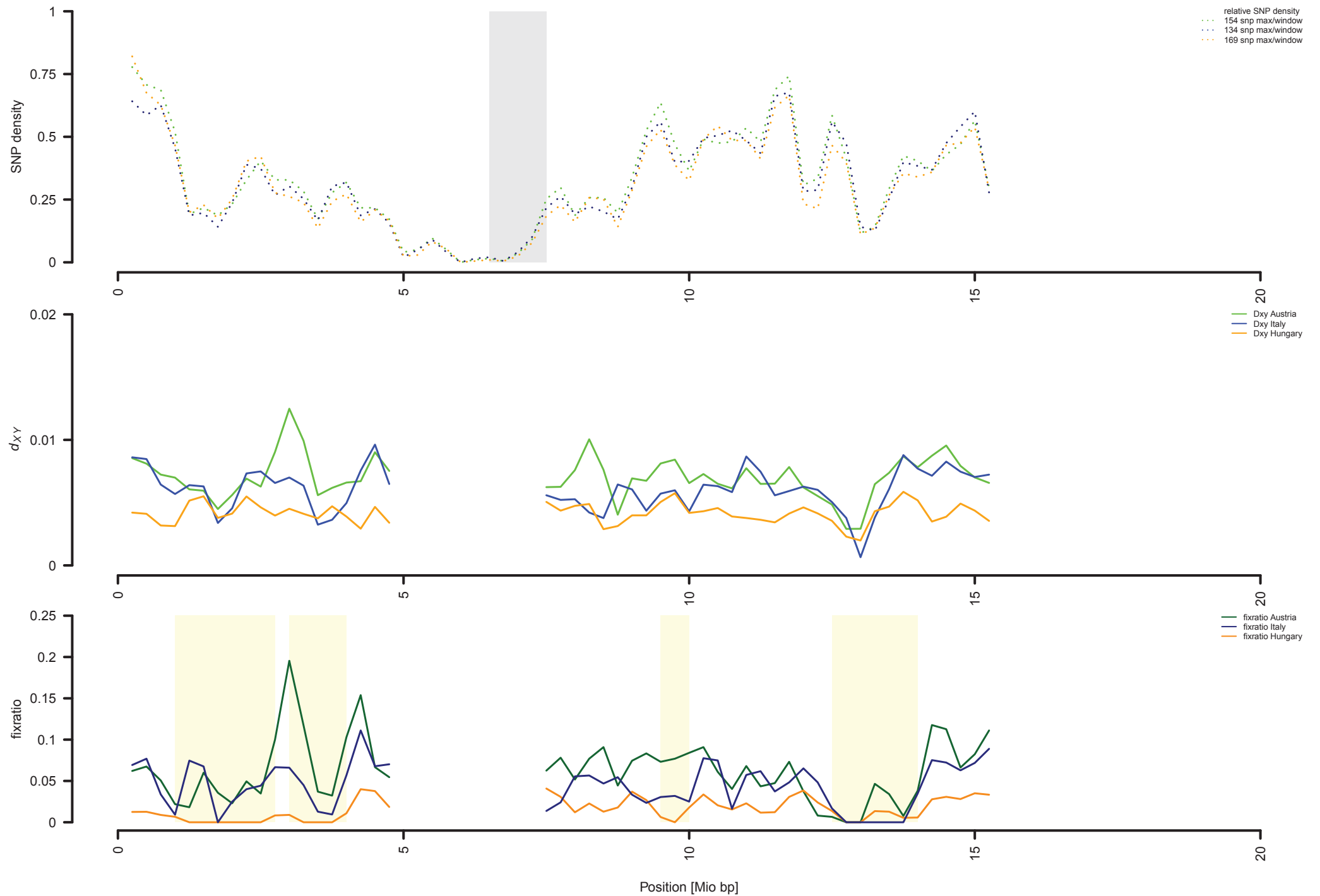




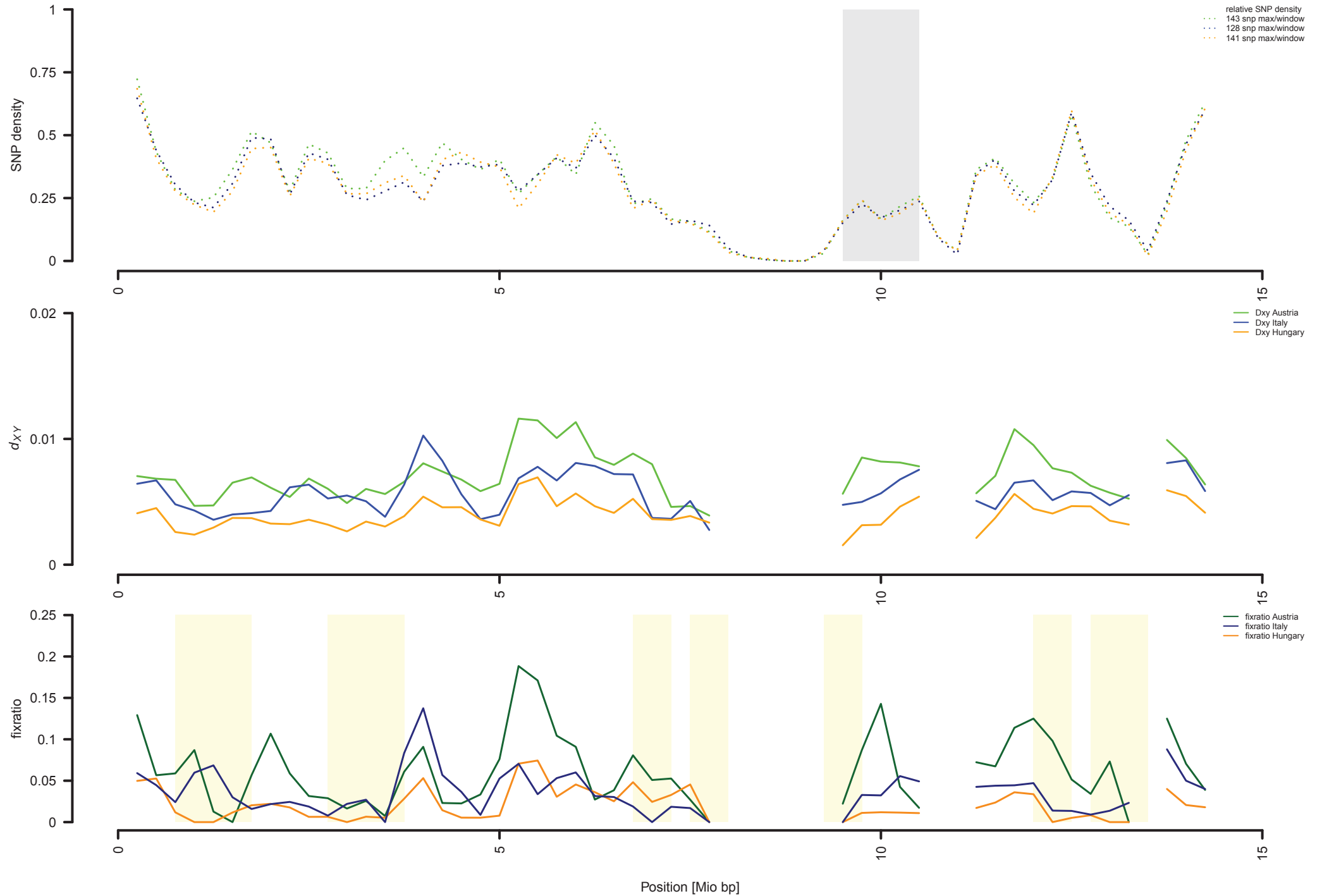
LG XIV

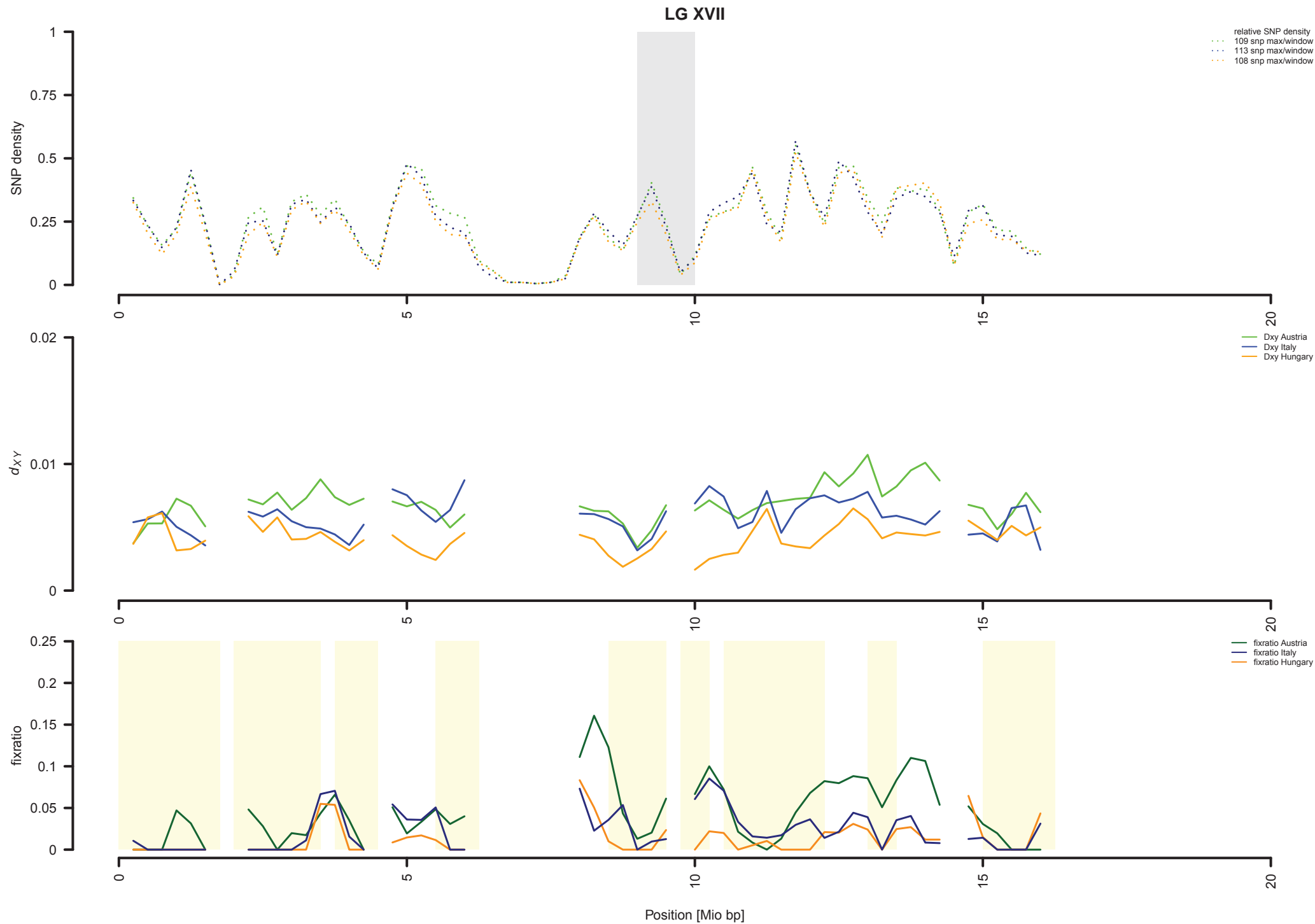


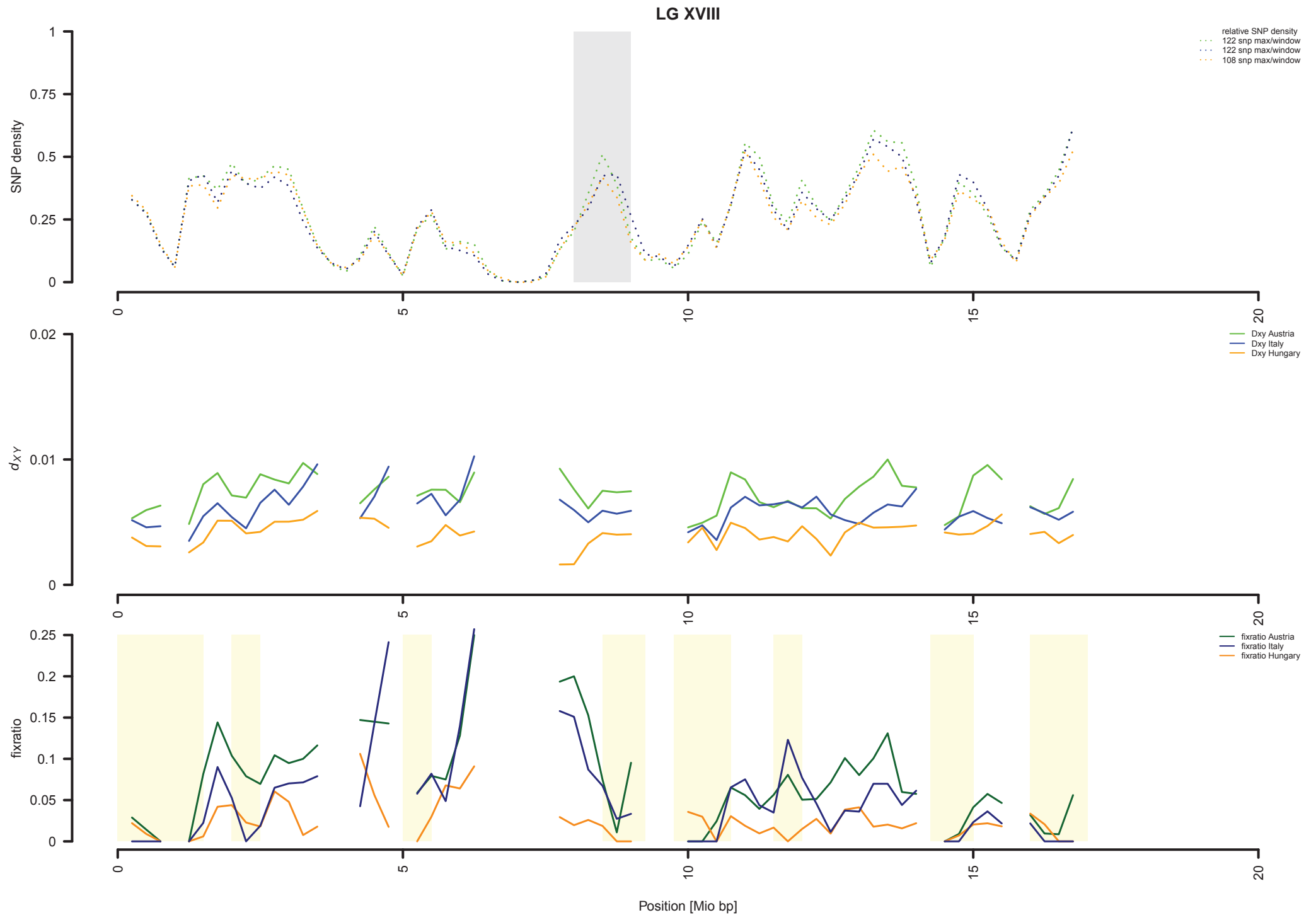
LG XV

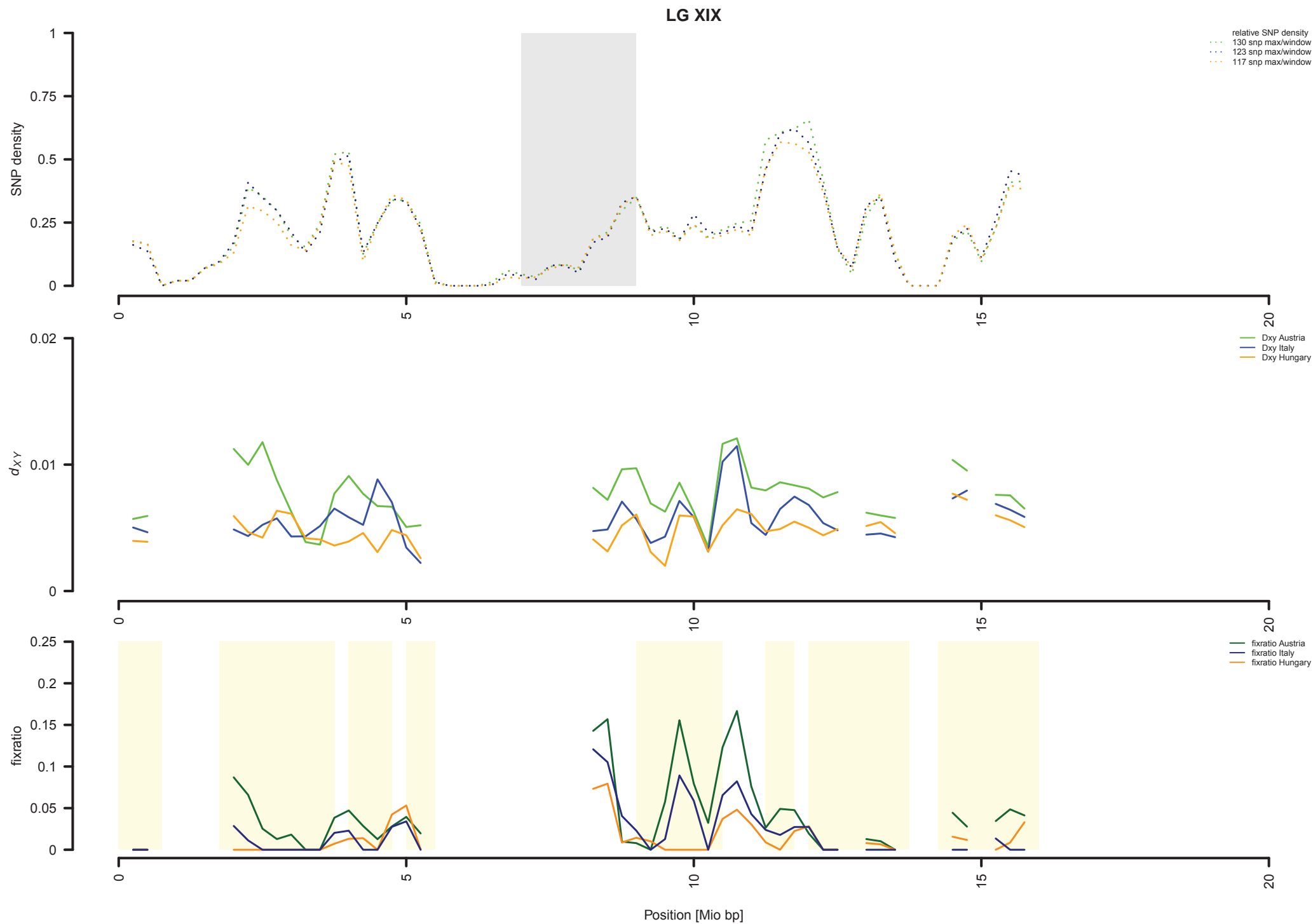


LG XVI

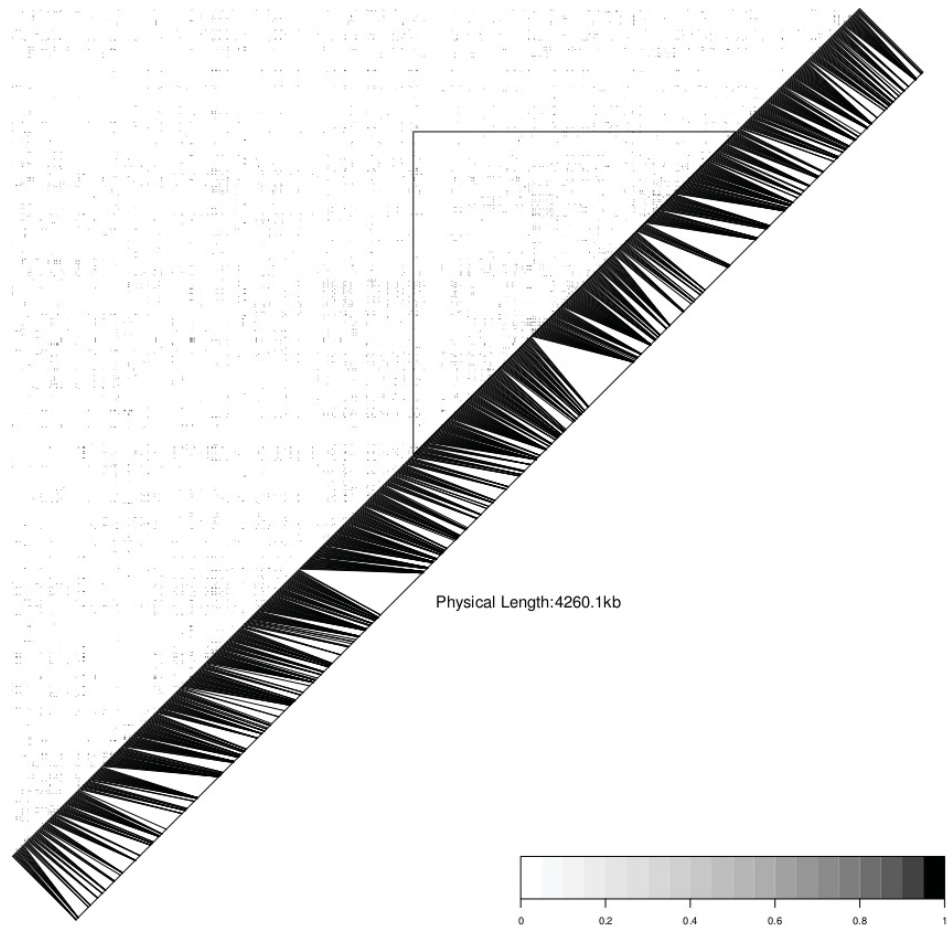




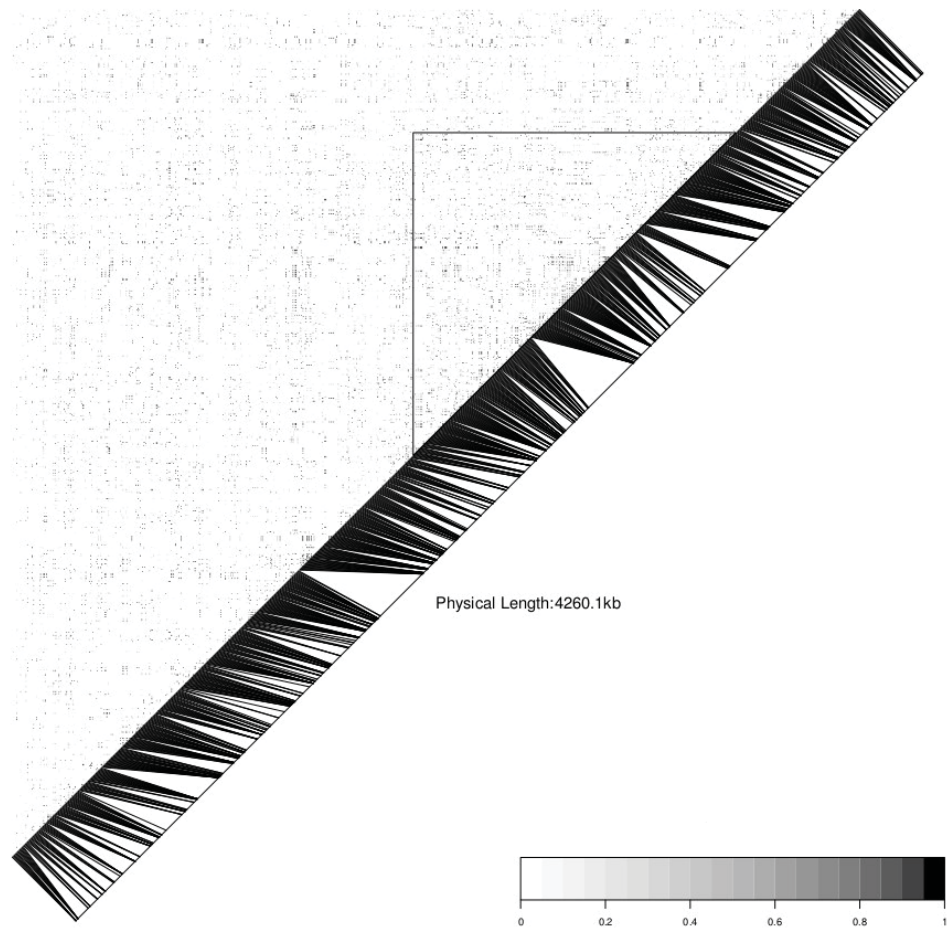




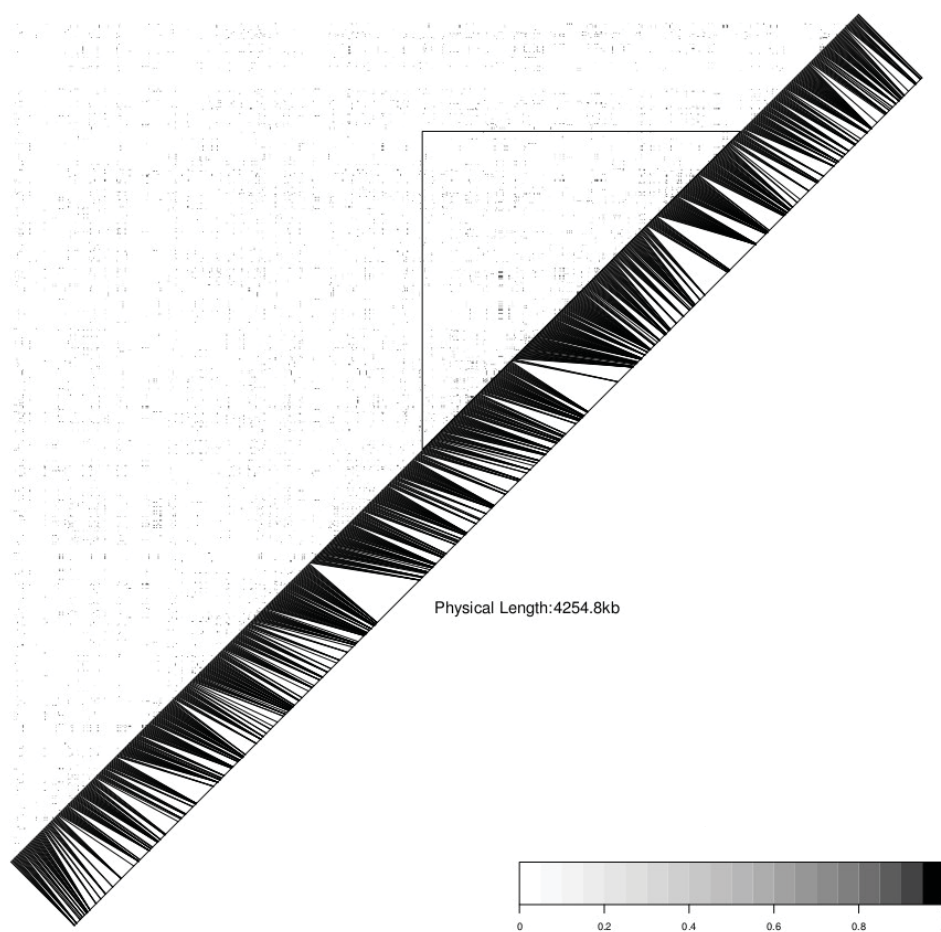
D' in Italy alba chr03 17.5–21.7 mio bp



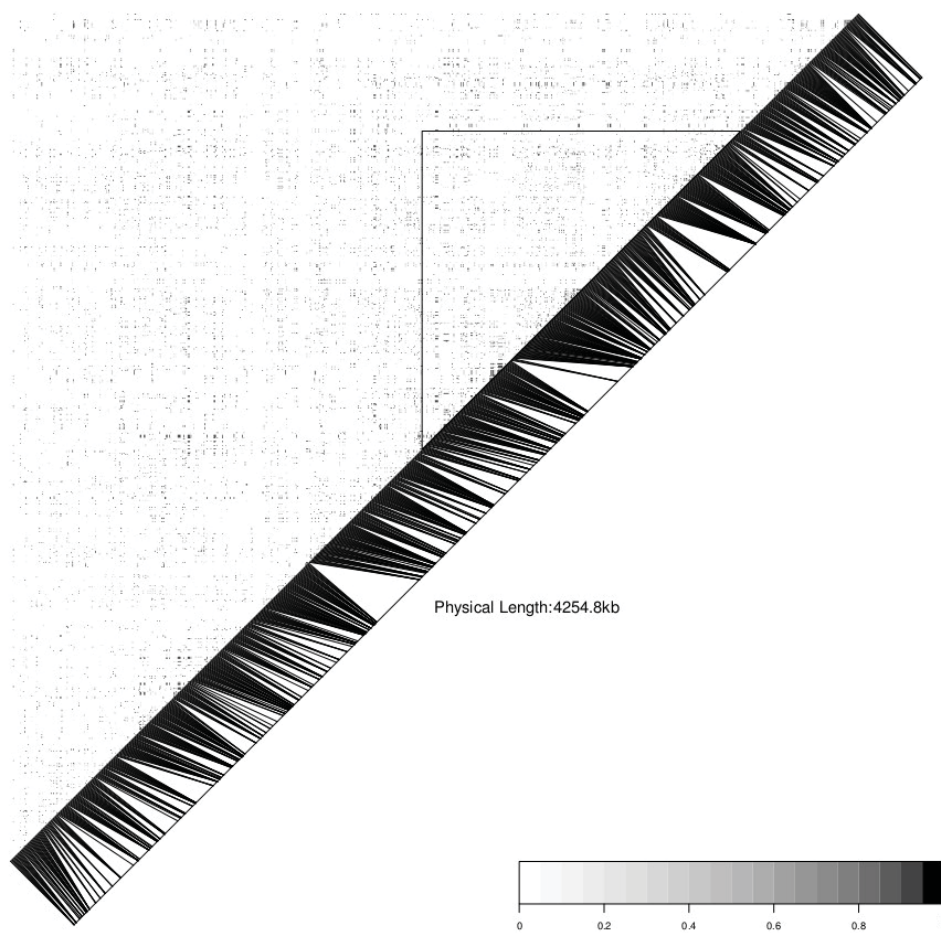
D' in Italy tremula chr03 17.5–21.7 mio bp



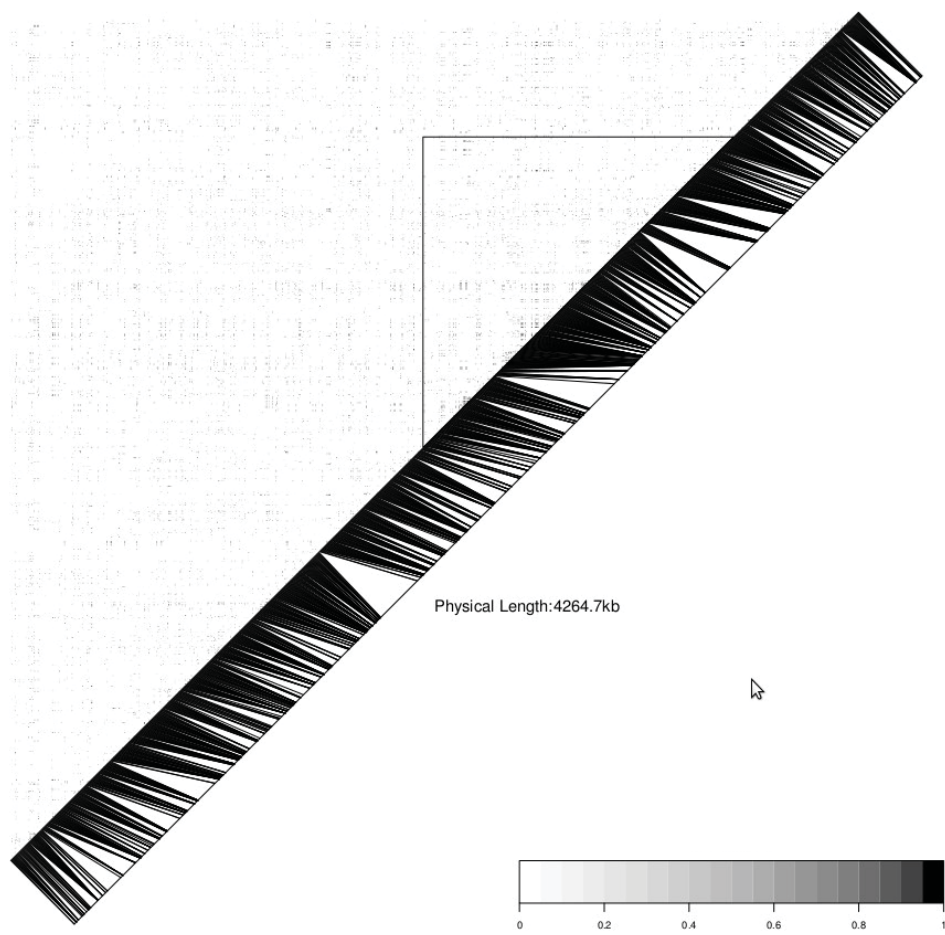
D' in *Austria alba* chr03 17.5–21.7 mio bp



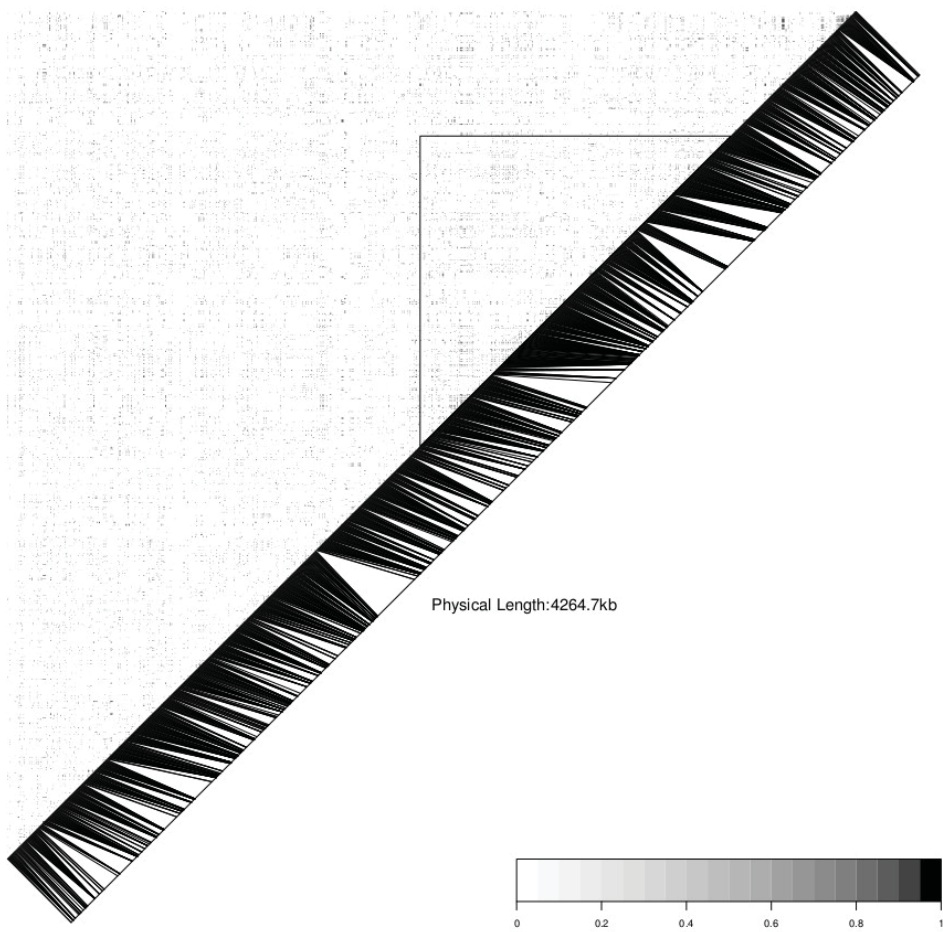
D' in *Austria tremula* chr03 17.5–21.7 mio bp



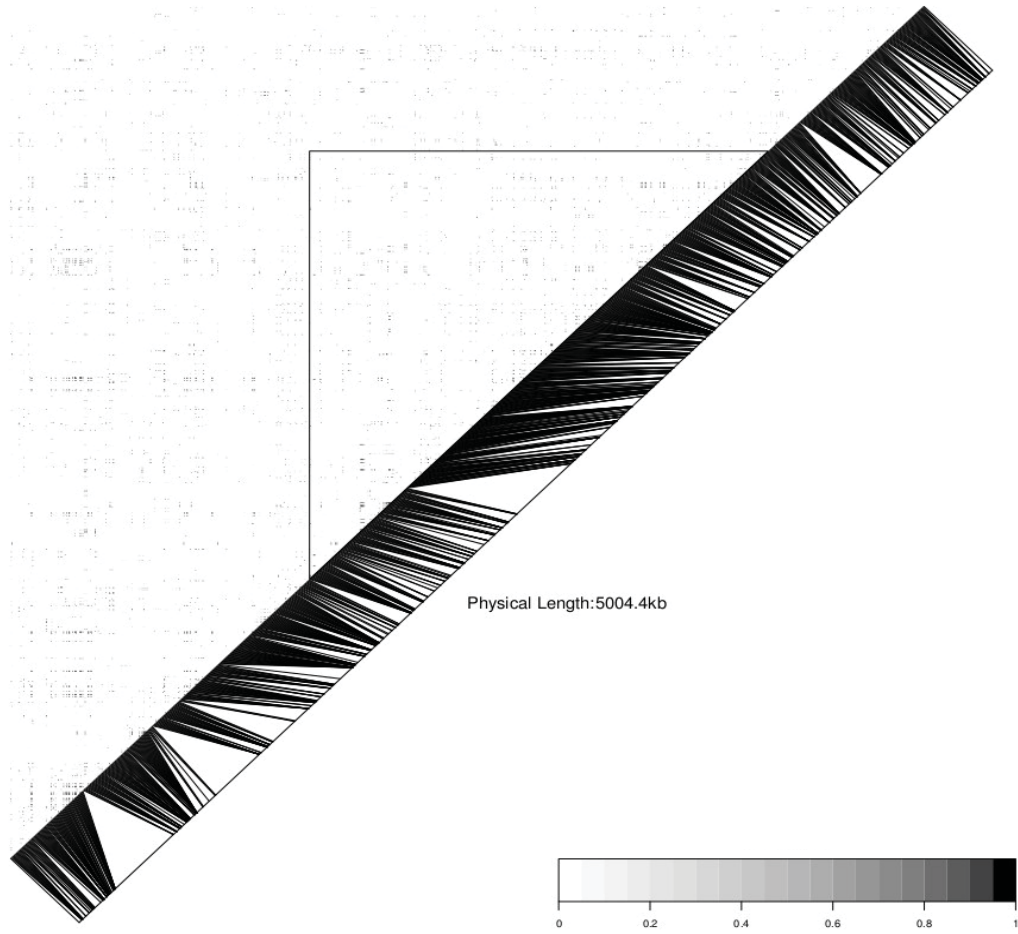
D' in Hungary alba chr03 17.5–21.7 mio bp



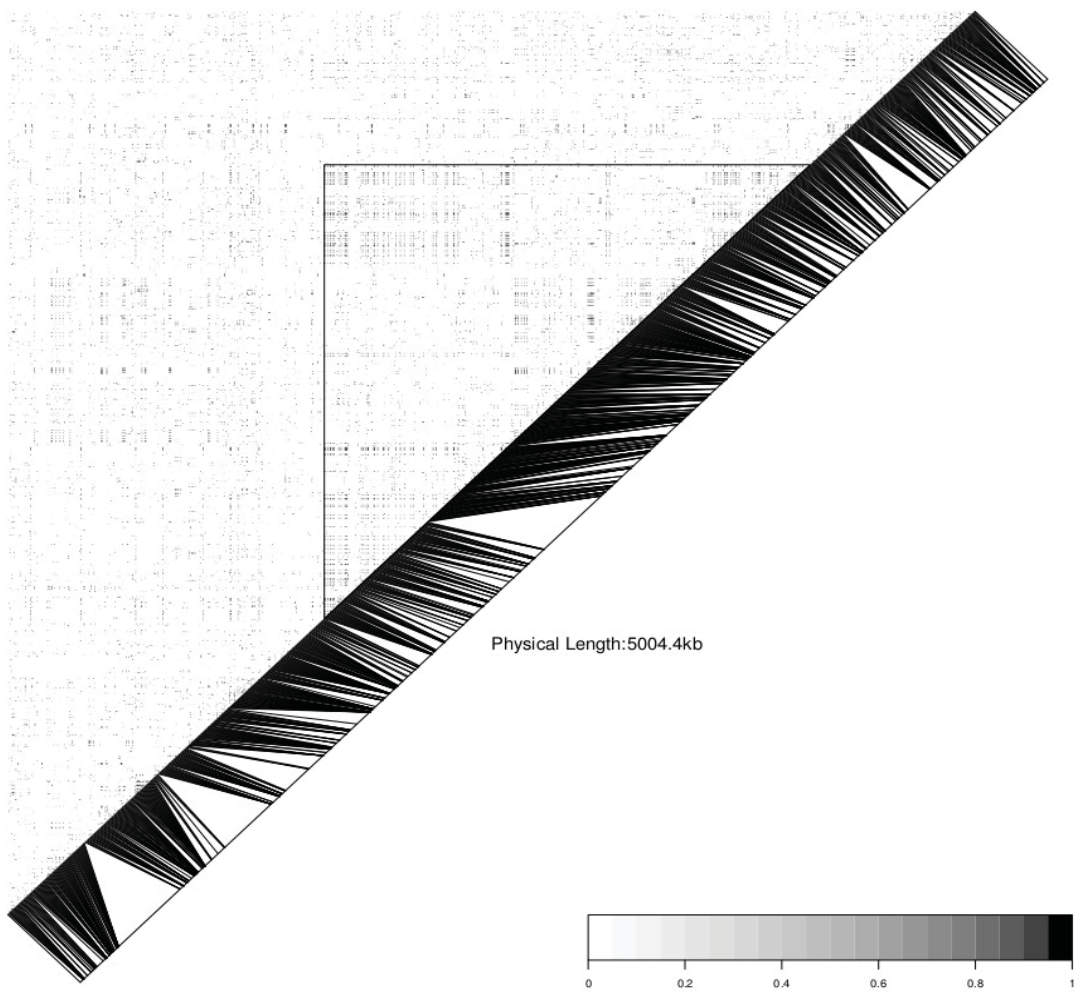
D' in Hungary tremula chr03 17.5–21.7 mio bp



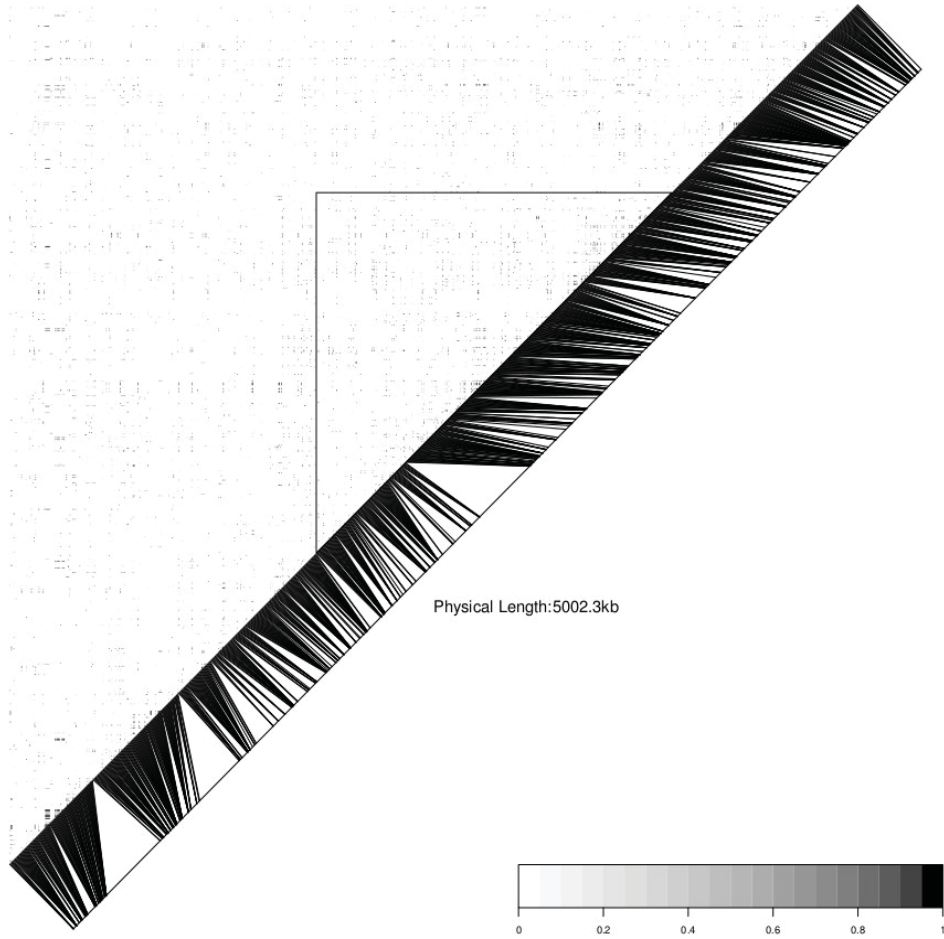
D' in Italy alba chr01 2.5–7.5 mio bp



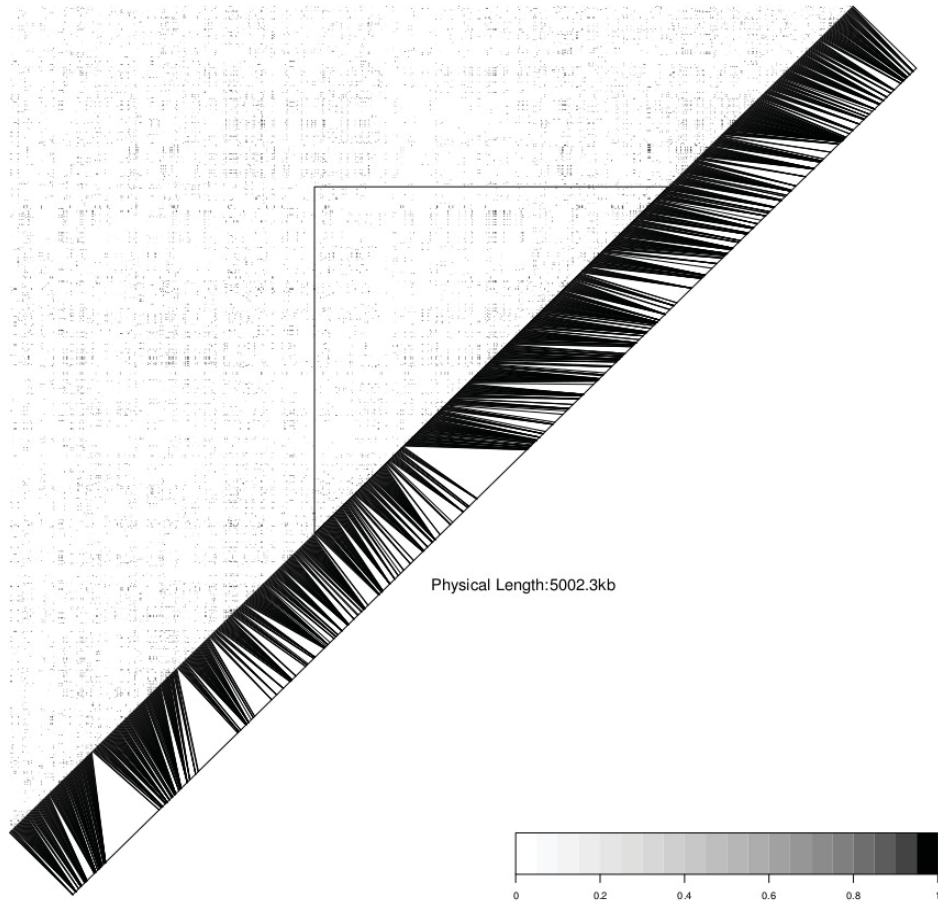
D' in Italy tremula chr01 2.5–7.5 mio bp



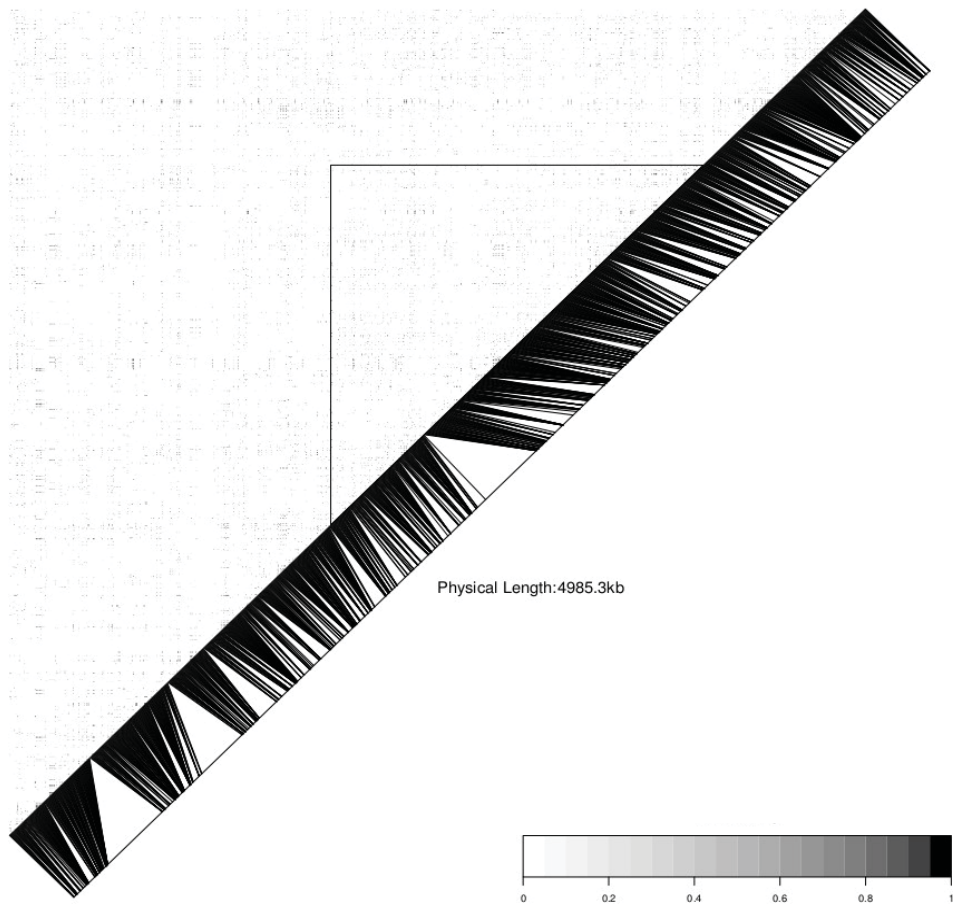
D' in Austria alba 2.5–7.5 mio bp



D' in Austria tremula 2.5–7.5 mio bp



D' in Hungary alba chr01 2.5–7.5 mio bp



D' in Hungary tremula chr01 2.5–7.5 mio bp

