The usefulness of multi-parent multi-environment QTL analysis: an illustration in different NAM populations - Supplementary material

Vincent Garin, Marcos Malosetti, Fred van Eeuwijk

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S1: Heritability computation

We computed the trait heritabilities on a line mean basis within the j^{th} cross using the formula 3 from Hung et al. (2012)

$$h^2 = \frac{\sigma^2_{g(cr_j)}}{\sigma^2_{g(cr_j)} + \frac{\sigma^2_{gxE(cr_j)}}{N_{env}} + \frac{\sigma^2_{e(cr_j)}}{N_{env} * N_{rep}}} \quad (1)$$

EU-NAM data

The model used for the computation of the heritabilities was the following:

$$\begin{aligned} y_{ijklm} =& \mu + env_k + rep_{l(k)} + block_{m(lk)} + cross_j + \\ & G_i(cross_j) + G_i(cross_j) * env_k + e_{ijklm} \end{aligned} \quad (2)$$

In model 2, all terms were random and the variance of the error term was cross-specific.

	σ^2_g	se(σ^2_g)	σ^2_{ge}	se(σ^2_{ge})	h^2(%)
D152	208.36	65.38	93.36	12.26	60.65
EC49A	0.11	93.29	80.07	63.86	0.04
EP44	257.42	139.59	29.11	176.75	66.64
EZ5	41.23	190.46	0.00		19.14
F03802	145.96	47.45	528.20	282.09	27.36
F2	302.10	79.10	75.92	66.24	71.97
F283	361.53	70.04	0.00		77.90
F64	222.86	80.69	100.30	61.39	60.92
UH006	224.28	60.12	138.10	81.42	57.17
UH009	20.12	37.53	75.42	62.59	12.70
DK105	270.12	75.60	106.34	61.26	59.76

US-NAM data

The model used for the computation of the heritabilities was the following:

$$
\begin{aligned}
y_{ijklmno} = &\mu + env_k + set_{l(k)} + block_{m(lk)} + row_{n(k)} + col_{o(k)} + cross_j + \\
&G_i(cross_j) + G_i * env_k + e_{ijklm}
\end{aligned}
\tag{3}
$$

In model 3, all terms were random and the variance of the error term was modelled by an environmental specific autoregressive correlation in the row and columns (AR1 x AR1) (Gilmour et al., 1997). For the USNAM data we were not able to fit a model with within cross genotype by environment variance term so we used an homogenous genotype by environment variance term.

	sigma.g	std.err	sigma.ge	std.err	heritability
B97	1.77	0.32	0.97	0.10	75.64
CML103	1.69	0.37	0.97	0.10	64.63
CML228	7.09	1.13	0.97	0.10	73.13
CML247	7.19	0.94	0.97	0.10	85.04
CML277	10.02	1.44	0.97	0.10	79.75
CML322	3.57	0.54	0.97	0.10	82.26
CML333	4.18	0.62	0.97	0.10	80.23
CML52	6.20	0.83	0.97	0.10	86.47
CML69	2.13	0.47	0.97	0.10	61.21
Hp301	3.24	0.44	0.97	0.10	86.98
IL14H	4.32	0.54	0.97	0.10	89.91
Ki11	6.50	1.06	0.97	0.10	70.35
Ki3	3.79	0.64	0.97	0.10	86.53
Ky21	1.64	0.32	0.97	0.10	71.62
M162W	3.09	0.50	0.97	0.10	78.33
M37W	3.09	0.53	0.97	0.10	73.31
Mo18W	4.84	0.76	0.97	0.10	76.34
MS71	2.22	0.32	0.97	0.10	82.07
NC350	2.77	0.51	0.97	0.10	70.48
NC358	1.67	0.33	0.97	0.10	73.09
Oh43	1.73	0.33	0.97	0.10	72.38
Oh7B	2.51	0.42	0.97	0.10	80.19
P39	5.47	0.68	0.97	0.10	91.86
Tx303	2.89	0.50	0.97	0.10	74.29
Tzi8	4.60	0.68	0.97	0.10	85.03

S2: List of detected QTLs

EU-NAM data

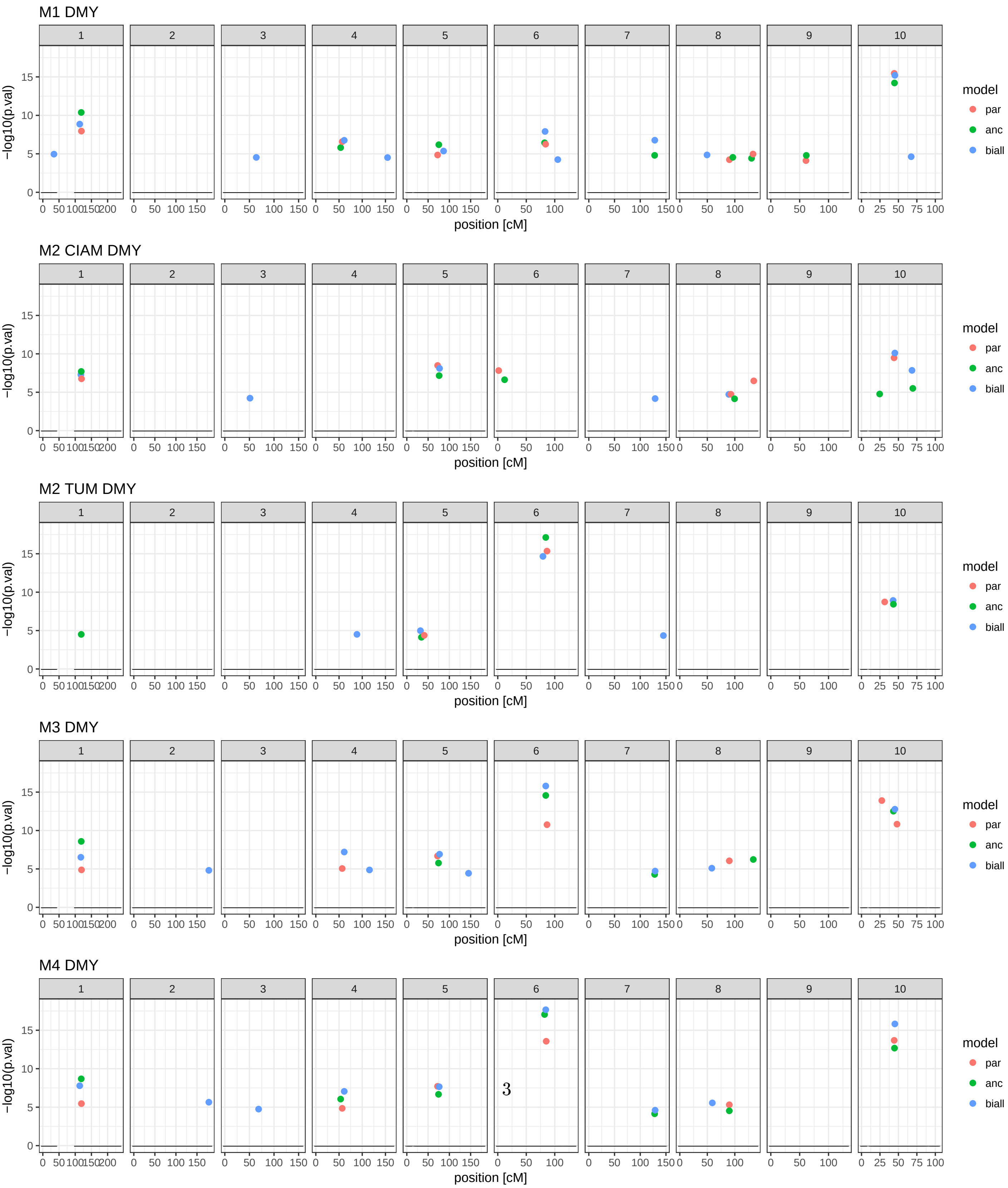

Figure 1: List of QTLs

EU-NAM M1

	n	Mk. names	chr	pos [cM]	-log10(pval)
parental					
	1	PZE.101146834	1	119.1	8
	2	PZE.104027223	4	56.8	6.6
	3	PZE.105054186	5	72.4	4.8
	4	PZE.106101027	6	83.9	6.3
	5	PZE.108099840	8	90.2	4.2
	6	PZE.108131921	8	133.1	5
	7	PZE.109054632	9	60.9	4.1
	8	PZE.110048720	10	44.3	15.5
ancestral					
	1	PZE.101144216	1	118.6	10.4
	2	PZE.104029507	4	53.5	5.8
	3	PZE.105068880	5	75.2	6.2
	4	PZE.106098066	6	82.1	6.4
	5	PZE.107128534	7	128	4.8
	6	PZE.108109731	8	96.4	4.5
	7	PZE.108131479	8	130.4	4.4
	8	PZE.109058296	9	61.5	4.8
	9	PZE.110049068	10	44.7	14.2
bi-allelic					
	1	PZE.101024519	1	34.3	5
	2	PZE.101143233	1	113.9	8.9
	3	PZE.103096063	3	64	4.5
	4	PZE.104052802	4	61.2	6.8
	5	PZE.104153023	4	154.5	4.5
	6	PZE.105103875	5	86.4	5.4
	7	PZE.106097991	6	83	7.9
	8	PZE.106114241	6	105.3	4.3
	9	PZE.107128336	7	128.3	6.8
	10	PZE.108027746	8	49.5	4.9
	11	PZE.110049474	10	45.2	15.2
	12	PZE.110086343	10	67.3	4.6

EU-NAM M2 CIAM

	n	Mk. names	chr	pos [cM]	-log10(pval)
parental					
	1	PZE.101147104	1	119.4	6.8
	2	PZE.105054186	5	72.4	8.5
	3	PZA00606.3	6	1.6	7.8
	4	PZE.108104106	8	92.9	4.7
	5	PZE.108133621	8	134.5	6.5
	6	PZE.110049572	10	44.1	9.5
ancestral					
	1	PZE.101144216	1	118.6	7.7
	2	PZE.105063383	5	76	7.2
	3	PZE.106009233	6	12	6.6
	4	PZE.108110343	8	99.5	4.1
	5	PZE.110009558	10	24.6	4.8
	6	PZE.110087849	10	69.5	5.5
bi-allelic					
	1	PZE.101144248	1	117.4	7.3
	2	PZE.103072486	3	51.2	4.2
	3	PZE.105074287	5	76.9	8.1
	4	PZE.107128846	7	128.9	4.2
	5	PZE.108099415	8	89.1	4.7
	6	PZE.110049474	10	45.2	10.1
	7	PZE.110088931	10	68.4	7.8

EU-NAM M2 TUM

	n	Mk. names	chr	pos [cM]	-log10(pval)
parental					
	1	PZE.105019465	5	40.9	4.4
	2	PZE.106102395	6	86.4	15.4
	3	PZE.110013764	10	31.4	8.7
ancestral					
	1	PZE.101144216	1	118.6	4.5
	2	PZE.105017551	5	34.3	4.1
	3	PZE.106101278	6	84.2	17.1
	4	PZE.110049040	10	43.2	8.4
bi-allelic					
	1	PZE.104094429	4	88.6	4.5
	2	PZE.105017975	5	32	5
	3	PZE.106095383	6	79.3	14.7
	4	PZE.107136612	7	145	4.4
	5	PZE.110049406	10	42.8	8.9

EU-NAM M3

	n	Mk. names	chr	pos [cM]	-log10(pval)
parental					
	1	PZE.101147104	1	119.4	4.9
	2	PZE.104027223	4	56.8	5.1
	3	PZE.105062183	5	72.2	6.7
	4	PZE.106102395	6	86.4	10.8
	5	PZE.108099425	8	90	6.1
	6	PZE.110010098	10	27.5	13.9
	7	PZE.110049922	10	48.1	10.8
ancestral					
	1	PZE.101144216	1	118.6	8.6
	2	PZE.105065789	5	74.5	5.8
	3	PZE.106101278	6	84.2	14.6
	4	PZE.107128534	7	128	4.3
	5	PZE.108133100	8	133.6	6.2
	6	PZE.110049040	10	43.2	12.5
bi-allelic					
	1	PZE.101144248	1	117.4	6.5
	2	PZE.102192367	2	178.2	4.8
	3	PZE.104052802	4	61.2	7.2
	4	PZE.104110016	4	115.6	4.9
	5	PZE.105074287	5	76.9	6.9
	6	PZE.105160757	5	145.1	4.4
	7	PZE.106101278	6	84.2	15.8
	8	PZE.107128846	7	128.9	4.7
	9	PZE.108057679	8	58.2	5.1
	10	PZE.110049474	10	45.2	12.8

EU-NAM M4

	n	Mk. names	chr	pos [cM]	-log10(pval)
parental					
	1	PZE.101146834	1	119.1	5.5
	2	PZE.104027223	4	56.8	4.9
	3	PZE.105062183	5	72.2	7.7
	4	PZE.106099144	6	85	13.6
	5	PZE.108099425	8	90	5.3
	6	PZE.110048720	10	44.3	13.7
ancestral					
	1	PZE.101144216	1	118.6	8.7
	2	PZE.104029507	4	53.5	6.1
	3	PZE.105065789	5	74.5	6.7
	4	PZE.106098066	6	82.1	17
	5	PZE.107128534	7	128	4.1
	6	PZE.108099425	8	90	4.5
	7	PZE.110049068	10	44.7	12.7
bi-allelic					
	1	PZE.101143233	1	113.9	7.8
	2	PZE.102192367	2	178.2	5.6
	3	PZE.103106593	3	68.7	4.8
	4	PZE.104052802	4	61.2	7.1
	5	PZE.105063758	5	76.1	7.7
	6	PZE.106101278	6	84.2	17.7
	7	PZE.107128846	7	128.9	4.6
	8	PZE.108058577	8	59.1	5.6
	9	PZE.110049474	10	45.2	15.8

US-NAM data

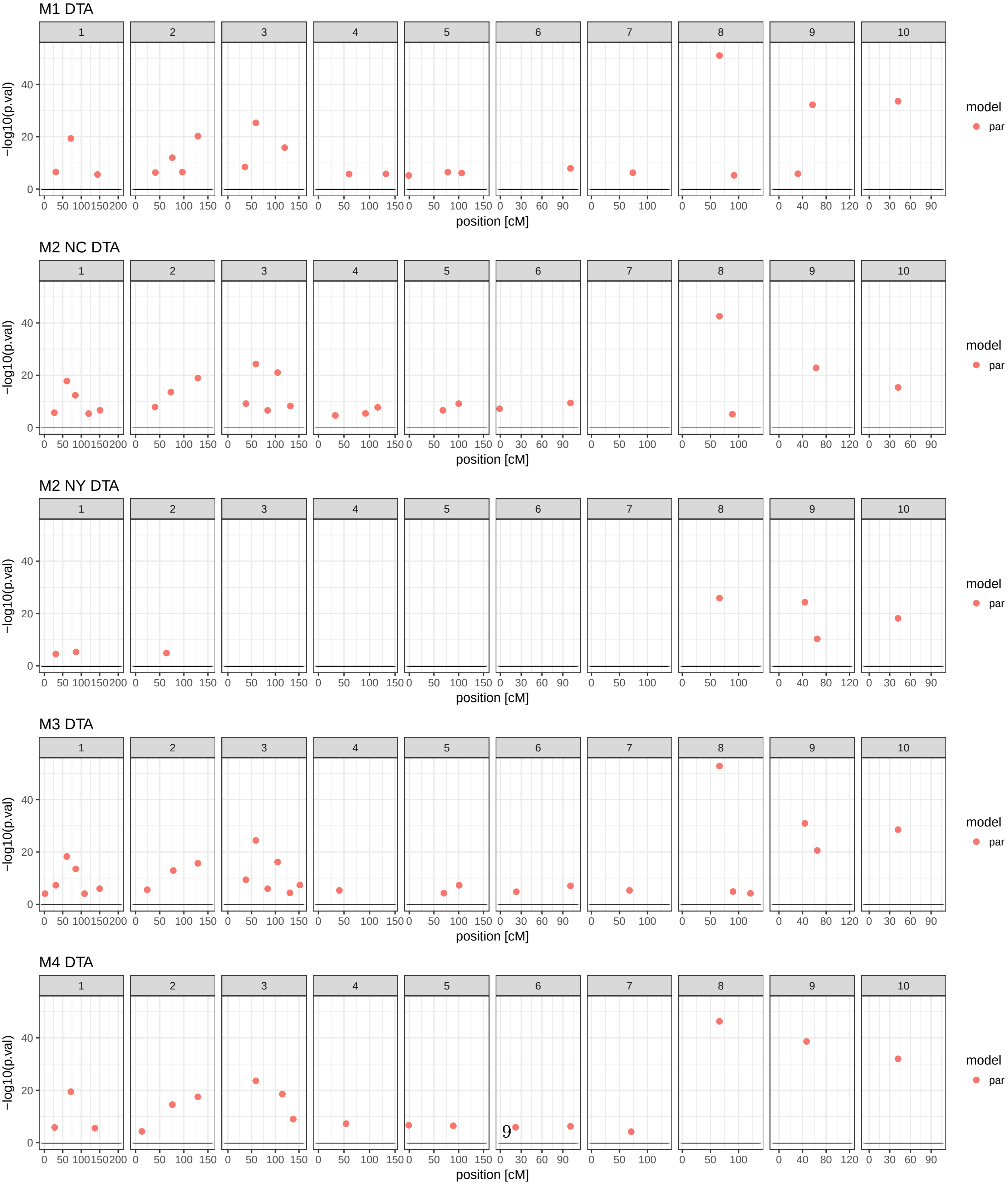

Figure 2: List of QTLs

US-NAM M1

	n	Mk. names	chr	pos [cM]	-log10(pval)
parental					
	1	m36	1	31	6.5
	2	m77	1	72	19.4
	3	m149	1	144	5.6
	4	m255	2	41	6.3
	5	m290	2	76	12
	6	m311	2	97	6.5
	7	m343	2	129	20.2
	8	m413	3	36	8.5
	9	m436	3	59	25.3
	10	m497	3	120	15.8
	11	m599	4	60	5.7
	12	m671	4	132	5.8
	13	m689	5	-1	5.2
	14	m768	5	78	6.5
	15	m796	5	106	6.2
	16	m947	6	101	7.9
	17	m1032	7	74	6.3
	18	m1162	8	66	51
	19	m1188	8	92	5.3
	20	m1275	9	32	5.9
	21	m1300	9	57	32.2
	22	m1414	10	42	33.5

US-NAM M2 New York

	n	Mk. names	chr	pos [cM]	-log10(pval)
parental					
	1	m32	1	27	5.7
	2	m66	1	61	17.8
	3	m89	1	84	12.3
	4	m125	1	120	5.3
	5	m156	1	151	6.6
	6	m254	2	40	7.8
	7	m287	2	73	13.5
	8	m343	2	129	18.9
	9	m415	3	38	9.1
	10	m436	3	59	24.3
	11	m461	3	84	6.5
	12	m482	3	105	21
	13	m509	3	132	8.2
	14	m572	4	33	4.6
	15	m631	4	92	5.4
	16	m655	4	116	7.7
	17	m758	5	68	6.6
	18	m790	5	100	9.1
	19	m845	6	-1	7.2
	20	m947	6	101	9.4
	21	m1162	8	66	42.6
	22	m1185	8	89	5.1
	23	m1306	9	63	22.9
	24	m1414	10	42	15.3

US-NAM M2 North Carolina

	n	Mk. names	chr	pos [cM]	-log10(pval)
parental					
	1	m36	1	31	4.5
	2	m91	1	86	5.3
	3	m278	2	64	4.9
	4	m1162	8	66	25.9
	5	m1287	9	44	24.3
	6	m1308	9	65	10.3
	7	m1414	10	42	18.2

US-NAM M3

	n	Mk. names	chr	pos [cM]	-log10(pval)
parental					
	1	m7	1	2	4.1
	2	m36	1	31	7.3
	3	m66	1	61	18.3
	4	m90	1	85	13.5
	5	m114	1	109	4.1
	6	m155	1	150	6
	7	m238	2	24	5.6
	8	m292	2	78	12.9
	9	m343	2	129	15.7
	10	m415	3	38	9.4
	11	m436	3	59	24.4
	12	m461	3	84	5.9
	13	m482	3	105	16.2
	14	m508	3	131	4.4
	15	m529	3	152	7.4
	16	m580	4	41	5.3
	17	m760	5	70	4.2
	18	m791	5	101	7.3
	19	m869	6	23	4.8
	20	m947	6	101	7.1
	21	m1026	7	68	5.3
	22	m1162	8	66	52.9
	23	m1186	8	90	4.9
	24	m1217	8	121	4.2
	25	m1287	9	44	31
	26	m1308	9	65	20.6
	27	m1414	10	42	28.6

US-NAM M4

	n	Mk. names	chr	pos [cM]	-log10(pval)
parental					
	1	m33	1	28	5.8
	2	m77	1	72	19.5
	3	m142	1	137	5.5
	4	m227	2	13	4.3
	5	m290	2	76	14.5
	6	m343	2	129	17.5
	7	m436	3	59	23.6
	8	m492	3	115	18.6
	9	m515	3	138	9
	10	m593	4	54	7.2
	11	m689	5	-1	6.6
	12	m779	5	89	6.4
	13	m868	6	22	5.9
	14	m947	6	101	6.3
	15	m1029	7	71	4.2
	16	m1162	8	66	46.3
	17	m1290	9	47	38.6
	18	m1414	10	42	32

S3: QTL additive effects allelic series

EU-NAM chr 6 82.1 cM

Table 1: QTL additive effects and standard deviations

	β_{M1}	β_{M4-E1}	β_{M4-E2}	sd(β_{M1})	sd(β_{M4-E1})	sd(β_{M4-E2})	β/sd(β)(M1)	β/sd(β)(M4-E1)	β/sd(β)(M4-E2)
UH007	0.00	0.00	0.00	0.00	0.00	0.00			
EZ5	0.00	0.00	0.00	0.00	0.00	0.00			
UH009	0.00	0.00	0.00	0.00	0.00	0.00			
D152	-4.07	-1.19	-7.20	0.71	0.83	0.83	-5.75	-1.42	-8.63
EC49A	-4.07	-1.19	-7.20	0.71	0.83	0.83	-5.75	-1.42	-8.63
F03802	-4.07	-1.19	-7.20	0.71	0.83	0.83	-5.75	-1.42	-8.63
F2	-4.07	-1.19	-7.20	0.71	0.83	0.83	-5.75	-1.42	-8.63
F283	-4.07	-1.19	-7.20	0.71	0.83	0.83	-5.75	-1.42	-8.63
UH006	-4.07	-1.19	-7.20	0.71	0.83	0.83	-5.75	-1.42	-8.63
DK105	-4.07	-1.19	-7.20	0.71	0.83	0.83	-5.75	-1.42	-8.63
F64	-6.13	0.52	-12.81	2.13	2.40	2.40	-2.88	0.22	-5.34
EP44	-0.94	5.65	10.19	5.72	5.63	5.63	-0.17	1.00	1.81

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Table 2: QTL additive effects and standard deviations

	β_{M1}	β_{M4-E1}	β_{M4-E2}	sd(β_{M1})	sd(β_{M4-E1})	sd(β_{M4-E2})	β/sd(β)(M1)	β/sd(β)(M4-E1)	β/sd(β)(M4-E2)
B73	0.00	0.00	0.00	0.00	0.00	0.00			
B97	0.04	0.09	0.08	0.14	0.14	0.14	0.27	0.66	0.56
CML103	0.29	0.55	0.21	0.16	0.16	0.16	1.83	3.46	1.31
CML228	0.36	0.43	0.45	0.22	0.22	0.22	1.61	1.92	2.03
CML247	0.54	0.45	0.55	0.17	0.20	0.20	3.12	2.28	2.83
CML277	-0.05	0.26	-0.17	0.22	0.18	0.18	-0.20	1.44	-0.94
CML322	0.41	0.68	0.60	0.16	0.18	0.18	2.62	3.91	3.45
CML333	0.60	0.69	0.62	0.17	0.19	0.19	3.55	3.65	3.30
CML52	0.59	0.47	0.52	0.22	0.19	0.19	2.65	2.49	2.73
CML69	0.61	0.67	0.64	0.16	0.16	0.16	3.70	4.16	3.98
Hp301	0.08	0.20	0.19	0.14	0.14	0.14	0.54	1.43	1.31
IL14H	-1.09	-0.82	-1.32	0.14	0.14	0.14	-7.56	-5.80	-9.35
Ki11	-0.11	-0.06	-0.52	0.19	0.20	0.20	-0.54	-0.29	-2.61
Ki3	0.17	0.44	-0.07	0.20	0.29	0.29	0.86	1.51	-0.23
Ky21	0.38	0.32	0.46	0.12	0.13	0.13	3.09	2.43	3.49
M162W	0.03	-0.03	-0.11	0.13	0.16	0.16	0.25	-0.17	-0.72
M37W	0.20	0.17	0.00	0.16	0.15	0.15	1.24	1.14	0.02
Mo18W	0.62	0.87	0.53	0.18	0.20	0.20	3.54	4.32	2.64
MS71	-1.06	-0.54	-1.17	0.14	0.13	0.13	-7.54	-4.13	-8.99
NC350	-0.27	0.56	-0.20	0.18	0.18	0.18	-1.45	3.11	-1.10
NC358	0.04	0.40	-0.05	0.12	0.14	0.14	0.32	2.81	-0.34
Oh43	-0.02	-0.07	0.12	0.13	0.14	0.14	-0.14	-0.50	0.81
Oh7B	0.15	0.22	0.20	0.15	0.16	0.16	1.01	1.33	1.24
P39	-1.32	-0.99	-1.56	0.18	0.18	0.18	-7.46	-5.48	-8.69
Tx303	0.48	0.64	0.44	0.17	0.20	0.20	2.87	3.24	2.23
Tzi8	-0.11	-0.23	-0.44	0.15	0.20	0.20	-0.74	-1.17	-2.18

US-NAM chr 9 47 cM

Table 3: QTL additive effects and standard deviations

	β_{M1}	β_{M4-E1}	β_{M4-E2}	sd(β_{M1})	sd(β_{M4-E1})	sd(β_{M4-E2})	β/sd(β)(M1)	β/sd(β)(M4-E1)	β/sd(β)(M4-E2)
B73	0.00	0.00	0.00	0.00	0.00	0.00			
B97	-0.05	0.01	0.02	0.12	0.13	0.13	-0.40	0.06	0.18
CML103	0.47	0.25	0.69	0.13	0.16	0.16	3.62	1.57	4.44
CML228	0.93	0.67	1.20	0.22	0.23	0.23	4.26	2.95	5.30
CML247	0.20	-0.29	0.71	0.16	0.19	0.19	1.25	-1.52	3.70
CML277	1.43	0.78	1.94	0.21	0.18	0.18	6.85	4.41	11.00
CML322	0.53	0.30	0.79	0.14	0.17	0.17	3.73	1.79	4.65
CML333	0.70	0.26	1.08	0.16	0.19	0.19	4.39	1.39	5.71
CML52	0.48	0.28	0.82	0.21	0.18	0.18	2.30	1.55	4.62
CML69	0.03	-0.36	0.18	0.14	0.15	0.15	0.19	-2.42	1.23
Hp301	0.46	0.51	0.41	0.13	0.14	0.14	3.42	3.56	2.81
IL14H	-0.05	0.15	0.18	0.14	0.14	0.14	-0.37	1.07	1.30
Ki11	1.10	0.28	1.85	0.20	0.21	0.21	5.43	1.33	8.70
Ki3	0.12	-0.19	0.20	0.18	0.27	0.27	0.65	-0.69	0.76
Ky21	0.23	0.29	0.18	0.10	0.13	0.13	2.23	2.20	1.35
M162W	0.36	-0.11	0.65	0.14	0.18	0.18	2.56	-0.62	3.66
M37W	-0.04	-0.02	0.15	0.15	0.16	0.16	-0.24	-0.13	0.96
Mo18W	0.16	-0.36	0.60	0.17	0.21	0.21	0.93	-1.72	2.82
MS71	0.31	0.04	0.46	0.13	0.13	0.13	2.42	0.32	3.58
NC350	-0.48	-0.57	-0.09	0.15	0.18	0.18	-3.28	-3.17	-0.51
NC358	-0.07	-0.29	0.36	0.12	0.15	0.15	-0.54	-2.01	2.47
Oh43	0.14	0.06	0.22	0.13	0.14	0.14	1.07	0.45	1.51
Oh7B	0.28	0.38	0.36	0.14	0.17	0.17	2.03	2.20	2.08
P39	0.28	0.55	0.50	0.17	0.19	0.19	1.62	2.85	2.57
Tx303	0.21	-0.09	0.51	0.15	0.20	0.20	1.42	-0.42	2.52
Tzi8	0.72	0.45	0.92	0.13	0.18	0.18	5.42	2.45	5.02

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Table 4: QTL additive effects and standard deviations

	β_{M1}	β_{M4-E1}	β_{M4-E2}	sd(β_{M1})	sd(β_{M4-E1})	sd(β_{M4-E2})	β/sd(β)(M1)	β/sd(β)(M4-E1)	β/sd(β)(M4-E2)
B73	0.00	0.00	0.00	0.00	0.00	0.00			
B97	-0.05	0.04	-0.20	0.13	0.13	0.13	-0.42	0.30	-1.49
CML103	-0.21	-0.20	-0.36	0.13	0.16	0.16	-1.61	-1.21	-2.21
CML228	1.34	0.66	2.10	0.21	0.22	0.22	6.27	2.98	9.41
CML247	-0.14	0.01	-0.47	0.16	0.19	0.19	-0.87	0.07	-2.43
CML277	2.03	1.39	2.99	0.22	0.18	0.18	9.22	7.54	16.26
CML322	-0.45	-0.26	-0.50	0.14	0.17	0.17	-3.22	-1.57	-2.97
CML333	0.16	-0.12	0.14	0.16	0.19	0.19	1.02	-0.66	0.74
CML52	0.29	0.41	0.22	0.20	0.19	0.19	1.45	2.20	1.21
CML69	-0.12	0.16	-0.26	0.14	0.16	0.16	-0.85	1.04	-1.63
Hp301	0.20	-0.01	0.41	0.14	0.15	0.15	1.44	-0.04	2.79
IL14H	-0.05	0.50	-0.02	0.13	0.14	0.14	-0.37	3.62	-0.15
Ki11	1.48	1.09	1.76	0.19	0.20	0.20	7.65	5.37	8.69
Ki3	-0.43	-0.55	-0.44	0.18	0.28	0.28	-2.38	-1.97	-1.57
Ky21	0.23	0.24	0.33	0.10	0.13	0.13	2.22	1.76	2.44
M162W	-0.08	-0.03	-0.37	0.14	0.18	0.18	-0.61	-0.20	-2.12
M37W	-0.17	-0.17	-0.23	0.14	0.15	0.15	-1.22	-1.13	-1.56
Mo18W	-0.21	-0.14	-0.42	0.17	0.21	0.21	-1.23	-0.65	-1.98
MS71	0.17	0.04	0.26	0.14	0.14	0.14	1.21	0.31	1.86
NC350	-0.20	-0.02	-0.07	0.15	0.18	0.18	-1.31	-0.13	-0.43
NC358	0.22	0.27	0.26	0.12	0.14	0.14	1.87	1.90	1.81
Oh43	-0.12	-0.05	-0.15	0.13	0.15	0.15	-0.91	-0.33	-1.01
Oh7B	-0.01	0.18	0.09	0.13	0.16	0.16	-0.07	1.11	0.53
P39	-0.15	0.00	-0.16	0.16	0.18	0.18	-0.90	0.02	-0.89
Tx303	-0.17	-0.04	-0.15	0.14	0.20	0.20	-1.19	-0.20	-0.75
Tzi8	-0.03	-0.08	-0.35	0.14	0.19	0.19	-0.23	-0.40	-1.82

S4: QTL additive effects allelic series

Comparison of the additive effect allelic series between M1, M2, M3 and M4 and two environments for two individual QTL positions. The upper panel contain the first environment result and the lower the one from the second environment.

EU-NAM ancestral model chr 6 82.1 cM

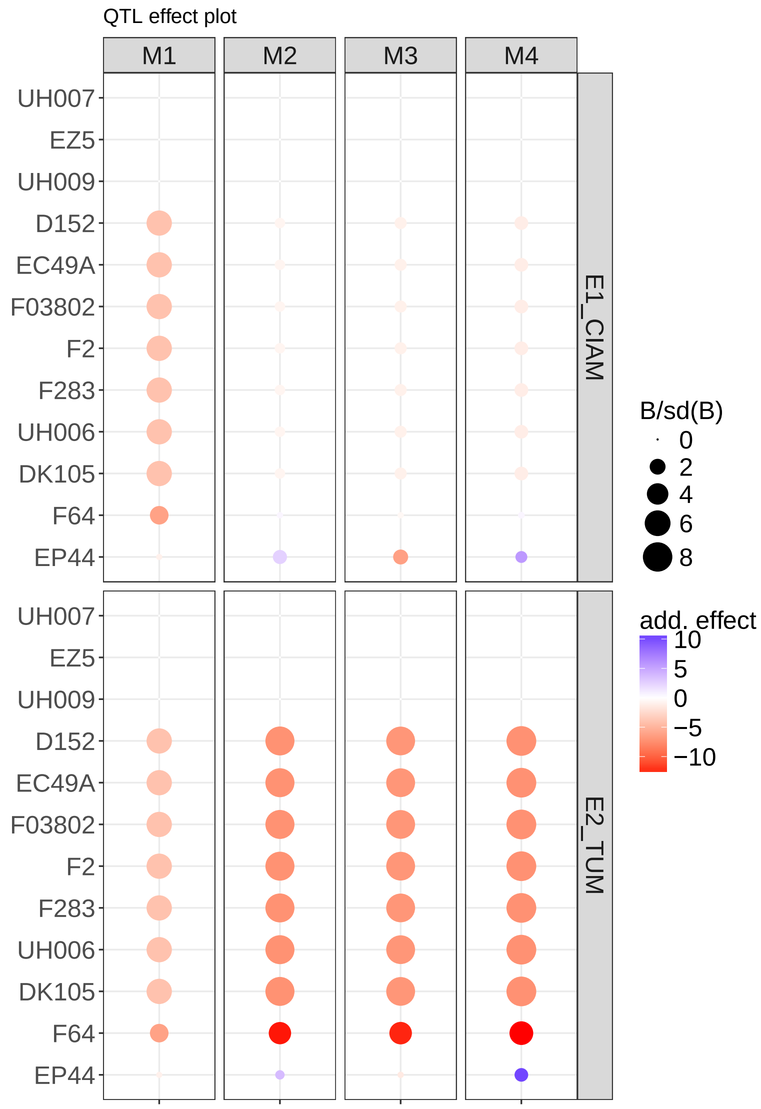

Figure 3: Comparison of the additive effect allelic series between M1, M2, M3 and M4 and two environments

US-NAM parental model chr 8 67 cM

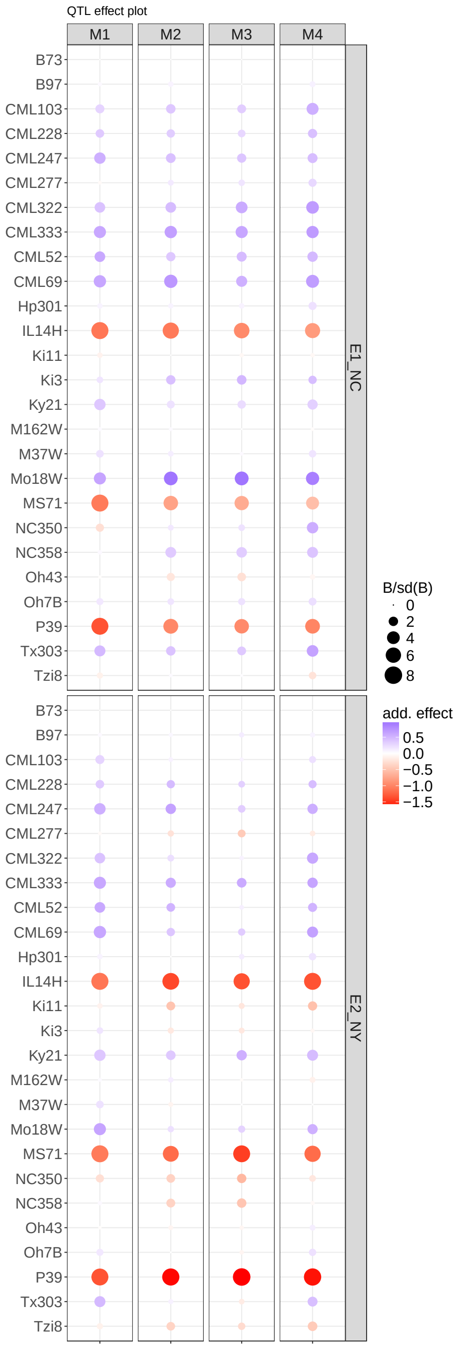

Figure 4: Comparison of the additive effect allelic series between M1, M2, M3 and M4 and two environments

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