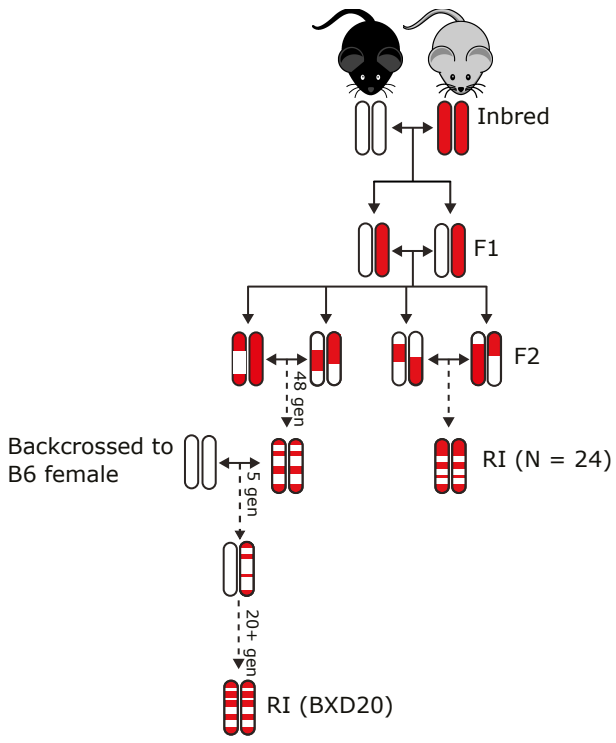
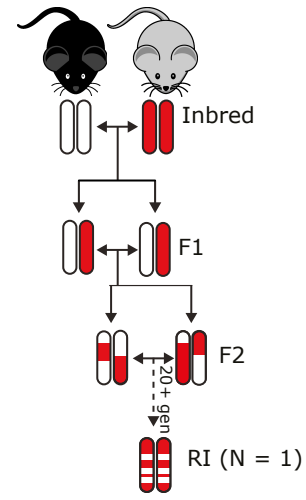
Figure S1: Production of each epoch of the BXD recombinant inbred family, related to Figure 1. The BXDs up to BXD220 have been produced in 6 epochs, with epoch 1 being started in 1971. Red coloring has been used to represent regions of the genome coming from the inbred C57BL/6J (B6) parental strain, whereas white coloring has been used to represent regions of the genome coming from the inbred DBA/2J (D2) strain. Solid lines have been used to represent a single generation, whereas dashed lines represent several generations, with the number of generations written along the line. During the 1970s to 2000s strains that were at risk of going extinct were often backcrossed to B6 in an attempt to rescue them, and this is shown in the figure. During epochs 4-6, strains at risk of extinction were sometimes crossed to other BXD RI strains in an attempt to rescue them. Beneath each epoch, the number of inbred strains begun, the number of strains with data within GeneNetwork, and the number of strains extant are shown. In cases where any crosses or backcrosses occurred these are also noted. Although the majority of the BXD family have the D2 Y-chromosome and B6 mitochondrial genome, this is not the case for all strains, and this is noted as well. Adapted from (Peirce et al., 2004; Williams and Auwerx, 2015). Full details can be found in Table S1.

Epoch 1a (1971)



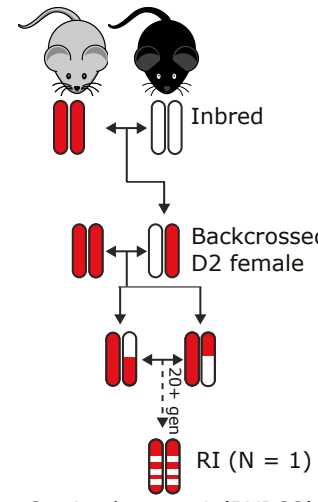
Strains begun: 30 (BXD1-30)
Strains with data: 26
Strains backcrossed to B6: 1 (BXD20)
Strain extant: 24 (BXD1,2,5,6,8,9,11-16, 18-22,24,24a,25,27–29,29a)

Epoch 1b (1970s)



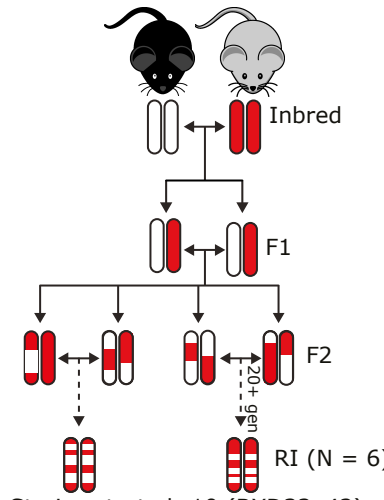
Strains begun: 1 (BXD31)
Strains with data: 1
Strain extant: 1 (BXD31)

Epoch 1c (1970s)



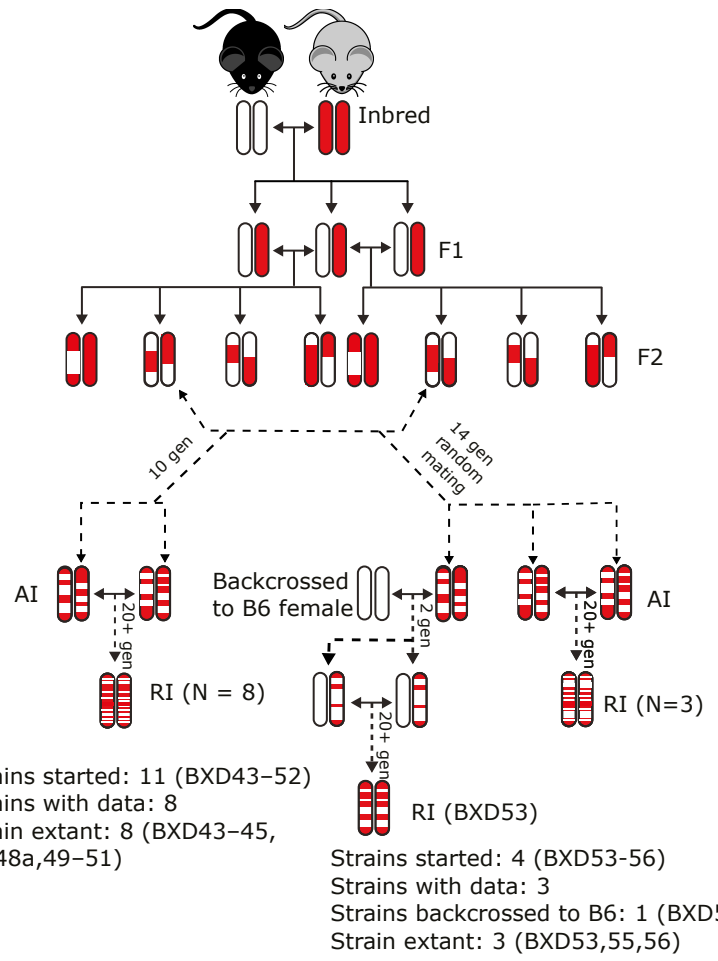
Strains begun: 1 (BXD32)
Strains with data: 1
Strain extant: 1 (BXD32)
Note: B6D2F1 was backcrossed to D2 mother

Epoch 2 (1990s)



Strains started: 10 (BXD33–42)
Strains with data: 7
Strain extant: 6 (BXD33,34, 38-40,42)

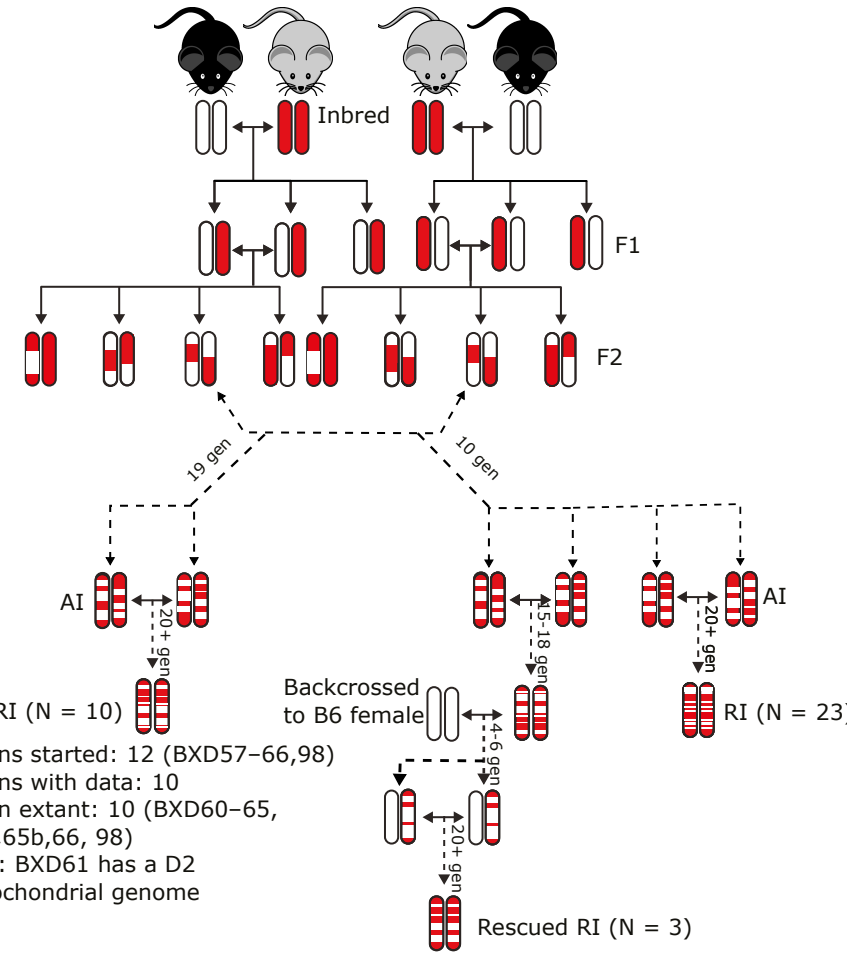
Epoch 3a (late 1990s)



Strains started: 11 (BXD43–52)
Strains with data: 8
Strain extant: 8 (BXD43–45, 48,48a,49–51)

Strains started: 4 (BXD53-56)
Strains with data: 3
Strains backcrossed to B6: 1 (BXD53)
Strain extant: 3 (BXD53,55,56)

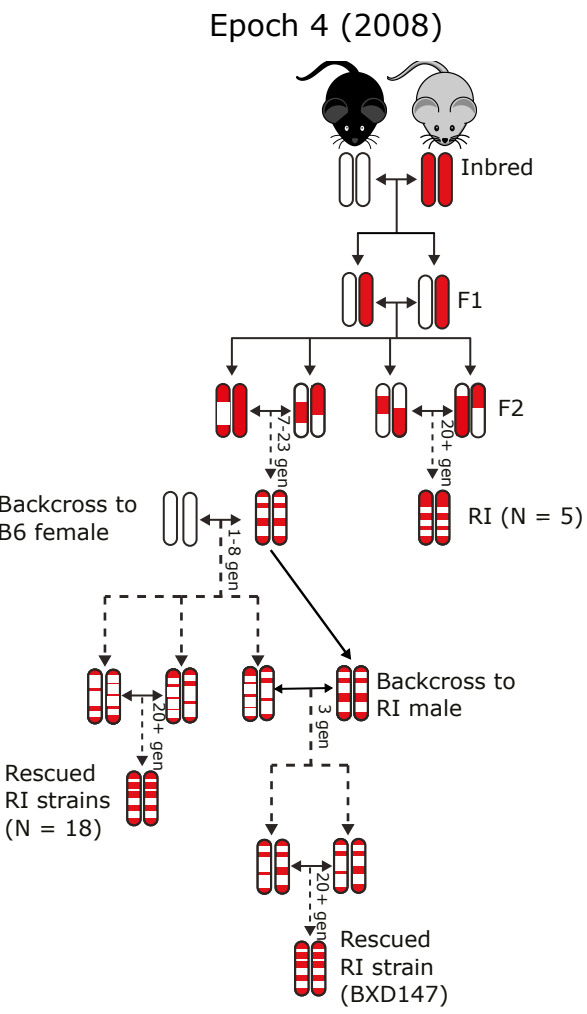
Epoch 3b (late 1990s)



Strains started: 12 (BXD57–66,98)
Strains with data: 10
Strain extant: 10 (BXD60–65, 65a,65b,66, 98)
Note: BXD61 has a D2 mitochondrial genome

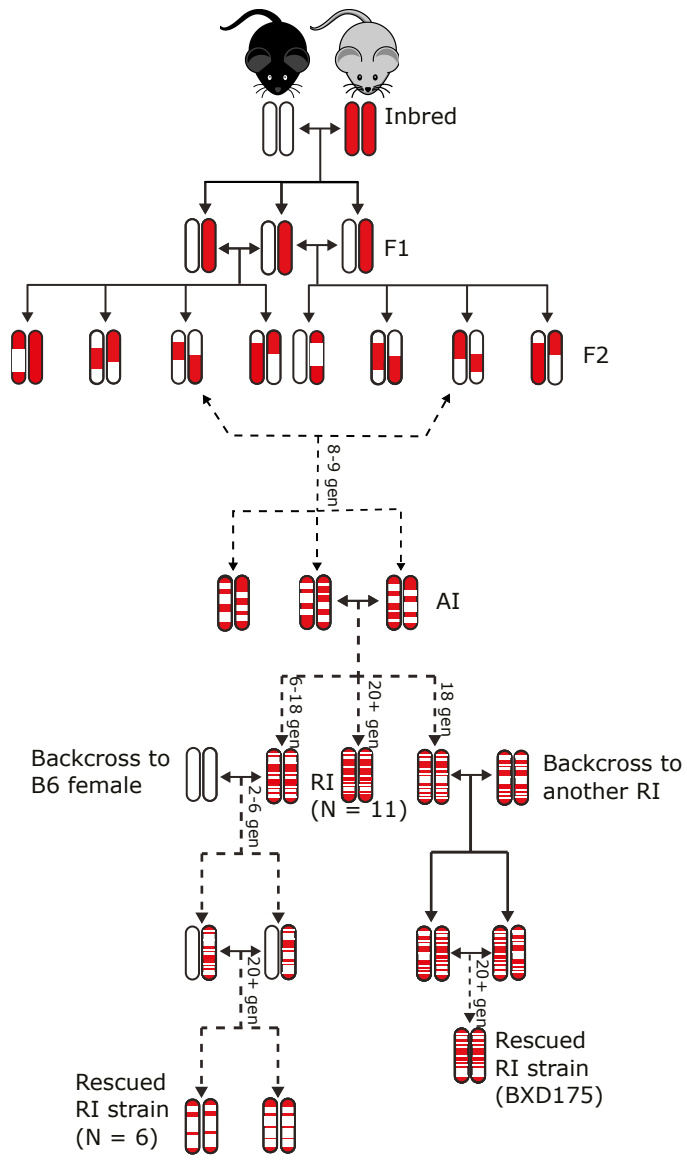
Strains started: 30 (BXD67–95,99–102)
Strains with data: 27
Strains backcrossed to B6: 3 (BXD78, 88, 91)
Strain extant: 26 (BXD67–71, 73,73a,73b,74,75,77-79,81,83–88,90,91,99–102)
Note: BXD74, 78, 90, 91, 95 and 99 have a D2 mitochondrial genome
Note: BXD71, 73,73a,73b,75,77,79,83–85,87,88, 95,102 have a B6 Y chromosome

Epoch 4 (2008)



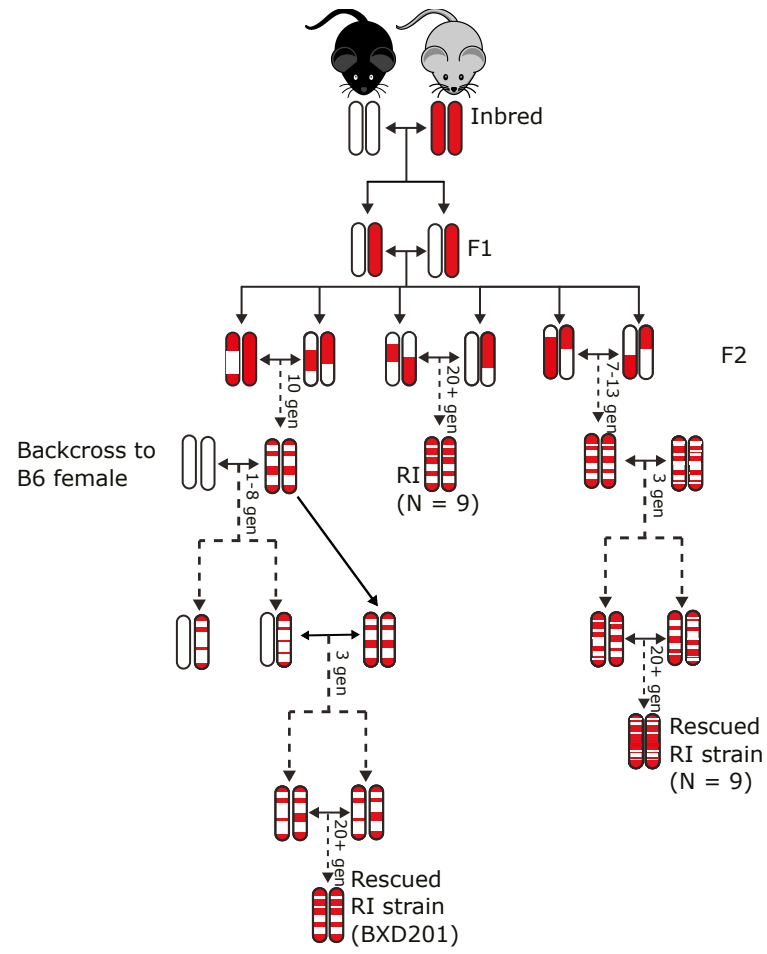
Strains started: 57 (BXD104–157)
Strains with data: 51
Strains backcrossed: 18 (BXD106, 109, 110, 112, 114, 116, 121, 127, 130, 131, 132, 134, 137, 139, 140, 146, 148, 149)
Strains with backcross to B6 then to RI: 1 (BXD147)
Strain extant: 24 (BXD111,113,114,122,123–125, 127,128,128a,131,137,139,144,147–152,154,156,157)

Epoch 5 (2010)



Strains started: 29 (BXD158–186)
Strains with data: 20
Strain backcrossed to B6: 6 (BXD162, 173, 174, 176, 181, 183)
Strains crossed to another RI: 1 (BXD175)
Strain extant: 18 (BXD160-162, 168–174,176–178,180,181, 183,184,186)

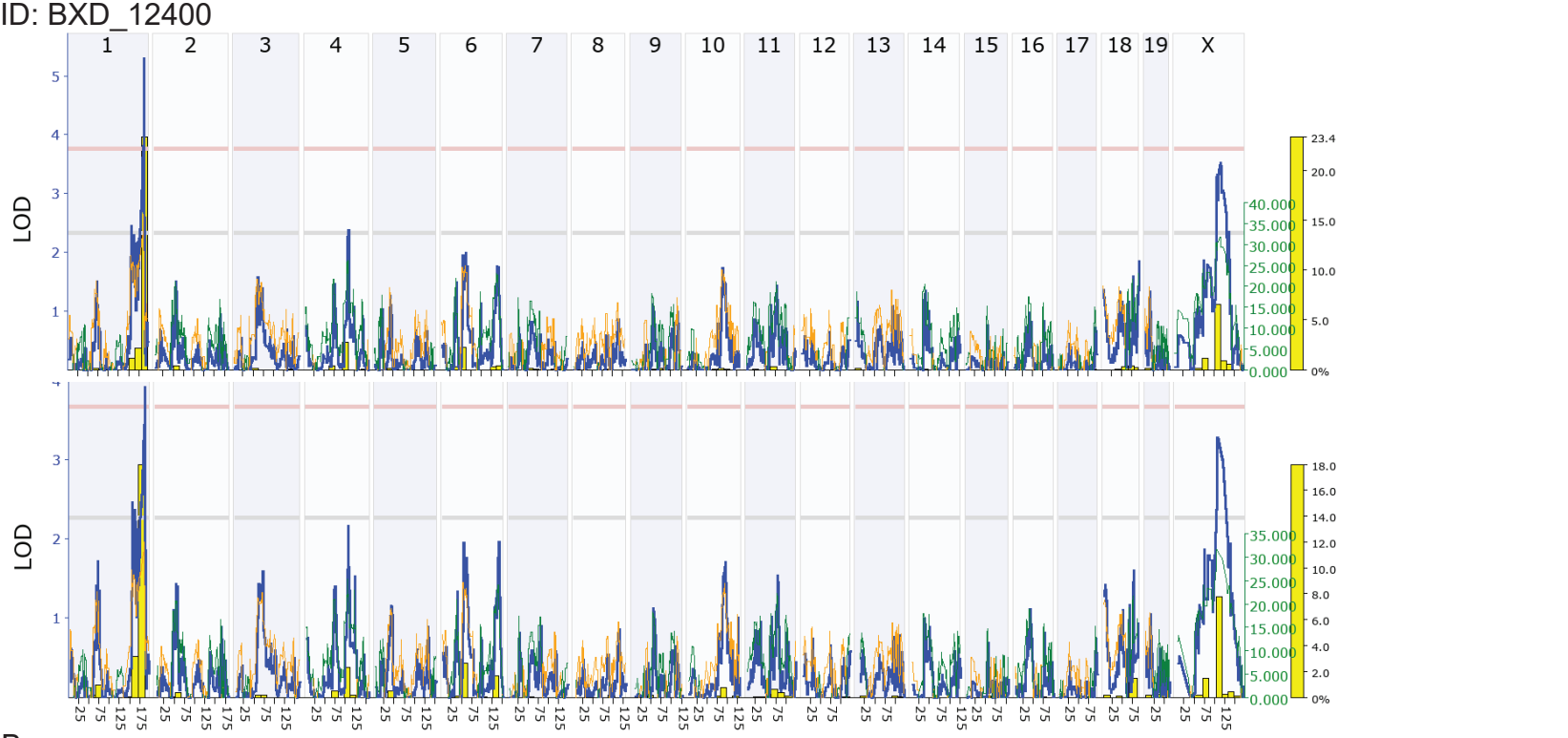
Epoch 6 (2014)



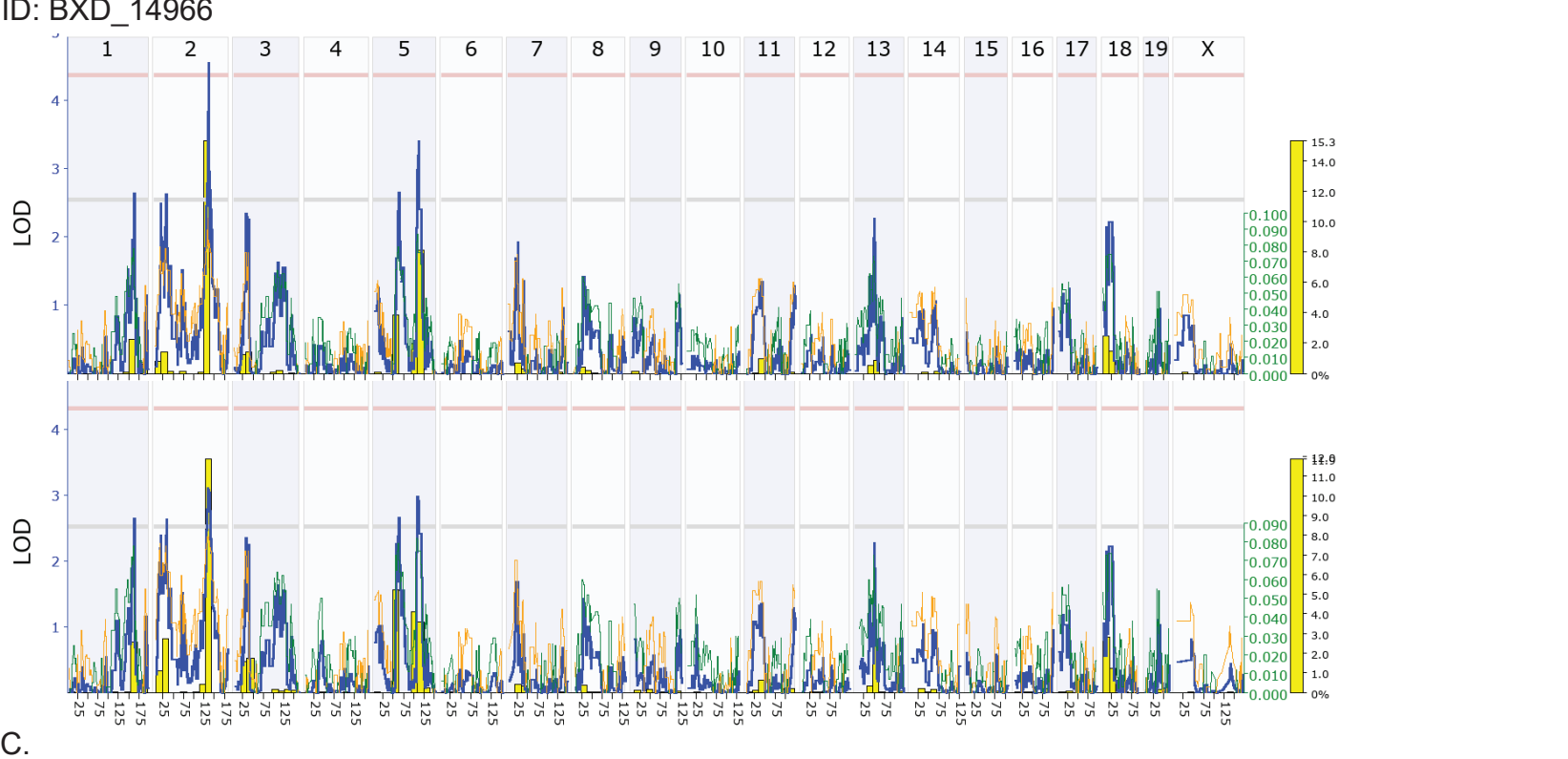
Strains started: 34 (BXD187–220)
Strains with data: 34
Strains crossed to another RI: 9 (BXD188, 189, 192, 196, 200, 206, 207, 209, 220)
Strain backcrossed then back to RI: 1 (BXD201)
Strain extant: 19 (BXD187,190,191, 194,195,199,202,204,205,210, 211,213–219)
Note: Epoch 7 still in development

Figure S2: The improved, denser, genotypes increase linkage in most cases, across chromosomes, decades of work (1984-2017), and number of strains used (n = 15 - 88), related to Figure 2. Phenotypes from GeneNetwork are shown where the peak LOD is improved by >1 using the current genotypes compared to the classic genotypes. For each phenotype the whole-genome QTL map is shown using the current or the classic genotype file. A full phenotype description, the year of publication (or submission to GN if unpublished), the number of strains analyzed, and the GeneNetwork ID for each phenotype is given. Phenotypes are **(A)** BXD_12400, **(B)** BXD_14966, **(C)** BXD_14968, **(D)** BXD_12956, **(E)** BXD_10463, **(F)** BXD_12846, **(G)** BXD_10666, **(H)** BXD_10043, **(I)** BXD_12889, **(J)** BXD_16305, **(K)** BXD_17177, **(L)** BXD_17733, **(M)** BXD_11336, **(N)** BXD_12899, **(O)** BXD_16185, **(P)** BXD_12506, **(Q)** BXD_12659 and **(R)** BXD_19312. For each QTL map the blue line shows the linkage between the trait and that position along the genome. The orange/green line represents the additive effect. The yellow bar shows the frequency of the LRS peak location from 2000 bootstraps of the data.

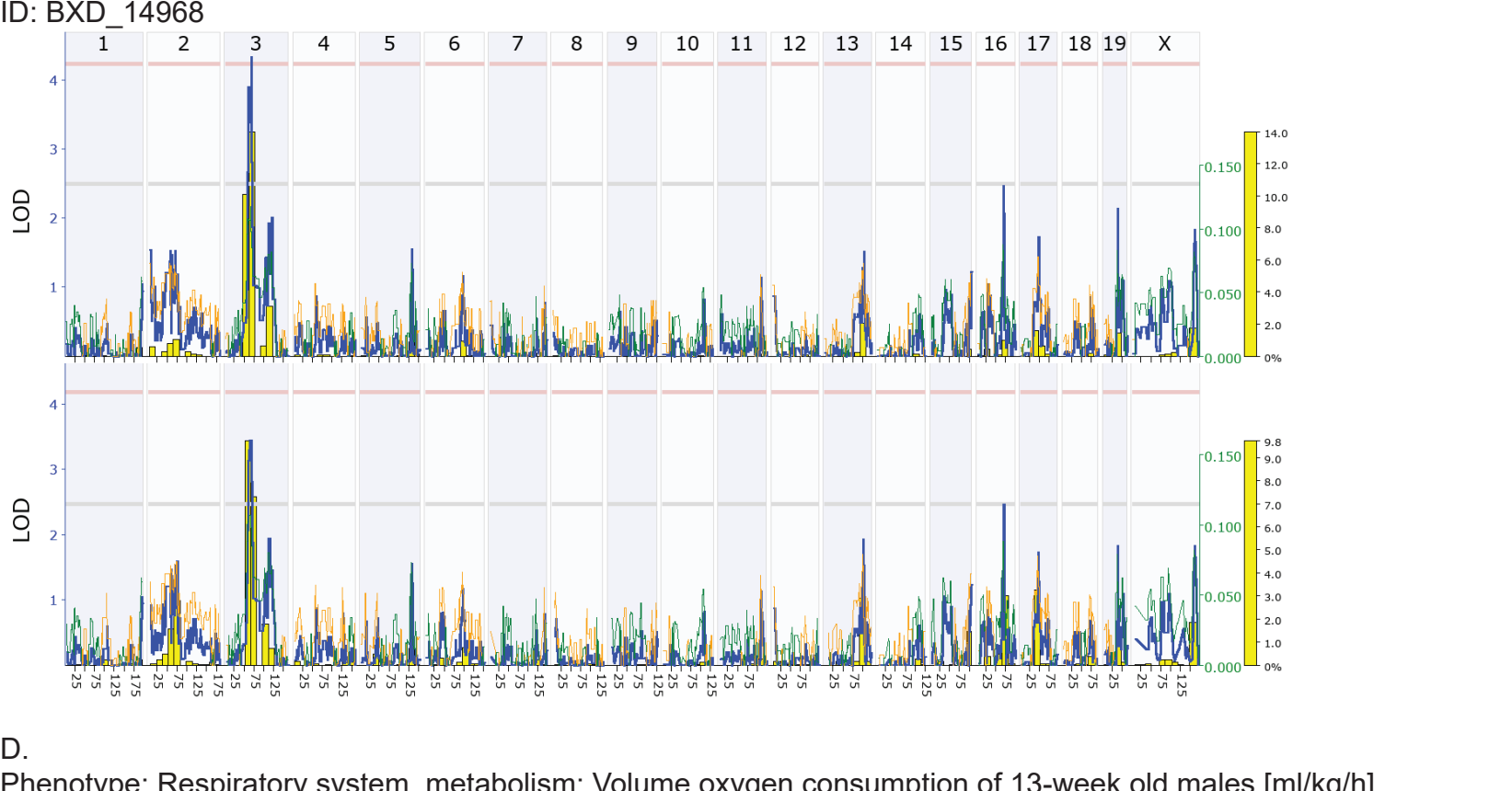
A.
Phenotype: Central nervous system, behavior: Anxiety assay, saline treated [0.18 ml/kg i.p.] (NOS group), activity in closed quadrants using an elevated zero maze in 60 to 120-day-old females only during last 5 min [n beam breaks]
Year of publication: 2009
n of strains: 71
ID: BXD_12400



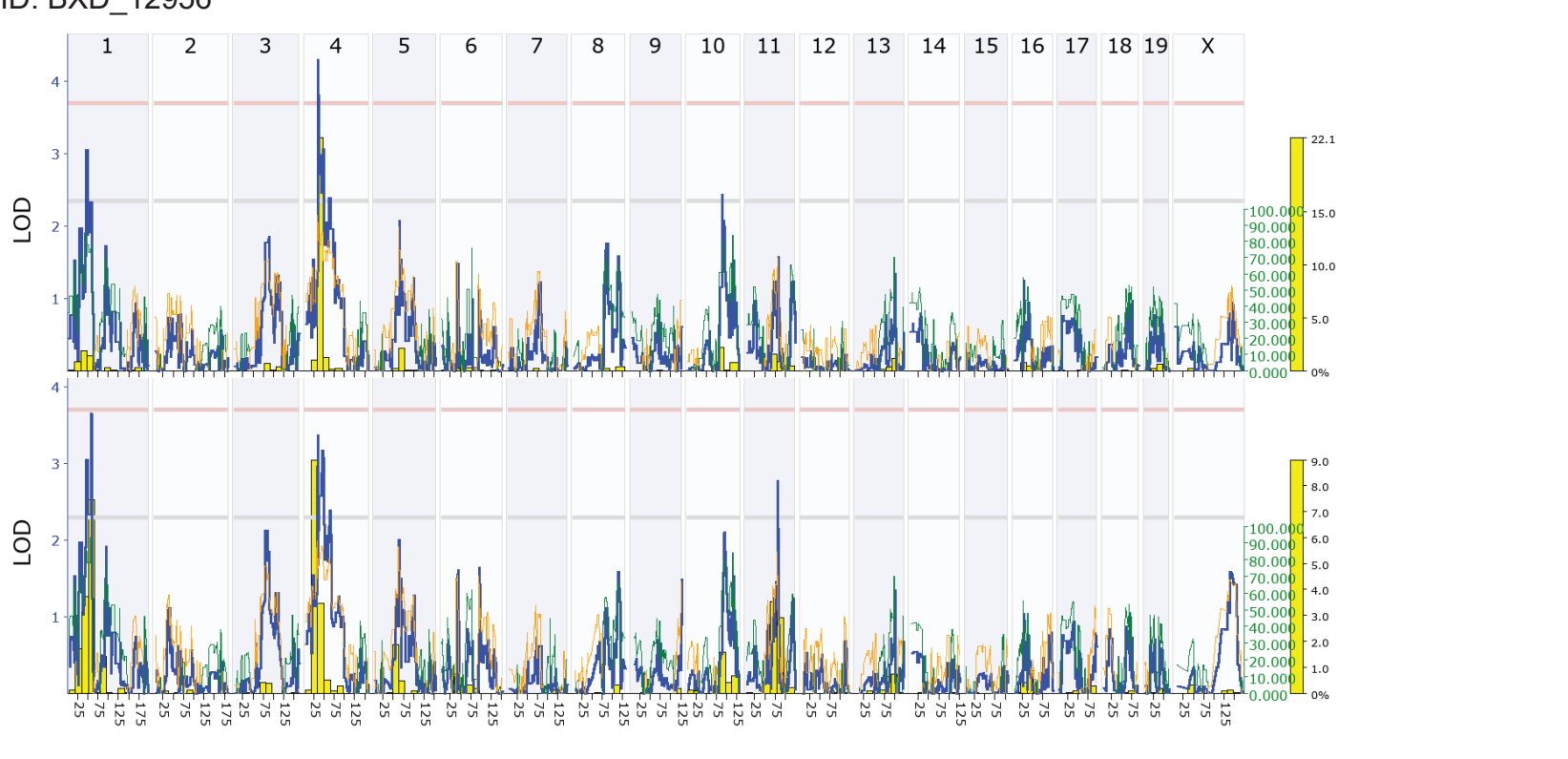
B.
Phenotype: Obesity, Brockmann (gudrun.brockmann@agrar.hu-berlin.de); High Fat Diet between 4 and 20 weeks (Ssniff(R) diet S8074-E010, Germany: The diet contained 20.7% crude protein, 25.1% crude fat, 5.0% crude fiber, 5.9% crude ash, 39.7% N-free extract, 20.0% starch, 17.5% sugar, vitamins, trace elements, amino acids, and minerals (19.1 MJ/kg metabolizable energy; thereof 45% energy from fat, 31% from carbohydrates, and 24% from proteins). The fat in the diet derived from coconut oil and suet.); Gluc70_10 in females + males
Year of publication: 2011
n of strains: 32
ID: BXD_14966



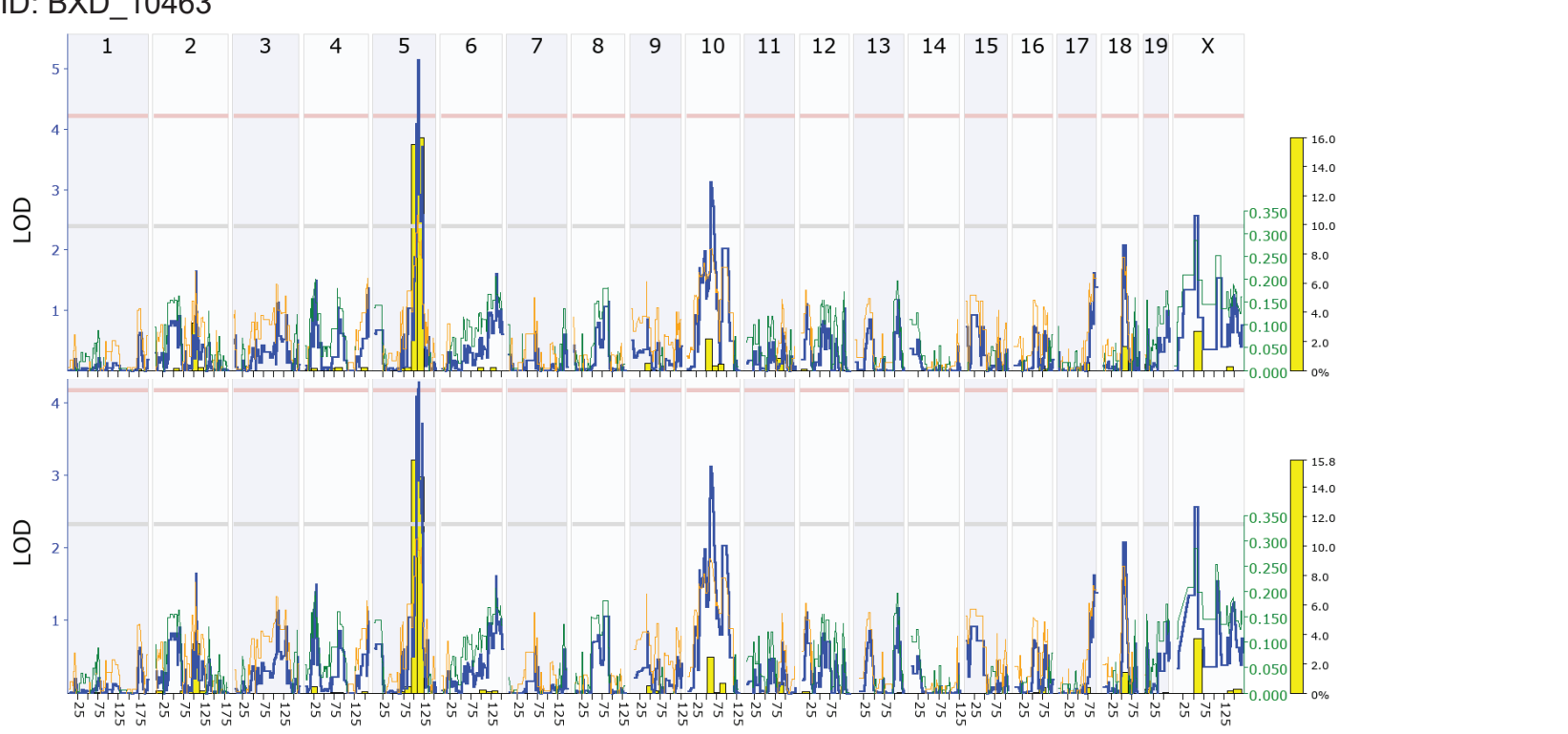
C.
Phenotype: Obesity, Brockmann (gudrun.brockmann@agrar.hu-berlin.de); High Fat Diet between 4 and 20 weeks (Ssniff(R) diet S8074-E010, Germany: The diet contained 20.7% crude protein, 25.1% crude fat, 5.0% crude fiber, 5.9% crude ash, 39.7% N-free extract, 20.0% starch, 17.5% sugar, vitamins, trace elements, amino acids, and minerals (19.1 MJ/kg metabolizable energy; thereof 45% energy from fat, 31% from carbohydrates, and 24% from proteins). The fat in the diet derived from coconut oil and suet.); FoodIntake7.10 in females + males
Year of publication: 2011
n of strains: 32
ID: BXD_14968



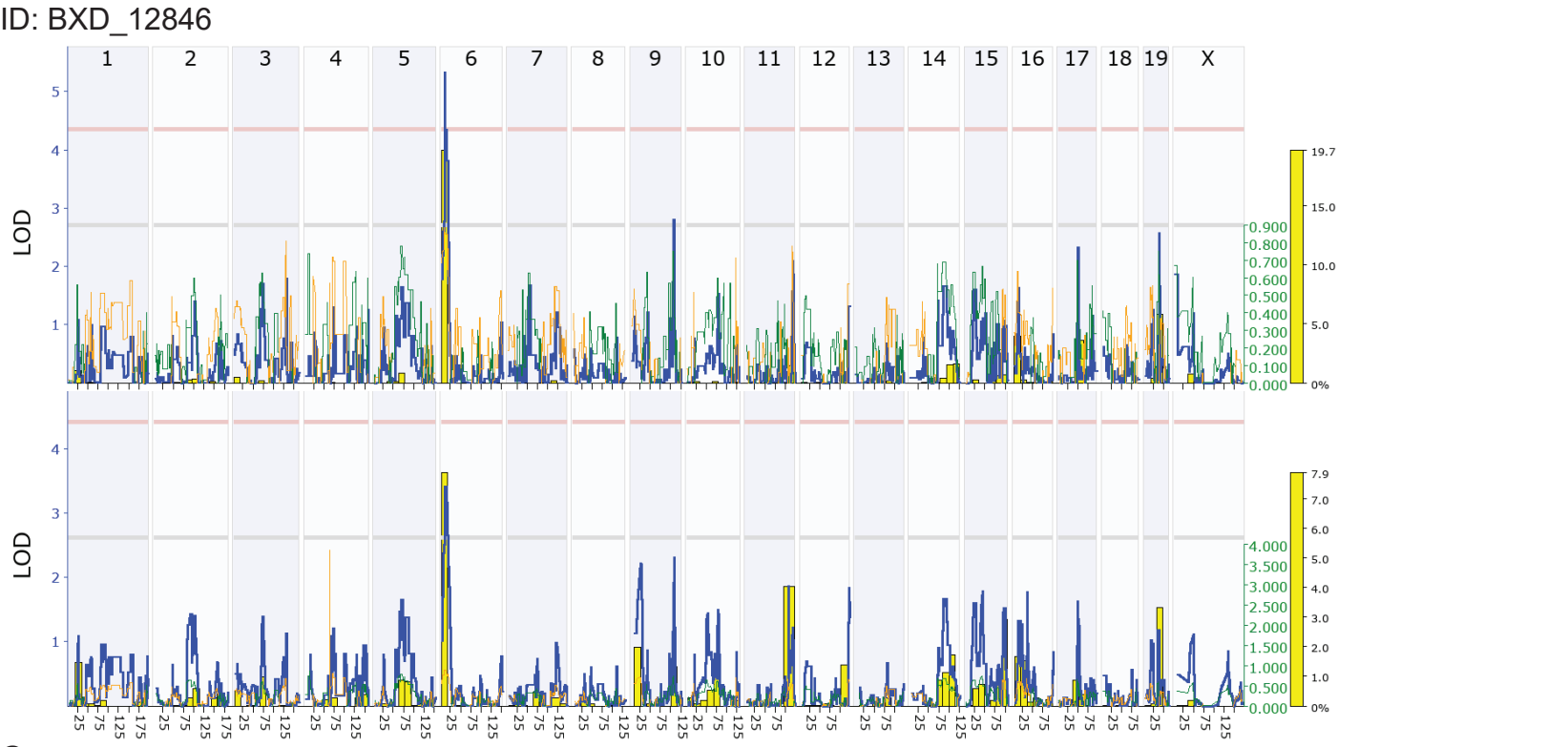
D.
Phenotype: Respiratory system, metabolism: Volume oxygen consumption of 13-week old males [ml/kg/h]
Year of publication: 2012
n of strains: 30
ID: BXD_12956



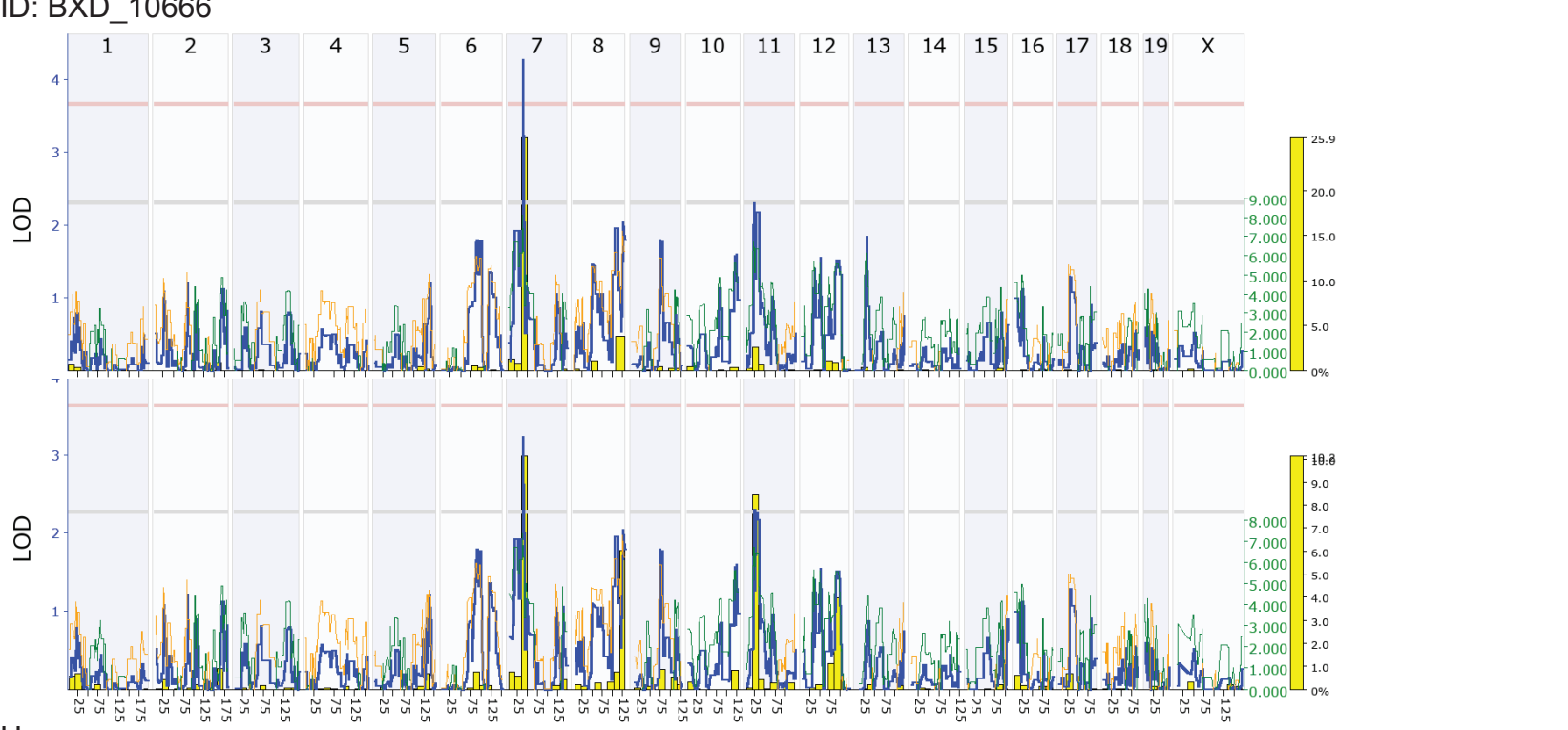
E.
Phenotype: Immune system, morphology: Thymus to body size ratio [x10^3]
Year of publication: 1984
n of strains: 20
ID: BXD_10463



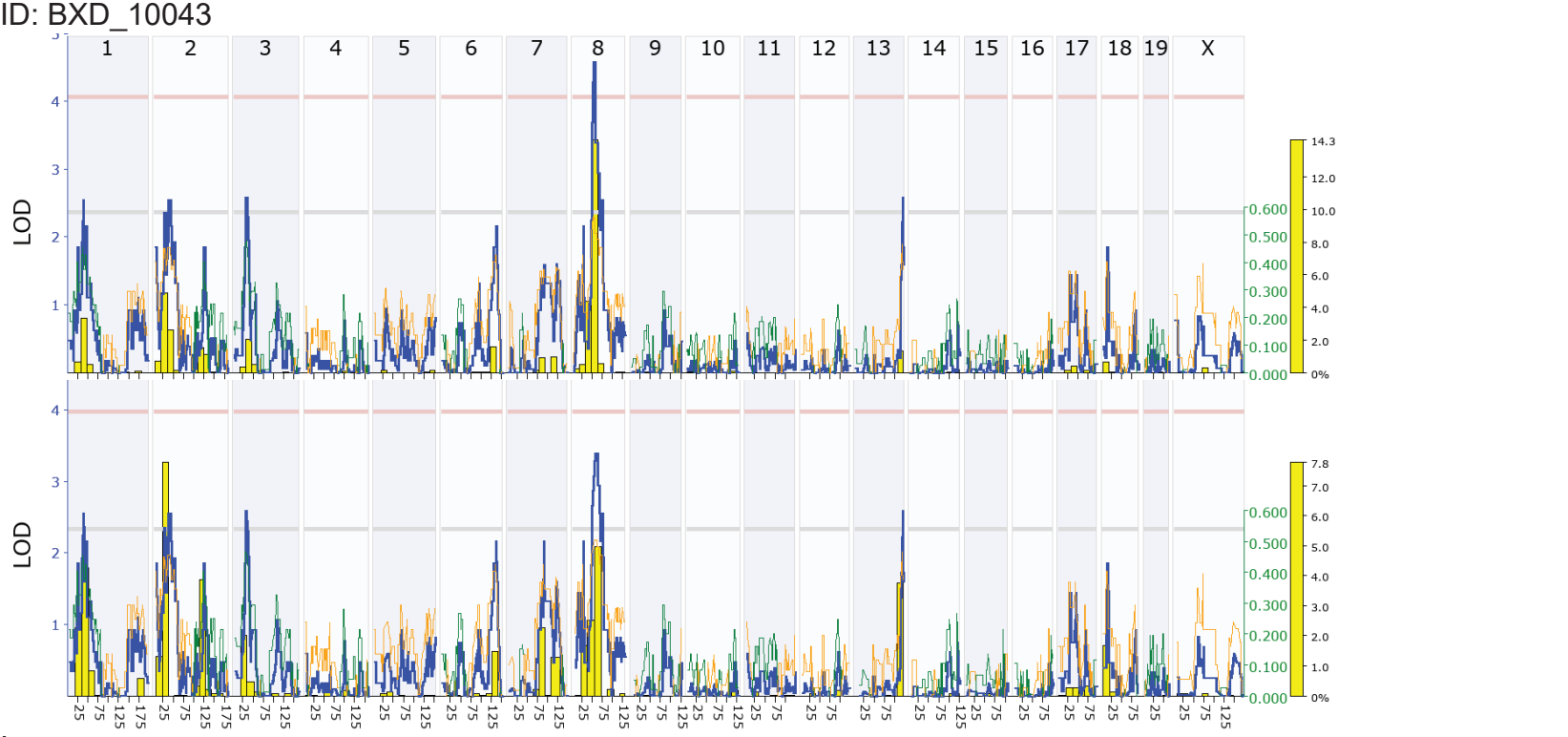
F.
Phenotype: Blood chemistry: Creatinine of 14-week old females [uM]
Year of publication: 2012
n of strains: 17
ID: BXD_12846



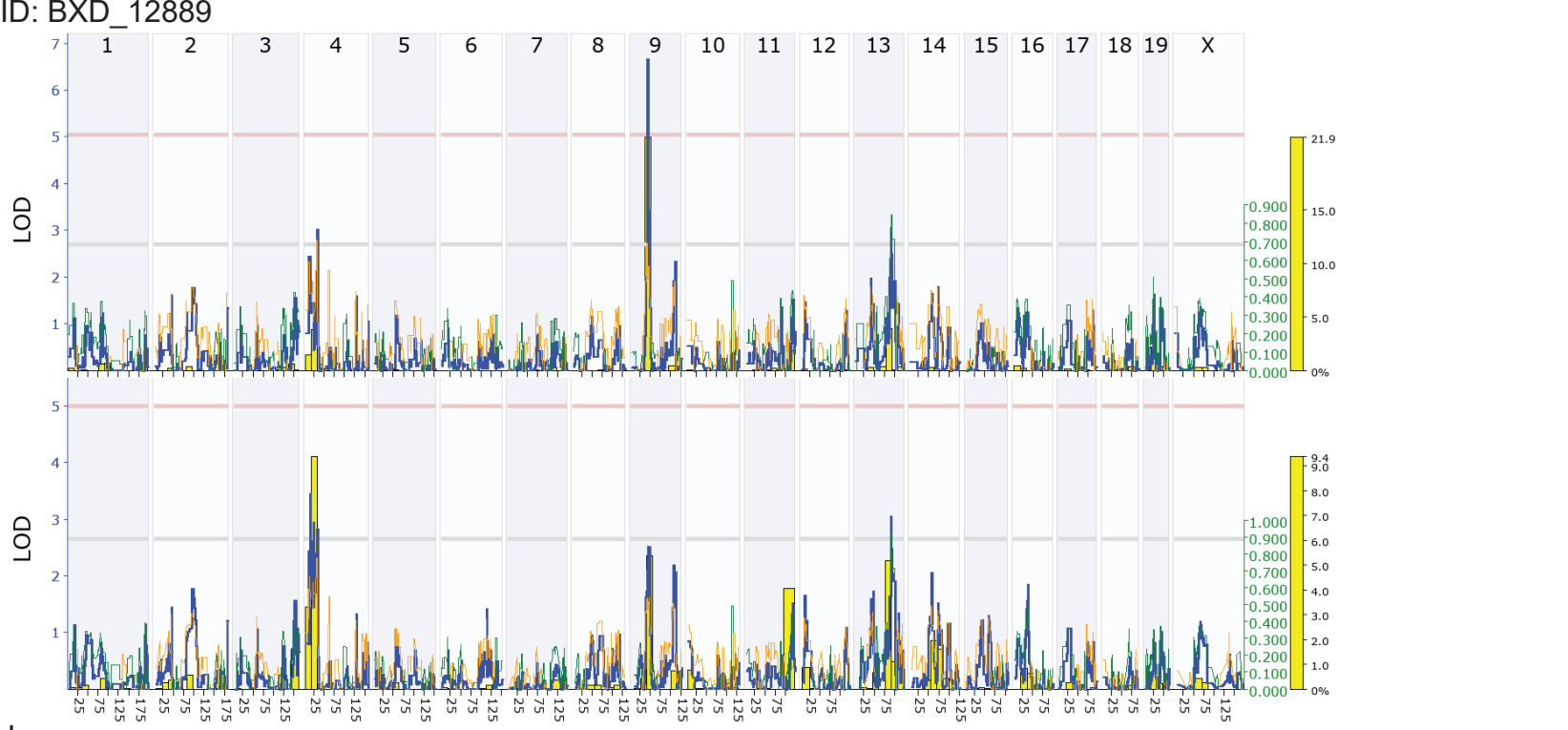
G.
Phenotype: Infectious disease, immune system: Cytotoxic T-cell (CTL) response (5 x 10^9 PFU AdLacZ iv), measured as interleukin 6 (IL-6) cytokine expression [pg/ml]
Year of publication: 2004
n of strains: 23
ID: BXD_10666



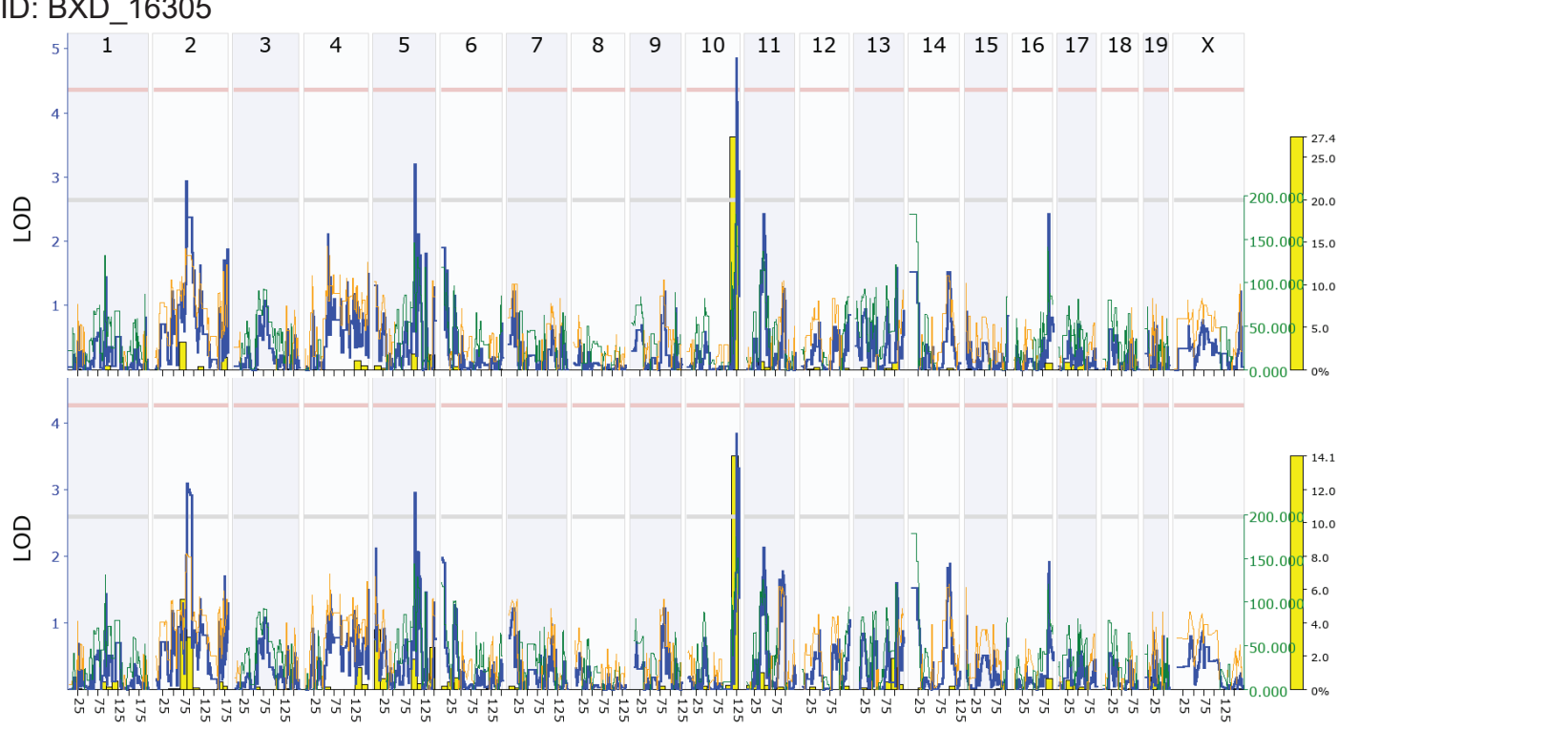
H.
Phenotype: Infectious disease, immune system: Ectromelia virus-induced mortality in males [3 = resistant, 2 = intermediate, 1 = susceptible, table 1 column 1, ordinal scale]
Year of publication: 1991
n of strains: 28
ID: BXD_10043



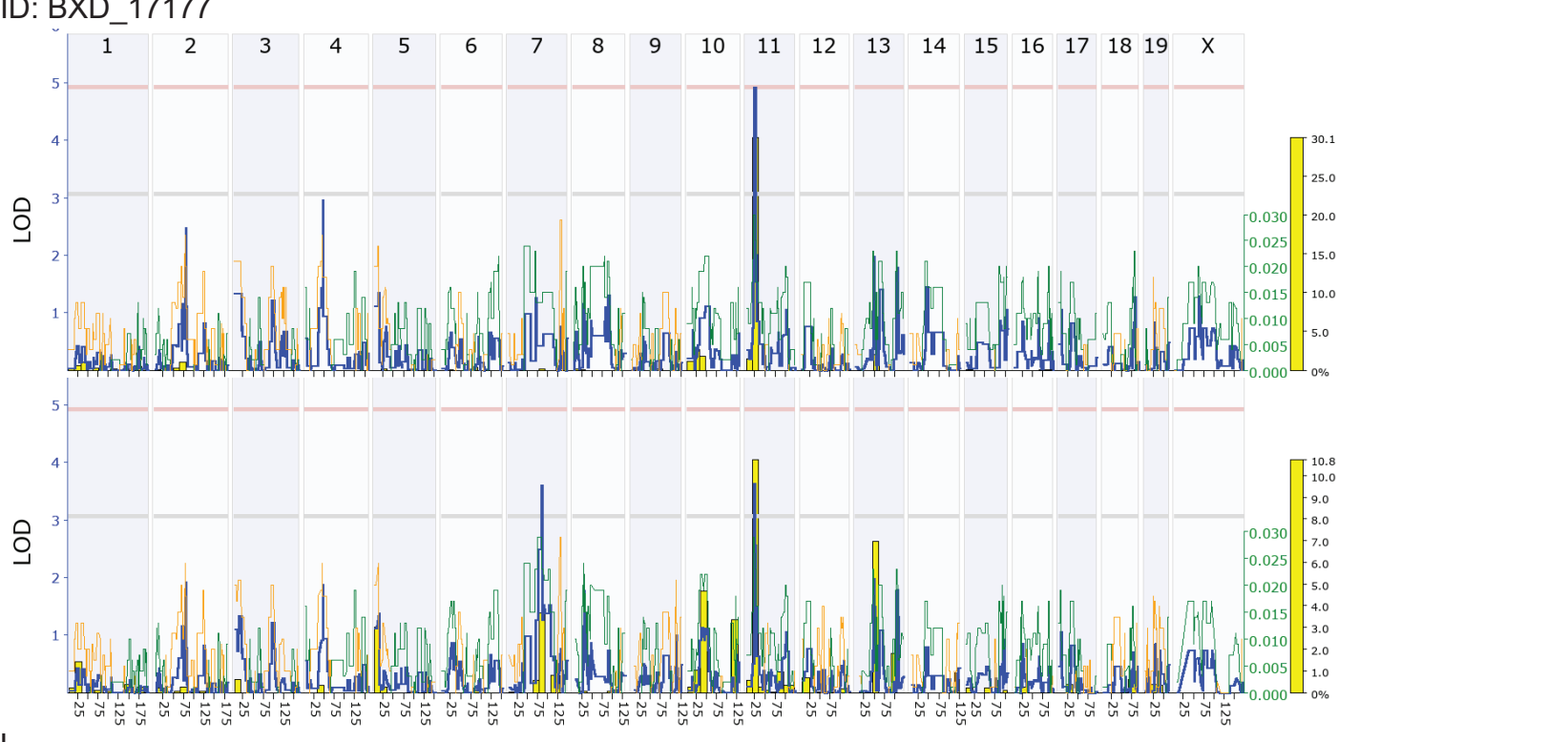
I.
Phenotype: Central nervous system, metabolism, behavior: Water intake of 13-week old females [ml/mouse/unit time]
Year of publication: 2012
n of strains: 19
ID: BXD_12889



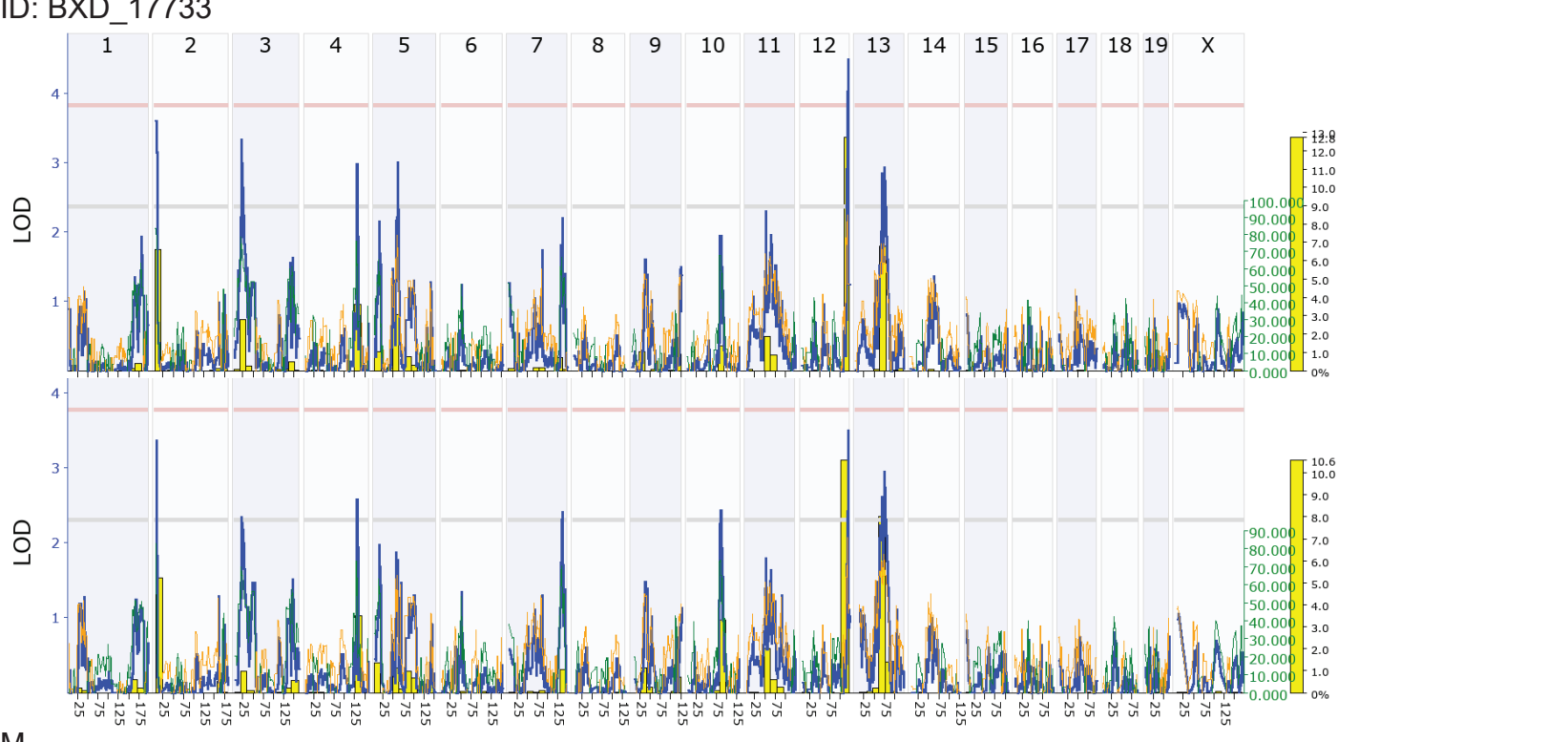
J.
Phenotype: Infectious disease, immune system: Herpes simplex virus type 1, serum neutralizing antibody titer in young adult females [unit]
Year of publication: 2013
n of strains: 20
ID: BXD_16305



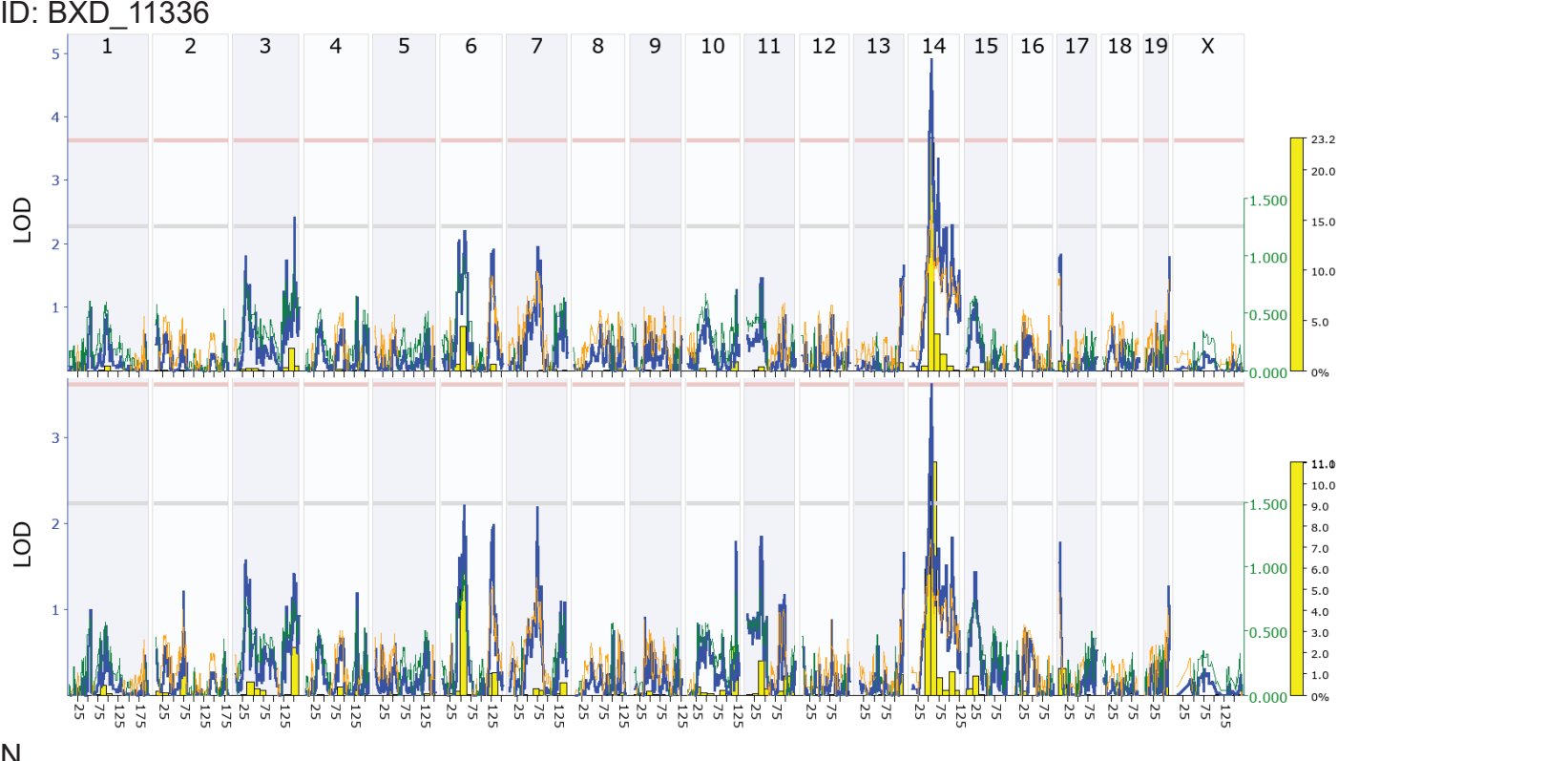
K.
Phenotype: Central nervous system, electrophysiology, behavior: Brain activity and coherence of electrical field oscillations, coherence at 71 Hz between local field potentials (LFP) at two sites (0.3 mm apart) in L2/3 of the primary whisker motor cortex in awake 6 to 12 month-old males and females (BXD73 and DBA/2J winsorized from 0.231 and 0.179 to 0.110 and 0.100, respectively) [coherence]
Year of publication: 2014
n of strains: 15
ID: BXD_17177



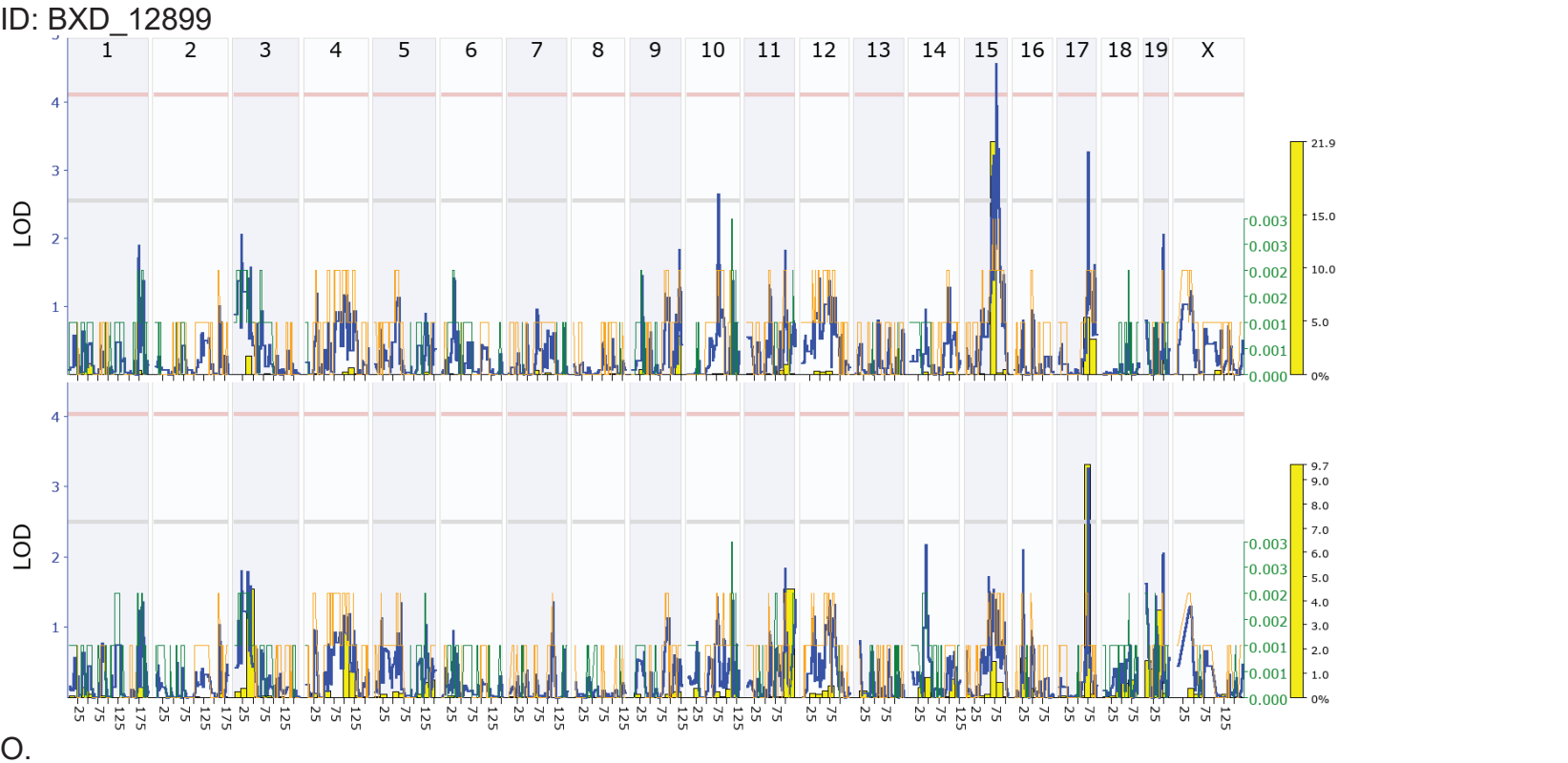
L.
Phenotype: Cardiovascular system, behavior: Run distance untrained, treadmill at 10 degree incline at 23 weeks of age, chow diet (6% kCal/fat Harlan.2918), males [m] (EPFL LISP3 Cohort)
Year of publication: 2016
n of strains: 52
ID: BXD_17733



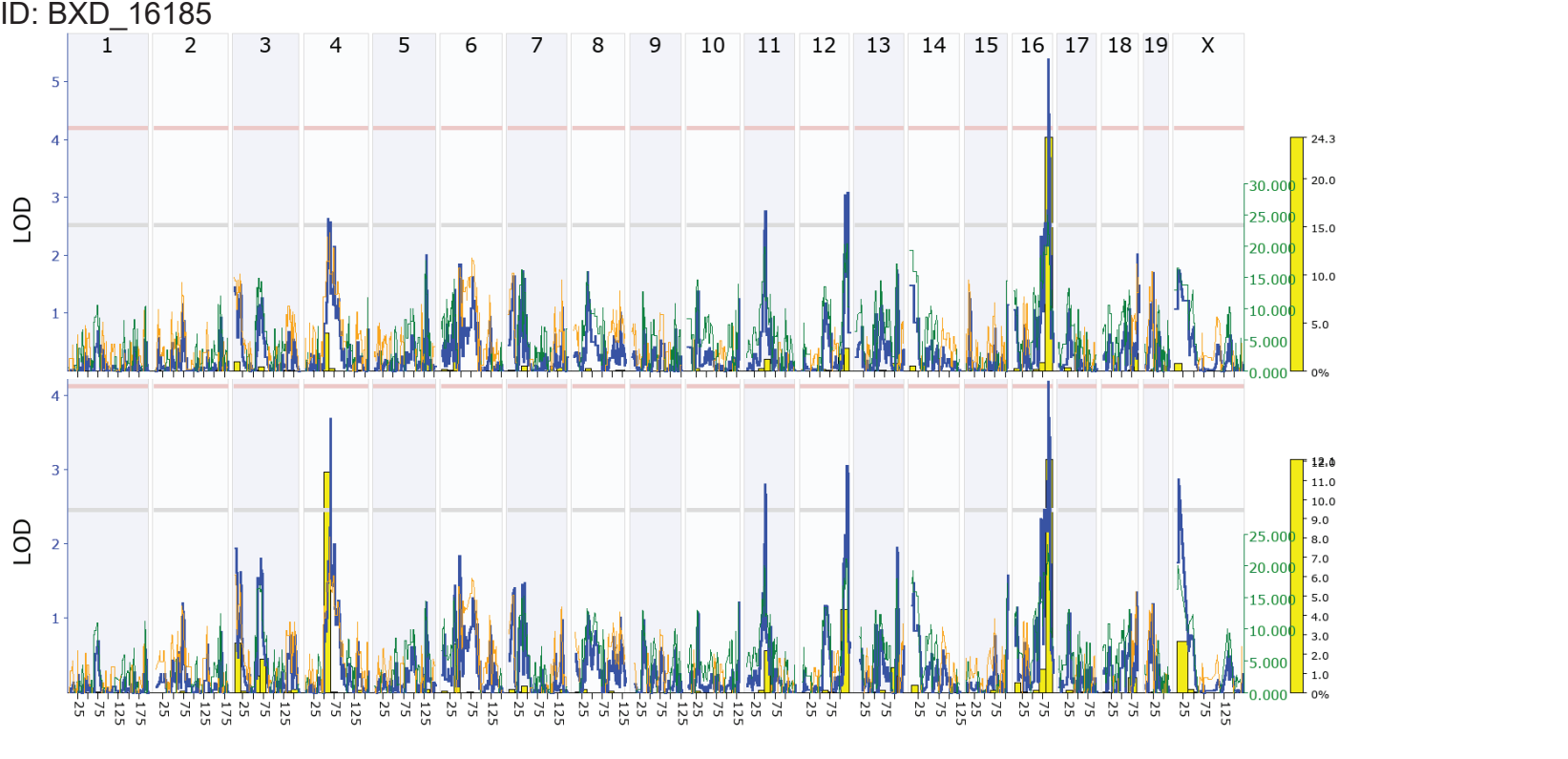
M.
Phenotype: Central nervous system, pharmacology, behavior: Naloxone-induced morphine withdrawal, naloxone (30 mg/kg ip) after morphine (50 mg/kg dose ip), number of jumps for males [n/15 min test]
Year of publication: 2010
n of strains: 63
ID: BXD_11336



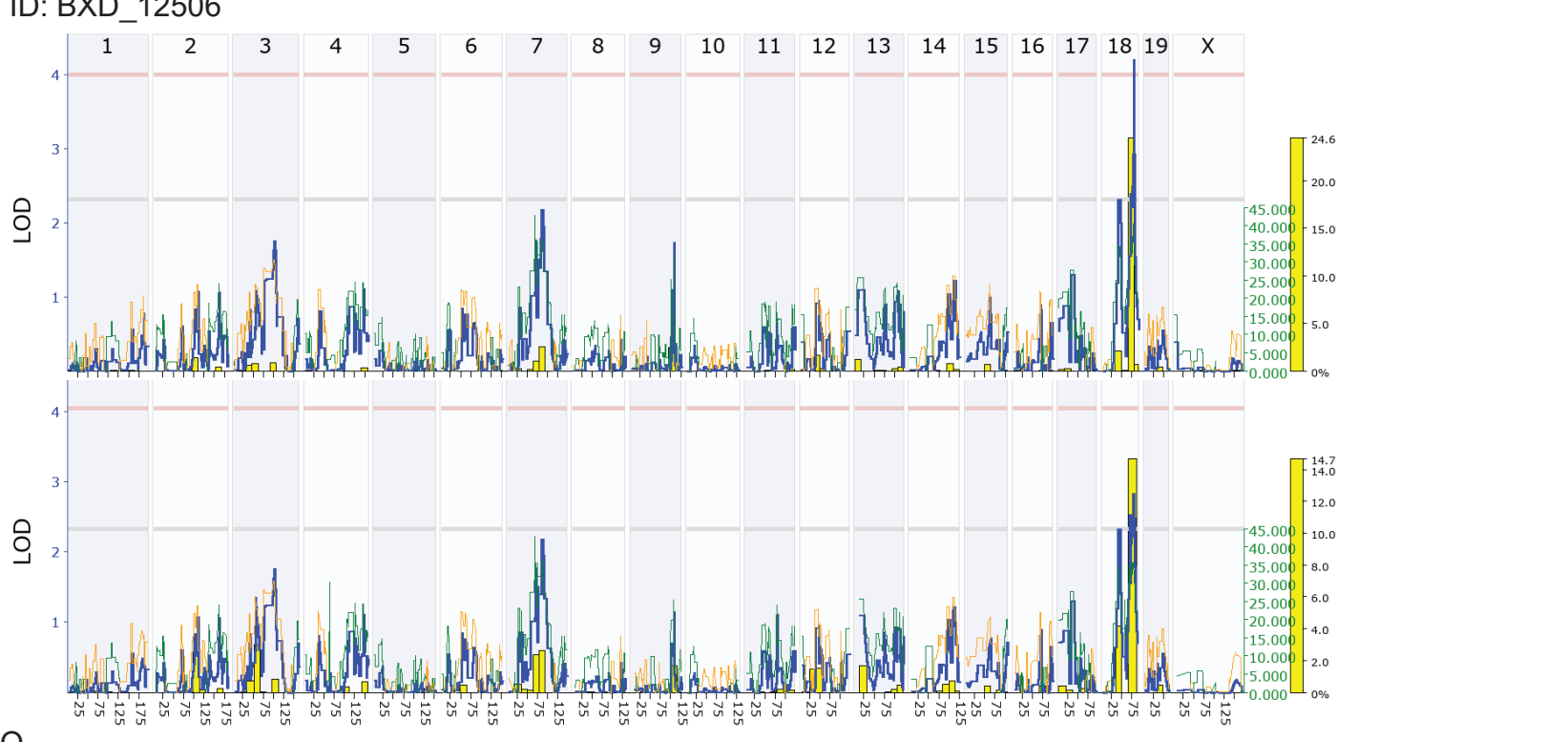
N.
Phenotype: Musculoskeletal system, metabolism: Bone mineral density for whole body by peripheral dual energy X-ray absorptiometer (PDEXA) of 19-week old males [g/cm2]
Year of publication: 2012
n of strains: 21
ID: BXD_12899



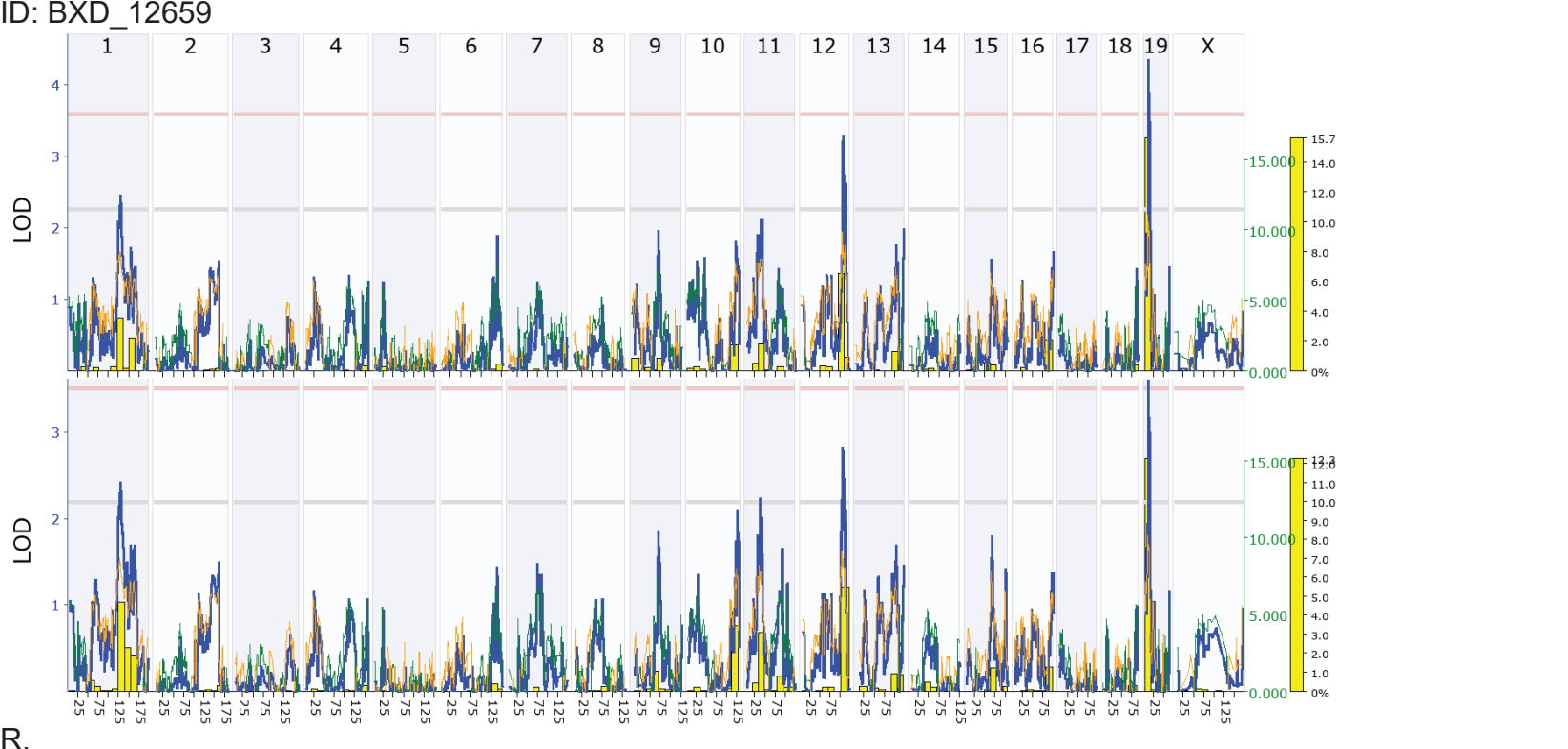
O.
Phenotype: Infectious disease, immune system: Herpes simplex virus type 1, virulence following corneal infection, young adult male and female (combined) [percent mortality]
Year of publication: 2013
n of strains: 35
ID: BXD_16185



P.
Phenotype: Liver, metabolism, nutrition: Iron level in liver of 120-day-old females fed 270 ppm Fe diet [µg/g]
Year of publication: 2012
n of strains: 24
ID: BXD_12506



Q.
Phenotype: Central nervous system, morphology: Brain weight, corrected only for dissection error (modified Feb2015 with new data for BXD79, from 374.017 to 432.7+/-10) [mg]
Year of publication: 2012
n of strains: 88
ID: BXD_12659



R.
Phenotype: Central nervous system, behavior, locomotor activity: Side-chamber entries during the 20-min habituation session in the 3-chamber social interaction task in adult males (non-winsorized values) [number].
Year of publication: 2015
n of strains: 50
ID: BXD_19312

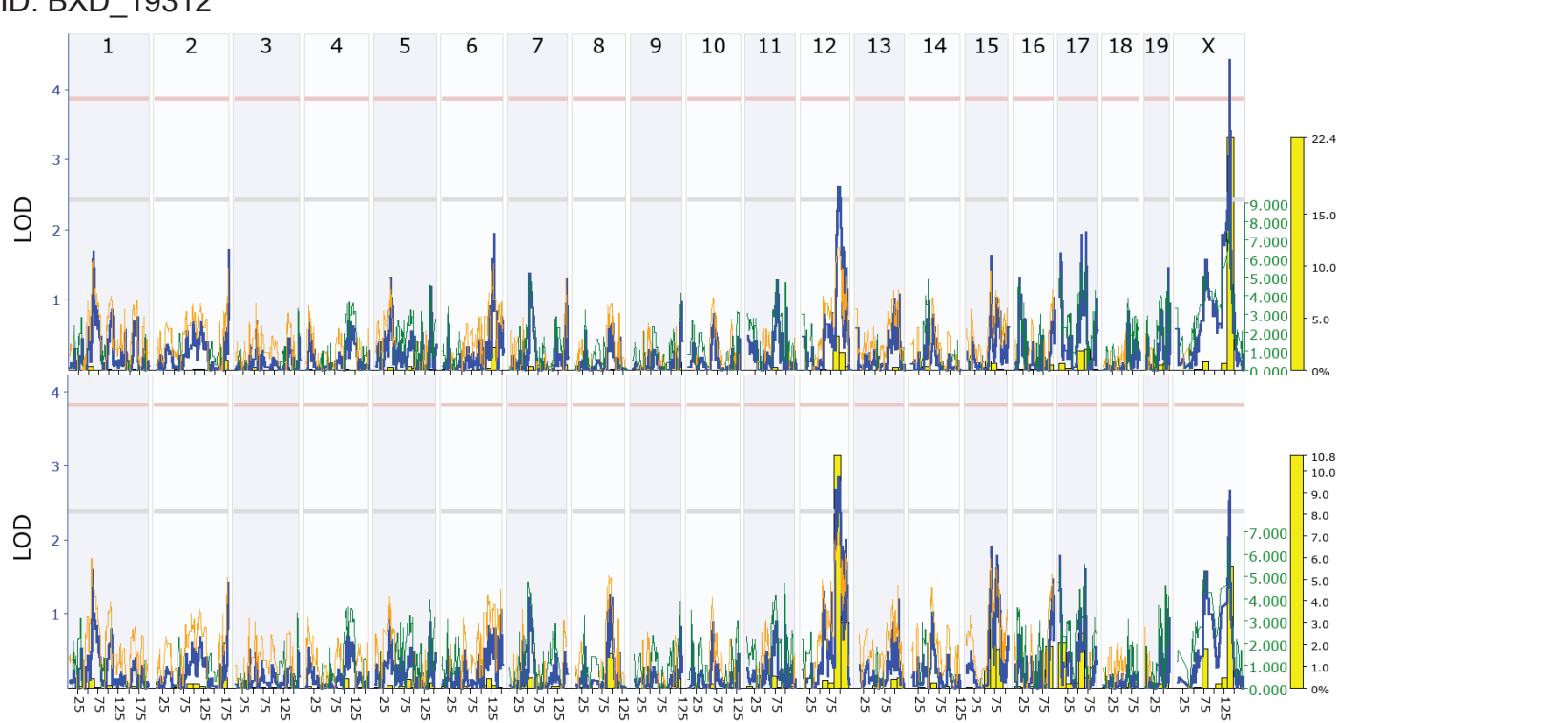


Figure S3: Effect sizes of *cis*-eQTL loci when gene expression per individual (red) is used, or mean gene expression per strain (blue), related to Figure 5. For a large midbrain gene expression array data set (55,683 probes, across 129 individuals of 37 BXD strains, with mean 3.5 replicates per strain, with a range of 1-5 replicates; VU BXD Midbrain Agilent SurePrint G3 Mouse GE (May12) Quantile; GN381) we mapped 6867 *cis*-eQTL. For each of these *cis*-eQTL, we calculated the proportion of variance in gene expression explained by the *cis*-eQTL marker using each individual or using strain means (i.e. biological replicates of the same genome). The density plot shows the density of *cis*-eQTLs with a given effect size when using individual (red) expression or strain mean expression (blue). The red and blue bars give the mean effect size for individuals (red) or strain means (blue). Datapoints for each *cis*-eQTL are shown, at 1/10 transparency.

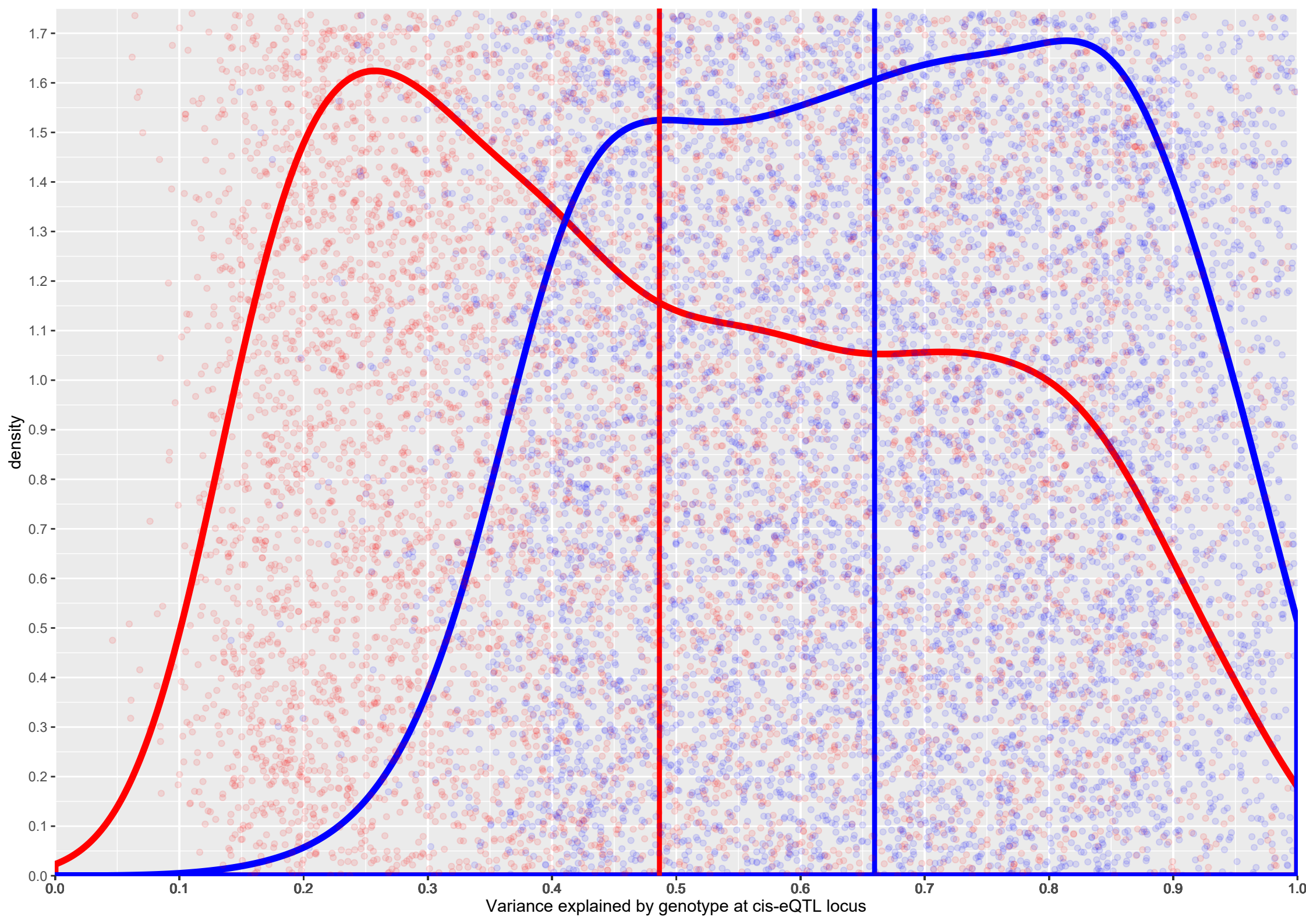
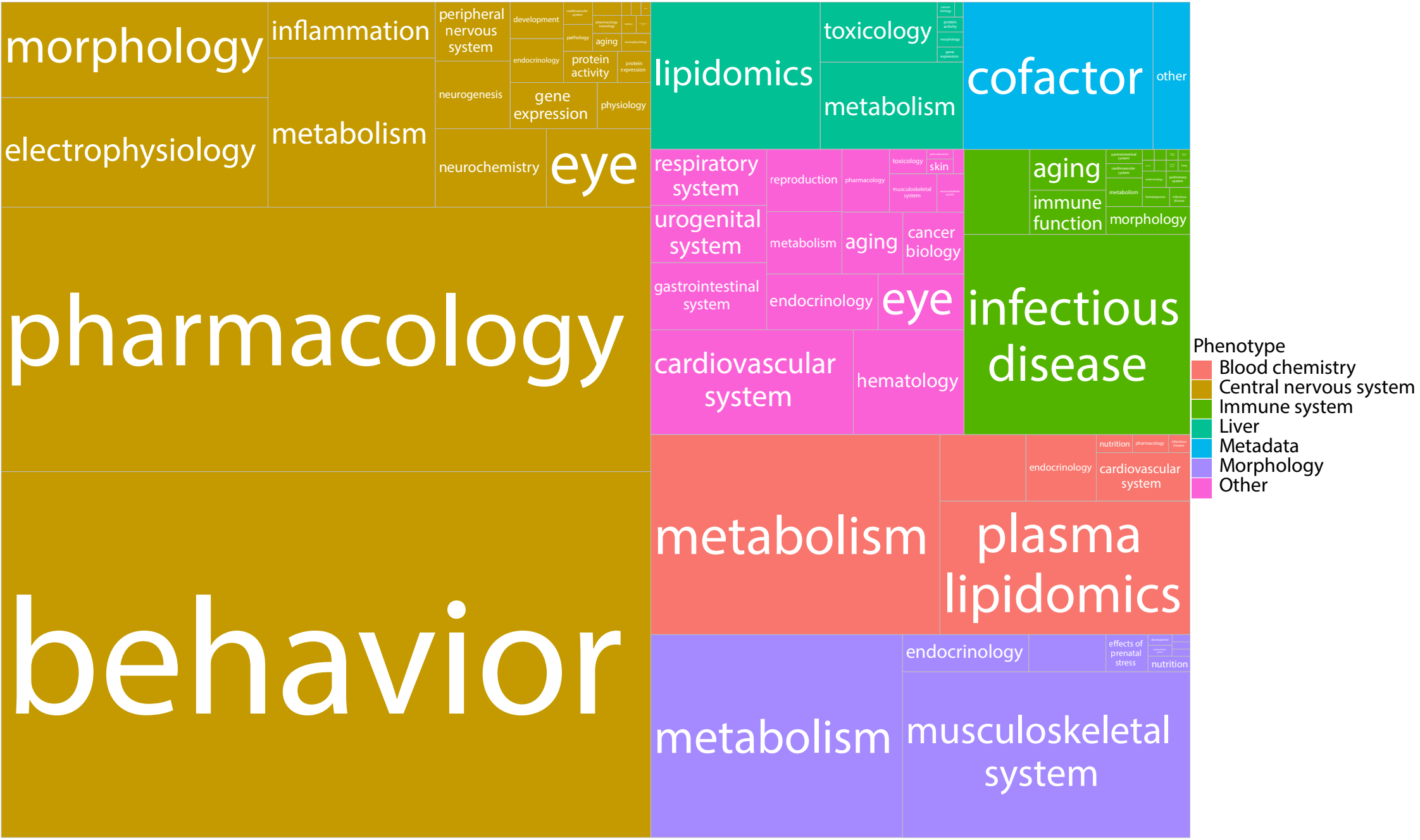

density
Variance explained by genotype at cis-eQTL locus
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7

Figure S4: A tree map, to give an overview of the BXD family phenotypes included in GeneNetwork, related to Figure 1. 7640 publicly available phenotypes measured across BXD strains were annotated to two levels, to allow easy visualization. This shows that approximately 50% of the phenotypes included in GeneNetwork are related to the central nervous system, due to the bias in researchers using the BXD family. Full details of the phenotypes can be found in Table S3.


morphology
inflammation
peripheral nervous system
development
pathology
aging
endocrinology
protein activity
protein expression
neurogenesis
gene expression
physiology
electrophysiology
metabolism
neurochemistry
eye
pharmacology
behavior
lipidomics
toxicology
metabolism
cofactor
other
respiratory system
reproduction
pharmacology
toxicology
skin
musculoskeletal system
urogenital system
metabolism
aging
cancer biology
gastrointestinal system
endocrinology
eye
cardiovascular system
hematology
aging
immune function
morphology
infectious disease
metabolism
plasma lipidomics
endocrinology
cardiovascular system
nutrition
pharmacology
metabolism
musculoskeletal system
endocrinology
effects of prenatal stress
nutrition
Phenotype
Blood chemistry
Central nervous system
Immune system
Liver
Metadata
Morphology
Other