Supporting Information for Fabian *et al.* "Genome-wide patterns of latitudinal differentiation among populations of *Drosophila melanogaster* from North America"

Additional supporting information may be found in the online version of this article.

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Table S1 Average nucleotide diversity π. Average π (average across SNP-wise π values) for each population and chromosomal arm. SE, standard error of the mean. Levels not connected by same letter are significantly different in a two-way ANOVA on rank-transformed means followed by a Tukey HSD posthoc test (details not shown). While chromosomal arms differed significantly in π, populations did not. Note, however, that the test of population variation, using ranks of means and only three populations, is not very powerful. To further examine whether populations differ in π we therefore applied a Kruskal-Wallis rank sum test on average π values estimated in non-overlapping 200 kb windows across the entire genome. Populations differed significantly in π, with Florida exhibiting higher diversity than Pennsylvania and Maine, while Pennsylvania and Maine did not differ from each other (Kruskal-Wallis $\chi^2 = 16.8$, df = 2, $P < 0.001$; followed by pairwise Wilcoxon rank sum posthoc tests, details not shown).

	FLORIDA[A]		**PENNSYLVANIA[A]**		**MAINE[A]**	
Chromosome	π	**SE**	π	**SE**	π	**SE**
X^A	0.0041	9.3×10^{-6}	0.0039	9.1×10^{-6}	0.0039	9.2×10^{-6}
$2L^B$	0.0075	1.2×10^{-5}	0.0071	1.2×10^{-5}	0.0070	1.2×10^{-5}
$2R^C$	0.0062	1.2×10^{-5}	0.0060	1.1×10^{-5}	0.0062	1.2×10^{-5}
$3L^C$	0.0066	1.1×10^{-5}	0.0060	1.1×10^{-5}	0.0060	1.1×10^{-5}
$3R^D$	0.0059	9.6×10^{-6}	0.0050	8.8×10^{-6}	0.0050	8.9×10^{-6}

Table S2 Average scaled population mutation rate θ_W. Average θ_W (average across SNP-wise θ_W values) for each population and chromosomal arm. SE, standard error of the mean. Levels not connected by same letter are significantly different in a two-way ANOVA on rank-transformed data followed by a Tukey HSD posthoc test (details not shown). While chromosomal arms differed significantly in θ_W, populations did not. Note, however, that the test of population variation, using ranks of means and only three populations, is not very powerful. To further examine whether populations differ in θ_W we therefore applied a Kruskal-Wallis rank sum test on average θ_W values estimated in non-overlapping 200 kb windows across the entire genome. Populations differed significantly in θ_W, with Florida exhibiting higher diversity than Pennsylvania and Maine, while Pennsylvania and Maine did not differ from each other (Kruskal-Wallis $\chi^2 = 22.3$, df = 2, $P < 0.001$; followed by pairwise Wilcoxon rank sum posthoc tests, details not shown).

	FLORIDA[A]		**PENNSYLVANIA[A]**		**MAINE[A]**	
Chromosome	θ_W	**SE**	θ_W	**SE**	θ_W	**SE**
X^A	0.0044	8.6×10^{-6}	0.0041	8.3×10^{-6}	0.0040	8.4×10^{-6}
$2L^B$	0.0077	1.1×10^{-5}	0.0072	1.1×10^{-5}	0.0071	1.1×10^{-5}
$2R^C$	0.0064	1.1×10^{-5}	0.0062	1.0×10^{-5}	0.0064	1.1×10^{-5}
$3L^C$	0.0070	1.0×10^{-5}	0.0062	9.6×10^{-6}	0.0062	9.6×10^{-5}
$3R^D$	0.0061	8.7×10^{-6}	0.0052	8.0×10^{-6}	0.0052	8.1×10^{-6}

Table S3 Average pairwise F_{ST} values. Average pairwise F_{ST} values (average across SNP-wise F_{ST} values) for each population comparison and chromosomal arm. SE, standard error of the mean. Levels not connected by same letter are significantly different in a two-way ANOVA on rank-transformed data followed by a Tukey HSD posthoc test. See text for further details.

	Florida - Maine[A]		**Florida - Pennsylvania[A]**		**Pennsylvania - Maine[B]**	
Chromosome	F_{ST}	**SE**	F_{ST}	**SE**	F_{ST}	**SE**
X^A	0.0380	8.4×10^{-5}	0.0381	8.3×10^{-5}	0.0307	8.0×10^{-5}
$2L^A$	0.0430	6.8×10^{-5}	0.0401	6.1×10^{-5}	0.0248	4.5×10^{-5}
$2R^A$	0.0370	6.4×10^{-5}	0.0365	6.2×10^{-5}	0.0253	5.3×10^{-5}
$3L^A$	0.0404	6.6×10^{-5}	0.0393	6.3×10^{-5}	0.0262	5.0×10^{-5}
$3R^A$	0.0633	1.0×10^{-4}	0.0606	9.8×10^{-5}	0.0258	4.8×10^{-5}

Table S4 Average pairwise F_{ST} in- and outside inversions. Average pairwise F_{ST} was estimated in- and outside four major cosmopolitan inversions for each population comparison, using 1 kb non-overlapping windows. "F_{ST} in": F_{ST} inside the inversion; "F_{ST} out", F_{ST} of the chromosomal arm outside the inversion. Significant differences between "F_{ST} in" and "F_{ST} out" in boldface (Wilcoxon Rank Sum Test, $\alpha <$ 0.01). SE, standard error of the mean.

	Florida – Maine				**Florida - Pennsylvania**				**Pennsylvania - Maine**			
Inversion	**F_{ST} in**	**SE**	**F_{ST} out**	**SE**	**F_{ST} in**	**SE**	**F_{ST} out**	**SE**	**F_{ST} in**	**SE**	**F_{ST} out**	**SE**
In(2L)t	**0.0448**	2.0×10^{-4}	**0.0484**	2.9×10^{-4}	**0.0406**	1.8×10^{-4}	**0.0441**	2.4×10^{-4}	**0.0263**	1.3×10^{-4}	**0.0251**	1.5×10^{-4}
In(2R)NS	**0.0368**	2.5×10^{-4}	**0.0394**	1.9×10^{-4}	**0.0359**	2.3×10^{-4}	**0.0384**	1.7×10^{-4}	0.0257	2.0×10^{-4}	0.0261	1.3×10^{-4}
In(3L)P	0.0426	2.0×10^{-4}	0.0462	3.4×10^{-4}	0.0417	1.9×10^{-4}	0.0441	3.1×10^{-4}	**0.0274**	1.3×10^{-4}	**0.0271**	1.9×10^{-4}
In(3R)P	**0.0880**	4.8×10^{-4}	**0.0623**	2.9×10^{-4}	**0.0841**	4.7×10^{-4}	**0.0609**	2.9×10^{-4}	0.0280	1.9×10^{-4}	0.0278	1.2×10^{-4}

Please see separate Excel (.xls) file:

Table S5 (Excel) Top candidate genes. Sheets FM (comparison Florida - Maine); FP (comparison Florida - Pennsylvania); PM (comparison Pennsylvania - Maine) show mean F_{ST} values for each candidate gene calculated using (a) candidate SNPs only and (b) all polymorphic SNPs. SE, standard error of the mean. Sheets A - G show the candidate genes in the different intersections of the Venn diagram shown in Fig. S4.

Table S6 Proportion of candidate SNPs within inversions. Enrichment was tested using one-sided FETs, with significant enrichment relative to the rest of the chromosomal arm shown in boldface. ov, overrepresented; un, underrepresented. FM, comparison Florida - Maine; FP, Florida - Pennsylvania; PM, Pennsylvania - Maine.

Inversion	**FM**	**FP**	**PM**
In(2L)t	**0.41^{un}**	**0.45^{un}**	0.70
In(2R)NS	**0.24^{un}**	**0.20^{un}**	**0.19^{un}**
In(3L)P	**0.84^{ov}**	**0.82^{ov}**	0.79
In(3R)P	**0.67^{ov}**	**0.68^{ov}**	0.41

Please see separate Excel (.xls) file:

Table S7 (Excel) Genome annotations of candidate genes. Candidate genes in significantly over- and underrepresented genome annotation categories for each pairwise population comparison. FM, comparison Florida - Maine; FP, Florida - Pennsylvania; PM, Pennsylvania - Maine.

Please see separate Excel (.xls) file:

Table S8 (Excel) Gene ontology (GO) analysis of candidate genes. Gene ontology (GO) analysis of candidate genes for each pairwise population comparison and the clinal ("plus_plus") genes. Columns contain: (1) GO category; (2) average number of genes obtained per simulation (10 million simulations); (3) candidate genes detected for the category; (4) empirical *P*-values; (5) false discovery rate (FDR); (6) total genes with at least one differentiated site; (7) total number of genes known in each category; (8) GO category name; (9) FBgn number of candidate genes detected for the category. FM, comparison Florida - Maine; FP, Florida - Pennsylvania; PM, Pennsylvania - Maine; "Plus_Plus", clinal genes. See text for further details.

Please see separate Excel (.xls) file:

Table S9 (Excel) Overlap between FM candidate genes and those identified by Kolaczkowski *et al.* (2011). This table shows the overlap between the candidate genes identified for the endpoints of the North American cline (Florida versus Maine) and the Australian cline (Queensland versus Tasmania; see Kolaczkowski *et al.* 2011), using a window-based approach (top 5% of non-overlapping 1 kb windows with highest F_{ST}). Details of the methods are given in the table; also see main text for further details.

Fig. S1 Average coverage of pool-seq samples. Average sequence coverage of Illumina-sequenced pooled samples for each chromosomal arm and population after processing of raw FASTA files. Error bars represent standard deviations (SD). Mean read depth of the euchromatic genome (*X*, *2L*, *2R*, *3L*, *3R*) was 47.4x in Florida, 45.3x in Pennsylvania, and 45.4x in Maine.

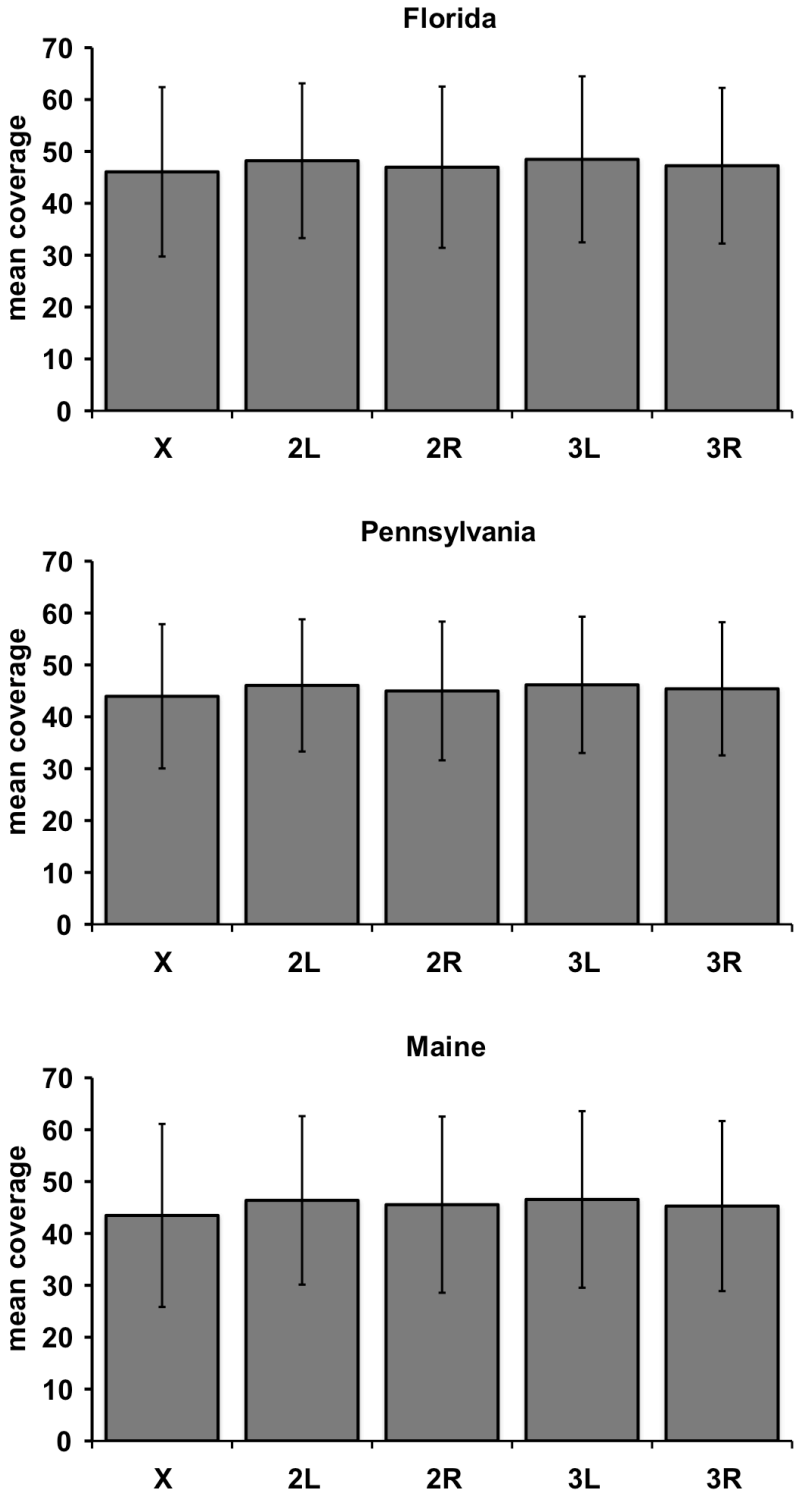


Fig. S2 Average scaled population mutation rate θ_W. Average θ_W was estimated over 200 kb non-overlapping windows and plotted separately for each chromosomal arm. Florida (black line); Pennsylvania (green); and Maine (red). Regions where the line is broken represent windows where the coverage was outside our predefined minimum/maximum coverage interval, i.e. windows with < 60% of the SNPs fulfilling our coverage criteria. The grey boxes indicate the approximate regions spanned by four major cosmopolitan inversions on the left and right arms of chromosome 2 (*In(2L)t* and *In(2R)NS*) and chromosome 3 (*In(3L)P* and *In(3R)P*). Note that the 4th (dot) chromosome is not shown.

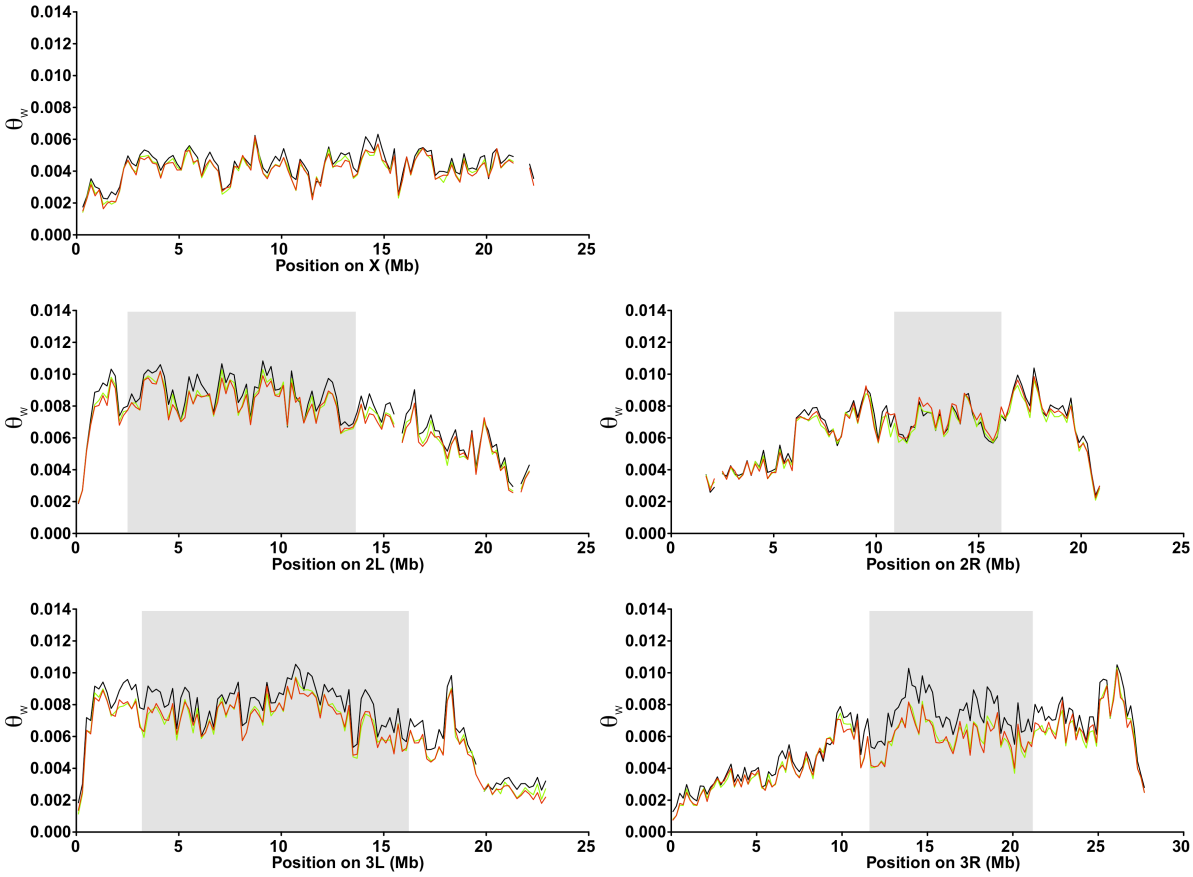


Fig. S3 Frequency of the major cosmopolitan inversion *In(3R)Payne*. We used four different, previously published molecular markers to estimate the frequency of *In(3R)P* in each population. (a) an indel marker in *hsr-omega* (see Anderson *et al.* 2003). (b) - (d) 3 SNP markers in *tolkin* from Matzkin *et al*. (2005): (b) T1444C, (c) C245T, and (d) T249C (see Matzkin *et al*. 2005 for details).

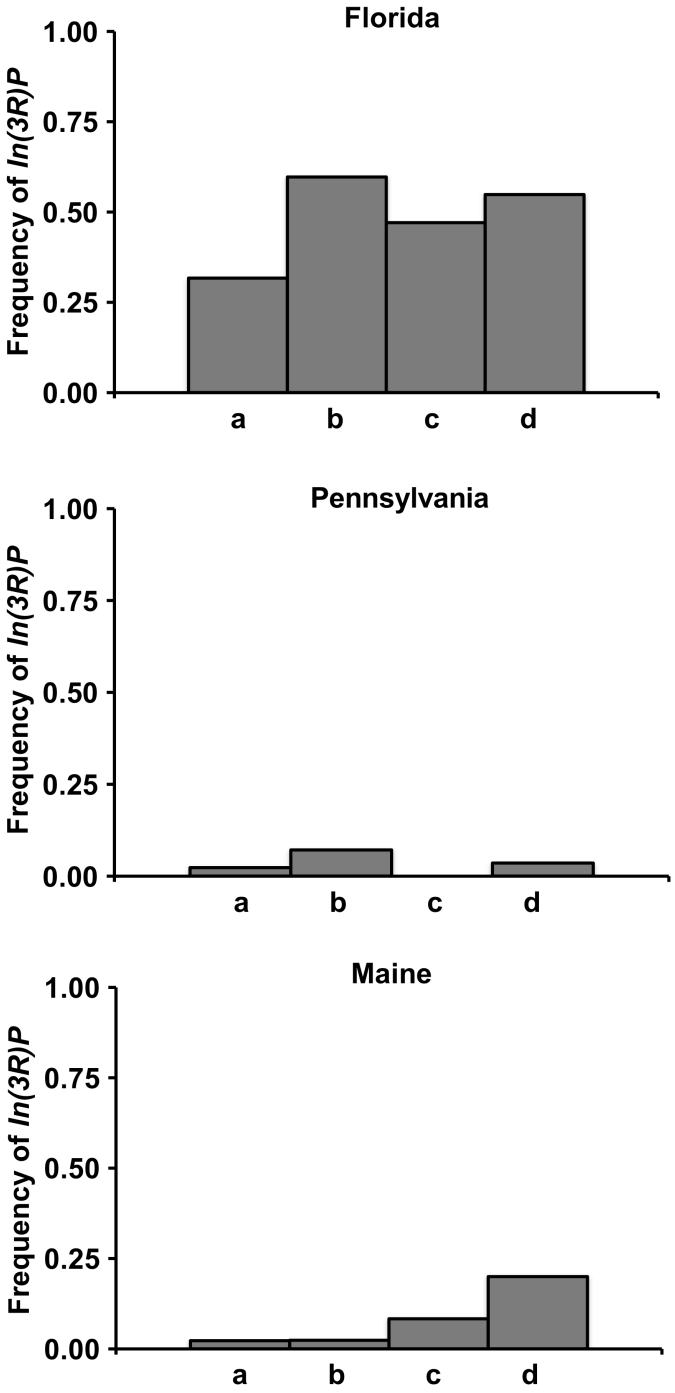


Fig. S4 Overlap of candidate genes among population comparisons. The Venn diagram shows the number of candidate genes for different intersections of the three pairwise population comparisons. FM (Florida - Maine; red), FP (Florida - Pennsylvania; blue); PM (Pennsylvania - Maine; green). (A) candidate genes unique for FM; (B) candidate genes unique for FP; (C) candidate genes unique for PM; (D) candidate genes overlapping only between FM and FP; (E) candidate genes overlapping only between FM and PM; (F) candidate genes overlapping only between FP and PM; (G) candidate genes occurring in all three comparisons. For lists of candidate genes see Table S6.

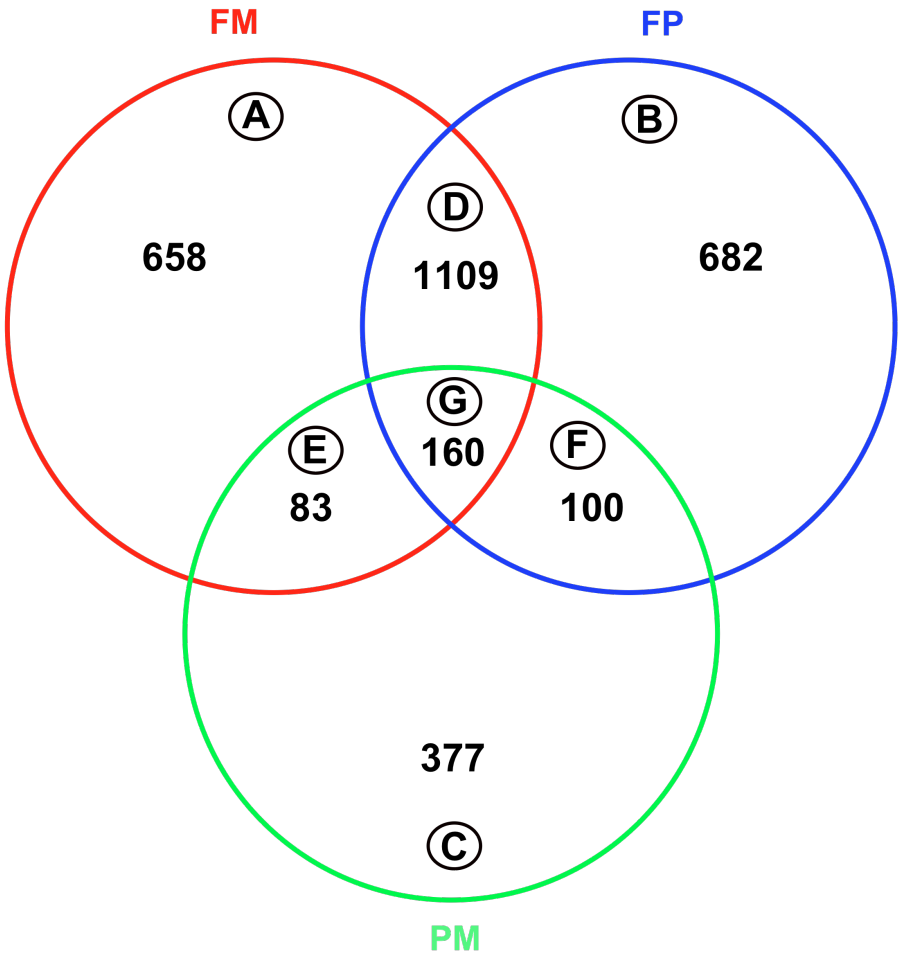
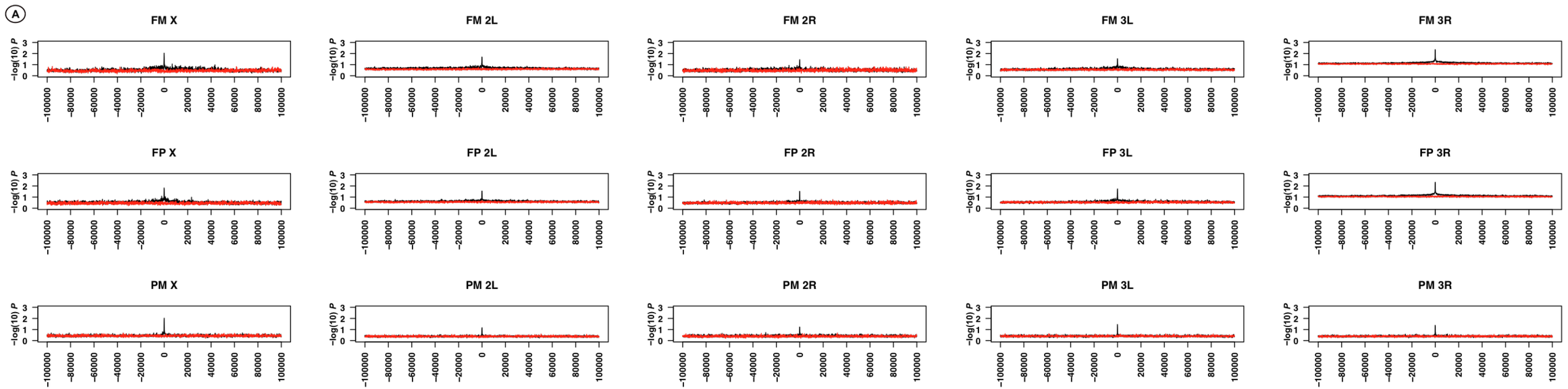


Fig. S5 Decay of statistical significance around candidate SNPs. Median -$\log_{10}(P)$-values from FET for SNPs flanking the candidate SNPs for all pairwise population comparisons, shown for (i) each chromosomal arm (A, B), (ii) the region spanned by *In(3R)P* (C, D), and (iii) averaged across all autosomes, without and without *3R* (E). FM, Florida - Maine; FP, Florida - Pennsylvania; PM, Pennsylvania - Maine. (A) – (B) Median estimated either using 100 bp windows (A) or 10 bp windows (B) and either covering a region of 100 kb (A) or 0.5 kb (B) upstream and downstream of candidate SNPs. Black line: median of SNPs flanking candidate SNPs; red line: median of SNPs ≥ 500 kb away from candidate SNPs. (C) – (D) Median estimated for *In(3R)P* either using 100 bp windows (C) or 10 bp windows (D) and either covering a region of 100 kb (C) or 0.5 kb (D) upstream and downstream of candidate SNPs. Dark blue line: median of SNPs flanking candidate SNPs inside the inversion *In(3R)P*; light blue line: median of SNPs flanking candidate SNPs outside the inversion *In(3R)P*; dark red line: median of SNPs inside the inversion *In(3R)P* and ≥ 500 kb away from candidate SNPs; light red line: median of SNPs outside the inversion *In(3R)P* and ≥ 500 kb away from candidate SNPs. (E) Median estimated across all autosomes including *3R* (left column) and without *3R* (right column) for each pairwise population comparison, using 100 bp windows. Dark line: median of SNPs flanking candidate SNPs; light lines: median of SNPs ≥ 500 kb away from candidate SNPs. See text for further details.

Note: Use the Zoom function in Adobe Acrobat to zoom into the individual figures and to view detailed aspects of the graphs.

Fig. S5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FM 2L
FM 2R
FM 3L
FM 3R
FP X
FP 2L
FP 2R
FP 3L
FP 3R
PM X
PM 2L
PM 2R
PM 3L
PM 3R
−log(10) P
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0
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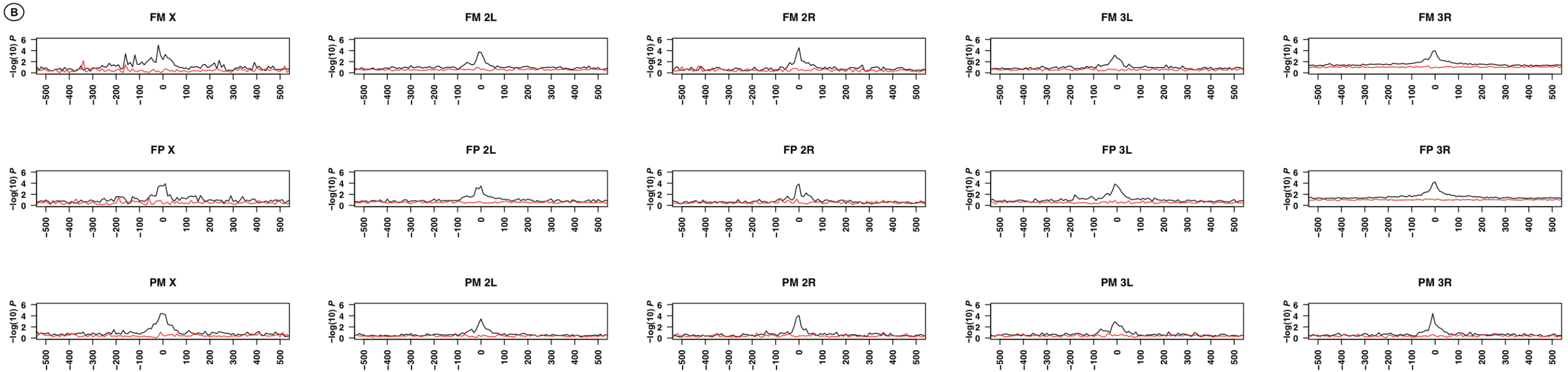
40000
60000
80000
100000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FM 2R
FM 3L
FM 3R
FP X
FP 2L
FP 2R
FP 3L
FP 3R
PM X
PM 2L
PM 2R
PM 3L
PM 3R
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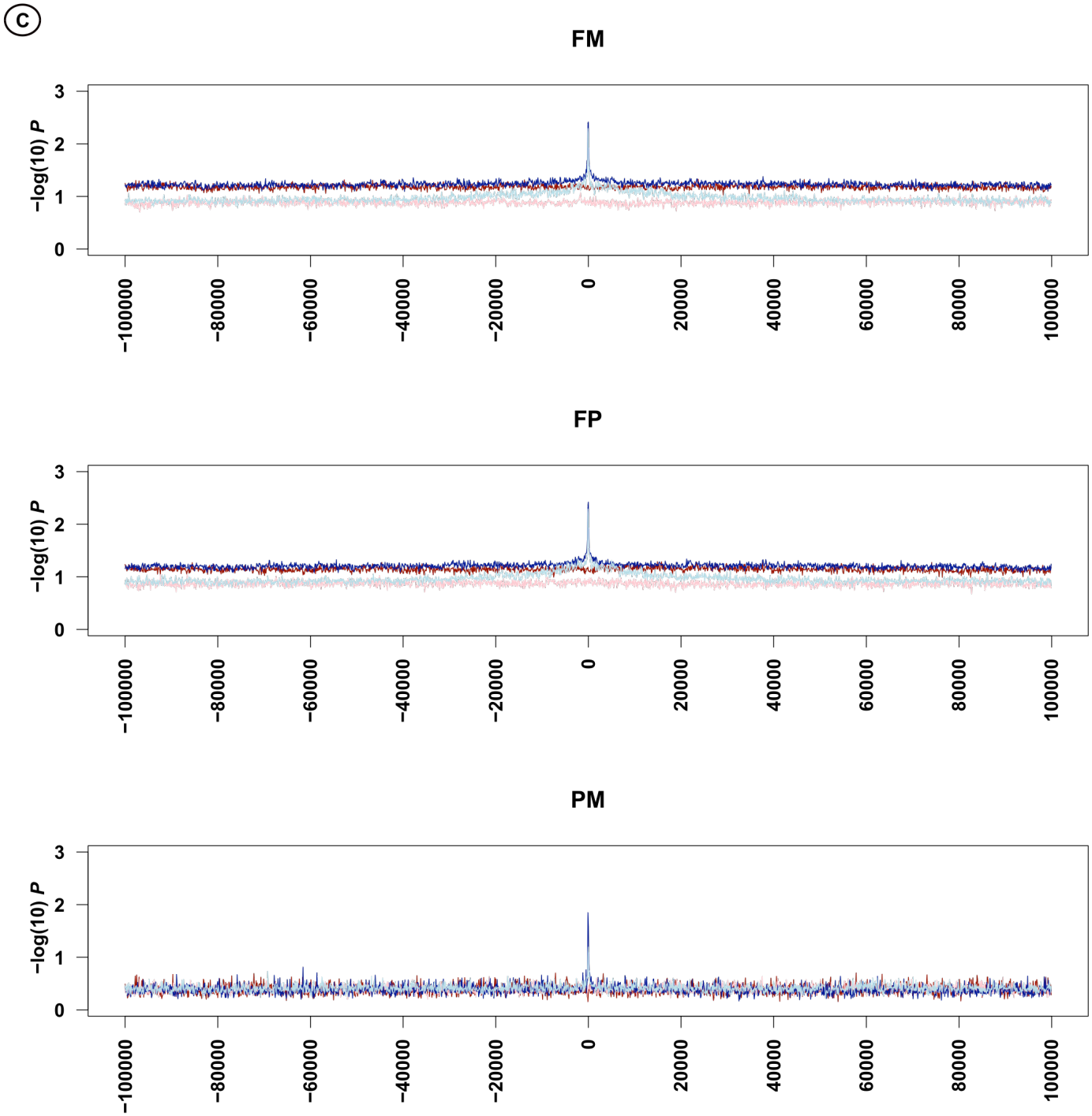
300
400
500


C
FM
FP
PM
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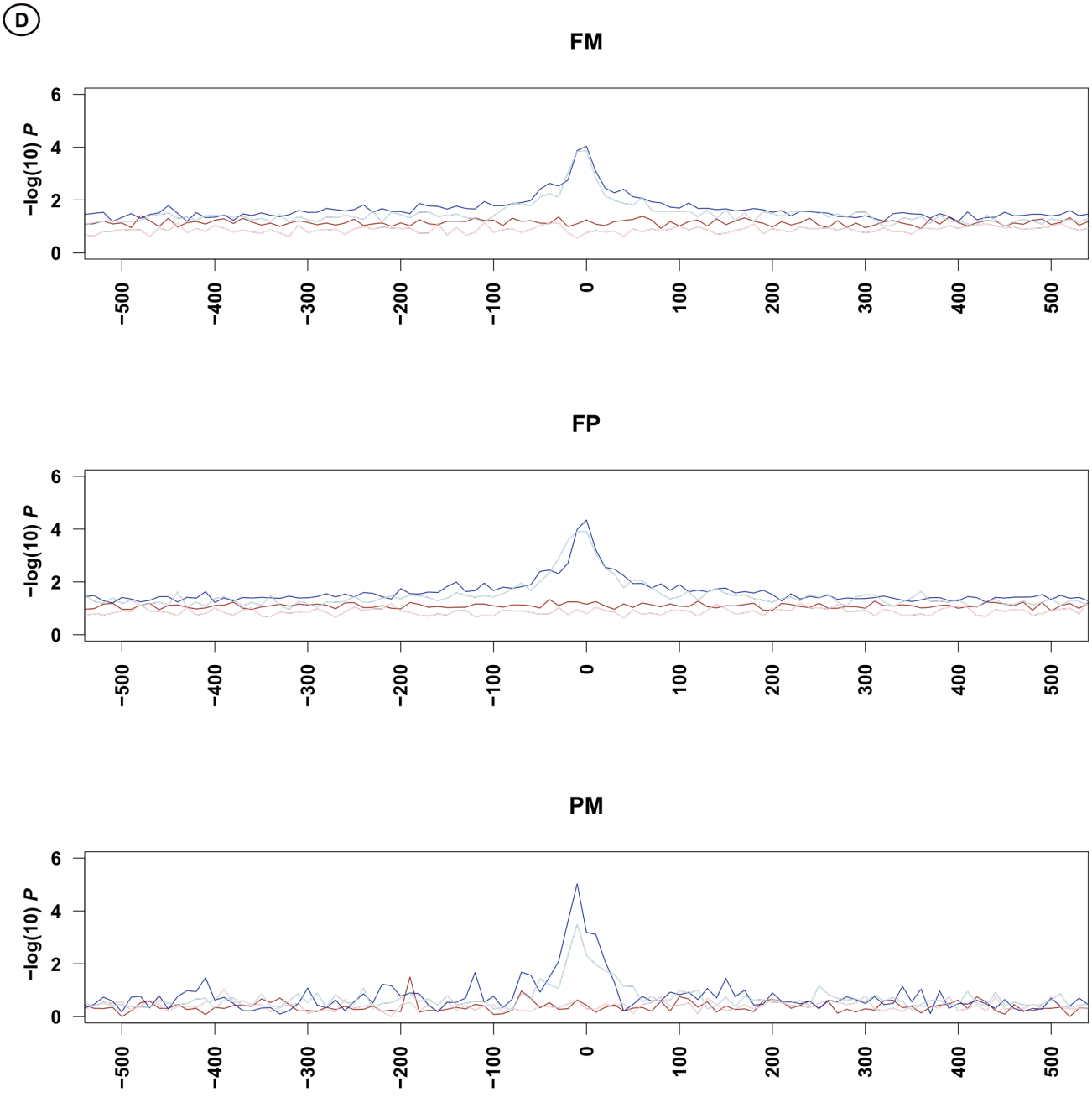
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FP
PM
−log(10) P
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−200
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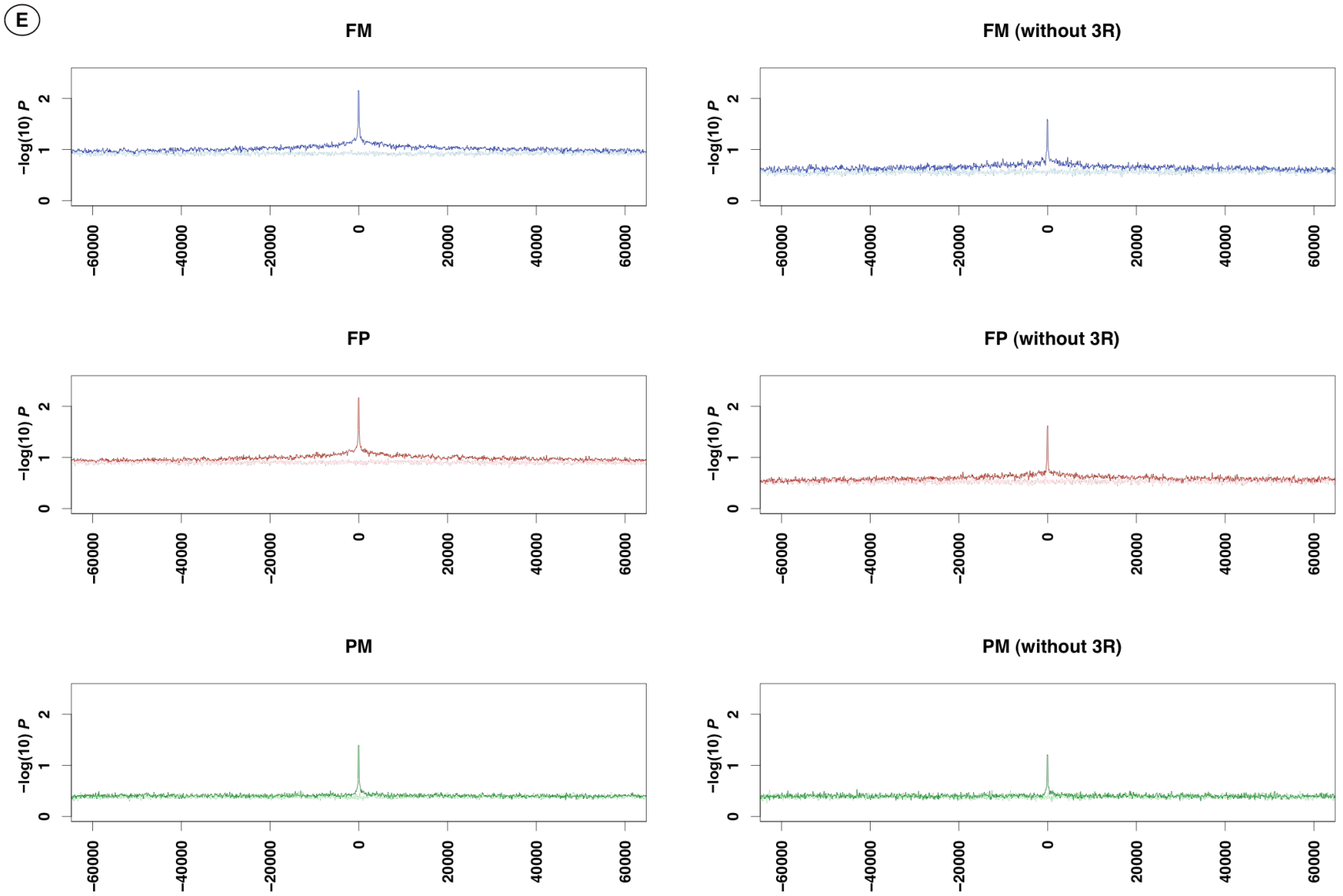
100
200
300
400
500


E
FM
FM (without 3R)
FP
FP (without 3R)
PM
PM (without 3R)
−log(10) P
0
1
2
−60000
−40000
−20000
0
20000
40000
60000

Appendix S1

Examples of candidate genes in the insulin/insulin-like growth factor (IIS) and target of rapamycin (TOR) signaling pathways[§]

14-3-3 epsilon[*1]

amon[2]

foxo[3]

Hmgcr[4]

Ilp3[5]

Ilp4[5]

Imp[6]

InR[*7]

Pi3K59F[8]

Pi3K92E[9]

tobi[10]

Tor[11]

Tsc1[11]

Shown are pairwise F_{ST} values of the candidate SNPs identified in the different population comparisons; red: Florida-Maine, blue: Florida-Pennsylvania, green: Pennsylvania-Maine.

Notes:

[§] IIS/TOR signaling affects many physiological and metabolic processes and functions, including growth; carbohydrate, lipid and amino acid metabolism; regulation of production of downstream secondary hormones such as juvenile hormone and ecdysone; body size, reproduction, reproductive dormancy, stress resistance, lifespan, and immune function. For reviews of these pathways, especially for their relevance in affecting lifespan, see Tatar *et al.* (2003) and Partridge *et al.* (2011).

* Also found by Kolaczkowski *et al.* (2011)

[1] Well known in *C. elegans* to affect lifespan via interaction with Daf-16/Foxo; also interacts with Foxo and affects lifespan in *Drosophila* (Nielsen *et al.* 2008). Also see: http://flybase.org/reports/FBgn0020238.html

[2] Potentially involved in pro-insulin cleavage and insulin production (Rayburn *et al.* 2003); implicated in the production of adipokinetic hormone (AKH, a

glucagon-like hormone in insects; Rhea *et al*. 2010). For further information see: http://flybase.org/reports/FBgn0023179.html

[3] *Foxo* is a forkhead box O transcription factor downstream of IIS; affects lifespan and other life history traits in *Drosophila* (e.g., Hwangbo *et al.* 2004); has also been shown to affect diapause/dormancy in mosquitos (Sim and Denlinger 2008). See: http://flybase.org/reports/FBgn0038197.html

[4] *Hmgcr* encodes a HMG (hydroxymethlyglutaryl) coenzyme A reductase; probably indirectly involved in IIS; affects the production of juvenile hormone production downstream of IIS (Belgacem *et al.* 2007). For further information see: http://flybase.org/reports/FBgn0263782.htm

[5] *Drosophila insulin-like peptides 3* and *5* (*ilp* or *dilp3* and *5*); peptide ligands for the insulin-like receptor *InR*; different *dilps* have been implicated in the regulation of growth, carbohydrate metabolism, and lifespan. See, for example, Grönke and Partridge (2010) and Grönke *et al.* (2010). For further information on *dilp3* see: http://flybase.org/reports/FBgn0044050.html; for *dilp5* see: http://flybase.org/reports/FBgn0044048.html

[6] *IGF-II mRNA binding protein*; might potentially be involved in IIS signaling. For further information see: http://flybase.org/reports/FBgn0262735.html

[7] *InR, insulin-like receptor*; known to affect body size, developmental time, fecundity and fertility, lifespan and the production of secondary hormones downstream of IIS such as juvenile hormone and ecdysone (see App. S2) in *Drosophila* (e.g., Tatar *et al.* 2001; also see Flatt *et al.* 2005 for a review); found to be strongly clinal along the east coast of the USA and Australia, with natural alleles affecting *Drosophila* life history traits (Paaby *et al.* 2010). Also see: http://flybase.org/reports/FBgn0013984.html

[8] *Pi3K59F* encodes *Phosphotidylinositol 3 kinase 59F*; might be involved in TOR signaling (e.g., Teleman *et al.* 2010). For further information see: http://flybase.org/reports/FBgn0015277.html

[9] Also known as *PI3K* or *Dp110* (http://flybase.org/reports/FBgn0015279.html); important in IIS: phosphatidylinositol-4,5-bisphosphate 3-kinase activity; has been found to be genetically associated with natural variation in the incidence of reproductive dormancy in *D. melanogaster* (Williams *et al.* 2006); genetically interacts with *cpo* (see App. S2; Schmidt 2011)

[10] *tobi*, *target of brain insulin*, is a relatively recently discovered gene involved in IIS/AKH (glucagon) signaling (Buch *et al.* 2008). For further information see: http://flybase.org/reports/FBgn0261575.html

[11] Both involved in target of rapamycin (TOR) signaling, with known effects on *Drosophila* lifespan and metabolism (e.g., Oldham and Hafen 2003; Kapahi *et al.* 2004; Luong *et al.* 2006; Partridge *et al*. 2011). For further details on *Tor* see: http://flybase.org/reports/FBgn0021796.html; for details on *Tsc1* see: http://flybase.org/reports/FBgn0026317.html

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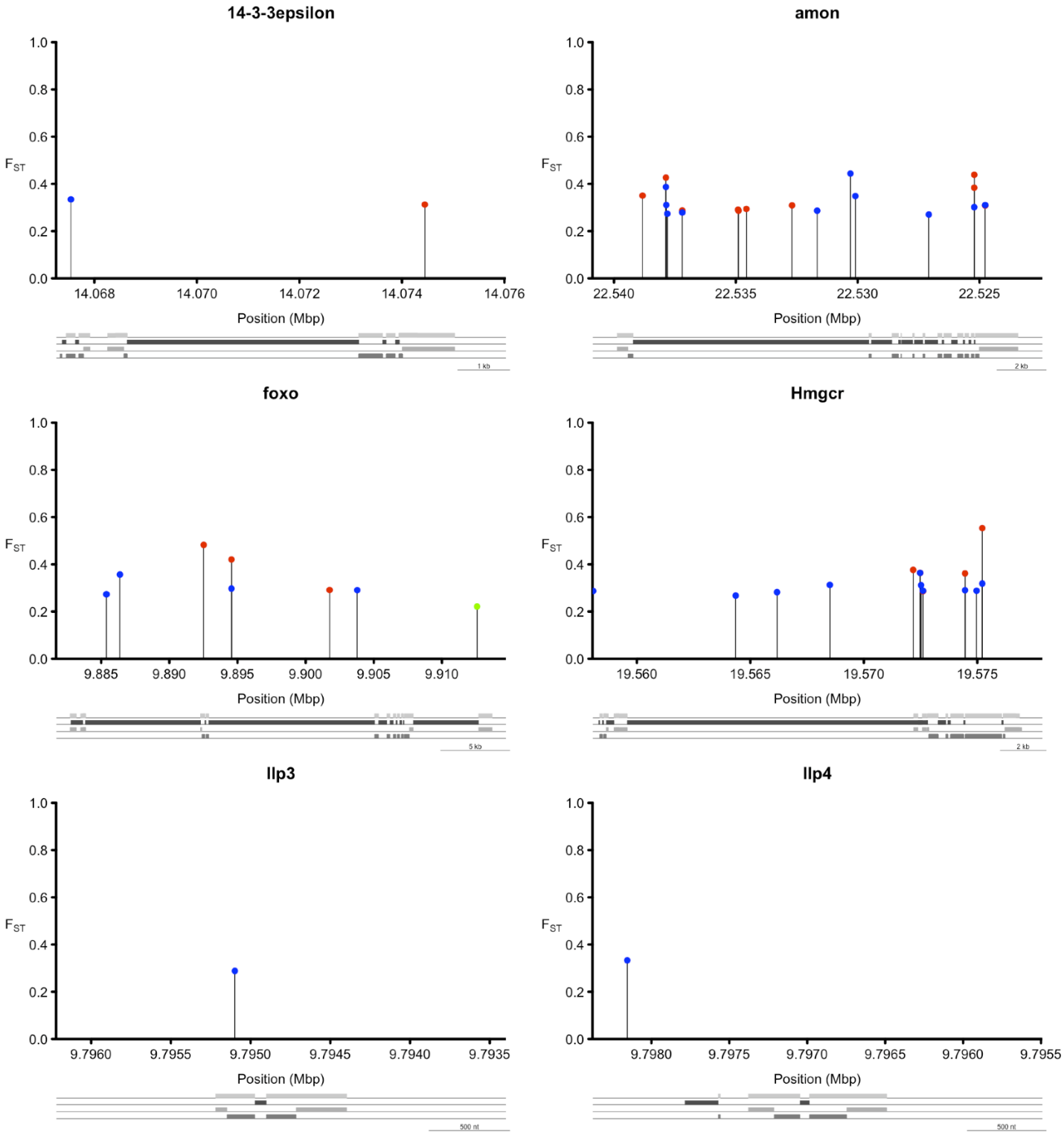
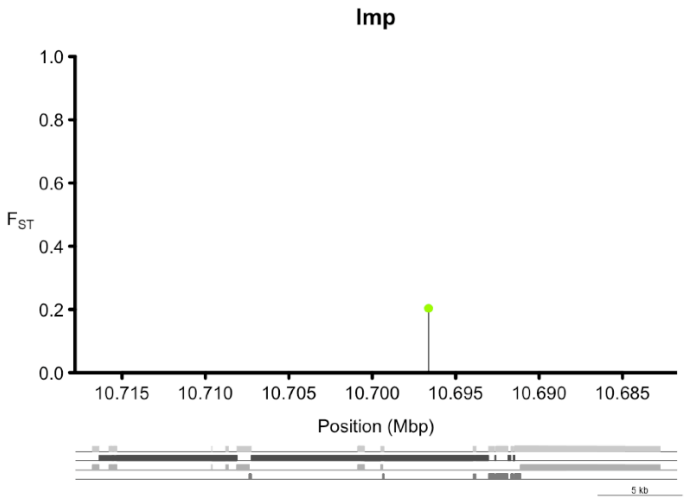

14-3-3epsilon
amon
foxo
Hmgcr
Ilp3
Ilp4
F_{ST}
Position (Mbp)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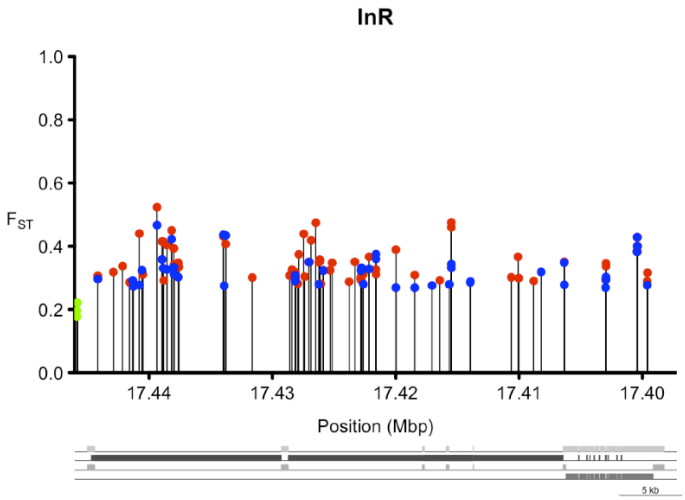
InR
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0.0
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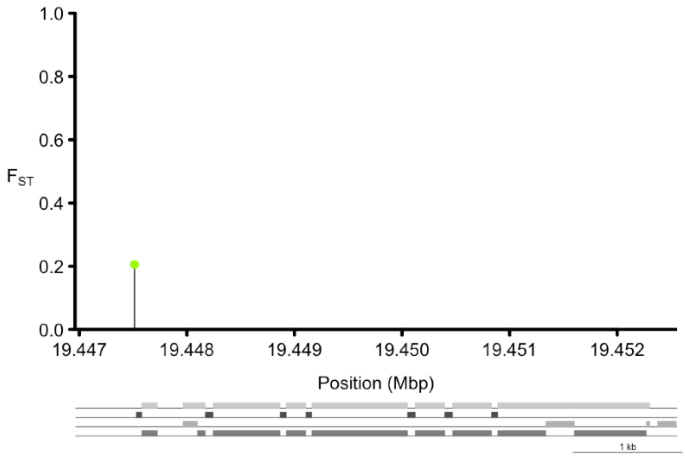
17.41
17.40
Position (Mbp)
5 kb


Pi3K59F
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19.452
Position (Mbp)
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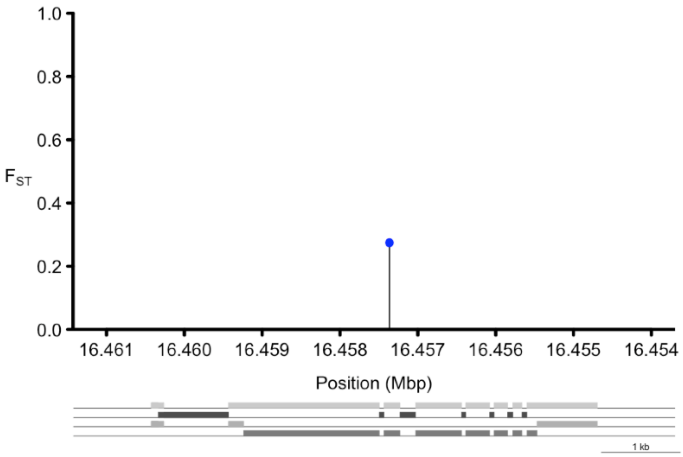
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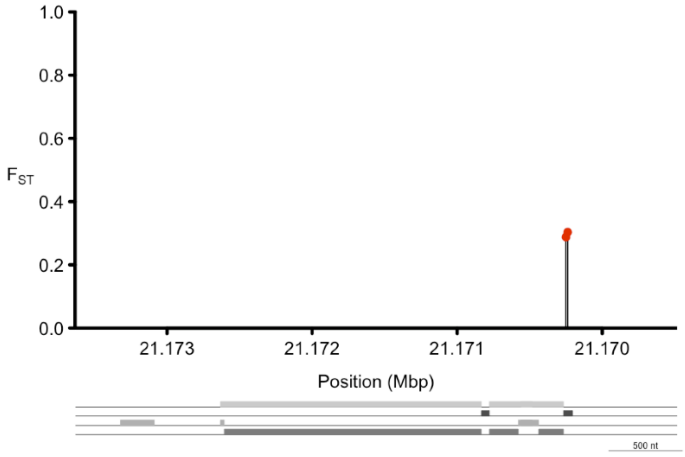
16.456
16.455
16.454
Position (Mbp)
1 kb


tobi
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0.8
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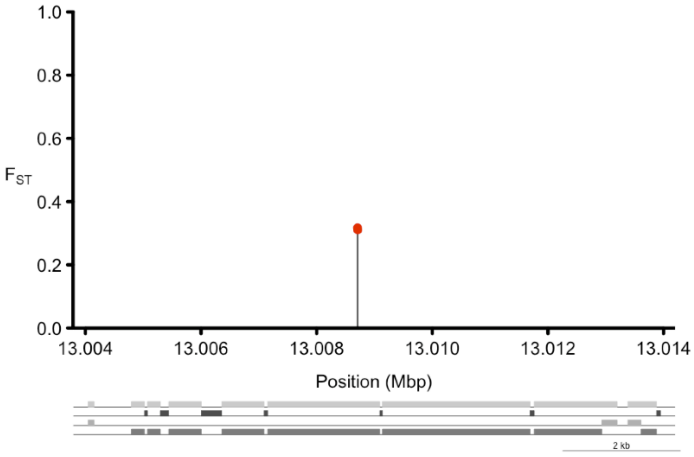
0.4
0.2
0.0
F_{ST}
21.173
21.172
21.171
21.170
Position (Mbp)
500 nt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Position (Mbp)
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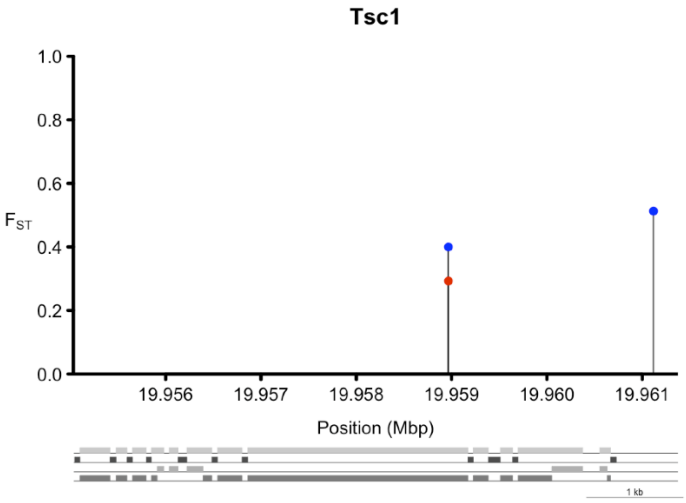
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F_{ST}
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19.957
19.958
19.959
19.960
19.961
Position (Mbp)
1 kb

Appendix S2

Examples of candidate genes involved in ecdysone biosynthesis and signaling[§]

cpo[*1]

dib[2]

dre4[#3]

Eig71Eb[4]

Eig71Ec[4]

Eip63E[*#4]

Eip74EF[*#4]

Eip75B[*#4,5]

Eip93F[*4]

Hr46[6]

Samuel[*7]

sec10[8]

svp[9]

woc[10]

Shown are pairwise F_{ST} values of the candidate SNPs identified in the different population comparisons; red: Florida-Maine, blue: Florida-Pennsylvania, green: Pennsylvania-Maine.

Notes:

[§] For reviews of this pathway see, for example, King-Jones and Thummel (2005) and Schwedes and Carney (2012). For a review of the biosynthesis of ecdysteroids see Gilbert *et al.* (2002). The active steroid hormone 20-hydroxy-ecdysone (20E; "ecdysone") has pervasive effects on various aspects of *Drosophila* life history, including effects on metamorphosis, growth, ovarian maturation, reproductive dormancy, lifespan, and immunity (e.g., see Kozlova and Thummel 2000; Simon *et al.* 2003; Flatt *et al.* 2005; King-Jones and Thummel 2005; Flatt *et al.* 2008; Tricoire *et al.* 2009; Galikova *et al*. 2011, and references therein)

* Also found by Kolaczkowski *et al.* (2011)

[#] Also found by Turner *et al*. (2011) in an artificial selection experiment on body size in *D. melanogaster*. It is particularly noteworthy that several ecdysone signaling genes were found by Turner *et al.* (2011) as well as in our study since ecdysone signaling affects many life history traits including growth

and body size (e.g., Colombani *et al.* 2005) and since body size is strongly clinal along the US east coast (e.g., Coyne and Beecham 1987)

[1] Found to be clinal along the US east coast and to affect the propensity of reproductive dormancy and related life history phenotypes by Schmidt *et al.* (2008); *cpo* contains a large number of ecdysone response elements (Schmidt *et al.* 2008) and is expressed in the larval ring gland, the main site of larval ecdysteroid production (Harvie *et al*. 1998); notably, reproductive dormancy is known to be affected ecdysteroids (e.g., Richard *et al.* 1998, 2001; Flatt *et al.* 2005). See: http://flybase.org/reports/FBgn0000363.html for further details

[2] *dib*, *disembodied*; involved in the biosynthesis of ecdysone (Chavez *et al*. 2000). For details see: http://flybase.org/reports/FBgn0000449.html

[3] Gene involved in chromatin binding; mutations in this gene cause ecdysteroid deficiency (Sliter and Gilbert 1992); for further information see: http://flybase.org/reports/FBgn0002183.html

[4] *Ecdysone-inducible proteins* (*Eip*) or *genes* (*Eig*); these are genes/proteins known to be transcriptionally induced/regulated by ecdysone; for detailed information on these genes see King-Jones and Thummel (2005) and http://flybase.org/reports/FBgn0004589.html; http://flybase.org/reports/FBgn0004590.html; http://flybase.org/reports/FBgn0005640.html; http://flybase.org/reports/FBgn0000567.html; http://flybase.org/reports/FBgn0000568.html http://flybase.org/reports/FBgn0013948.html

[5] *Eip75B* has been found to be associated with the startle response by Yamamoto *et al.* (2008) and in a GWAS study based on the DGRP lines by Mackay *et al*. (2012); it has also been identified in a P-element insertion screen to affect lifespan (Magwire *et al*. 2010)

[6] *Hormone receptor 46*; also known as *dHR3*; a nuclear hormone receptor; known to affect embryogenesis and growth; also interacts with IIS (App. S1) (Carney *et al*. 1997; King-Jones and Thummel 2005; Montagne *et al.* 2010). For details see: http://flybase.org/reports/FBgn0000448.html

[7] Also known as *moses*; found by Kolaczkowski *et al.* (2011) to be clinal; interacts with *Hormone receptor 78* (*Hr78*) and thus with ecdysone signaling (Baker *et al*. 2007). See: http://flybase.org/reports/FBgn0032330.html

[8] Might be involved in ecdysteroid secretion (Andrews *et al*. 2002). See: http://flybase.org/reports/FBgn0027103.html

[9] *Svp, seven up*; uclear hormone receptor (NHR) known to interact with ecdysone signaling (see Gates *et al.* 2004; King-Jones and Thummel 2005). See: http://flybase.org/reports/FBgn0003651.html

[10] *woc, without children*; found by Turner *et al.* (2008) to be clinal; *woc* mutants are ecdysteroid deficient (Warren *et al*. 2001). For further information see: http://flybase.org/reports/FBgn0010328.html

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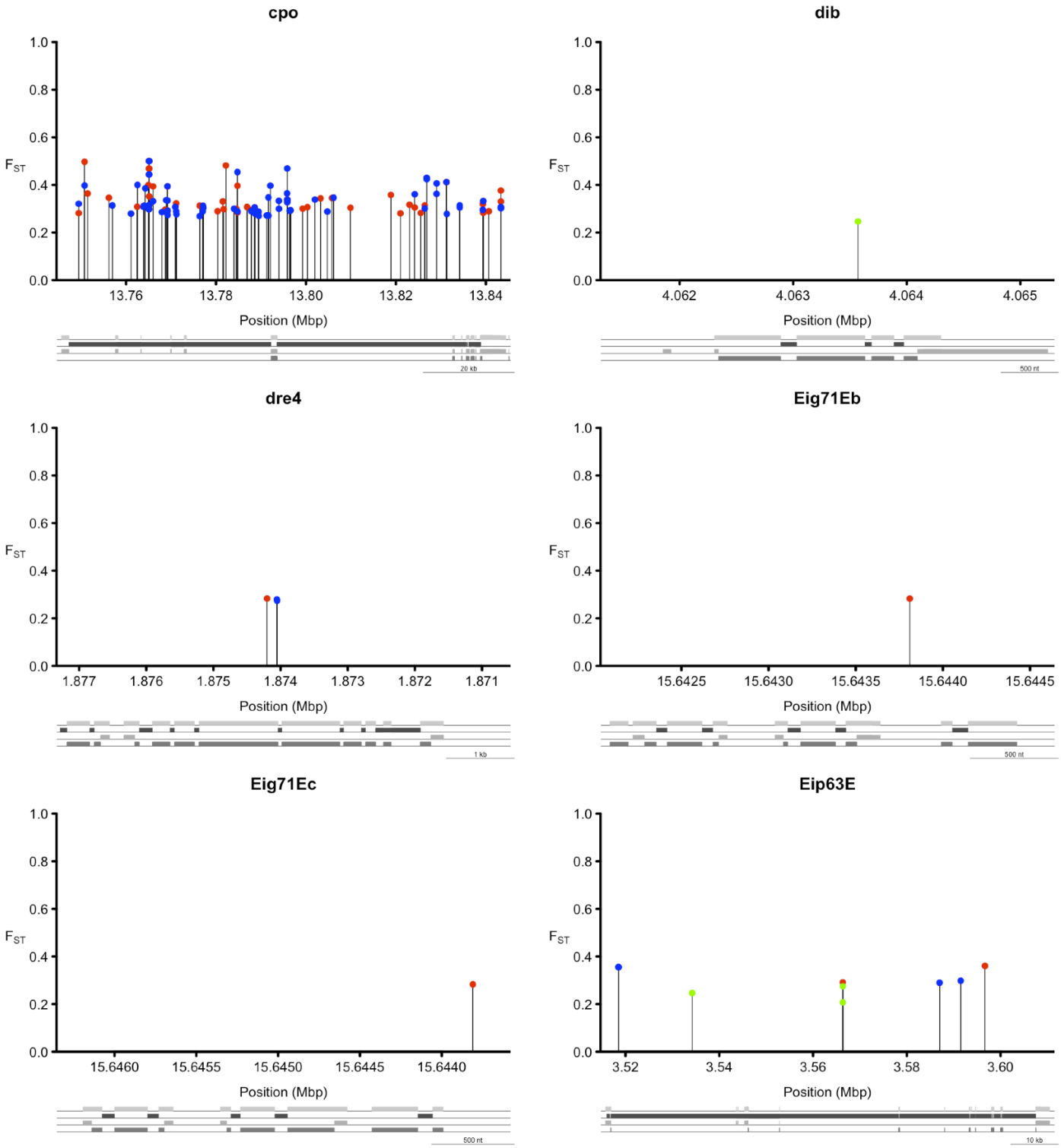
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Eig71Ec
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13.80
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4.063
4.064
4.065
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1.876
1.875
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1.873
1.872
1.871
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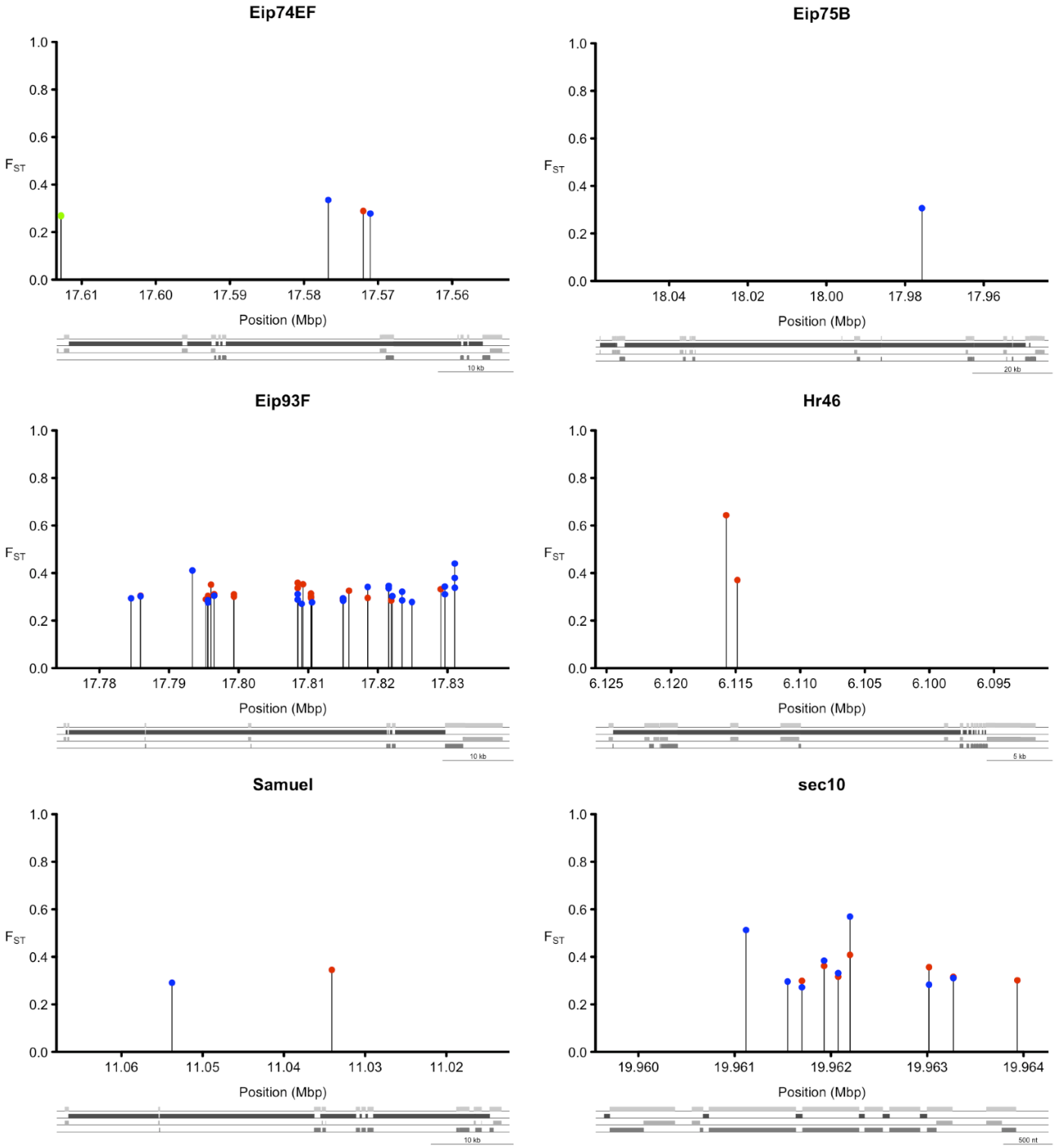
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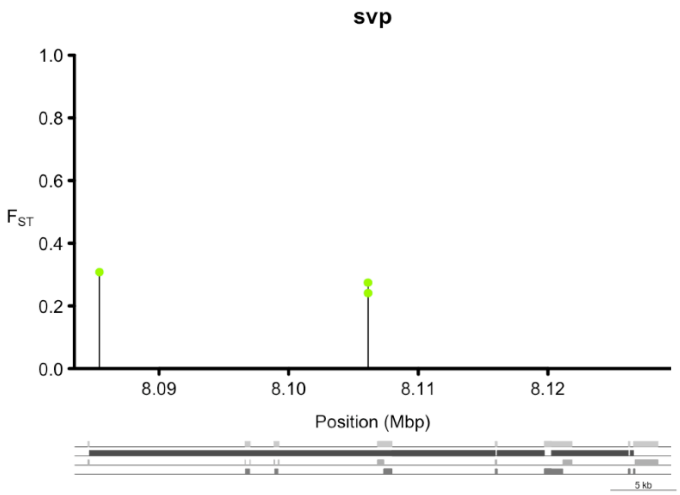
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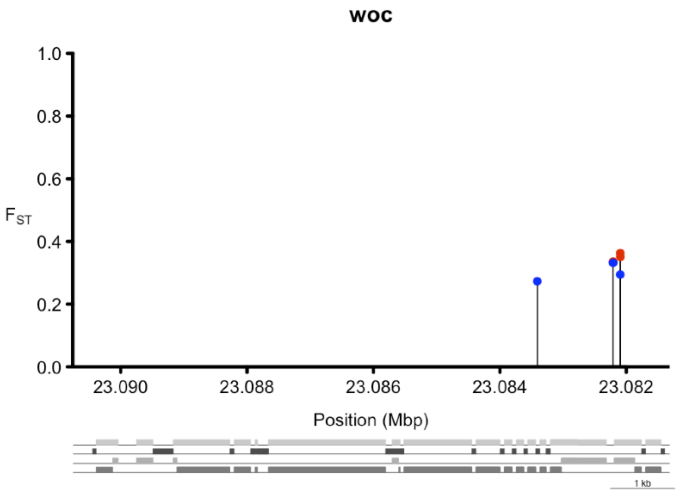
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23.082
Position (Mbp)
1 kb

Appendix S3

Examples of candidate genes involved in nuclear hormone receptor (NHR) signaling and other endocrine pathways[§]

ETHR[1]

GRHR[2]

Hr46[3]

Hr96[4]

svp[5]

Shown are pairwise F_{ST} values of the candidate SNPs identified in the different population comparisons; red: Florida-Maine, blue: Florida-Pennsylvania, green: Pennsylvania-Maine.

Notes:

[§] Nuclear hormone receptor (NHR) signaling and endocrine signaling in general is critically important for coordinating many aspects of development, growth and metabolism; for a review see, for example, King-Jones and Thummel (2005). For other candidate genes involved in hormonal signaling pathways not shown here see App. S1 (IIS/TOR) and App. S2 (ecdysone)

[1] *ETHR, eclosion triggering hormone receptor*; Kolaczkowski *et al.* (2011) do not find the receptor but find the gene that encodes the ligand for ETHR, i.e. *eclosion hormone* (*Eh*). See: http://flybase.org/reports/FBgn0038874.html

[2] *GRHR, gonadotropin-releasing hormone receptor*, also known as *adipokinetic hormone receptor (AKHR)*; functional analog of mammalian glucagon receptor; involved in fat storage and mobilization and carbohydrate metabolism (e.g., Grönke *et al*. 2007; Bharucha *et al*. 2008). For details see: flybase.org/reports/FBgn0025595.html

[3] *Hr46, hormone receptor 46*, a nuclear hormone receptor (NHR); also known as dHR3; involved in ecdysone signaling (e.g., King-Jones and Thummel 2005); also see App. S2. See: http://flybase.org/reports/FBgn0000448.html

[4] *Hr96, hormone receptor 96*, a nuclear hormone receptor (NHR); involved in triacylglycerol and cholesterol metabolism as well as the xenobiotic response (King-Jones *et al.* 2006; Horner *et al*. 2009; Sieber *et al*. 2009). For details see: http://flybase.org/reports/FBgn0015240.html

[5] *svp, seven up*; a nuclear hormone receptor (NHR); also involved in ecdysone signaling (see App. S2); for details see King-Jones and Thummel (2005) and: http://flybase.org/reports/FBgn0003651.html

References:

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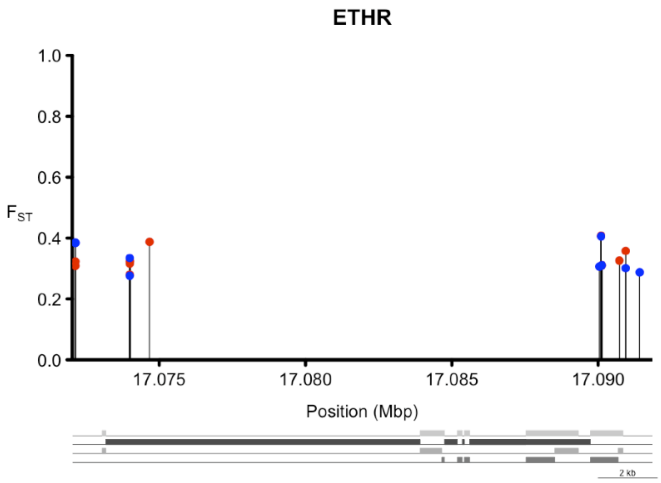
King-Jones K, Thummel CS (2005) Nuclear receptors - A perspective from *Drosophila. Nature Reviews Genetics* **6**, 311-323.

King-Jones K, Horner MA, Lam G, Thummel CS (2006) The DHR96 nuclear receptor regulates xenobiotic responses in *Drosophila*. *Cell Metabolism* **4**, 37-48.

Kolaczkowski B, Kern AD, Holloway AK, Begun DJ (2010) Genomic Differentiation Between Temperate and Tropical Australian Populations of *Drosophila melanogaster*. *Genetics* **187**, 245-260.

Sieber MH, Thummel CS (2009) The DHR96 Nuclear Receptor Controls Triacylglycerol Homeostasis in *Drosophila*. *Cell Metabolism* **10**, 481-490.


ETHR
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F_{ST}
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0.0
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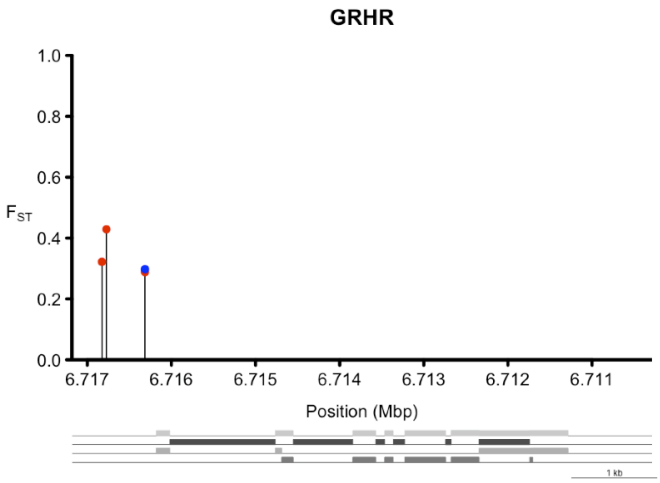
17.080
17.085
17.090
Position (Mbp)
2 kb


GRHR
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0.8
0.6
F_{ST}
0.4
0.2
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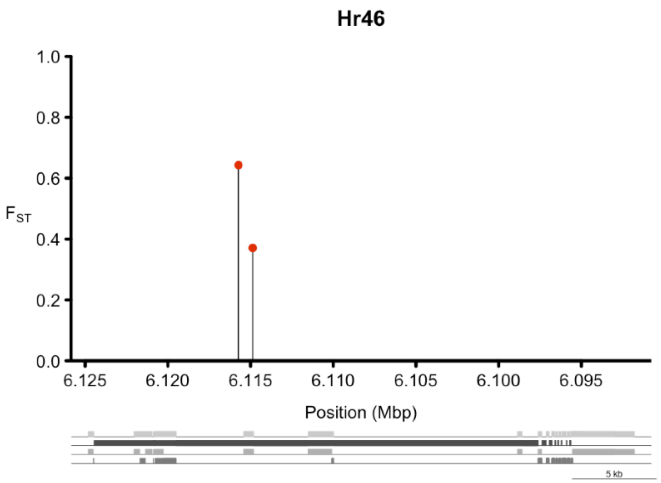
6.716
6.715
6.714
6.713
6.712
6.711
Position (Mbp)
1 kb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F_{ST}
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6.105
6.100
6.095
Position (Mbp)
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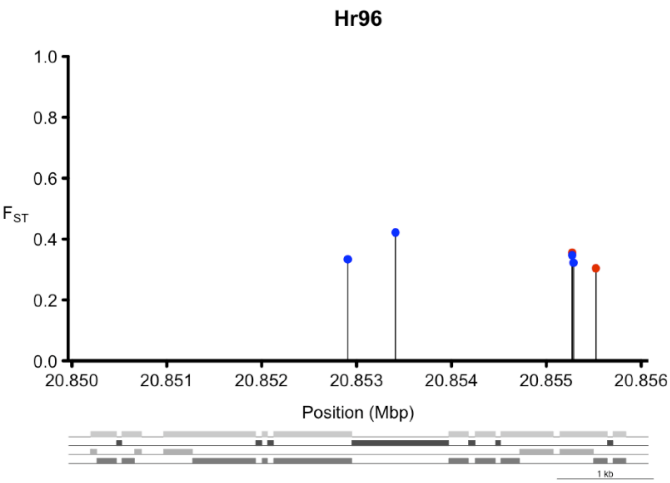
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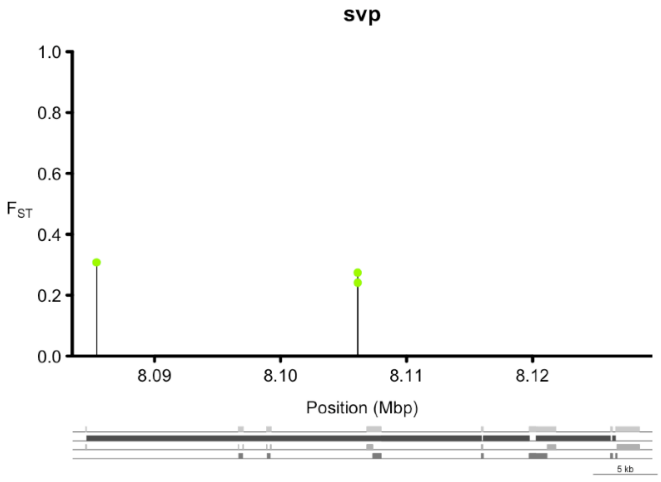
F_{ST}
0.4
0.2
0.0
20.850
20.851
20.852
20.853
20.854
20.855
20.856
Position (Mbp)
1 kb


svp
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0.8
0.6
F_{ST}
0.4
0.2
0.0
8.09
8.10
8.11
8.12
Position (Mbp)
5 kb

Appendix S4

Examples of candidate genes involved in lipid metabolism[§]

Lip2[1]

Lip3[2]

Lsd-1[3]

Lsd-2[4]

Lsp1alpha[5]

Shown are pairwise F_{ST} values of the candidate SNPs identified in the different population comparisons; red: Florida-Maine, blue: Florida-Pennsylvania, green: Pennsylvania-Maine.

Notes:

[§] For a review of lipid metabolism in *Drosophila* see Baker and Thummel (2007). It is important to note that high triglyceride content is associated with both improved resistance to starvation stress and with the expression of reproductive dormancy; natural diapause genotypes have higher triglyceride content than non-diapause lines; and flies from northern populations from the east coast of the US (Vermont) have a higher triglyceride content than flies from southern populations (Florida) (Schmidt *et al.* 2005)

[1] *Lip2, Lipase 2*; triglyceride lipase activity; for detailed information see: http://flybase.org/reports/FBgn0024740.html

[2] *Lip3, Lipase 3;* triglyceride lipase activity; for detailed information see: http://flybase.org/reports/FBgn0023495.html

[3] *Lsd-1, Lipid storage droplet-1*; regulation of lipid storage; see further information at: http://flybase.org/reports/FBgn0039114.html

[4] *Lsd-2, Lipid storage droplet-2*; functional homolog of human Perilipin/ADRP; involved in the regulation of lipid storage; mutants have reduced lipid levels and starvation sensitive; gain of function/overexpression increased lipid levels and resistance to starvation (Teixeira *et al.* 2003, Grönke *et al*. 2003, Baker and Thummel 2007). See: http://flybase.org/reports/FBgn0030608.html

[5] *Lsp1 alpha*, *Larval serum protein 1 α*; *Lsp1α*, together with the β and γ subunits of *Lsp1*, is expressed in the larval fat body and induced by ecdysone (see App. S2); it is a storage protein complex, associated with lipid droplets, that serves as a reservoir for amino acids and energy during metamporphosis (Burmester *et al.* 1999, Beller *et al*. 2006). For further information see: http://flybase.org/reports/FBgn0002562.html

References:

Baker KD, Thummel CS (2007) Diabetic larvae and obese flies - Emerging studies of metabolism in Drosophila. *Cell Metabolism* **6**, 257-266.

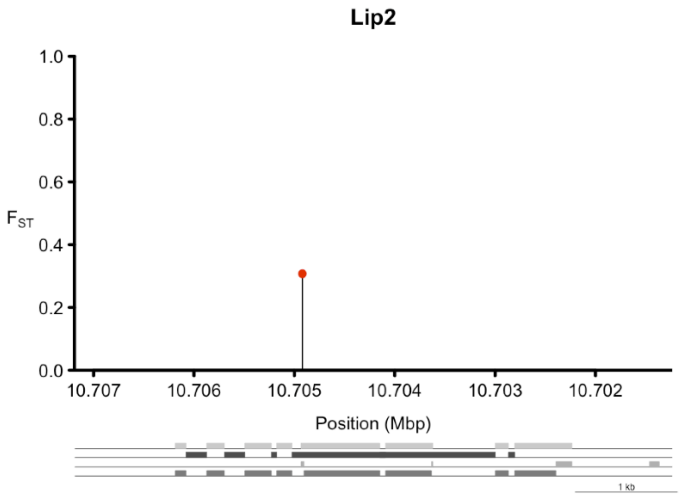
Beller M, Riedel D, Jansch L, Dieterich G, Wehland J, Jackle H, Kuhnlein RP (2006) Characterization of the *Drosophila* lipid droplet subproteome. *Molecular and Cellular Proteomics* **5**, 1082-1094.

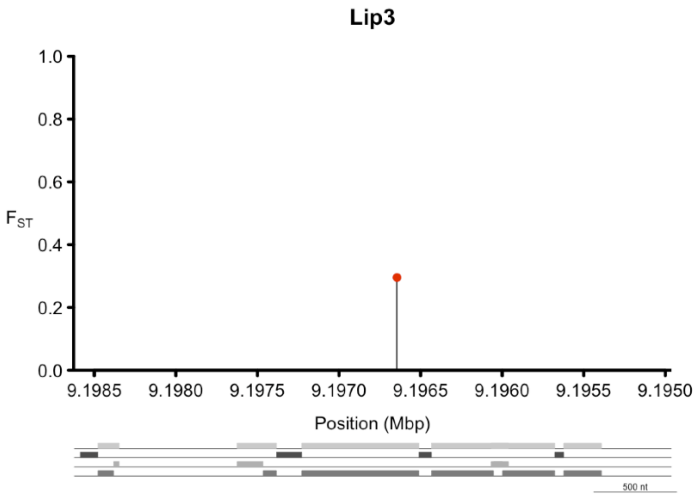
Burmester T, Antoniewski C, Lepesant JA (1999) Ecdysone-regulation of synthesis and processing of fat body protein 1, the larval serum protein receptor of *Drosophila melanogaster*. *European Journal of Biochemistry* **262,** 49-55.

Grönke, S., Beller, M., Fellert, S., Ramakrishnan, H., Jackle, H., and Kuhnlein, R.P. (2003) Control of fat storage by a *Drosophila* PAT domain protein. *Current Biology* **13**, 603–606.

Schmidt P, Matzkin L, Ippolito M, Eanes W (2005) Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster. Evolution* **59**, 1721-1732.

Teixeira, L., Rabouille, C., Rorth, P., Ephrussi, A., and Vanzo, N.F. (2003) *Drosophila* Perilipin/ADRP homologue Lsd2 regulates lipid metabolism. *Mechanisms of Development* **120**, 1071–1081.


Lip2
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
10.707
10.706
10.705
10.704
10.703
10.702
Position (Mbp)
1 kb


Lip3
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
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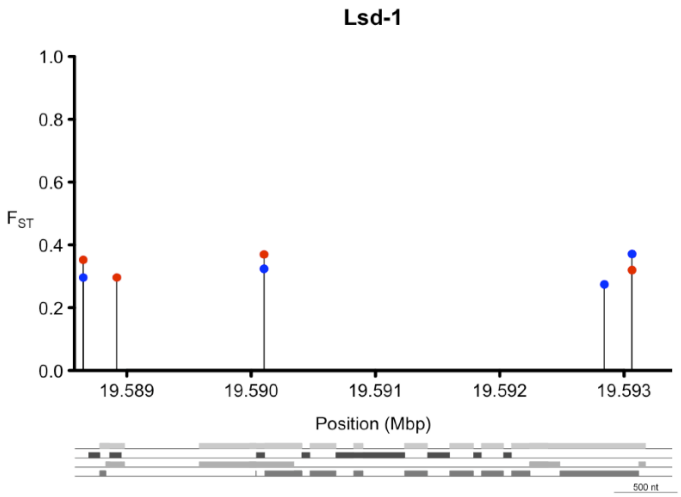
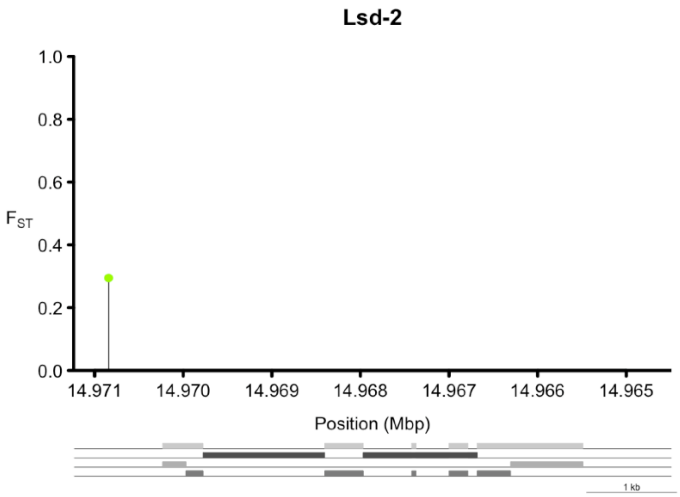
9.1980
9.1975
9.1970
9.1965
9.1960
9.1955
9.1950
Position (Mbp)
500 nt


Lsd-1
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
19.589
19.590
19.591
19.592
19.593
Position (Mbp)
500 nt


Lsd-2
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
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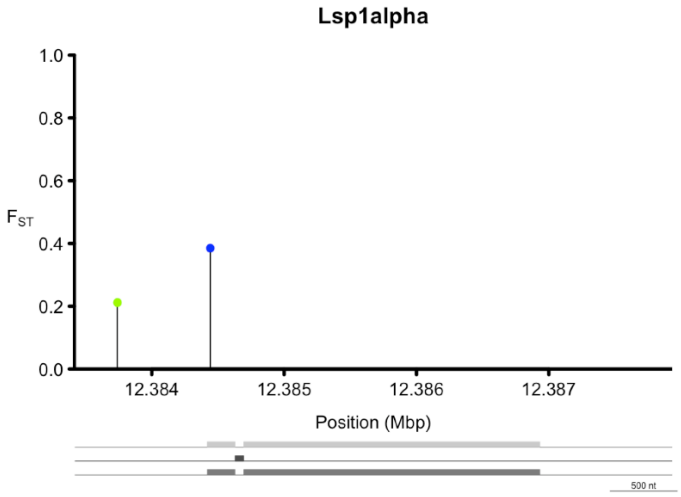
14.970
14.969
14.968
14.967
14.966
14.965
Position (Mbp)
1 kb


Lsp1alpha
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
12.384
12.385
12.386
12.387
Position (Mbp)
500 nt

Appendix S5

Examples of candidate genes involved in innate immunity (Toll/Imd signaling)[§]

AttA[1]

AttB[2]

Dif[3]

Dpt[4]

DptB[5]

Dro[6]

imd[7]

*Irc**[8]

PGRP-LA[9]

PGRP-LC[10]

PGRP-LF[11]

*sick**[12]

Tl[13]

Toll-4[14]

Tollo[15]

TotF[16]

Shown are pairwise F_{ST} values of the candidate SNPs identified in the different population comparisons; red: Florida-Maine, blue: Florida-Pennsylvania, green: Pennsylvania-Maine.

Notes:

[§] For reviews of innate immunity in *Drosophila* see Hoffmann and Reichhart (2002), Hoffmann (2003), Kim and Kim (2005), Kaneko and Silverman (2005), and Ferrandon *et al.* (2007). Genes involved in innate immunity are known to exhibit a lot of genetic variation in natural populations, to be under strong natural selection, and to evolve rapidly (Lesser *et al.* 2006; Lazzaro 2008; Obbard *et al.* 2009). Both Turner *et al.* (2008) and Kolaczkowski *et al*. (2011) find an overrepresentation of genes involved in innate immune response ("defense responses") in their analyses of clinal variation in *D. melanogaster*; this suggests that there might exist pervasive latitudinal variation in selection pressure imposed by pathogens. It also interesting to note that innate immunity in *Drosophila* is hormonally regulated by ecdysone signaling (Flatt *et al.* 2008) (see App. S2) and IIS/Foxo (Becker *et al.* 2010) (see App. S1).

* Also found by Kolaczkowski *et al.* (2011)

[1] *AttA, Attacin A*; encodes an antimicrobial peptide; for more information see http://flybase.org/reports/FBgn0012042.html

[2] *AttB, Attacin B*; encodes an antimicrobial peptide; for more information see http://flybase.org/reports/FBgn0041581.html

[3] *Dif, dorsal-related immunity factor,* a NF-κB-like transcription factor in the Toll signaling pathway; see http://flybase.org/reports/FBgn0011274.html

[4] *Dpt, Diptericin*; encodes an antimicrobial peptide; for more information see http://flybase.org/reports/FBgn0004240.html

[5] *DptB, Diptericin B*; encodes an antimicrobial peptide; see http://flybase.org/reports/FBgn0034407.html

[6] *Dro, Drosocin*; encodes an antimicrobial peptide; see http://flybase.org/reports/FBgn0010388.html

[7] *imd, immune deficiency*; protein of central function in the Imd signaling pathway; see http://flybase.org/reports/FBgn0013983.html

[8] *Irc, immune-regulated catalase*; a gene involved in a key host defense system required during host-microbe interactions in the gastrointestinal tract (Ha *et al.* 2005 a,b); also found to vary clinally by Kolaczkowski *et al.* (2011); also see http://flybase.org/reports/FBgn0038465.html

[9-11] *PGRP, peptidoglycan recognition proteins*; major proteins that detect bacterial cell wall peptidoglycans; acting upstream of the Imd and Toll in the Imd/Toll immune pathways (e.g., Kaneko and Silverman 2005). Also see: http://flybase.org/reports/FBgn0035975.html; http://flybase.org/reports/FBgn0035976.html; http://flybase.org/reports/FBgn0035977.html

[12] *sick, sickie*; involved in the response to Gram-negative bacteria (Foley and Farrell 2004); also found to be clinal by Kolaczkowski *et al.* (2001). See: http://flybase.org/reports/FBgn0263873.html

[13] *Tl, Toll*; defining member of the Toll immune signaling pathway; critical for anti-microbial peptide gene expression after Gram-positive and fungal infections; activates Toll signaling by binding to Spätzle ligand (e.g., Kaneko and Silverman 2005). See: http://flybase.org/reports/FBgn0262473.html

[14] *Toll-4*; potential role in innate immunity unclear. For further information see http://flybase.org/reports/FBgn0032095.html

[15] *Tollo,* or *Toll-8*; negative regulator of the antimicrobial response (Akhouyari *et al*. 2011). See: http://flybase.org/reports/FBgn0029114.html

[16] *TotF, Turandot F*; a stress induced humoral factor; role in immunity somewhat unclear although has been found to induced by infection. See http://flybase.org/reports/FBgn0044811.html

References:

Becker T, Loch G, Beyer M*, et al.* (2010) FOXO-dependent regulation of innate immune homeostasis. *Nature* **463**, 369-373.

Akhouayri I, Turc C, Royet J, Charroux B (2011) Toll-8/Tollo Negatively Regulates Antimicrobial Response in the *Drosophila* Respiratory Epithelium. *PLoS Pathogens* **7**, e1002319.

Ferrandon D, Imler J, Hetru C, Hoffmann J (2007) The *Drosophila* systemic immune response: sensing and signaling during bacterial and fungal infections. *Nature Reviews Immunology* **7**, 862-874.

Flatt T, Heyland A, Rus F*, et al.* (2008) Hormonal regulation of the humoral innate immune response in *Drosophila melanogaster*. *Journal of Experimental Biology* **211**, 2712-2724.

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Ha E-M, Oh C-T, Bae YS, Lee W-J (2005a) A Direct Role for Dual Oxidase in *Drosophila* Gut Immunity. *Science* **310**, 847-850.

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Obbard DJ, Welch JJ, Kim K-W, Jiggins FM (2009) Quantifying Adaptive Evolution in the *Drosophila* Immune System. *PLoS Genetics* **5**, e1000698.

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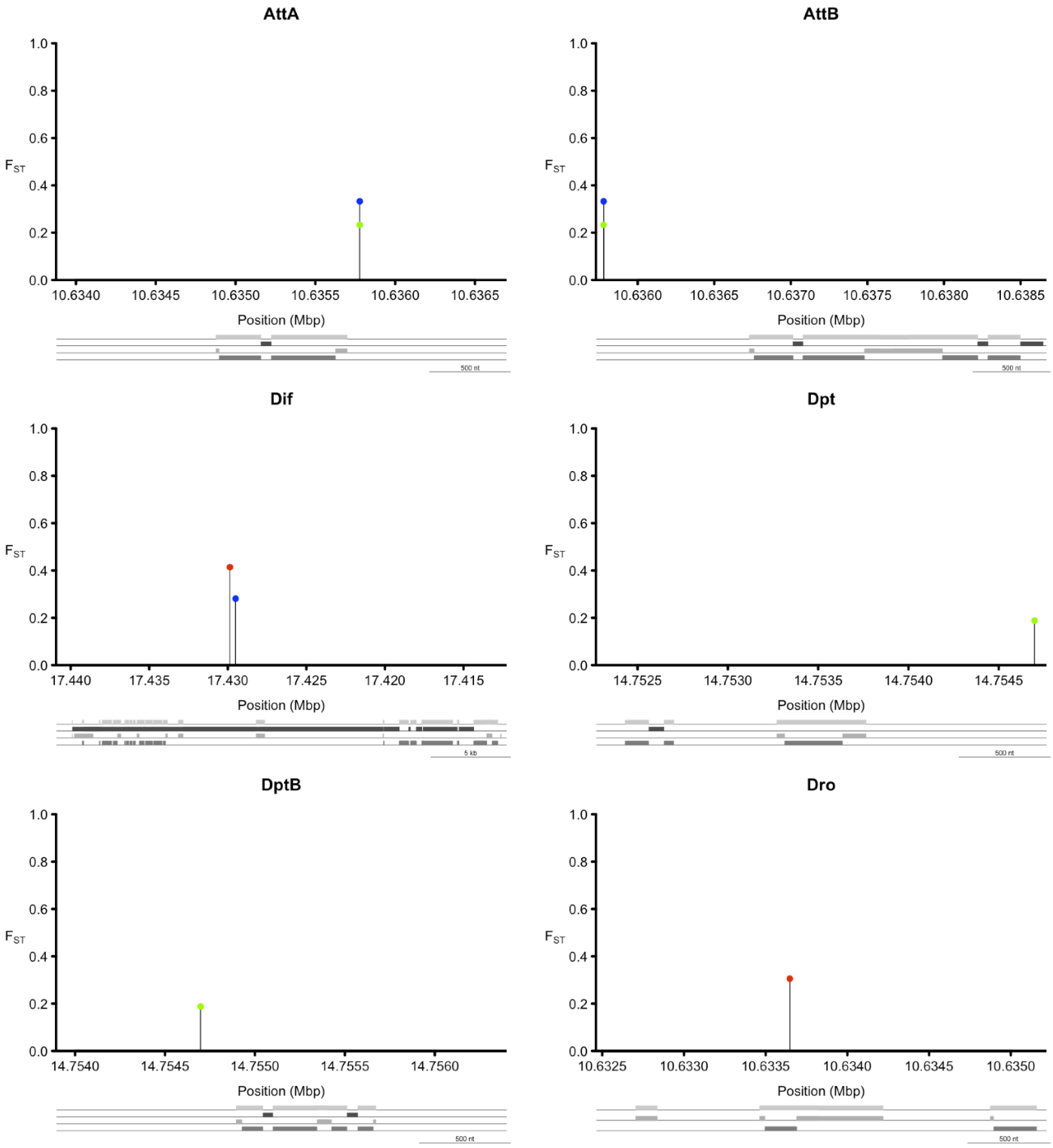
AttA
AttB
Dif
Dpt
DptB
Dro
F_{ST}
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0.8
0.6
0.4
0.2
0.0
10.6340
10.6345
10.6350
10.6355
10.6360
10.6365
10.6360
10.6365
10.6370
10.6375
10.6380
10.6385
17.440
17.435
17.430
17.425
17.420
17.415
14.7525
14.7530
14.7535
14.7540
14.7545
14.7540
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14.7550
14.7555
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10.6335
10.6340
10.6345
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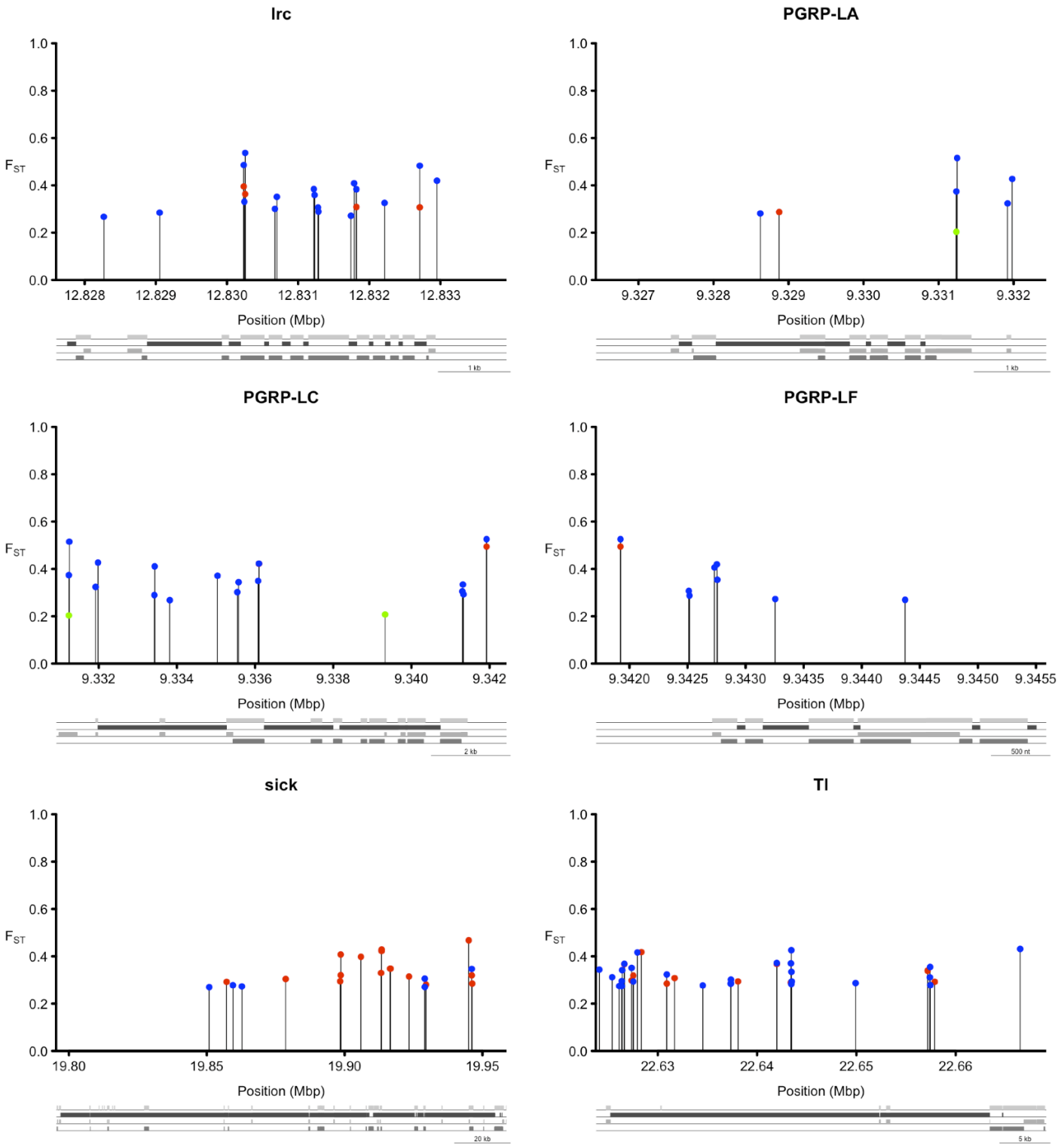
Position (Mbp)
500 nt
5 kb


Irc
PGRP-LA
PGRP-LC
PGRP-LF
sick
TI
F_{ST}
Position (Mbp)
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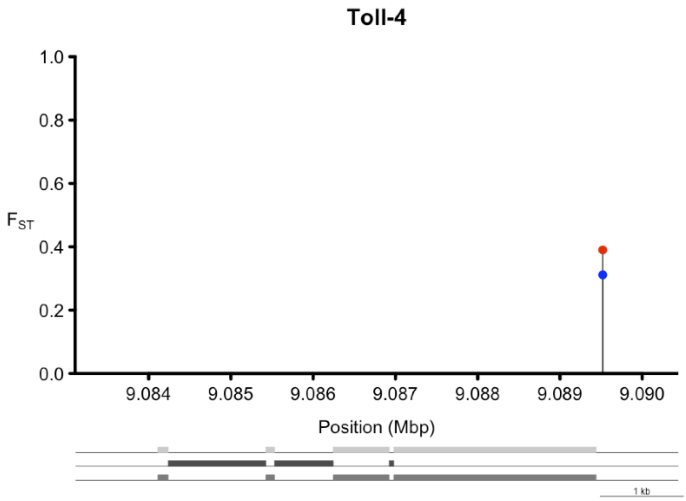
0.8
0.6
0.4
0.2
0.0
12.828
12.829
12.830
12.831
12.832
12.833
9.327
9.328
9.329
9.330
9.331
9.332
9.332
9.334
9.336
9.338
9.340
9.342
9.3420
9.3425
9.3430
9.3435
9.3440
9.3445
9.3450
9.3455
19.80
19.85
19.90
19.95
22.63
22.64
22.65
22.66
1 kb
2 kb
500 nt
20 kb
5 kb


Toll-4
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0.8
0.6
F_{ST}
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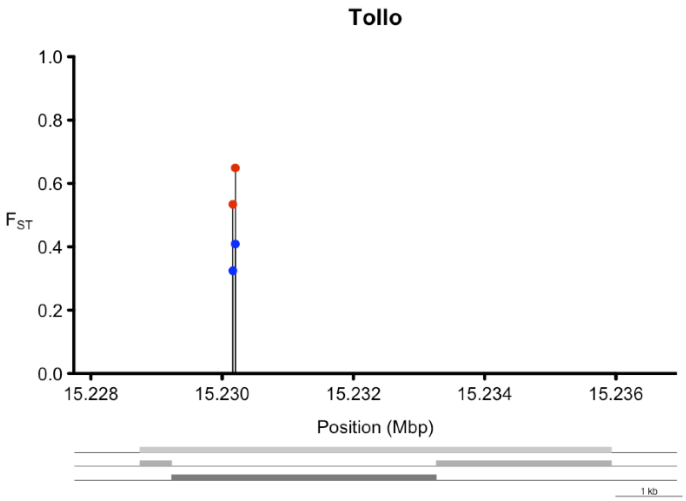
0.2
0.0
9.084
9.085
9.086
9.087
9.088
9.089
9.090
Position (Mbp)
1 kb


Tollo
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0.6
F_{ST}
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0.2
0.0
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15.232
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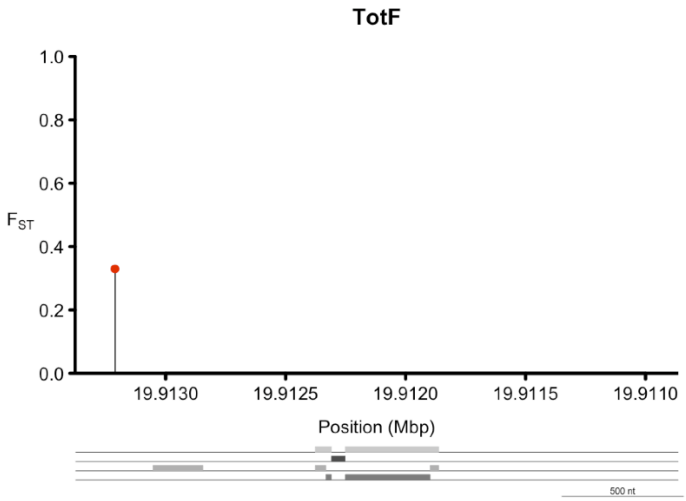
15.236
Position (Mbp)
1 kb


TotF
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0.6
F_{ST}
0.4
0.2
0.0
19.9130
19.9125
19.9120
19.9115
19.9110
Position (Mbp)
500 nt

Appendix S6

Examples of candidate genes involved in epidermal growth factor (EGFR) signaling[§]

*ed**[1]

EGFR[2]

*S**[3]

Shown are pairwise F_{ST} values of the candidate SNPs identified in the different population comparisons; red: Florida-Maine, blue: Florida-Pennsylvania, green: Pennsylvania-Maine.

Notes:

[§] EGFR signaling is of major importance for the regulation of differentiation of many tissues during *Drosophila* development; for a review of EGFR signaling see, for example, Schweizer and Shilo (1997) and Shilo *(*2003)

* Also found by Kolaczkowski *et al*. (2011)

[1] *ed, echinoid*; encodes a transmembrane protein and cell adhesion molecule that antagonizes EGFR signaling; for further information see: http://flybase.org/reports/FBgn0000547.html

[2] *EGFR, epidermal growth factor receptor*; the defininig member of the EGFR pathway; also found, along with other genes in this pathway, by Turner *et al*. (2011) in a study of artificial selection for body size. Might also be involved in starvation stress resistance (Mackay *et al*. 2012). For further details see: http://flybase.org/reports/FBgn0003731.html

[3] *S, Star*; essential gene implicated in trafficking of ligands for EGFR signaling. For details see: http://flybase.org/reports/FBgn0003310.html

References:

Kolaczkowski B, Kern AD, Holloway AK, Begun DJ (2010) Genomic Differentiation Between Temperate and Tropical Australian Populations of *Drosophila melanogaster*. *Genetics* **187**, 245-260.

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Schweitzer R, Shilo BZ (1997) A thousand and one roles for the *Drosophila* EGF receptor. *Trends in Genetics* **13**, 191-196.

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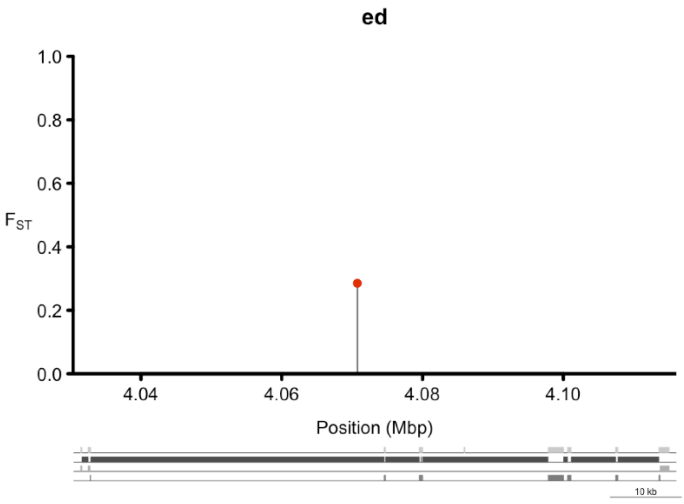
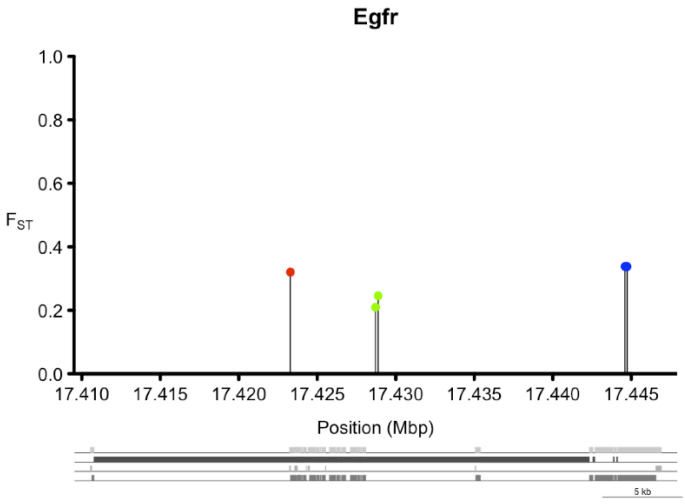
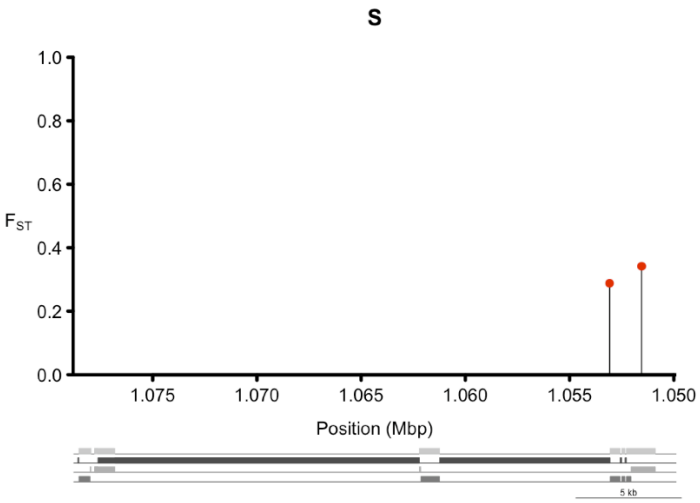
Turner TL, Stewart AD, Fields AT, Rice WR, Tarone AM (2011) Population-Based Resequencing of Experimentally Evolved Populations Reveals the Genetic Basis of Body Size Variation in *Drosophila melanogaster*. *PLoS Genetics* **7**, e1001336.


ed
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
4.04
4.06
4.08
4.10
Position (Mbp)
10 kb


Egfr
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
17.410
17.415
17.420
17.425
17.430
17.435
17.440
17.445
Position (Mbp)
5 kb


S
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
1.075
1.070
1.065
1.060
1.055
1.050
Position (Mbp)
5 kb

Appendix S7

Examples of candidate genes involved in JAK/STAT signaling[§]

*crb**[1]

*CycE**[2]

*Ptp61F**[3]

*Stat92E**[4]

*tkv**[5]

*upd2**[6]

upd3[7]

Shown are pairwise F_{ST} values of the candidate SNPs identified in the different population comparisons; red: Florida-Maine, blue: Florida-Pennsylvania, green: Pennsylvania-Maine.

Notes:

[§] For reviews of JAK/STAT signaling in *Drosophila* see, for example, Zeidler *et al*. (2000) and Arbouzova and Zeidler (2006). JAK-STAT signaling plays a major role in the signal transduction of cytokine and growth factor signals; the pathway is involved in a variety of developmental and physiological processes and functions, for example, in the regulation of cell proliferation, stem cell maintenance, immunity, sex determination, embryonic segmentation, larval hematopoiesis, and ommatidia polarity, etc. (Zeidler *et al*. 2000; Arbouzova and Zeidler 2006). It is noteworthy that Kolaczkowski *et al.* (2011) find strong differentiation along the Australian cline for several genes in this pathway and that we find the same pattern for the US east coastal cline.

* Also found by Kolaczkowski *et al.* (2011)

[1] *crb, crumbs*; a modifier of JAK/STAT signaling; also involved in salvador/hippo/warts signaling; identified by Kolaczkowski *et al*. (2011) to be clinal as well as by Turner *et al*. (2011) in an artificial selection experiment on body size. See: http://flybase.org/reports/FBgn0259685.html

[2] *CycE, Cyclin E*; interacts with *Stat92E* (cf. Arbouzova and Zeidler 2006); also found by Kolaczkowski *et al*. (2011) to be clinal. For further details see: http://flybase.org/reports/FBgn0010382.html

[3] *Ptp61F, Protein tyrosine phosphatase 61F*; acts as a suppressor of STAT92E-dependent transcription (cf. Arbouzova and Zeidler 2006); also identified by Kolaczkowski *et al*. (2011) to be clinal. For more information see http://flybase.org/reports/FBgn0003138.html

[4] *Stat92E;* major transcription factor involved in JAK/STAT signaling (cf. Arbouzova and Zeidler 2006); also identified by Kolaczkowski *et al*. (2011). For more information see: http://flybase.org/reports/FBgn0016917.html

[5] *tkv, thickveins*; involved in TGFβ/BMP signaling (see App. S8), a modifier of JAK/STAT signaling; also identified by Kolaczkowski *et al*. (2011). For more information see: http://flybase.org/reports/FBgn0003716.html

[6-7] *upd2* and *upd3, unpaired 2* and *unpaired 3*; together with *upd*, the genes *upd2* and *upd3* encode the three JAK/STAT ligands; *upd2* seems to be a freely diffusible ligand of the JAK/STAT receptor *dome*; *upd3* is less well understood but expressed in the developing gonads, the larval lymph and in circulating haemocytes following septic injury (see Arbouzova and Zeidler 2006). Interestingly, fat body specific activation of JAK/STAT signaling results in the expression of several antimicrobial peptides (see App. S5) and requires *upd3*, but not *upd* or *upd2* (cf. Arbouzova and Zeidler 2006). For details see: http://flybase.org/reports/FBgn0030904.html; http://flybase.org/reports/FBgn0053542.html

References:

Arbouzova NI, Zeidler MP (2006) JAK/STAT signalling in *Drosophila*: insights into conserved regulatory and cellular functions. *Development* **133**, 2605-2616.

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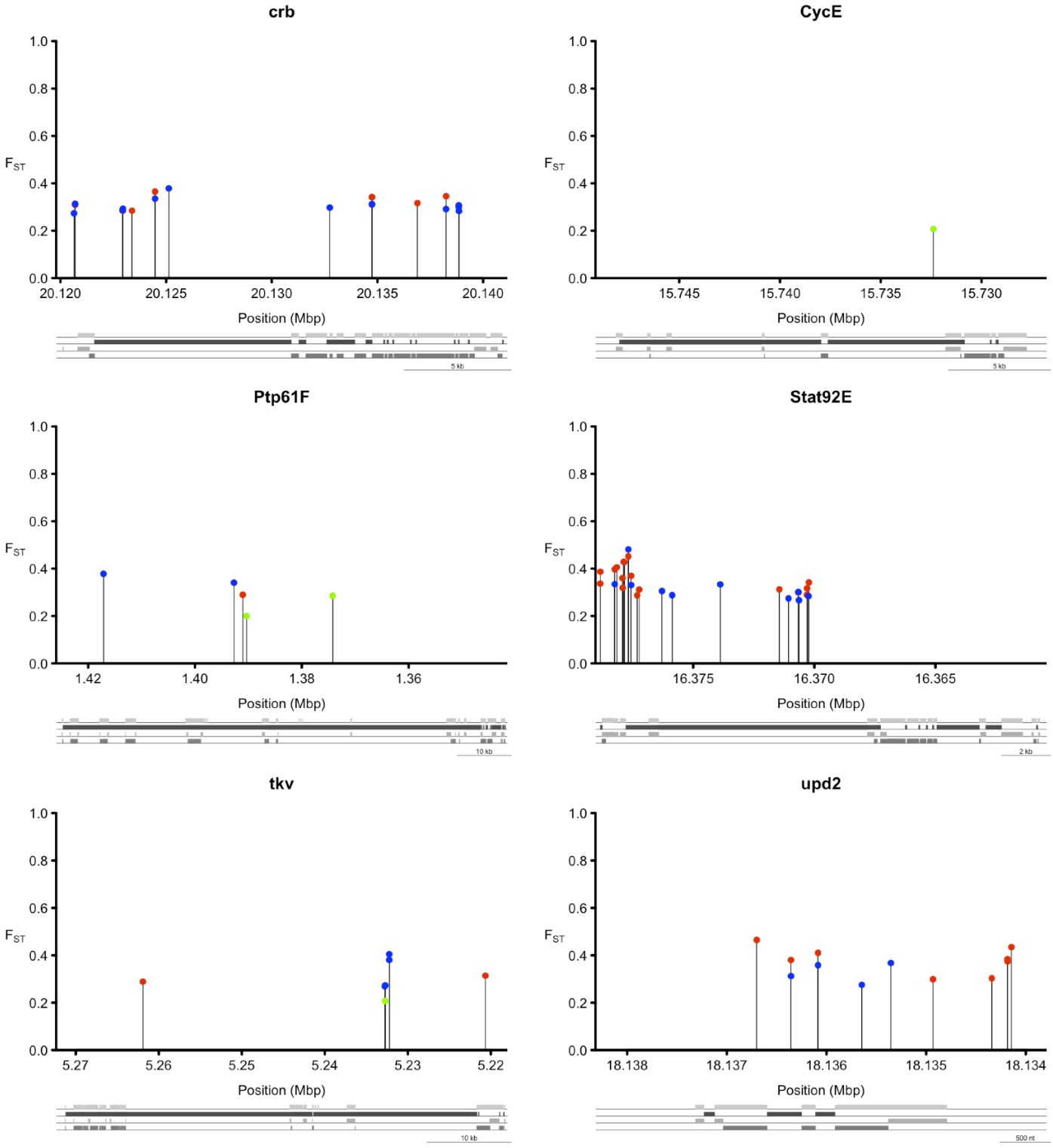
Turner TL, Stewart AD, Fields AT, Rice WR, Tarone AM (2011) Population-Based Resequencing of Experimentally Evolved Populations Reveals the Genetic Basis of Body Size Variation in *Drosophila melanogaster*. *PLoS Genetics* **7**, e1001336.

Zeidler MP, Bach EA, Perrimon N (2000) The roles of the *Drosophila* JAK/STAT pathway. *Oncogene* **19**, 2598-2606.


crb
CycE
Ptp61F
Stat92E
tkv
upd2
F_{ST}
Position (Mbp)
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0.8
0.6
0.4
0.2
0.0
20.120
20.125
20.130
20.135
20.140
15.745
15.740
15.735
15.730
1.42
1.40
1.38
1.36
16.375
16.370
16.365
5.27
5.26
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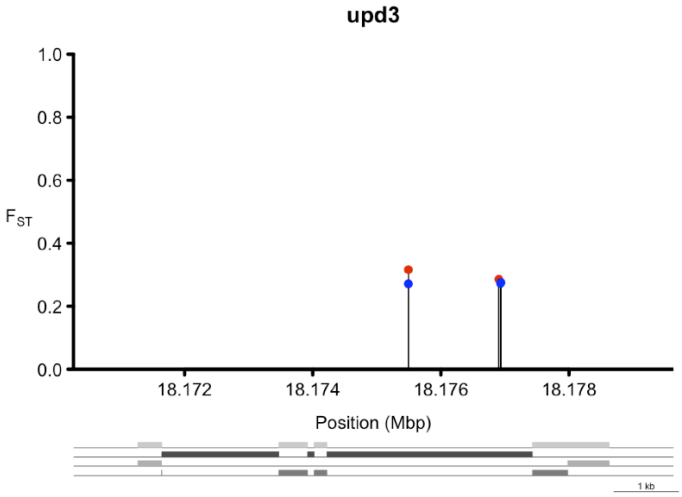
5 kb
10 kb
2 kb
500 nt


upd3
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
18.172
18.174
18.176
18.178
Position (Mbp)
1 kb

Appendix S8

Examples of candidate genes involved in TGF-β/BMP signaling[§]

Dad[1]

*dally**[2]

*dpp**[3]

*tkv**[4]

Shown are pairwise F_{ST} values of the candidate SNPs identified in the different population comparisons; red: Florida-Maine, blue: Florida-Pennsylvania, green: Pennsylvania-Maine.

Notes:

[§] For a review of this pathway see Derynck and Miyazono (2008). The TGF-β/BMP superfamily represents a major signaling pathway which regulates many developmental and cellular processes, including early axis specification, cell shape and proliferation, apoptosis, and differentiation (Derynck and Miyanozo 2008). The ligand superfamily consists of two main subfamilies, the BMP and the TGF-β/activin subfamily (Feng and Derynck 2005).

* Also found by Kolaczkowski *et al.* (2011)

[1] *Dad, daughters against dpp*; antagonizes *dpp* (*decapentaplegic*) and *Mad* (*mothers against dpp*); see: http://flybase.org/reports/FBgn0020493.html

[2] *dally, division abnormally delayed*; also found by Turner *et al*. (2011) in an artificial selection experiment on body size in *D. melanogaster* and by Kolaczkowski *et al.* (2011) to vary clinally. See: http://flybase.org/reports/FBgn0263930.html

[3] *dpp, decapentaplegic*; a BMP (bone morphogenetic protein) 2,4 ortholog and ligand; found by Kolaczkowski *et al.* (2011) to vary clinally. For details see: http://flybase.org/reports/FBgn0000490.html

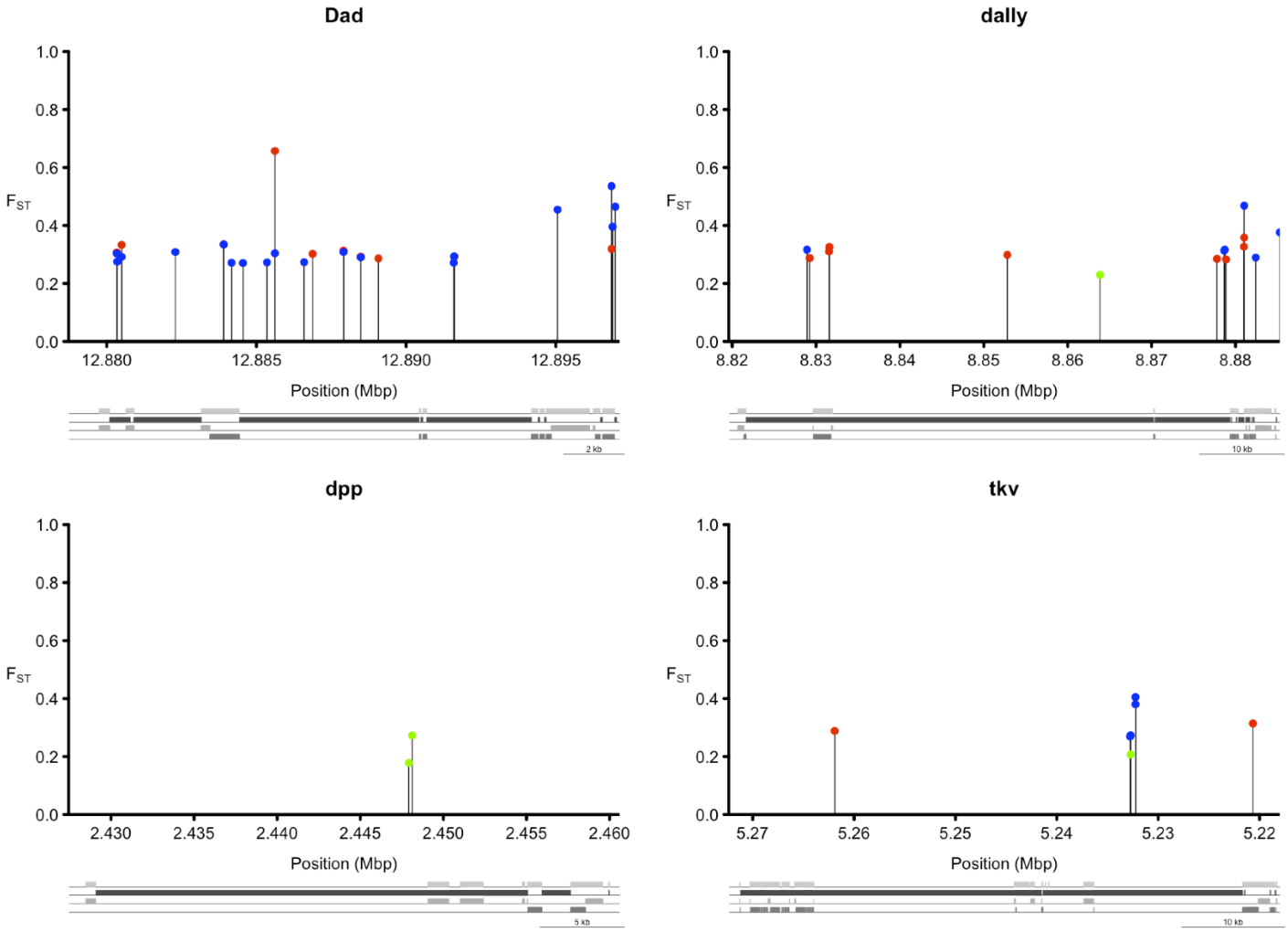
[4] *tkv, thickveins*; a type 1 TGF-β/BMP receptor; found by Kolaczkowski *et al.* (2011) to vary clinally. See: http://flybase.org/reports/FBgn0003716.html

References:

Derynck R, Miyazono K (2008) *The TGF-β Family*. Cold Spring Harbor Laboratory Press.
Feng XH, Derynck R (2005) Specificity and versatility in TGF-beta signaling through Smads. *Annual Review of Cell and Developmental Biology* **21**, 659-693.

Kolaczkowski B, Kern AD, Holloway AK, Begun DJ (2011) Genomic Differentiation Between Temperate and Tropical Australian Populations of *Drosophila melanogaster*. *Genetics* **187**, 245-260

Turner TL, Stewart AD, Fields AT, Rice WR, Tarone AM (2011) Population-Based Resequencing of Experimentally Evolved Populations Reveals the Genetic Basis of Body Size Variation in *Drosophila melanogaster*. *PLoS Genetics* **7**, e1001336.


Dad
dally
dpp
tkv
F_{ST}
Position (Mbp)
12.880
12.885
12.890
12.895
8.82
8.83
8.84
8.85
8.86
8.87
8.88
2.430
2.435
2.440
2.445
2.450
2.455
2.460
5.27
5.26
5.25
5.24
5.23
5.22
2 kb
10 kb
5 kb

Appendix S9

Examples of candidate genes involved in torso signaling[§]

pnt[*1]

tld[*2]

tup[*3]

Shown are pairwise F_{ST} values of the candidate SNPs identified in the different population comparisons; red: Florida-Maine, blue: Florida-Pennsylvania, green: Pennsylvania-Maine.

Notes:

[§] For reviews of torso signaling see, for example, Duffy and Perrimon (1994) and Li (2005). The *torso* (*tor*) gene, the defining member of the pathway, is a maternally contributed receptor tyrosine kinase (RTK) required, together with its ligand *trunk*, for cell fate specification in the terminal regions (head and tail) of the early *Drosophila* embryo (e.g., Li 2005). The pathway is also known to regulate metamorphosis and body size. Interestingly, the embryonic trunk ligand is related to prothoracicotropic hormone (PTTH), a hormone that regulates ecdysone production (see App. S2) in the larval prothoracic gland and that is crucially important for metamorphosis; PTTH has been found to initiate metamorphosis by activation of the Torso/ERK pathway, and torso turns out to be the PTTH receptor (Rewitz *et al.* 2009).

* Also found by Kolaczkowski *et al.* (2011)

[1] *pnt, pointed;* found to be clinal by Turner *et al.* (2008) and by Kolaczkowski *et al.* (2011); has also been found in a screen of *P*-element insertion lines and in a GWAS study based on the DGRP lines to affect starvation resistance (Harbison et al. 2004). See: http://flybase.org/reports/FBgn0003118.html

[2] *tld, tolloid;* also interacts with TGF-β/BMP signaling (App. S8); found to be clinal by Kolaczkowski *et al.* (2011); see: http://flybase.org/reports/FBgn0003719.html

[3] *tup, tailup;* found to be clinal by Kolaczkowski *et al.* (2011). For details see: http://flybase.org/reports/FBgn0003896.html

References:

Duffy JB, Perrimon N (1994) The Torso Pathway in *Drosophila*: Lessons on Receptor Tyrosine Kinase Signaling and Pattern Formation. *Developmental Biology* **166**, 380-395.

Harbison ST, Yamamoto AH, Fanara JJ, Norga KK, Mackay TFC (2004) Quantitative Trait Loci Affecting Starvation Resistance in *Drosophila melanogaster*. *Genetics* **166**, 1807-1823.

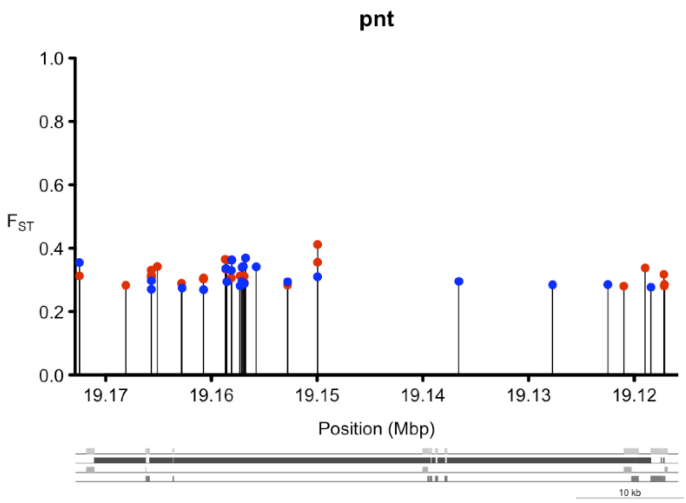
Kolaczkowski B, Kern AD, Holloway AK, Begun DJ (2011) Genomic Differentiation Between Temperate and Tropical Australian Populations of *Drosophila melanogaster*. *Genetics* **187**, 245-260

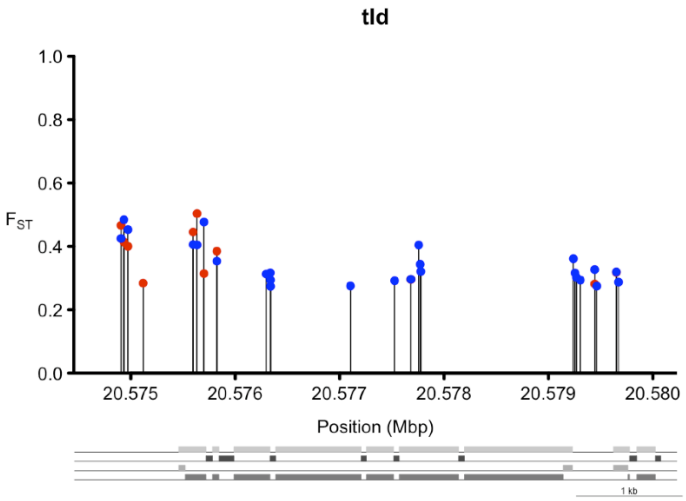
Mackay TFC, Richards S, Stone EA*, et al.* (2012) The *Drosophila melanogaster* Genetic Reference Panel. *Nature* **482**, 173-178.

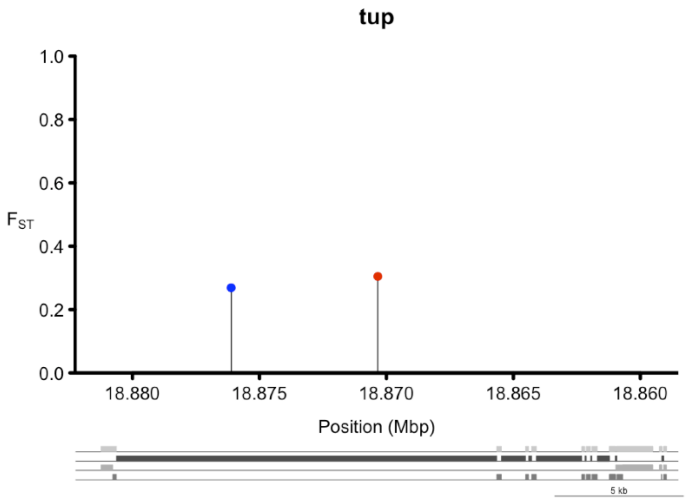
Li WX (2005) Functions and mechanisms of receptor tyrosine kinase Torso signaling: Lessons from *Drosophila* embryonic terminal development. *Developmental Dynamics* **232**, 656-672.

Rewitz KF, Yamanaka N, Gilbert LI, O'Connor MB (2009) The Insect Neuropeptide PTTH Activates Receptor Tyrosine Kinase Torso to Initiate Metamorphosis. *Science* **326**, 1403-1405.

Turner TL, Levine MT, Eckert ML, Begun DJ (2008) Genomic Analysis of Adaptive Differentiation in *Drosophila melanogaster*. *Genetics* **179**, 455-473.


pnt
1.0
0.8
0.6
0.4
0.2
0.0
F_{ST}
19.17
19.16
19.15
19.14
19.13
19.12
Position (Mbp)
10 kb


tld
1.0
0.8
0.6
0.4
0.2
0.0
F_{ST}
20.575
20.576
20.577
20.578
20.579
20.580
Position (Mbp)
1 kb


tup
1.0
0.8
0.6
0.4
0.2
0.0
F_{ST}
18.880
18.875
18.870
18.865
18.860
Position (Mbp)
5 kb

Appendix S10

Examples of candidate genes involved in the circadian clock / rhythm[§]

Clk[1]

*cry**[2]

*timeless**[3]

*timeout**[4]

Shown are pairwise F_{ST} values of the candidate SNPs identified in the different population comparisons; red: Florida-Maine, blue: Florida-Pennsylvania, green: Pennsylvania-Maine.

Notes:

[§] For reviews of the circadian clock, its molecular genetics, physiology, and environmental implications, see Justin (2001), Hardin (2005), Kyriacou *et al.* (2008) and Emerson *et al.* (2009). Interestingly, multiple studies have found that genes in this pathway vary strongly clinally across latitude (see Costa *et al.* 1992, Sawyer *et al.* 1997, Sandrelli *et al*. 2007, Tauber *et al*. 2007, Turner *et al.* 2008, Kolaczkowski *et al.* 2011) and to be involved in the photoperiodic regulation of reproductive dormancy (ovarian diapause) in *D. melanogaster* (see Sandrelli *et al*. 2007, Tauber *et al*. 2007, Emerson *et al.* 2009, Schmidt 2011). Consistent with previous findings (Sandrelli *et al*. 2007, Tauber *et al*. 2007, Turner *et al.* 2008, Kolaczkowski *et al.* 2011) we find evidence for clinal variation at the *timeless*, *timeout* and *cryptrochrome* loci and we provide new evidence for potential clinal variation at the *clock* locus. However, in contrast to several previous studies (e.g., Costa *et al.* 1992, Sawyer *et al.* 1997, Turner et al. 2008), we do not observe significant clinal variation for *period* (*per*). However, this might be due to our rather stringent criteria for defining candidate SNPs/genes: the highest F_{ST} values for individual SNPs in *period* in our data are: (a) one SNP with F_{ST} = 0.32 (Pennsylvania-Maine), (b) 0.25 (Florida-Pennsylvania), and (c) 0.2 (Florida-Maine) – these are substantial F_{ST} values indicative of strong differentiation. However, although all three SNPs have significant FET *P*-values in our analysis, they are all above our FDR threshold; moreover, two of them (b, c) do not fulfill our 0.5% F_{ST} outlier threshold (cutoff: F_{ST} = 0.26 for b, and F_{ST} = 0.28 for c).

* Also found by Kolaczkowski *et al.* (2011)

[1] *Clk, clock*; no clinal variation found in this gene for the Australian cline (Weeks *et al.* 2006). See: http://flybase.org/reports/FBgn0023076.html

[2] *cry, cryptochrome*; affects circadian resetting and photosensitivity; found to be clinal by Kolaczkowski *et al*. (2011). For detailed information see: http://flybase.org/reports/FBgn0025680.html

[3] *tim, timeless*; found to be clinal by Sandrelli *et al*. (2007), Tauber *et al.* (2007), and Kolaczkowski *et al.* (2011); known to affect reproductive (ovarian) dormancy (Sandrelli *et al*. 2007, Tauber *et al.* 2007). For details see: http://flybase.org/reports/FBgn0014396.html

[4] *timeout,* also known as *timeless2*; involved in circadian photoreception (Benna *et al.* 2010); found to be clinal by Turner *et al.* (2008) and to harbor clinal copy number variation by Kolaczkowski *et al*. (2011). For details see: http://flybase.org/reports/FBgn0038118.html

References:

Benna C, Bonaccorsi S, Wülbeck C*, et al.* (2010) *Drosophila timeless2* Is Required for Chromosome Stability and Circadian Photoreception. *Current Biology* **20**, 346-352.

Costa R, Peixoto AA, Barbujani G, Kyriacou CP (1992) A Latitudinal Cline in a *Drosophila* Clock Gene. *Proceedings: Biological Sciences* **250**, 43-49.

Emerson KJ, Bradshaw WE, Holzapfel CM (2009) Complications of complexity: integrating environmental, genetic and hormonal control of insect diapause. *Trends in Genetics* **25**, 217-225.

Hardin PE (2005) The Circadian Timekeeping System of *Drosophila. Current Biology* **15**, R714-R722.

Justin B (2001) The *Drosophila* circadian clock: what we know and what we don't know. *Seminars in Cell and Developmental Biology* **12**, 287-293.

Kolaczkowski B, Kern AD, Holloway AK, Begun DJ (2011) Genomic Differentiation Between Temperate and Tropical Australian Populations of *Drosophila melanogaster*. *Genetics* **187**, 245-260.

Kyriacou CP, Peixoto AA, Sandrelli F, Costa R, Tauber E (2008) Clines in clock genes: fine-tuning circadian rhythms to the environment. *Trends in Genetics* **24**, 124-132.

Sandrelli F, Tauber E, Pegoraro M*, et al.* (2007) A molecular basis for natural selection at the *timeless* locus in *Drosophila melanogaster*. *Science* **316**, 1898-1900.

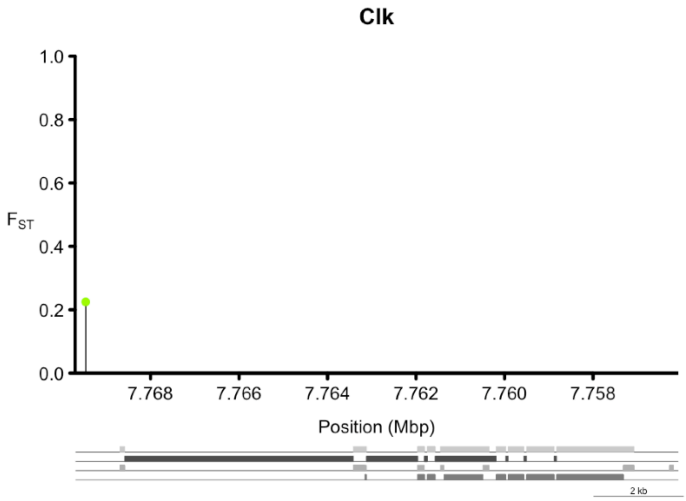
Sawyer LA, Hennessy JM, Peixoto AA*, et al.* (1997) Natural Variation in a *Drosophila* Clock Gene and Temperature Compensation. *Science* **278**, 2117-2120.

Schmidt PS (2011) Evolution and mechanisms of insect reproductive diapause: a plastic and pleiotropic life history syndrome. In Mechanisms of Life History Evolution (T Flatt, A Heyland, eds.). Pp. 230-242. Oxford: Oxford University Press.

Tauber E, Zordan M, Sandrelli F*, et al.* (2007) Natural selection favors a newly derived timeless allele in *Drosophila melanogaster*. *Science* **316**, 1895-1898.

Turner TL, Levine MT, Eckert ML, Begun DJ (2008) Genomic Analysis of Adaptive Differentiation in *Drosophila melanogaster*. *Genetics* **179**, 455-473.

Weeks AR, McKechnie SW, Hoffmann AA (2006) In search of clinal variation in the period and clock timing genes in Australian *Drosophila melanogaster* populations. *Journal of Evolutionary Biology* **19**, 551-557.


Clk
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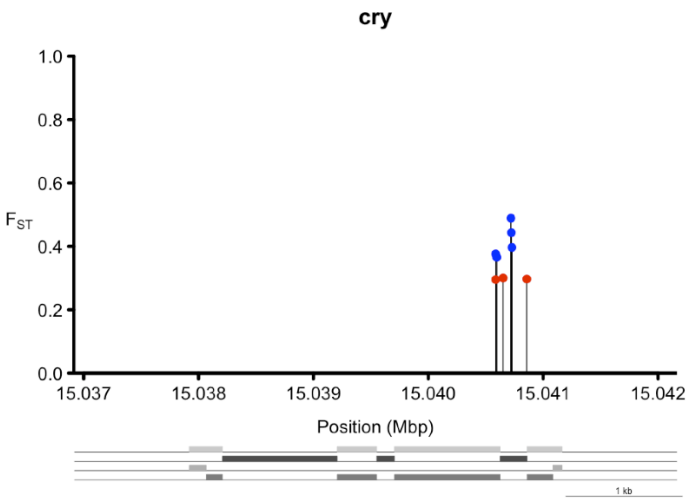
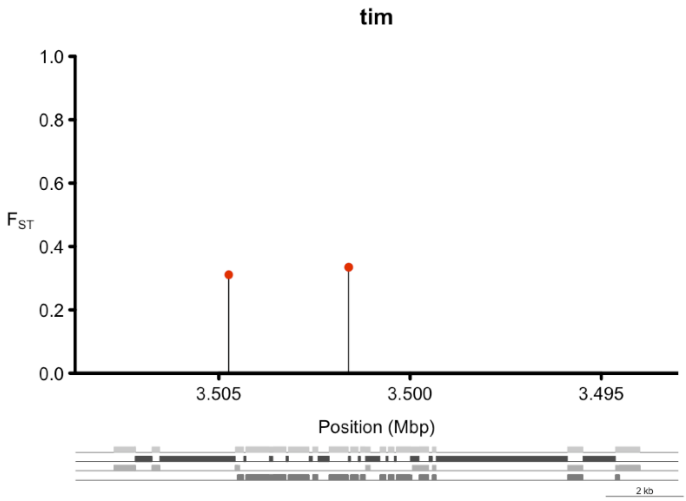
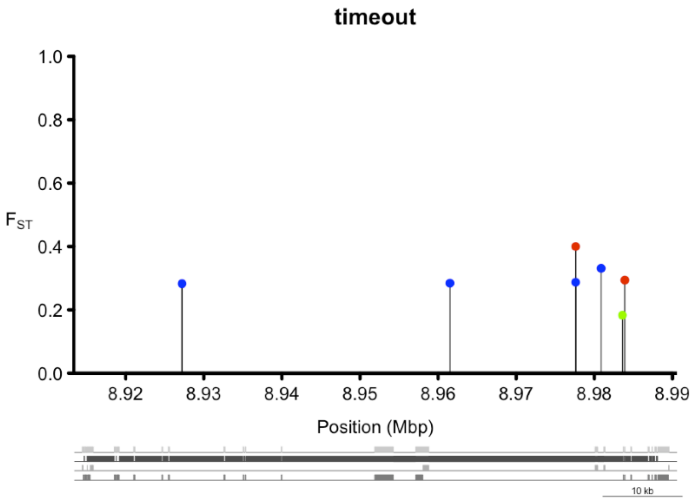
0.8
0.6
F_{ST}
0.4
0.2
0.0
7.768
7.766
7.764
7.762
7.760
7.758
Position (Mbp)
2 kb


cry
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
15.037
15.038
15.039
15.040
15.041
15.042
Position (Mbp)
1 kb


tim
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
3.505
3.500
3.495
Position (Mbp)
2 kb


timeout
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
8.92
8.93
8.94
8.95
8.96
8.97
8.98
8.99
Position (Mbp)
10 kb

Appendix S11

Examples of candidate genes involved in learning and memory[§]

dnc[1]

DopR[2]

drl[3]

Fas3[4]

for[5]

Nf1[6]

sra[7]

Shown are pairwise F_{ST} values of the candidate SNPs identified in the different population comparisons; red: Florida-Maine, blue: Florida-Pennsylvania, green: Pennsylvania-Maine.

Notes:

[§] For reviews of learning and memory in *Drosophila* see, for example, Keene and Waddell (2007) and Busto *et al.* (2010). The candidate genes shown below are all involved in associative odor learning and memory and typically expressed in the mushroom bodies (corpora peduncalata); some of them are also involved in locomotory behavior, determination of adult lifespan, synaptic transmission, and axon guidance. It is interesting to note that Kolaczkowski *et al.* (2011) find an enrichment of clinally varying candidate genes in the gene ontology (GO) category "mushroom body development".

[1] *dnc, dunce;* a famous gene involved in learning (Dudai *et al.* 1976); apart from learning deficiencies mutants also display sexual hyperactivity and reduced lifespan (Bellen *et al.* 1987). For further information see: http://flybase.org/reports/FBgn0000479.html

[2] *DopR* (= *dDA1*), *Dopamine receptor*; required in mushroom body neurons for aversive and appetitive learning (Kim *et al.* 2007). For further information see: http://flybase.org/reports/FBgn0011582.html

[3] *drl* (= *lio*), *derailed*; also known as *linotte*; mutants deficient in olfactory avoidance response (Dura *et al.* 1993). For further information see: http://flybase.org/reports/FBgn0015380.html

[4] *Fas3, Fasciclin 3*; learning and memory mutant reported by Dubnau *et al.* (2003; see their supplementary material). For further information see: http://flybase.org/reports/FBgn0000636.html

[5] *for, foraging;* a gene that encodes a cGMP-dependent protein kinase; known to harbor a naturally occuring behavioral polymorphism, the so-called sitter-rover larval polymorphism that affects larval foraging behavior (e.g., Osborne *et al.* 1997); interestingly, this polymorphism also affects learning and memory (Mery *et al.* 2007). Moreover, the *foraging* locus is involved in the physiological response to food deprivation and has been shown to interact with several components of IIS, including *InR* and *PI3K* (*Dp110*) (see App. S1) (Kent *et al.* 2009). Turner *et al.* (2008) also have identified this gene to vary clinally. See: http://flybase.org/reports/FBgn0000721.html

[6] *Nf1, Neurofibromin 1*; involved in learning (see Guo *et al.* 2000). For further information see: http://flybase.org/reports/FBgn0015269.html

[7] *sra* (= *nla*)*, sarah*; involved in *Drosophila* learning, with the human homolog being involved in Down syndrome (see Chang *et al.* 2003). For further information see: http://flybase.org/reports/FBgn0086370.html

References:

Bellen HJ, Kiger JA (1987) Sexual hyperactivity and reduced longevity of dunce females of *Drosophila melanogaster*. *Genetics* **115**, 153-160.

Busto GU, Cervantes-Sandoval I, Davis RL (2010) Olfactory Learning in *Drosophila*. *Physiology* **25**, 338-346.

Chang KT, Shi Y-J, Min K-T (2003) The *Drosophila* homolog of Down's syndrome critical region 1 gene regulates learning: Implications for mental retardation. *Proceedings of the National Academy of Sciences* **100**, 15794-15799.

Dubnau J, Chiang A-S, Grady L*, et al.* (2003) The staufen/pumilio Pathway Is Involved in *Drosophila* Long-Term Memory. *Current Biology* **13**, 286-296.

Dudai Y, Jan YN, Byers D, Quinn WG, Benzer S (1976) dunce, a mutant of *Drosophila* deficient in learning. *Proceedings of the National Academy of Sciences* **73**, 1684.

Dura JM, Preat T, Tully T (1993) Identification of linotte, a new gene affecting learning and memory in *Drosophila melanogaster*. *Journal of neurogenetics* **9**, 1-14.

Guo H-F, Tong J, Hannan F, Luo L, Zhong Y (2000) A neurofibromatosis-1-regulated pathway is required for learning in *Drosophila*. *Nature* **403**, 895-898.

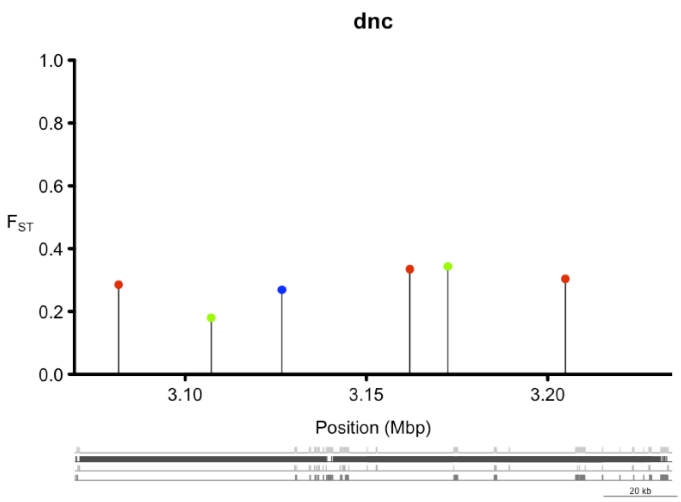
Keene AC, Waddell S (2007) *Drosophila* olfactory memory: single genes to complex neural circuits. *Nature Reviews Neuroscience* **8**, 341-354.

Kent CF, Daskalchuk T, Cook L, Sokolowski MB, Greenspan RJ (2009) The *Drosophila* foraging gene mediates adult plasticity and gene-environment interactions in behaviour, metabolites, and gene expression in response to food deprivation. *PLoS Genetics* **5**, e1000609.

Kim Y-C, Lee H-G, Han K-A (2007) D1 Dopamine Receptor dDA1 Is Required in the Mushroom Body Neurons for Aversive and Appetitive Learning in *Drosophila*. *The Journal of Neuroscience* **27**, 7640-7647.

Mery F, Belay AT, So AK-C, Sokolowski MB, Kawecki TJ (2007) Natural polymorphism affecting learning and memory in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **104**, 13051-13055.

Osborne KA, Robichon A, Burgess E*, et al.* (1997) Natural Behavior Polymorphism Due to a cGMP-Dependent Protein Kinase of *Drosophila*. *Science* **277**, 834-836.


dnc
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0.6
F_{ST}
0.4
0.2
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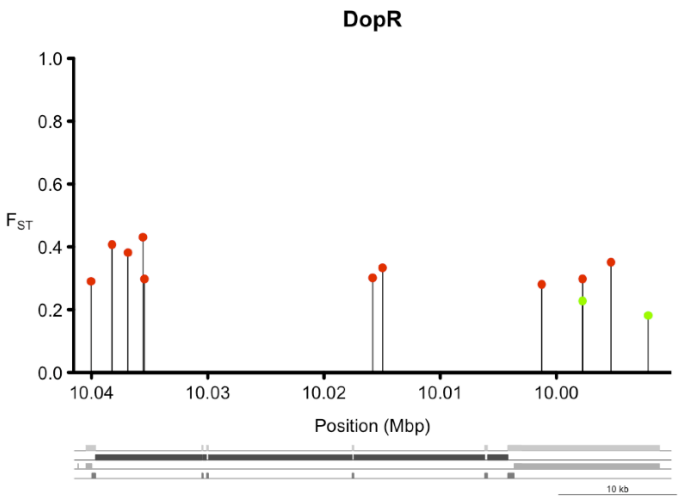
3.20
Position (Mbp)
20 kb


DopR
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0.6
F_{ST}
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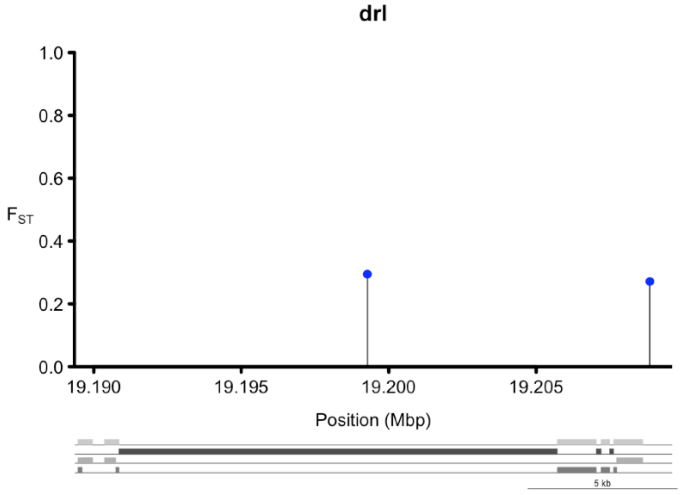
0.2
0.0
10.04
10.03
10.02
10.01
10.00
Position (Mbp)
10 kb


drl
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F_{ST}
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0.2
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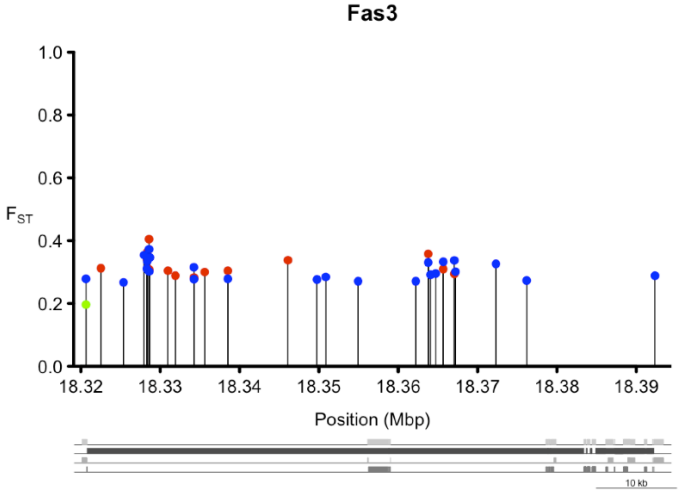
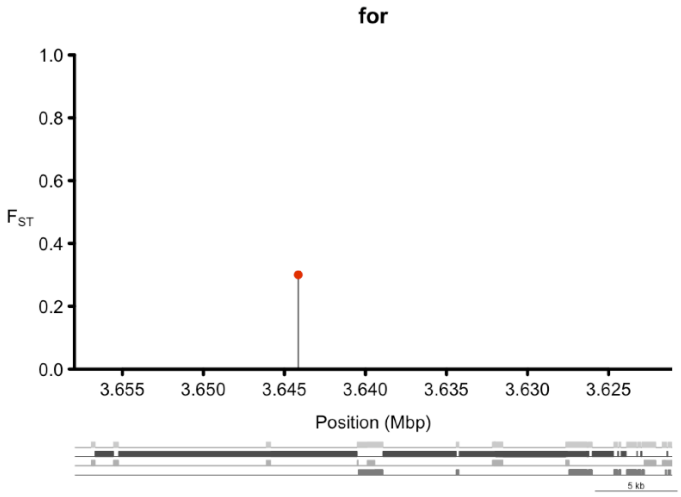
19.195
19.200
19.205
Position (Mbp)
5 kb


Fas3
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
18.32
18.33
18.34
18.35
18.36
18.37
18.38
18.39
Position (Mbp)
10 kb


for
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
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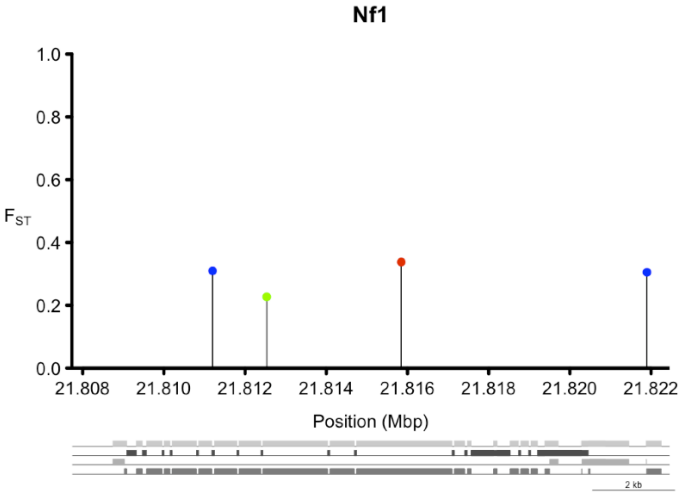
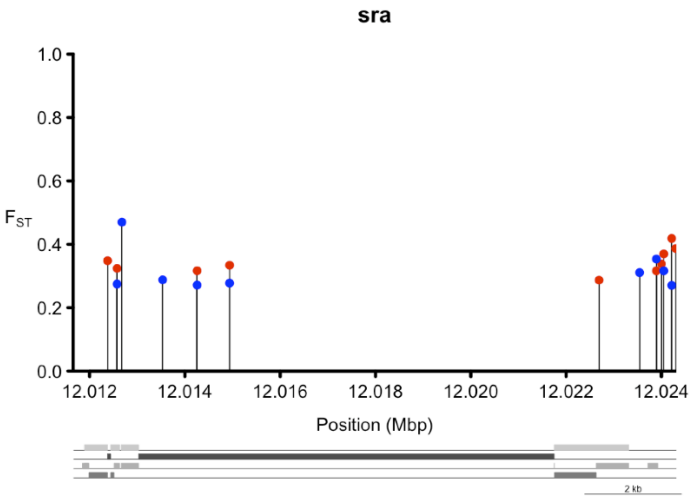
3.650
3.645
3.640
3.635
3.630
3.625
Position (Mbp)
5 kb


Nf1
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
21.808
21.810
21.812
21.814
21.816
21.818
21.820
21.822
Position (Mbp)
2 kb


sra
1.0
0.8
0.6
F_{ST}
0.4
0.2
0.0
12.012
12.014
12.016
12.018
12.020
12.022
12.024
Position (Mbp)
2 kb

Appendix S12

Examples of some transcription factor candidate genes

*14-3-3 epsilon**[1]

abd-A[2]

Abd-B[3]

bab1[4]

*E(Pc)**[5]

fru[6]

*Ino80**[7]

*Sfmbt**[8]

Ubx[9]

Shown are pairwise F_{ST} values of the candidate SNPs identified in the different population comparisons; red: Florida-Maine, blue: Florida-Pennsylvania, green: Pennsylvania-Maine.

Notes:

* Also found by Kolaczkowski *et al.* (2011)

[1] *14-3-3 epsilon*; involved in IIS by interacting with *Foxo* (see App. S1); found to be clinal by Kolaczkowski *et al.* (2011); for further information see: http://flybase.org/reports/FBgn0020238.html

[2] *abd-A, Abdominal A*; a major Hox gene. For further details see: http://flybase.org/reports/FBgn0000014.html

[3] *Abd-B, Abdominal B*; a major Hox gene. For further details see: http://flybase.org/reports/FBgn0000015.html

[4] *bab1, bric a brac 1*; important in multiple developmental processes, including gonad development. See: http://flybase.org/reports/FBgn0004870.html

[5] *E(Pc), Enhancer of Polycomb*; found to be clinal by Kolaczkowski *et al.* (2011). See: http://flybase.org/reports/FBgn0000581.html

[6] *fru, fruitless*; important gene in the regulation of sex-specific (male) courtship behavior. For details see: http://flybase.org/reports/FBgn0004652.html

[7] *Ino80;* found to be clinal by Kolaczkowski *et al.* (2011). For details see: http://flybase.org/reports/FBgn0086613.html

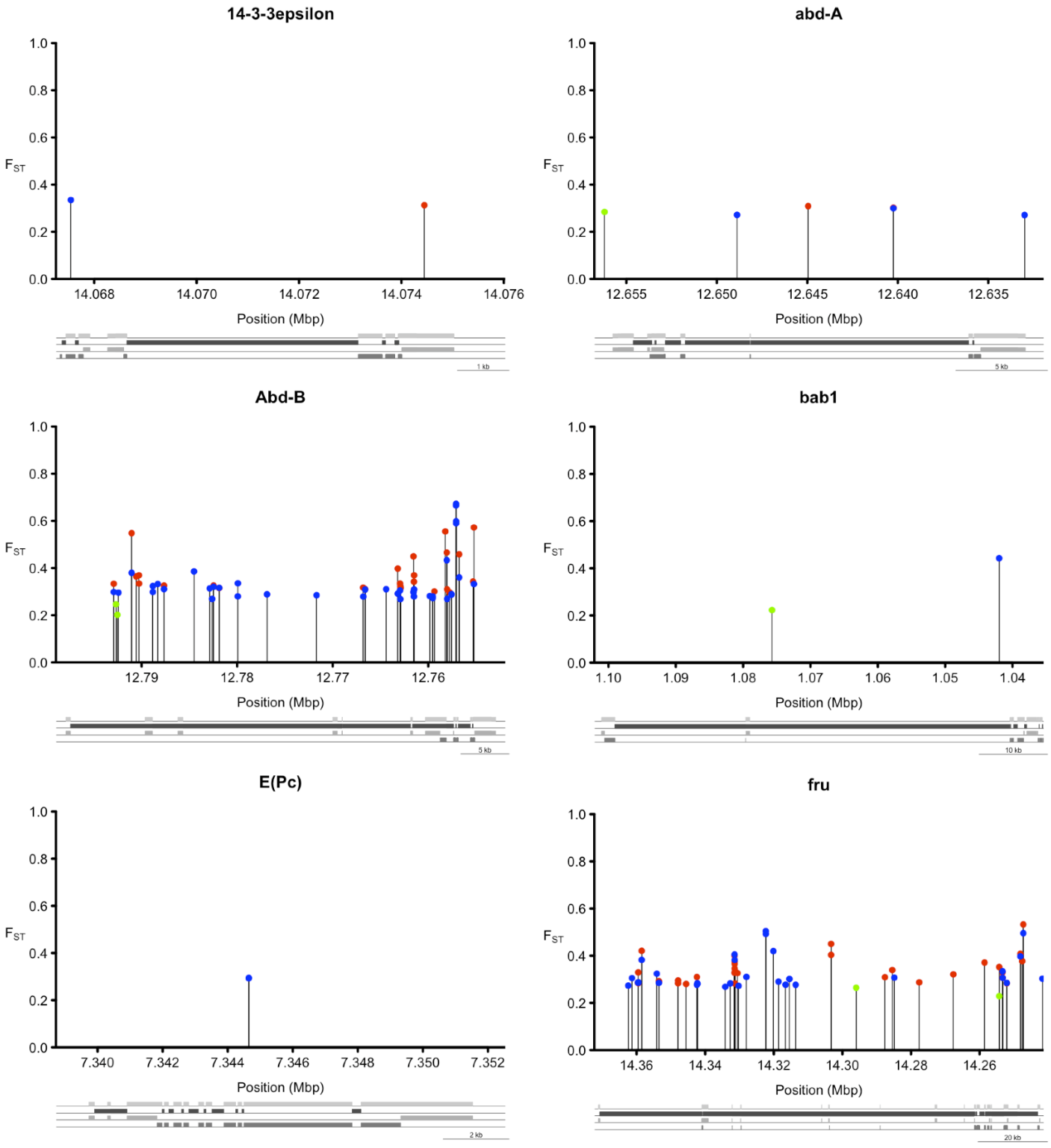
[8] *Sfmbt, Scm-related gene containing four mbt domains*; a chromatin-binding protein involved in gene regulation; a polycomb group repressor (Grimm *et al*. 2009); found to vary clinally by Kolaczkowski *et al.* (2011). For detailed information see: http://flybase.org/reports/FBgn0032475.html

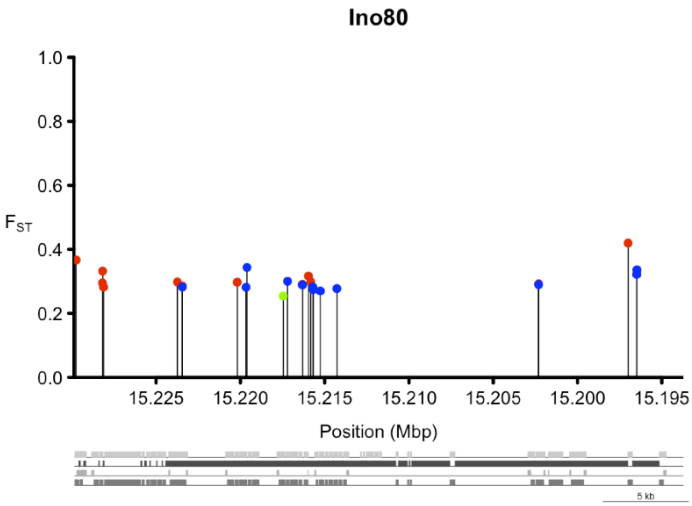
[9] *Ubx, Ultrabithorax*; a major Hox gene. For further details see: http://flybase.org/reports/FBgn0003944.html

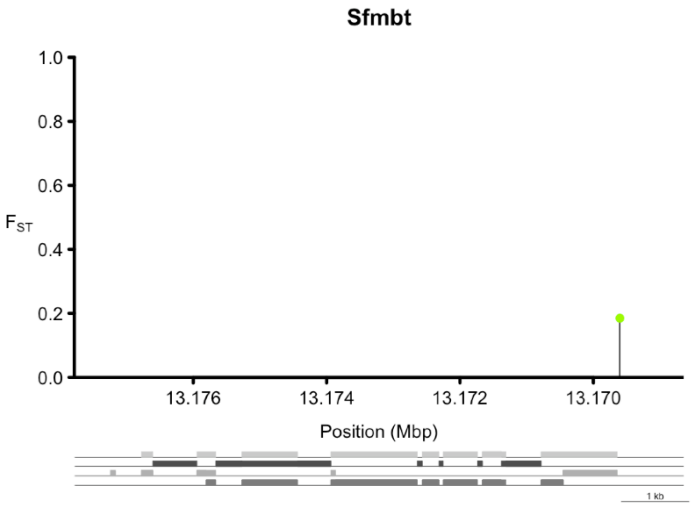
References:

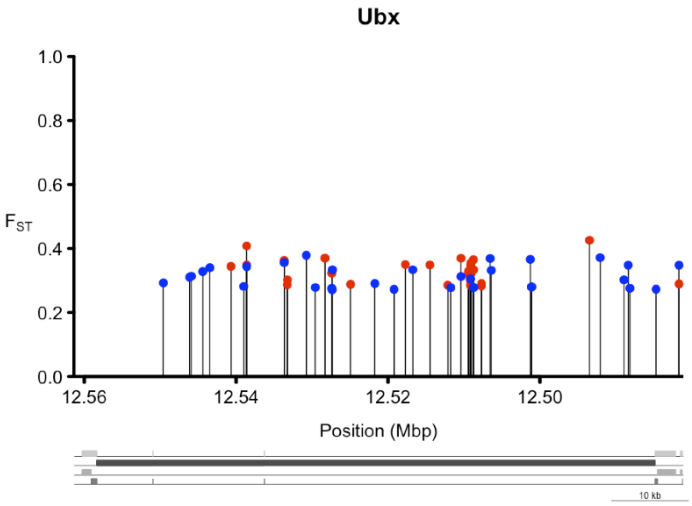
Grimm C, Matos R, Ly-Hartig N*, et al.* (2009) Molecular recognition of histone lysine methylation by the Polycomb group repressor dSfmbt. *EMBO Journal* **28**, 1965-1977.

Kolaczkowski B, Kern AD, Holloway AK, Begun DJ (2011) Genomic Differentiation Between Temperate and Tropical Australian Populations of *Drosophila melanogaster*. *Genetics* **187**, 245-260.


14-3-3epsilon
abd-A
Abd-B
bab1
E(Pc)
fru
F_{ST}
Position (Mbp)


Ino80
1.0
0.8
0.6
0.4
0.2
0.0
F_{ST}
15.225
15.220
15.215
15.210
15.205
15.200
15.195
Position (Mbp)
5 kb


Sfmbt
1.0
0.8
0.6
0.4
0.2
0.0
F_{ST}
13.176
13.174
13.172
13.170
Position (Mbp)
1 kb


Ubx
1.0
0.8
0.6
0.4
0.2
0.0
F_{ST}
12.56
12.54
12.52
12.50
Position (Mbp)
10 kb

Please see the separate folder with individual files:

Appendix S13 (folder with word files and Python scripts) Folder containing a description of our bioinformatic analysis pipeline, including Python scripts.

Please see the separate folder with individual files:

Appendix S14 (folder with individual pdf files) Folder containing clinal candidate gene list and plots for each clinal ("plus_plus") candidate gene, showing the changes in allele frequency for the candidate gene as a function of latitude.