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 $\pi$. Average $\pi$ (average across SNP-wise $\pi$ 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 $\pi$, 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 $\pi$ we therefore applied a Kruskal-Wallis rank sum test on average $\pi$ values estimated in non-overlapping 200 kb windows across the entire genome. Populations differed significantly in $\pi$, 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).

| Chromosome | FLORIDA$^A$ | PENNSYLVANIA$^A$ | MAINE$^A$ |
|------------|-------------|------------------|------------|
|            | $\pi$       | SE               | $\pi$      | SE          | $\pi$      | SE          |
| X$^A$      | 0.0041      | 9.3x10$^{-6}$    | 0.0039     | 9.1x10$^{-6}$| 0.0039     | 9.2x10$^{-6}$|
| 2L$^B$     | 0.0075      | 1.2x10$^{-5}$    | 0.0071     | 1.2x10$^{-5}$| 0.0070     | 1.2x10$^{-5}$|
| 2R$^C$     | 0.0062      | 1.2x10$^{-5}$    | 0.0060     | 1.1x10$^{-5}$| 0.0062     | 1.2x10$^{-5}$|
| 3L$^C$     | 0.0066      | 1.1x10$^{-5}$    | 0.0060     | 1.1x10$^{-5}$| 0.0060     | 1.1x10$^{-5}$|
| 3R$^D$     | 0.0059      | 9.6x10$^{-6}$    | 0.0050     | 8.8x10$^{-6}$| 0.0050     | 8.9x10$^{-6}$|
Table S2  Average scaled population mutation rate $\theta_W$. Average $\theta_W$ (average across SNP-wise $\theta_W$ 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 $\theta_W$, 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 $\theta_W$ we therefore applied a Kruskal-Wallis rank sum test on average $\theta_W$ values estimated in non-overlapping 200 kb windows across the entire genome. Populations differed significantly in $\theta_W$, 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).

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

| Chromosome | Florida - Maine$^A$ | Florida - Pennsylvania$^A$ | Pennsylvania - Maine$^B$ |
|------------|--------------------|---------------------------|-------------------------|
| X$^A$      | 0.0380 8.4x10$^{-5}$ | 0.0381 8.3x10$^{-5}$     | 0.0307 8.0x10$^{-5}$    |
| 2L$^A$     | 0.0430 6.8x10$^{-5}$ | 0.0401 6.1x10$^{-5}$     | 0.0248 4.5x10$^{-5}$    |
| 2R$^A$     | 0.0370 6.4x10$^{-5}$ | 0.0365 6.2x10$^{-5}$     | 0.0253 5.3x10$^{-5}$    |
| 3L$^A$     | 0.0404 6.6x10$^{-5}$ | 0.0393 6.3x10$^{-5}$     | 0.0262 5.0x10$^{-5}$    |
| 3R$^A$     | 0.0633 1.0x10$^{-4}$ | 0.0606 9.8x10$^{-5}$     | 0.0258 4.8x10$^{-5}$    |
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.

| Inversion | Florida – Maine | Florida - Pennsylvania | Pennsylvania - Maine |
|-----------|-----------------|------------------------|----------------------|
|           | $F_{ST}$ in     | SE                     | $F_{ST}$ out        | SE                     | $F_{ST}$ in     | SE                     | $F_{ST}$ out        | SE                     |
| $In(2L)t$ | 0.0448          | 2.0x10^{-4}            | 0.0484              | 2.9x10^{-4}            | 0.0406          | 1.8x10^{-4}            | 0.0441              | 2.4x10^{-4}            | 0.0263          | 1.3x10^{-4}            | 0.0251              | 1.5x10^{-4}            |
| $In(2R)NS$| 0.0368          | 2.5x10^{-4}            | 0.0394              | 1.9x10^{-4}            | 0.0359          | 2.3x10^{-4}            | 0.0384              | 1.7x10^{-4}            | 0.0257          | 2.0x10^{-4}            | 0.0261              | 1.3x10^{-4}            |
| $In(3L)P$ | 0.0426          | 2.0x10^{-4}            | 0.0462              | 3.4x10^{-4}            | 0.0417          | 1.9x10^{-4}            | 0.0441              | 3.1x10^{-4}            | 0.0274          | 1.3x10^{-4}            | 0.0271              | 1.9x10^{-4}            |
| $In(3R)P$ | 0.0880          | 4.8x10^{-4}            | 0.0623              | 2.9x10^{-4}            | 0.0841          | 4.7x10^{-4}            | 0.0609              | 2.9x10^{-4}            | 0.0280          | 1.9x10^{-4}            | 0.0278              | 1.2x10^{-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 | 0.45 | 0.70 |
| In(2R)NS  | 0.24 | 0.20 | 0.19 |
| In(3L)P   | 0.84 | 0.82 | 0.79 |
| In(3R)P   | 0.67 | 0.68 | 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 $\theta_W$. Average $\theta_W$ 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 -log10(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
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
amon²
foxo³
Hmgcr⁴
Ilp³⁵
Ilp⁴⁵
Imp⁶
InR⁷
Pi3K59F⁸
Pi3K92E⁹
tobi¹⁰
Tor¹¹
Tsc¹¹

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 (hydroxymethylglutaryl) 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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Rayburn LYM, Gooding HC, Choksi SP, et al. (2003) amontillado, the Drosophila Homolog of the Prohormone Processing Protease PC2, is Required During Embryogenesis and Early Larval Development. Genetics 163, 227-237.

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Appendix S2

Examples of candidate genes involved in ecdysone biosynthesis and signaling

- cpo
- dib
- dre4
- Eig71Eb
- Eig71Ec
- Eip63E
- Eip74EF
- Eip75B
- Eip93F
- Hr46
- Samuel
- sec10
- svp
- woc

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-hydroxyecdysone (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; nuclear 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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Examples of candidate genes involved in nuclear hormone receptor (NHR) signaling and other endocrine pathways

ETHR
GRHR
Hr46
Hr96
svp

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
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ETHR

GRHR

Hr46

Hr96

svp
Appendix S4

Examples of candidate genes involved in lipid metabolism

\[ \text{Lip}2^1 \]
\[ \text{Lip}3^2 \]
\[ \text{Lsd-1}^3 \]
\[ \text{Lsd-2}^4 \]
\[ \text{Lsp1alpha}^5 \]

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:

$^5$ 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](http://flybase.org/reports/FBgn0024740.html)

$^2$ *Lip3, Lipase 3*; triglyceride lipase activity; for detailed information see: [http://flybase.org/reports/FBgn0023495.html](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](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](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 metamorphosis (Burmester *et al.* 1999, Beller *et al.* 2006). For further information see: [http://flybase.org/reports/FBgn0002562.html](http://flybase.org/reports/FBgn0002562.html)
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Appendix S5

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

\begin{align*}
&\text{AttA}^1 \\
&\text{AttB}^2 \\
&\text{Dif}^3 \\
&\text{Dpt}^4 \\
&\text{DptB}^5 \\
&\text{Dro}^6 \\
&\text{imd}^7 \\
&\text{Irc}^8 \\
&\text{PGRP-LA}^9 \\
&\text{PGRP-LC}^{10} \\
&\text{PGRP-LF}^{11} \\
&\text{sick}^*^{12} \\
&\text{Tl}^3 \\
&\text{Toll-4}^{14} \\
&\text{Tollo}^{15} \\
&\text{TotF}^{16}
\end{align*}

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 \textit{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 \textit{et al.} 2006; Lazzaro 2008; Obbard \textit{et al.} 2009). Both Turner \textit{et al.} (2008) and Kolaczkowski \textit{et al.} (2011) find an overrepresentation of genes involved in innate immune response ("defense responses") in their analyses of clinal variation in \textit{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 \textit{Drosophila} is hormonally regulated by ecdysone signaling (Flatt \textit{et al.} 2008) (see App. S2) and IIS/Foxo (Becker \textit{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](http://flybase.org/reports/FBgn0012042.html)

2. **AttB, Attacin B;** encodes an antimicrobial peptide; for more information see [http://flybase.org/reports/FBgn0041581.html](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](http://flybase.org/reports/FBgn0011274.html)

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

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

6. **Dro, Drosocin;** encodes an antimicrobial peptide; see [http://flybase.org/reports/FBgn0010388.html](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](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](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/FBgn0035975.html); [http://flybase.org/reports/FBgn0035976.html](http://flybase.org/reports/FBgn0035976.html); [http://flybase.org/reports/FBgn0035977.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](http://flybase.org/reports/FBgn0263873.html)

13. **Ti, 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](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](http://flybase.org/reports/FBgn0032095.html)

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

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Appendix S6

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

$ed^*$

$EGFR^2$

$S^3$

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:

$\S$ 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 defining 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

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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.
Examples of candidate genes involved in JAK/STAT signaling[^5]

- *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:**

[^5]: 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.

[^1]: Also found by Kolaczkowski *et al.* (2011)

[^2]: *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](http://flybase.org/reports/FBgn0259685.html)

[^3]: *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](http://flybase.org/reports/FBgn0010382.html)

[^4]: *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](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

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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:**

\(^5\) 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 Miyazono 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* (decapentaplegic) and *Mad* (mothers against dpp); see: [http://flybase.org/reports/FBgn0020493.html](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 clinically. See: [http://flybase.org/reports/FBgn0263930.html](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 clinically. For details see: [http://flybase.org/reports/FBgn0000490.html](http://flybase.org/reports/FBgn0000490.html)

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

**References:**

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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

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

¹ 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 *cryptochrome* 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](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](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 *timeless*2; 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

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Appendix S11

Examples of candidate genes involved in learning and memory

\[ \text{dnc}^4 \]
\[ \text{DopR}^2 \]
\[ \text{drl}^3 \]
\[ \text{Fas3}^4 \]
\[ \text{for}^5 \]
\[ \text{Nf1}^6 \]
\[ \text{sra}^7 \]

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:

\[ ^5 \text{For reviews of learning and memory in Drosophila see, for example, Keene and Waddell (2007) and Busto et al. (2010). The candidate genes shown below are all involved in associative odor learning and memory and typically expressed in the mushroom bodies (corpora peduncalata); some of them are also involved in locomotory behavior, determination of adult lifespan, synaptic transmission, and axon guidance. It is interesting to note that Kolaczkowski et al. (2011) find an enrichment of clinally varying candidate genes in the gene ontology (GO) category "mushroom body development".} \]

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

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

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

\[ ^4 \text{Fas3, Fasciclin 3; learning and memory mutant reported by Dubnau et al. (2003; see their supplementary material). For further information see:} \]
\[ \text{http://flybase.org/reports/FBgn0000636.html} \]
for, foraging; a gene that encodes a cGMP-dependent protein kinase; known to harbor a naturally occurring 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 clinically. See: http://flybase.org/reports/FBgn0000721.html

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

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

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Appendix S12

Examples of some transcription factor candidate genes

14-3-3 epsilon*

abd-A

Abd-B

bab

E(Pc)*

fru

Ino80*

Sfmbt*

Ubx

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
Starting text...
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.