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Evidence of Spatially Varying Selection Acting on Four Chromatin-Remodeling Loci in *Drosophila melanogaster*

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ABSTRACT

The packaging of DNA into proper chromatin structure contributes to transcriptional regulation. This packaging is environment sensitive, yet its role in adaptation to novel environmental conditions is completely unknown. We set out to identify candidate chromatin-remodeling loci that are differentiated between tropical and temperate populations in *Drosophila melanogaster*, an ancestrally equatorial African species that has recently colonized temperate environments around the world. Here we describe sequence variation at seven such chromatin-remodeling loci, four of which (*chd1*, *ssrp*, *chm*, and *glu*) exhibit strong differentiation between tropical and temperate populations. An in-depth analysis of *chm* revealed sequence differentiation restricted to a small portion of the gene, as well as evidence of clinal variation along the east coasts of both the United States and Australia. The functions of *chd1*, *chm*, *ssrp*, and *glu* point to several novel hypotheses for the role of chromatin-based transcriptional regulation in adaptation to a novel environment. Specifically, both stress-induced transcription and developmental homeostasis emerge as potential functional targets of environment-dependent selection.

THE level, timing, and spatial distribution of gene expression vary both within and between species (Oleksiak *et al.* 2002; Meiklejohn *et al.* 2003; Rifkin *et al.* 2003; Nuzhdin *et al.* 2004; Gilad *et al.* 2006). Recent work on the evolution of gene expression has focused largely on local regulatory elements (*cis*-regulation; Gompel *et al.* 2005; Wittkopp 2006) or on the expression/activity of proteins that interact with such sequences (*trans*-regulation; Wittkopp *et al.* 2004; Wang *et al.* 2007). These DNA-transcription factor interactions, however, compose only one component of gene regulation. The packaging of DNA into proper chromatin structure provides an additional level of transcriptional regulation (Elgin and Workman 2001). For example, variation in chromatin state (*i.e.*, permissive or restrictive) may affect access of *cis*-regulatory sequence by transcription factors. Recent chromatin research, buttressed by new tools and key insights, is rapidly revealing the complex and elegant mechanisms of chromatin remodeling and their transcriptional consequences (Allis *et al.* 2007). Evolutionary biologists now have the unprecedented opportunity to explore how this fundamental component of gene regulation contributes to adaptation.

The packaging of DNA into chromatin is environment sensitive. Early Drosophila work demonstrated the temperature sensitivity of two classic chromatin-related phenotypes—position-effect variegation (PEV) (Gowen 1933) and polytene chromosome "puff" induction (Ashburner 1967). More recent studies demonstrate that silencing maintained by the Polycomb group genes (PcGs), which remodel chromatin at developmentally important loci to maintain developmental trajectories (Lewis 1978; Schwartz and Pirrotta 2007), increases with increasing temperature (Fauvarque and Dura 1993). Moreover, stress induces global histone hypoacetylation that results in a coordinated downregulation of transcription (Berthiaume *et al.* 2006), particularly at components of the translation apparatus (Causton *et al.* 2001). The promoters of stress response genes, in contrast, experience chromatin remodeling that results in transcriptional activation (yeast: Zhao *et al.* 2005; Uffenbeck and Krebs 2006; Drosophila: Leibovitch *et al.* 2002). Finally, stress responders such as heat-shock proteins (which are generalized environmental-response molecules) co-immunoprecipitate with such known chromatin-remodeling complexes as TAC1 (Smith *et al.* 2004) and the PcG complex, PRC1 (Wang and Brock 2003). Given that chromatin dynamics are environment sensitive, the evolution of proteins that remodel chromatin may contribute to adaptation to novel habitats.

Tolerance of environmental stresses varies both within and between species. The low-latitude species *Drosophila virilis*, for example, maintains both elevated thermotolerance and an elevated heat-shock protein (hsp) induction threshold relative to the closely related, high-latitude species, *D. lummei* (Garbuz *et al.* 2003). Similar variation in environmental tolerance occurs within

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species. For example, *D. melanogaster*, an ancestrally equatorial African species, has recently adapted to temperate environments (Lachaise *et al.* 1988). Krebs and Feder (1997) document natural variation in thermotolerance and hsp70 induction thresholds in *D. melanogaster* populations found in the midwestern United States (Krebs and Feder 1997). The association of genetic differentiation and environmental tolerance is perhaps most obvious in the context of variation along latitudinal clines, one of the most thoroughly documented cases of natural variation associated with habitat heterogeneity at both the genotypic (allozyme: Knibb *et al.* 1981; DNA: Verrelli and Eanes 2001 and Sezgin *et al.* 2004; inversions: Knibb *et al.* 1981) and phenotypic levels. Phenotypic clinal variation includes variation in tolerance of several environmental conditions, such as temperature tolerance (Hoffmann *et al.* 2002), starvation resistance (Karan *et al.* 1998), and dehydration resistance (Karan *et al.* 1998; but see Hoffmann *et al.* 2001). This clinal variation provides an opportunity to identify chromatin-regulated biological processes and the associated chromatin machinery, which may underlie phenotypic differences between populations experiencing different environmental stresses.

To search for candidate, clinally varying loci that function in chromatin/histone remodeling (hereafter, "chromatin remodeling"), we took advantage of a whole-genome tiling array analysis that used DNA hybridization patterns to describe, on a genomic scale, candidate regions of differentiation among tropical and temperate populations of *D. melanogaster* from multiple continents (Turner *et al.* 2008, accompanying article in this issue). Here we describe DNA sequence variation in tropical and temperate population samples from Australia for a set of chromatin-remodeling genes that exhibited tropical–temperate differences in hybridization patterns on tiling arrays. We explore one candidate locus, the PcG *chameau* (*chm*), in greater depth to delineate the physical extent of tropical–temperate differentiation and to test for clinal variation in both Australia and the United States. Our results raise several new hypotheses about how chromatin dynamics may contribute to adaptation.

MATERIALS AND METHODS

Generating a list of candidate tropical–temperate differentiated chromatin genes: Here we briefly describe the tiling array experiment, details for which can be found in Turner *et al.* (2008, accompanying article). Female flies derived from isofemale lines from two northern Australian populations (Queensland) were pooled. DNA was extracted, fragmented, and hybridized to four replicate Affymetrix tilling arrays. The procedure was repeated for population samples from southern Australia (Tasmania) and for U. S. population samples from Maine and Florida (latitudes of population samples can be found in supplemental Table S1). Following normalization and *t*-tests, each array feature was assigned a *q*-value (false discovery rate). To generate windows of significant differentiation, the average *q*-value was calculated along each chromosome arm in windows of 20, 50, and 100 probes (~800, 2000, 4000 bp, respectively), moving 10% of the window size at each measurement. Significance thresholds were determined by permutation test. Finally, the union of significant windows among all window sizes was determined. The resulting "regions of differentiation" spanned, on average, one to two genes (Turner *et al.* 2008). Using the FlyBase gene summaries (http://www.flybase.org), a list of chromatin-associated genes located within significant windows (supplemental Table S2) was compiled. Only windows that overlapped two or fewer genes, and for which the focal chromatin gene was located near the midpoint of the window, were considered further (but see below for one exception). This reduced set of windows contained eight chromatin genes. To provide finer-scale analysis of differentiation on the basis of the array data, a 20-probe (~800 bp) sliding-window analysis was conducted to determine if variation in putative differentiation varied across the window, and if so, whether the focal chromatin gene overlapped the most differentiated region (data not shown). The window containing the chromatin gene *Bj1* and its neighbor, CG33993, exhibited hybridization differences restricted largely to gene CG33993 (data not shown). Therefore, *Bj1* was not considered for further sequence analysis. Table 1 shows the final set of seven candidates. The U. S. window containing *chm* contained three genes but was retained because it partially overlapped the Australian window.

Sequencing-based validation of candidate windows: The goal of the DNA sequencing analysis was to document the magnitude and physical extent of genetic differentiation between tropical and temperate populations for the seven candidate genes. The differentiated regions detected in the tiling array analysis could be due to a small, highly differentiated region of DNA or to a larger, less dramatically differentiated region. Moreover, some regions, especially those at the less conservative significance thresholds (*i.e.*, $P = 0.05$), may be false discoveries. Strong tropical–temperate sequence differentiation over a small physical scale that includes a candidate gene, however, would support the hypothesis that the gene is influenced by spatially varying selection.

For the single-gene windows, three regions were amplified: a single 1-kb region inside the window, and two 1-kb regions outside (one 5′, one 3′ of the window). If the window contained two genes, four 1-kb regions were amplified—two within the window and two outside the window. Sequence data were collected from 16 tropical (~17° latitude, two populations) and 16 temperate (~45° latitude, two populations) Australian isofemale lines. For *chm* only, we also sequenced 16 (sub)tropical (~25° latitude) and 16 temperate (~45° latitude) U. S. isofemale lines (supplemental Table S1). Multi-fly DNA preps were PCR amplified, cloned (TOPO TA kit, Invitrogen, San Diego), and sequenced with M13 primers. Rare errors introduced by Taq polymerase will not affect conclusions regarding geographic differentiation. Primer pairs used to generate all sequence data can be found in supplemental Table S4. Sequences have been submitted to GenBank under accession nos. EU414997–EU416170.

In-depth investigation of the *chm* locus—extent/magnitude of (sub)tropical–temperate differentiation, clinal variation, and linkage to *In(2L)t*: As we describe below (results), the *chm* locus was differentiated in both the United States and Australia. We further investigated patterns of allele-frequency variation at *chm* by additional sequencing both inside and outside the gene region to determine more precisely the extent and magnitude of tropical and temperate sequence differentiation. We sequenced the same 64 isofemale lines described

TABLE 1

Culled list of significantly differentiated windows from hybridization experiment and associated chromatin-remodeling genes

Chromosome	Window coordinates	Window size	Continent	Gene
X	20,213,337–20,218,718	5381	Australia	*HERC2*
X	1,738,978–1,742,532	3554	Australia	*trr*
2L	16,731,317–16,737,494	6177	Australia	*glu*
3L	19,816,835–19,818,616	1781	Australia	*Mi-2*
2L	2,982,496–2,986,379	3883	Australia	*chd1*
2L	7,410,897–7,416,684	5787	Australia	*chm*
2L	7,408,650–7,416,684	8034	United States	*chm*
2R	19,311,720–19,315,687	3967	Australia	*ssrp*

Coordinates were obtained from *D. melanogaster* reference genome release 4.3.

above (32 from Australia, 32 from the United States). Within the *chm* gene region, we amplified a total of five regions that each spanned between 1 and 1.3 kb and covered all eight exons. Outside *chm*, we sequenced a single 1-kb region 8 kb downstream in both the United States and Australia. We also sequenced two 1-kb regions 8 and 12 kb upstream of *chm* in the U. S. samples. In Australia, where upstream differentiation was more variable, we sequenced four 1-kb regions 4, 8, 12, and 17 kb 5′ of *chm* (see Figure 1). Because we sequenced cloned products from isofemale lines, we were unable to assess linkage disequilibrium among sites on different amplicons. Finally, one of the two significantly differentiated replacement sites shared among Australia and the United States (arginine/praline; see RESULTS) was assayed in 10 *D. melanogaster* alleles from Zimbabwe (Begun and Aquadro 1993).

We also assessed clinal variation at *chm* by measuring allele-frequency variation in additional populations at several intermediate latitudes (with respect to the tropical and temperate samples, see supplemental Table S1) in the United States and Australia. A single clinal SNP was assayed from nine Australia populations that span 15°–45° latitude and seven U. S. populations that span 25°–45° latitude (names, latitudes, and sample sizes are listed in supplemental Table S1). To genotype the target SNP from clinal samples, a single male from each isofemale line was crossed to flies from the *y;cn,bw;sp* stock corresponding to the reference genome. A single offspring from each cross was assayed for the state of the target variant inherited from its father by PCR–restriction fragment length polymorphism (RFLP). The most significantly differentiated site in Australia that could be assayed by RFLP analysis was not the most significantly differentiated site in the United States that could be assayed with this method; therefore a different SNP was assayed on each continent. The Australian SNP (2L: 7,413,926, v. 4.3; $F_{ST} = 0.36$), a silent site in the coding region of *chm*, was assayed using *Hga*I (New England Biolabs, Ipswich, MA). The U. S. SNP (2L: 7,413,468, v. 4.3; $F_{ST} = 0.20$), also a silent site in the coding region of *chm*, was assayed using *Bst*UI (New England Biolabs).

chm is located 5.7 Mb from the breakpoint of *In(2L)t* (http://www.flybase.org), a known clinal inversion (Mettler *et al.* 1977). To determine whether allele-frequency variation is explained by linkage to *In(2L)t*, the same chromosomes assayed for the target SNP were also assayed by PCR for the presence/absence of *In(2L)t* (Andolfatto and Kreitman 2000).

Analysis: Multi-site F_{ST}, π, linkage disequilibrium, and Tajima's *D* estimates were made in DNAsp (Rozas *et al.* 2003). Site-by-site F_{ST} estimates and Monte Carlo sampling to determine *P*-values (following Berry and Kreitman 1993) were run using Python scripts. All other statistical tests were run in JMP (v. 5.1, SAS Institute, Cary, NC). The standard error for the allele-frequency estimates was estimated by $\sqrt{(p(1-\mathrm{p})/n)}$.

RESULTS

Chromatin/histone-remodeling genes *ssrp*, *glu*, *chd1*, and *chm* exhibit tropical–temperate sequence differentiation: Seventeen significant windows ($P \leq 0.05$) identified by the whole-genome tiling array analysis (Turner *et al.* 2008, accompanying article), representing regions of differential hybridization patterns of tropical–temperate population samples (see MATERIALS AND METHODS), overlapped chromatin-remodeling genes (supplemental Table S2). Ten of those windows overlapped only a small portion of the chromatin-remodeling gene or contained three or more genes and were not considered further. The remaining seven windows and associated chromatin-remodeling genes (Table 1) were retained for DNA sequence analysis.

We found low levels of polymorphism in three of the seven regions (*Mi-2*, *HERC*, *trr*, see Table 2), all three of which had low estimates of F_{ST}. These three regions are located in areas of low crossing over (Kliman and Hey 1993), which is consistent with the observed lack of polymorphism (Begun and Aquadro 1992). We observed no sequence differentiation in the genes, suggesting that these three loci were false discoveries in the array experiment. Note that our list of candidate genes was inferred from the least conservative FDR class.

The four remaining regions, which contained genes *ssrp*, *glu*, *chd1*, and *chm*, showed average levels of polymorphism (Shapiro *et al.* 2007) and significant sequence differentiation between tropical and temperate populations (Table 2). The F_{ST} estimates at the chromatin-associated loci range from 0.10 to 0.15, which are consistently elevated relative to regions immediately outside the putatively significant windows and relative to random regions of the genome (United States: $F_{ST} = 0.040$; Australia: $F_{ST} = 0.036$; Turner *et al.* 2008, this issue). Importantly, high levels of sequence differentiation

TABLE 2

Estimates of sequence differentiation (F_{ST}) and polymorphism (π) for amplified regions inside window, overlapping chromatin gene, and outside window

Focal region	Amplicon (midpoint)	Amplicon size	F_{ST}	π (all)	π (tropical/temperate)
chd1	2L: 2980275	936	0.030	0.005	0.005/0.004
	2L: 2985171	***1405***	***0.170***	***0.008***	***0.008/0.007***
	2L: 2989305	1426	0.001	0.007	0.008/0.005
chm (Australia)	2L: 7407345	1044	0.180	0.015	0.014/0.031
	2L: 7412208	**1250**	**0.151**	**0.015**	**0.017/0.010**
	2L: 7414710	***1251***	***0.422***	***0.011***	***0.015/0.003***
	2L: 7417734	1326	0.041	0.012	0.015/0.009
chm (United States)	2L: 740735	1044	0.000	0.013	0.015/0.013
	2L: 7412208	**1250**	**0.007**	**0.015**	**0.015/0.017**
	2L: 7413459	***1251***	***0.100***	***0.012***	***0.012/0.015***
	2L: 7417734	1326	0.045	0.004	0.015/0.012
glu	NA	NA	NA	NA	NA
	2L: 16732566	***1358***	***0.120***	***0.010***	***0.010/0.008***
	2L: 16735626	**1113**	**0.090**	**0.009**	**0.008/0.010**
	2L: 16738423	943	0.070	0.004	0.004/0.003
ssrp	2R: 19308927	1099	0.001	0.008	0.009/0.008
	2R: 19312582	**1139**	**0.018**	**0.012**	**0.013/0.011**
	2R: 19315342	***1000***	***0.130***	***0.005***	***0.007/0.004***
	2R: 19320319	1121	0.001	0.003	0.003/0.004
Mi-2	3L: 19813845	1072	—	0.000	0.003/0.007
	3L: 19816984	***1248***	***0.002***	***0.001***	***0.001/0.001***
	3L: 19822410	932	0.000	0.001	0.001/0.001
HERC	X: 20211332	1014	0.015	0.005	0.004/0.006
	X: 20216217	***1194***	—	***0.000***	***0.000/0.000***
	X: 20221822	1039	0.018	0.005	0.005/0.005
trr	X: 1737499	1089	0.012	0.002	0.002/0.002
	X: 1741745	***929***	***0.001***	***0.002***	***0.002/0.002***
	X: 1745278	1083	0.028	0.002	0.002/0.002

Boldface type indicates amplified regions inside window and italics indicate the region overlapping the chromatin gene. Locations are from *D. melanogaster* reference genome release 4.3.

were restricted to the focal gene, even in the two cases where two gene regions overlapped the window (*ssrp*, *glu*) (Table 2—the 5′ region outside the window containing *glu* was recalcitrant to amplification). The *chm* locus exhibits the most dramatic differentiation in Australia ($F_{ST} = 0.42$). F_{ST} at *chm* differs substantially between the two continents, with the Australian populations exhibiting considerably higher levels of differentiation, in agreement with the genomewide pattern (Turner *et al.* 2008, accompanying article in this issue). The elevated F_{ST} in both Australia and the United States inspired a more in-depth analysis of this gene region.

Tropical–temperate differentiation is restricted to the *chm* gene region: The U. S. and Australian multi-site F_{ST} estimates peak in the first part of *chm* (Figure 1). Moreover, the fraction of significantly differentiated SNPs ($P \leq 0.05$ by permutation test) also peaks in this 5′ region of *chm* (Table 3). Tropical–temperate differentiation is restricted to the *chm* gene region and is largely overlapping on the two continents (Figure 1), strongly suggesting that geographic variation at this chromatin-remodeling gene is the result of selection rather than demographic history.

The total number of SNPs detected across the *chm* gene region is similar for Australia and the U. S. (Table 3), while the magnitude of F_{ST} and fraction of significantly differentiated SNPs differs substantially across the two continents (see *y*-axis of Figure 1). More specifically, of the 61 SNPs significantly differentiated between tropical and temperate zones (the union of all Australian and U. S. SNPs across *chm*—Table 3, boldface rows), 50 occur in Australia only, 3 in the U. S. only, and 8 are shared. In many cases SNPs differentiated only in Australia also show allele-frequency differences between Maine and Florida in the expected direction, but of smaller magnitude (supplemental Table S3). One exception is peak two (Figure 1), which is entirely intergenic and shows strong differentiation in Australia but not in the U. S. The different magnitudes of F_{ST} and fraction of differentiated SNPs on the two continents are

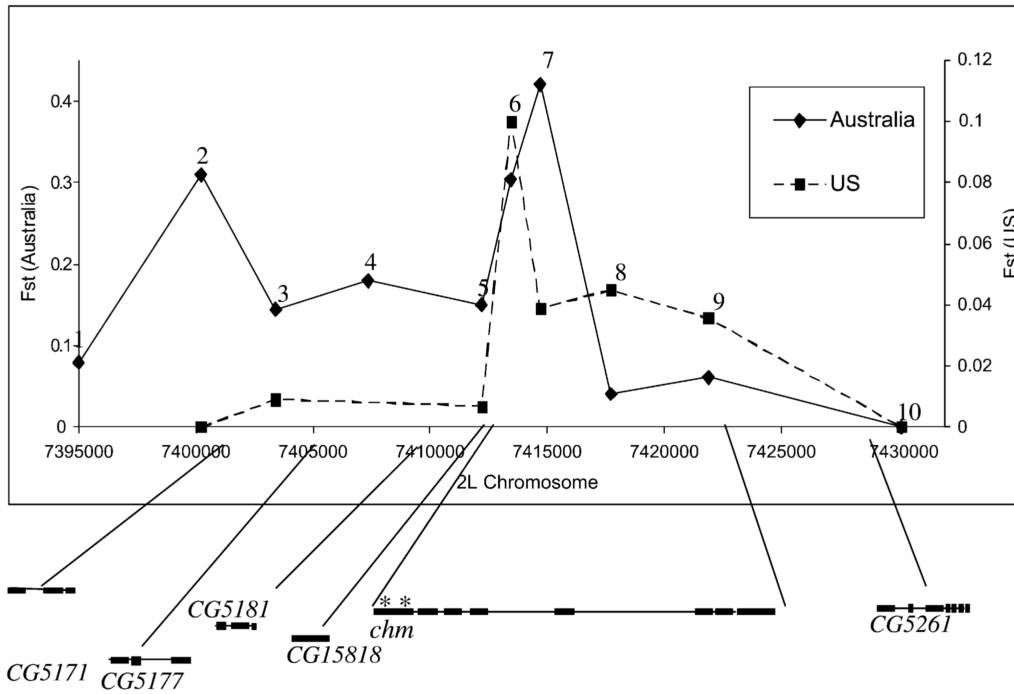

FIGURE 1.—F_{ST} across a 35-kb region (*D. melanogaster* reference genome release 4.3) containing the *chm* locus. Each point corresponds to the midpoint of a single amplicon (see MATERIALS AND METHODS) listed in supplemental Table S2, with the exception of peak seven, which corresponds to a mosaic of two amplicons. All genes located in this chromosomal region are indicated. The two significant replacement SNPs are in exon 1 (indicated with asterisks), although they occur in different amplicons. The serine/proline polymorphism occurs at peak five, while the arginine-proline amino acid polymorphism occurs at peak six.

consistent with differences observed at the genomewide scale (TURNER *et al.* 2008, this issue) and likely reflect the larger latitudinal span in Australia.

Two of the eight SNPs that are significantly differentiated on both continents occur in the 5′-end of the first large intron and the remaining six occur in the *chm* coding region. Two of these coding variants are replacement SNPs. Intriguingly, none of the other 59 significantly differentiated SNPs are replacement variants (supplemental Table S3).

The first replacement site is similarly differentiated in Australia and the United States (F_{ST} = 0.18 and 0.20,

TABLE 3

Polymorphism estimates and breakdown of SNP number and class across 35-kb region near *chm*, corresponding to Figure 1

Peak no.	Continent	Amplicon (bp)	No. of SNPs (diff/total)	Noncoding/ coding (diff)	π (tropical/ temperate)
1	Australia	1010	3/38	3/0	0.015/0.010
2	Australia	1010	13/33	13/0	0.016/0.006
	United States		0/29	0/0	0.014/0.017
3	Australia	1044	7/25	5/2	0.009/0.008
	United States		1/32	1/0	0.014/0.014
4	Australia	1020	7/29	7/0	0.014/0.010
5	**Australia**	**1250**	**11/34**	**9/2**[a]	**0.017/0.010**
	United States		**2/33**	**1/1**[a]	**0.015/0.017**
6	**Australia**	**1251**	**19/30**	**6/13**[a]	**0.014/0.007**
	United States		**5/37**	**0/5**[a]	**0.011/0.015**
7	**Australia**	**1251**	**23/34**	**17/6**	**0.015/0.003**
	United States		**2/39**	**2/0**	**0.016/0.014**
8	**Australia**	**1320**	**1/40**	**1/0**	**0.015/0.009**
	United States		**2/34**	**2/0**	**0.015/0.012**
9	**Australia**	**1250**	**4/26**	**4/0**	**0.009/0.009**
	United States		**0/26**	**0/0**	**0.009/0.009**
10	Australia	1080	0/21	0/0	0.009/0.011
	United States		0/18	0/0	0.010/0.011

For each amplicon ("peak") from Figure 1, the table lists the fraction of SNPs significantly differentiated between tropical and temperate regions ($P \leq 0.05$ by permutation test; see MATERIALS AND METHODS), as well as the number of SNPs significantly differentiated in the noncoding and coding regions of the amplicon. The peaks that overlap the *chm* gene region are in boldface. diff, significantly differentiated at the $P \leq 0.05$ level.

[a] Single replacement SNPs.

respectively), but is not among the most differentiated sites for either continent. Nevertheless, this proline/serine polymorphism may be functionally significant, as the derived proline variant is a radical amino acid change from the ancestral serine allele, which is conserved in *D. simulans*, *D. sechelia*, and *D. erecta* (all species for which sequence was available and alignable; supplemental Figure S1).

The second replacement site is one of the most significantly differentiated sites in the United States ($F_{ST} = 0.23$), although not in Australia ($F_{ST} = 0.22$). The proline variant is also a radical amino acid change from the ancestral arginine allele, which is conserved in *D. sechellia*, *D. yakuba*, *D. erecta*, *D. ananassae*, and *D. persimilis* (supplemental Figure S2). Unfortunately, these two replacement sites were assayed on different amplicons, which precluded rigorous assessment of linkage disequilibrium between them (see MATERIALS AND METHODS). Interestingly, variants that are virtually fixed in the temperate zones for both replacement sites correspond to the ancestral state (see supplemental Figures S1 and S2).

The most significantly differentiated sites in Australia occur in the first, large intron of *chm* (supplemental Table S3, three sites with $F_{ST} = 0.66$). These sites, however, are not significantly differentiated in the United States (although site 772 exhibits variation in the same direction; supplemental Table S3). The dominant temperate variant at site 772, like the amino acid variants described above, represents the ancestral state. Such a variant, if functionally significant, may affect adaptive gene expression variation across this latitudinal gradient. Interestingly, one of the best-studied clinally varying genes, *Adh*, also appears to harbor both selected amino acid and gene expression variants in *D. melanogaster* (summarized in BERRY and KREITMAN 1993). Moreover, *Ldh* in *Fundulus heteroclitus* exhibits both amino acid and gene expression variants along a north–south gradient (PLACE and POWERS 1979; SCHULTE *et al.* 2000).

Differentiated site in *chm* exhibits linear cline along both continents independent of *In(2L)t*: A single SNP was chosen for RFLP analysis in additional populations sampled along the east coasts of Australia and the United States (different SNPs were used on each continent; see MATERIALS AND METHODS). We observed a significant correlation between allele frequency and latitude in Australia ($R^2 = 0.76$, $P < 0.001$; Figure 2a) and in the U. S. ($R^2 = 0.79$, $P < 0.007$; Figure 2b) for these SNPs. Analysis of standard [*i.e.*, non-*In(2L)t*] chromosomes only also reveals a significant correlation with latitude, despite a reduced data set (Australia: $R^2 = 0.66$, $P = 0.007$; United States: $R^2 = 0.47$, $P = 0.08$). These data support previous findings that inversions explain clinal variation only at sites relatively close to the breakpoints (KENNINGTON *et al.* 2006). Finally, both SNPs show strong linkage disequilibrium with the

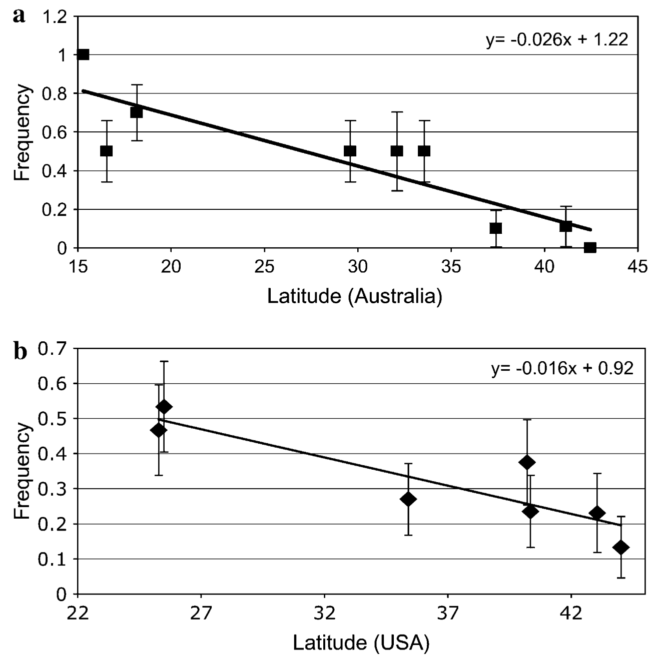

FIGURE 2.—Clinal variation at a differentiated SNP across latitudinal gradients in Australia (a) and the US (b), and equations for the regression lines.

arginine/proline replacement SNP discussed above ($D' = 1.00$ for both regions and continents; see supplemental Table S5 for haplotype frequencies), supporting the inference that the radical arginine/proline polymorphism is clinal on both continents.

Population genetic evidence of selection at *chm* in temperate populations: In both the tropical and temperate Australia data, Tajima's D (TAJIMA 1983) is near zero outside the region of significantly large F_{ST} values (Figure 3). Within the region associated with large F_{ST} values, however, Tajima's D is strongly negative in the temperate population and relatively positive (although not significantly so) in the tropical population in Australia. This pattern is not apparent in the United States, where differentiation is substantially smaller (data not shown). The skew toward rare variants in temperate Australia is consistent with a model in which the temperate population has a temperate-adapted, high-frequency haplotype and rare, less fit tropical haplotypes. These data create a puzzle when considered in combination with the observation of two, strongly differentiated, radical amino acid variants for which the common allele in temperate regions is the ancestral state. The classic model describes a derived, low-frequency sub-Saharan African variant rising to high frequency in a New World temperate zone (*e.g.*, SCHMIDT *et al.* 2000; SEZGIN *et al.* 2004). If the dominant temperate *chm* haplotype in southern Australian populations derives from a random sample of temperate haplotypes (defined by the presence of the putative selected ancestral variant) currently segregating in

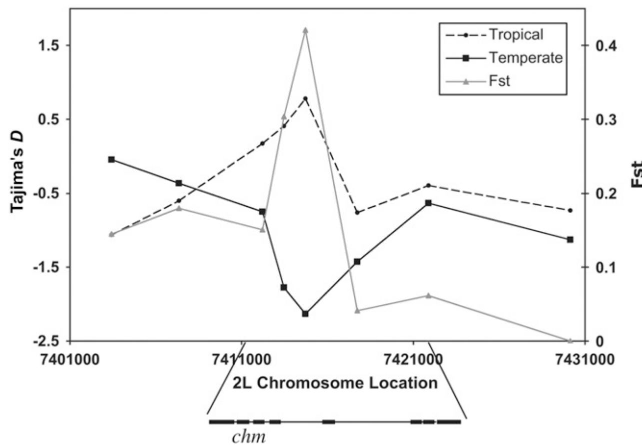

FIGURE 3.—Tajima's D across 35-kb region (*D. melanogaster* reference genome release 4.3) containing the *chm* locus in temperate and tropical Australia population samples. Each point corresponds to the midpoint of a single 1- to 1.3-kb amplicon.

tropical populations, we would expect similar estimates of Tajima's D for both the temperate population and the "temperate haplotypes" segregating in tropical populations. Tajima's D for the latter population, however, is close to zero ($D = 0.30$). One possibility is the stochastic nature of selection associated with standing variation, such that a small subset of favored haplotypes may be disproportionately represented after selection (ORR and BETANCOURT 2001). Alternatively, there may be fitness differences among the haplotypes containing a putative favored, ancestral variant, such that only a small subset of such haplotypes are strongly favored in a novel environment. Epistatic fitness variation among ancestral variants during colonization of novel environments could, in principle, contribute to such dynamics. Several well-studied loci, such as *Adh* in *D. melanogaster* and *Ldh* in *F. heterclitus*, harbor multiple sites under selection along latitudinal clines (PLACE and POWERS 1979; BERRY and KREITMAN 1993; SCHULTE *et al.* 2000).

A comparison of differentiation between Australian and U. S. temperate zones and between their tropical zones further supports the idea that selection has acted more strongly in the temperate zone in the recent evolutionary history of these *D. melanogaster* populations (Table 4). If temperate-adapted alleles were driven to high frequency independently on the two continents and the selected site(s) was segregating at a non-negligible frequency in the ancestral populations, different haplotypes could rise to high frequency in the Australian and U. S. temperate populations. Our observations are consistent with this hypothesis: F_{ST} calculated from the U. S. temperate region *vs.* the Australian temperate region is larger than the F_{ST} generated from the equivalent tropical comparison (Table 4). Moreover, we observed this difference only at the *chm* locus where the tropical–temperate F_{ST} is high.

TABLE 4

Estimates of differentiation among similar climatic regions on different continents

Peak no.	F_{ST} (temperate United States–temperate Australia)	F_{ST} (tropical United States–tropical Australia)
2	0.120	0.063
3	0.052	0.046
5	0.040	0.000
6	**0.160**	**0.021**
7	*0.260*	*0.000*
8	0.036	0.041
9	0.005	0.000
10	0.010	0.000

Boldface type indicates peak U. S. differentiation; italics indicate peak Australian differentiation.

DISCUSSION

We have reported evidence of tropical–temperate differentiation at four chromatin-remodeling gene regions. A detailed analysis of one gene, *chm*, revealed that elevated geographic differentiation in the coding region was restricted to the 5′ end and that two replacement variants, both of which are radical changes, are significantly differentiated in both Australia and the United States. The amino acid variants at both replacement sites (see supplemental Figures S1 and S2) are virtually fixed in temperate zones of both continents, but occur at intermediate frequencies in the tropical zones. Interspecific comparisons (supplemental Figures S1 and S2) revealed that the high-frequency temperate variants are the ancestral rather than the derived states. Both variants at the arginine/proline replacement site were found in an African sample from Zimbabwe, with the ancestral variant occurring in 9 of the 10 alleles surveyed (data not shown).

Why might an ancestral variant (or variants), which is typically thought of as adapted for tropical environments, spread to high frequency under selection in temperate populations? One interesting possibility is selection associated with karyotypic variation. The ancestral karyotypic state (standard chromosomes) in *D. melanogaster* is found at high frequency in the temperate region (METTLER *et al.* 1977). Molecular data near inversion breakpoints suggest that the inversions are recent on the timescale of molecular evolution in *D. melanogaster* (HASSON and EANES 1996; ANDOLFATTO and KREITMAN 2000; MATZKIN *et al.* 2005) and that much of the polymorphism in the species is older than the inversions, despite the fact that the inversions are more abundant in equatorial Africa than in the recently established populations. This suggests the possibility that cases such as *chm*, in which temperate non-African populations have an ancestral variant(s) at high frequency while tropical non-African populations do not

(Braverman *et al.* 2005), may be subject to selection pressures also associated with karyotypic variants.

The two continents do not share their respective, most significantly differentiated sites. This observation may be attributed to a combination of power differences (possibly related to the smaller latitudinal span in the United States) and difference in the organization of ancestral variation found among the chromosomes associated with the invasion of each continent. This lack of overlap, combined with evidence of elevated F_{ST} across the U. S. and Australian temperate zones (Table 4), support independent invasions of these two continents.

Previous work on evolution at chromatin-remodeling loci has focused largely on proteins that localize to the heterochromatin (Malik *et al.* 2002; Vermaak *et al.* 2005). These proteins are evolving adaptively perhaps in response to endogenous evolutionary forces such as centromeric drive. The group of genes identified in this study offers a less biased description (with respect to the diversity of chromatin functions) of how euchromatic chromatin remodeling may contribute to adaptation in response to exogenous forces, such as those found in a novel environment. However, these four loci by no means represent an exhaustive list of chromatin/histone-remodeling genes that may be influenced by spatially varying selection (see Turner *et al.* 2008 for limitations of hybridization technology).

CHD1, or *c*hromo-ATPase/*h*elicase-*D*NA-binding protein *1*, is an ATP-utilizing chromatin assembly factor that acts on nucleosome spacing to confer a transcriptionally active chromatin conformation (Lusser *et al.* 2005; see Table 5). Stokes *et al.* (1996) document a nonrandom distribution of CHD1 on polytene chromosomes (Stokes *et al.* 1996), where CHD1 is restricted largely to a subset of interbands and puffed regions. CHD1 localizes specifically to developmental puffs (especially ecdysone-sensitive puffs) and heat-shock-induced puffs (Stokes *et al.* 1996).

Remarkably, CHD1 both colocalizes and interacts *in vivo* with SSRP1 (Kelley *et al.* 1999), another chromatin-remodeling protein identified in this study. SSRP1, or *s*tructure-*s*pecific *r*ecognition *p*rotein *1*, is an HMG-box family protein (Table 5) that complexes with Spt16 to form the FACT complex (Orphanides *et al.* 1999). This complex localizes to genes actively transcribed by PolII and removes the histone dimer H2A-H2B as PolII passes (Belotserkovskaya *et al.* 2003). Like CHD1, the FACT complex is recruited to heat-shock loci following stress (Saunders *et al.* 2003) and to critical development loci during metamorphosis (Andrulis *et al.* 2000). Like CHM (discussed below), during embryogenesis the FACT complex modulates expression of several Hox genes (Shimojima *et al.* 2003).

The third gene identified in this study, *chm*, dominantly suppresses PEV and maintains Hox gene silencing by other Polycomb group proteins (Grienenberger *et al.* 2002). Specifically, *chm* dominantly enhances the aberrant sex-comb phenotype of Pc^{-} mutants, designating *chm* as a Polycomb group gene. Like CHD1 and the FACT complex, Polycomb group proteins are also recruited to critical developmental loci during embryogenesis and metamorphosis (Ringrose and Paro 2004). Unlike CHD1 and FACT, however, Polycomb group proteins generally act as transcriptional repressors rather than activators. Nevertheless, the PcG CHM also activates transcription, specifically promoting histone 4 (H4) acetylation (Table 5) of AP-1 sites (dJun, cJun) associated with the JNK pathway (Miotto *et al.* 2006). This transcriptional activation enhances JNK pathway activity, which stimulates dorsal thorax closure during metamorphosis. In addition to this developmental role, the JNK pathway also contributes to stress response in both Drosophila (Wang *et al.* 2003) and mice (Tournier *et al.* 2000). More specifically, the interaction of *chm* with DFos and/or Djun is essential for JNK pathway response to both chemical and osmotic stress (Miotto and Struhl 2006). Finally, activation of JNK signaling in *D. melanogaster* inhibits the insulin/IGF signaling (IIS) pathway, resulting in increased life span (Wang *et al.* 2005). Intriguingly, longevity is one of several life-history traits known to vary along latitudinal clines (Schmidt *et al.* 2005).

TABLE 5

Protein names, domain names, and functions encoded by the loci identified in this study

Protein	Domain	Function
CHD1	Chromodomain	Chromatin compaction, gene silencing (also found in HP1, PC)
	DNA binding	Binds (A + T) minor grooves
	ATPase/helicase	Activates transcription via chromatin conformational changes
SSRP1	HMG box	DNA/chromatin binding
CHM	MYST	Acetyltransferase (transfers acetyl groups to histone tails)
SMC4	ATPase	Chromatin condensation (in combination with other condensing components)
	ATP binding	Chromatin condensation (in combination with other condensing components)

Our final tropical–temperate differentiated locus, *glu*, codes for SMC4, an ATP-binding/ATPase that, as part of the condensin complex, contributes to chromosome condensation during the prometaphase of mitosis (Hirano and Mitchison 1994; Steffensen *et al.* 2001) and likely meiosis as well (Steffensen *et al.* 2001). By ensuring proper condensation, sister chromatids resolve appropriately prior to segregation, thereby minimizing nondisjunction. We have long understood that chromosome segregation in Drosophila is temperature

sensitive, with low temperatures associated with elevated rates of meiotic nondisjunction in females (Tokunaga 1970a,b). Tropical–temperate sequence differentiation at the *glu* locus may contribute to faithful sister-chromatid segregation in the face of novel environmental conditions in recently established populations. Moreover, increased rates of nondisjunction are also associated with autosomal inversions (Roberts 1962). The likelihood of nondisjunction, potentially generated by clinal inversions [*In(2L)t, In(3R)P, In(3L)P, In(2R)NS* (Stalker 1976)] or due to temperature may be modulated by evolution at the *glu* locus. In addition to its role in prometaphase chromosome condensation, the condensin complex colocalizes with PcG proteins to their binding sites (Polycomb response elements) during interphase (Lupo *et al.* 2001). Localization with Polycomb group proteins suggests that, like CHD1, SSRP1, and CHM, SMC4 may carry out a regulatory role in development.

The regulation of developmental trajectories emerges as an unexpected biological process potentially targeted by spatially varying selection. All four chromatin-remodeling candidates investigated in this study either localize to developmental puffs and/or interact with the Polycomb group, which maintains long-term epigenetic silencing (through mitosis) of essential developmental regulators (Ringrose and Paro 2004). Long-term silencing, at least at Polycomb protein targets, is positively correlated with temperature (Fauvarque and Dura 1993). Given that cold temperatures (and possibly other stressors) disrupt the integrity of this chromatin-based silencing, it is tempting to speculate that evolution at chromatin proteins buffers these developmental trajectories from perturbations by novel environmental conditions. Moreover, low temperatures may also perturb transcriptional regulation at development puffs independently of the Polycomb group (Ashburner 1970), suggesting yet another potential source of selection driving the evolution at chromatin-remodeling proteins. At the phenotypic level, environment-induced developmental mistakes are common and are observed across diverse taxa, such as phenocopying in flies (Gloor 1947; Santamaria 1979; Gibson and Hogness 1996; Roberts and Feder 1999), temperature-induced neural tube defects in developing human fetuses (Chambers *et al.* 1998), and developmental instability as measured by fluctuating asymmetry across many taxa (*e.g.*, Badyaev *et al.* 2000; Milton *et al.* 2003; Chang *et al.* 2007). Future research may determine that chromatin-associated aberrant transcription may underlie these environment-induced developmental mistakes found in naive populations.

In addition to developmental homeostasis, the four identified chromatin-remodeling loci reveal that stress response machinery, particularly those proteins associated with heat-shock gene transcription, may be targeted by spatially varying selection. Heat-shock genes, found at heat-shock puffs, are generalized stress responders to varied stimuli, such as osmotic stress, UV exposure, and cold stress (in addition to high temperature; Lindquist 1986). These proteins are remarkably well conserved across distantly related taxa (Voellmy 1984), which is consistent with the idea that selection on heat-shock protein function would likely occur at the level of transcriptional regulation rather than (or at least in addition to) the modification of chaperone function (Frydenberg *et al.* 2003). Promoter regions of many stress-induced and heat-shock loci have common *cis*-regulatory elements (Uffenbeck and Krebs 2006), further underscoring the potential for evolution in *trans*-activation. Such *trans*-activation at the level of chromatin organization provides coordinated, global gene regulation among disparate loci. Recent evolution at CHM, CHD1, and SSRP1 suggest that coordinately modulating disparate stress-response loci may be essential for maintaining a nonstressed biological state despite novel environmental conditions.

Adaptation to a novel habitat requires at least in part the buffering of environment-sensitive processes in the face of novel conditions. Given that chromatin packaging is environment sensitive, we expect many loci regulated by chromatin proteins to be aberrantly expressed in organisms exposed to novel environments. A small number of evolutionary changes in a few key chromatin-remodeling complexes may modulate the genomewide adverse effects of an (initially) suboptimal environment, thereby buffering critical biological processes and facilitating the invasion of novel habitat.

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