Review

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Revisiting classic clines in *Drosophila melanogaster* in the age of genomics

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Adaptation to spatially varying environments has been studied for decades, but advances in sequencing technology are now enabling researchers to investigate the landscape of genetic variation underlying this adaptation genome wide. In this review we highlight some of the decades-long research on local adaptation in *Drosophila melanogaster* from well-studied clines in North America and Australia. We explore the evidence for parallel adaptation and identify commonalities in the genes responding to clinal selection across continents as well as discussing instances where patterns differ among clines. We also investigate recent studies utilizing whole-genome data to identify clines in *D. melanogaster* and several other systems. Although connecting segregating genomic variation to variation in phenotypes and fitness remains challenging, clinal genomics is poised to increase our understanding of local adaptation and the selective pressures that drive the extensive phenotypic diversity observed in nature.

The clinal genomic framework

Despite vast phenotypic and genetic diversity in the tree of life, species often appear precisely adapted to their local environment, suggesting strong selection for DNA variants that underlie local adaptation. Evolutionary biologists have long sought to connect this genetic variation to variation in phenotypes and fitness within natural populations. One fruitful approach has been to sample individuals along geographic transects – such as latitude, longitude, or altitude – that vary predictably in abiotic (e.g., temperature, precipitation, UV radiation) and biotic (e.g., species biodiversity, levels of competition) conditions. Evaluation of variation along such transects enables the identification of clines, broadly defined as a predictable geographic gradient in a measurable genotypic (e.g., allozyme or allele frequencies) or phenotypic (e.g., body size, thermal tolerance) character [1]. Two types of clines – those situated along discrete environments and those along continuous environments – have been historically evaluated theoretically and empirically (Box 1).

Sampling along clines provides unique benefits and can potentially attenuate some of the confounding effects of demography that may be difficult to control for when sampling populations from patchy landscapes. For example, gene flow should be more predictable along clines, thus making it easier to identify adaptive from nonadaptive differentiation [2]. Clines are often predictable and replicable to a degree that variation sampled from patchy landscapes is not; for example, a cline along a coastal latitudinal transect can potentially be replicated on multiple continents. Such patterns of differentiation repeated among clines provide evidence of parallel adaptation. Finally, properties of a cline – such as the width, slope, and shape – can also inform inferences about underlying demographic and selective forces [1–5].

Although adaptation to spatially varying selection has been evaluated for decades using phenotypic data and genetic data from a small number of candidate loci, the recent abundance of whole-genome data provides an opportunity to discover novel causative variants beyond those previously identified by candidate gene studies. Moreover, the discovery of novel clines allows researchers to ask fundamental questions about natural selection and the genetic basis of adaptation. What are the genomic targets of spatially varying selection and how do they facilitate adaptation to the local environment? What are the molecular mechanisms underlying local adaptation? How widely distributed across the genome are loci with alleles under clinal selection and what does this imply about the genetic basis of adaptive traits? As homologous characters may exhibit parallel responses to similar underlying selection pressures, how often does adaptation occur in parallel – within and between species – among clines? Here we highlight some of the decades-long research on local adaptation in *D. melanogaster* from a group of particularly well-studied clines in North America and Australia. We explore the evidence for parallel adaptation and identify commonalities in the genes responding to clinal selection among continents. We also highlight cases where patterns are not repeatable among clines. Finally, we explore recent studies utilizing whole-genome data that have just begun to identify the targets of selection along clines in *D. melanogaster* and in other species.

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Box 1. Genetic models underlying discrete- and continuous-environment clines

Two major types of clinal pattern have historically been studied in natural populations: clines where two discrete environments meet in a tension zone (or sometimes a hybrid zone) and clines where populations are locally adapted along a continuous environment (Figure I). Clines in tension zones are often sharp, narrow, and centered on an ecotone – the transition between two biomes. The exact shape of discrete-environment clines is determined by a balance between selection against maladapted alleles – due to either intrinsic or extrinsic incompatibilities in the tension zone – and dispersal distance [3,4,128]. Individuals in the tension zone should therefore have lower fitness relative to their 'pure' counterparts in the tails of the cline. Discrete-environment clines have been well studied both theoretically [4,5,128–130] and empirically, with some of the best examples coming from studies of three-spined sticklebacks [131,132], mice [133,134], *Heliconius* butterflies [135,136], and fire-bellied toads [4,5]. The genetic model underlying discrete-environment clines can be contrasted with that of continuous-environment clines – clines arising due to adaptation to continuously varying local environments – which are the primary focus of this review. Relative to discrete-environment clines, continuous-environment clines are found in a single species where populations are connected by high levels of gene flow. In contrast to the stepped fitness function of discrete-environment clines, fitness optima of continuous-environment clines gradually shift with the environmental gradient, and selection favors locally adapted alleles at all positions along the geographic transect. While continuous-environment clines are often broader than their discrete-environment counterparts, their shape should parallel changes in the environment, leading to sharp clines under certain environmental conditions. In continuous-environment clines, causative variants are expected to closely track their environmental selection pressures while clines of neutral variants should not. Despite these expectations, distinguishing causal variants from background noise remains a challenge. The underlying genetic model of continuous-environment clines suggests that these clinal variants will have a quantitative genetic basis. Whether all variants underlying such quantitative traits will track the environmental gradient equally well remains an outstanding theoretical and empirical question.

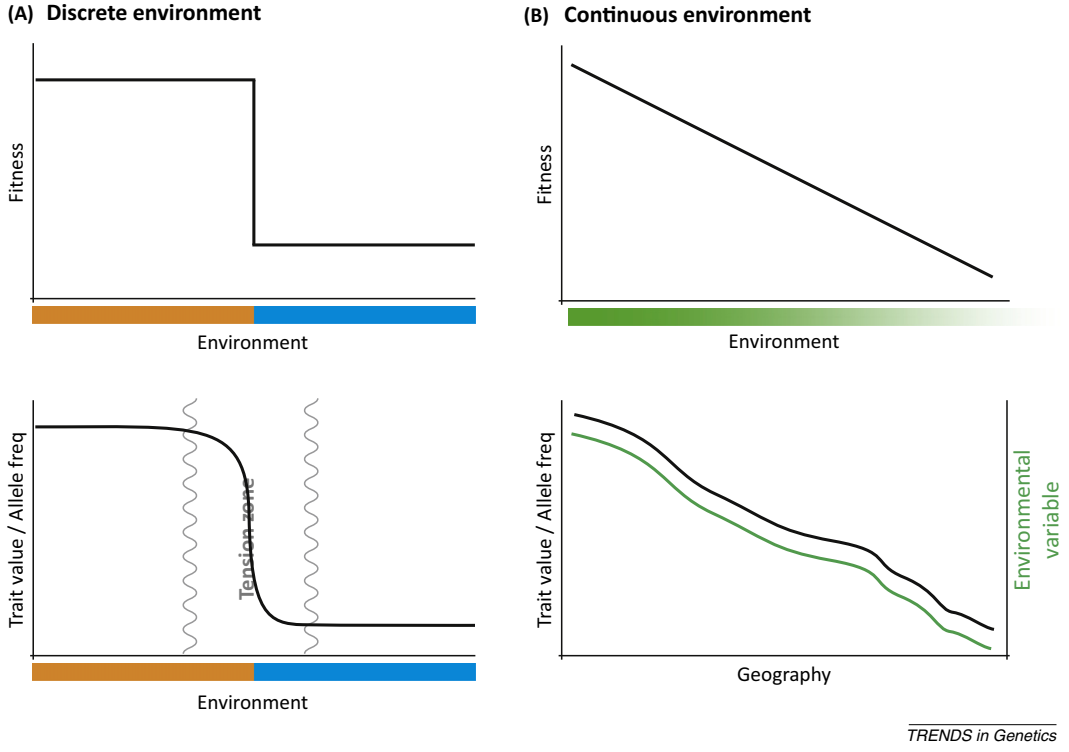

Figure I. A simplified fitness landscape contrasting discrete- and continuous-environment clines, as well as their expected shapes. **(A)** In discrete-environment clines, the fitness landscape (top: pattern shown for the orange population; blue population would be a mirror image) is often represented by a step function with two fitness optima, where alleles from one species are selected against as they introgress away from their home population. Consequently, the slope of the resulting trait/allele frequency cline (bottom) is relatively shallow in the tails and transitions sharply through the tension zone, although the exact shape is dependent on the strength of selection and dispersal distance. **(B)** The fitness landscape of a continuous-environment cline (top: pattern shown for leftmost population) represents a shifting fitness optimum along a continuous-environmental gradient. The resulting trait/allele frequency cline (bottom: black line) may be less steep than a discrete-environment cline and should closely track the environmental selection pressure (green line).

Phenotypic, genetic, and genomic variation in *D. melanogaster* clines

Expansion of D. melanogaster *out of equatorial Africa*

Decades of careful study has made *D. melanogaster* the most extensively explored system for elucidating phenotypic, genetic, and genomic divergence among natural populations. Genetic data suggest that *D. melanogaster* expanded out of its native range in equatorial Africa into Eurasia approximately 10 000–20 000 years ago and that this expansion was associated with a severe population bottleneck [6]. Changes in climatic conditions during the late Pleistocene period are likely to have facilitated migration out of Africa [7,8], but adaptation to numerous ecological factors that vary with latitude has been required in the derived high-latitude populations that now extend as far north as Finland (64°N) and as far south as Tasmania (43°S) [9]. More recently, *D. melanogaster* invaded North America and Australia, and was first collected within only

Table 1. Clinal patterns among American *Drosophila melanogaster* populations

Trait/genetic marker		Location	Clinal pattern	Refs
Genome wide		Eastern NA	Higher sequence diversity and most negative Tajima's *D* in lowest-latitude population, *In(3R)P* at highest frequency in the lowest-latitude population	[50]
		Eastern NA	Higher sequence diversity and lower ratio of X-to-autosomal-linked variation in low-latitude population; greater skew toward high-frequency alleles in high-latitude population; *In(3R)Mo, In(2L)t, In(2R)NS, In(3L)Payne, In(3R)Payne* frequencies decrease with latitude	[52]
		Eastern NA and Panama City, Panama	Differential gene expression: highly expressed genes in high-latitude populations of *D. melanogaster* are also highly expressed in high-latitude populations of *Drosophila simulans*	[12]
Genes	*Adh*	Eastern NA and western SA	*Adh-F* increases with latitude	[33,36,38]
	Aldh	Eastern NA	*Aldh-Phe* increases with latitude	[39]
	cpo	Eastern NA	Various clinal SNPs	[40,41]
	Est6	Eastern NA	$Est6^{1.00}$, $Est6^{3}$, and *Est6-S* increase with latitude	[30,33,137]
	EstC	Ontario, Canada; MA, USA; TX, USA	$EstC^{3}$ allele generally decreases with latitude	[33]
	G6pd	NA	*G6pd-F* and *G6pd* increase with latitude	[30,31,33]
	Gpdh	Eastern NA, western SA	*Gpdh-S* increases with latitude	[30,38]
	InR	Eastern NA	InR^{short} increases with latitude	[138,139]
	LapD	Ontario, Canada; MA, USA; TX, USA	$LapD^{3}$ allele generally decreases with latitude	[33]
	Odh	NA	*Odh-S* decreases with latitude	[30,32]
	Pgd	NA	*Pgd-F* increases with latitude	[30,31,33]
	Tpi	NA	*Tpi-F* increases with latitude	[140]
Inversions	*In(2L)t, In(2R)NS, In(3L)Payne, In(3R)Payne*	NA	Frequency decreases with latitude	[47,48]
	In(2L)t, In(3L)Payne, In(3R)Payne, In(3R)Mo	NA	Inversions differ	[46]
TEs	Family: *Rt1b, invader4, pogo, Doc, S-element, BS, 1360*	Eastern NA	Families differ	[72,141]
Phenotype	Cell membrane plasticity	Eastern NA	Highest in high-latitude population	[142]
	Diapause incidence and ovariole number	Eastern NA	Increases with latitude	[25]
	Various organs	Western SA	Size increase with latitude	[23]
	Egg size	Western SA	Increases with latitude	[26]
	Ethanol tolerance	Western NA	Ethanol resistance increases with latitude	[143]
	Female fresh weight and ovariole number	NA and SA	Increases with latitude	[24]
	Lifetime fecundity	Eastern NA	Decreases with latitude	[27]
	Lifespan, heat and cold resistance	Eastern NA	Increases with latitude	[27]
	Nighttime locomotor activity	Eastern NA	Increases with latitude	[11]
	Per capita fecundity	Eastern NA	Varies with age	[25]
	Sleep bout duration	Eastern NA	Decreases with latitude	[11]
	Wing area, cell size, and cell number	Western SA	Increases with latitude	[21]
	Wing length and bristle number	Eastern NA	Increases with latitude	[22]

the past 150 years on each continent; populations of *D. melanogaster* along the eastern coasts of North America and Australia have since been independently sampled and evaluated for decades [9,10] (Table 1).

North American and Australian clines

Although clines in individual traits or alleles are typically treated separately, for simplicity we refer to the collective of clines along the same transect as a singular entity ('the cline'). The North American cline along the Atlantic seaboard has been heavily sampled over 18° of latitude from southern Florida, USA (25°N) to Vermont and Maine, USA (44°N), although recent studies have extended this cline by 22° latitude in the south to include a population of *D. melanogaster* from Panama City, Panama (9°N) [11,12]. The Australian cline has been heavily sampled over 28° of latitude from northern Queensland (15°S) to Tasmania (43°S). Although both clines are coastal, longitude varies more than 10° along the North American cline but remains relatively constant in Australia [13,14]

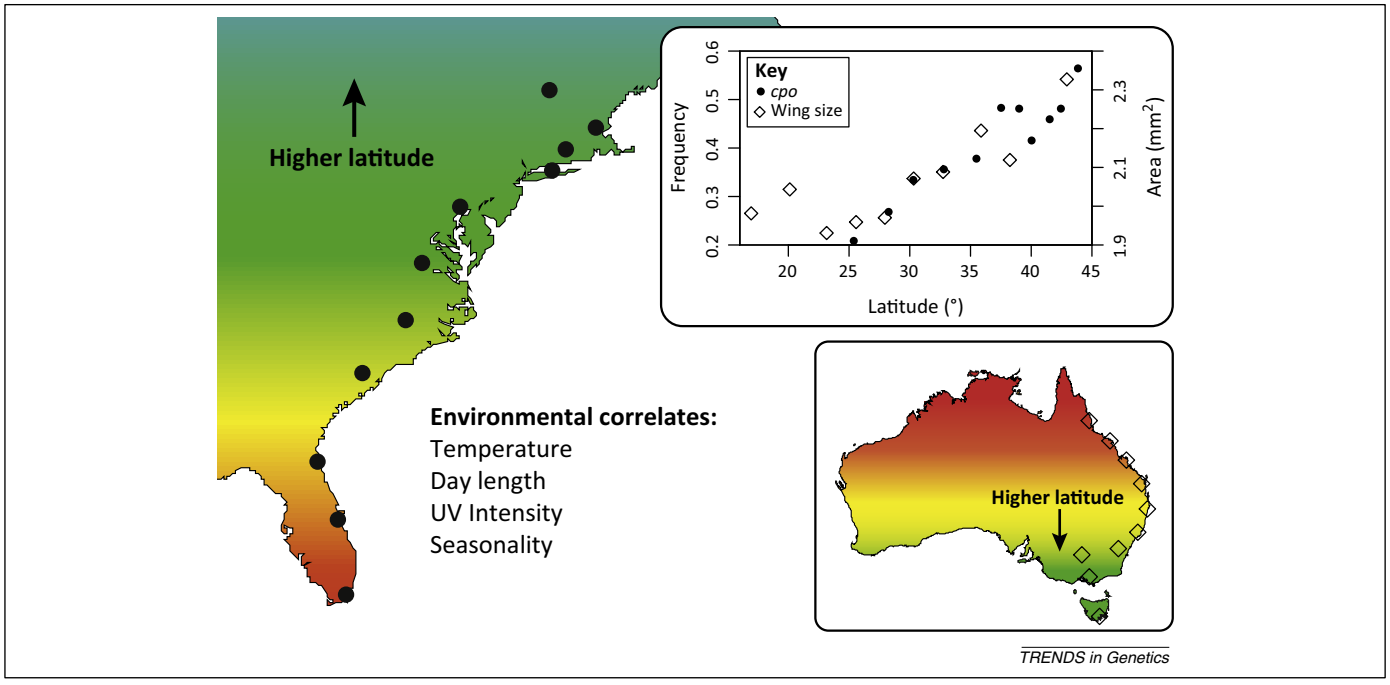

Figure 1. Phenotypes and alleles often respond to clinal selection in predictable ways. For example, both the allele frequency of the SNP corresponding to amino acid residue 356 in *couch potato* (*cpo*) sampled from North America (circles; modified from [40]) and the wing area of flies sampled from eastern Australia (diamonds; modified from [13]) increase with latitude. Selection on these traits could be mediated by multiple environmental factors that correlate with latitude.

(Figure 1). Environmental conditions vary greatly – although often predictably – along these clines. High-latitude populations on both continents consistently experience lower mean temperatures, greater variance in temperatures across seasons, and reduced UV light exposure [10,15–17]. As an example, over the past 30 years mean monthly temperatures averaged 24.1 °C in southern Florida (Station ID 087020) and 5.5 °C in central Vermont (Station ID 431360) [18]. Perhaps even more notable, the average maximum monthly temperature during this period in Vermont (12.6 °C) was 5.9 °C lower than the average minimum monthly temperature in Florida (18.5 °C). Many other environmental factors vary along the North American and Australian clines that generate spatial differentiation of phenotypes and genotypes and it should be noted that many of these factors also covary with altitude, such that similar clines are found along individual mountain ranges [19,20].

Phenotypic variation

Phenotypic differentiation along latitudinal transects has been shown for various traits in *D. melanogaster* and many patterns are recapitulated among continents (Table 1). One of the most striking patterns is the size difference in flies from different locations: populations of flies from high latitudes generally display larger body size and a concomitant increase in wing size [13,21], bristle number [22], and organ size [23]. Ovariole number [24,25] and egg size also increase with latitude [26], although lifetime fecundity decreases with latitude [27]. Adaptation to colder temperature reveals itself as phenotypic differentiation in several traits not directly related to size. For example, in the eastern USA, populations of *D. melanogaster* from higher latitudes display greater diapause incidence at low temperatures [25] and have greater cold tolerance [27]. High-latitude populations of *D. melanogaster* in Australia also have greater cold tolerance, but lower heat tolerance, than their lower-latitude conspecifics [10,28]. In contrast to the North American cline, the incidence of diapause expression [29] and the number of ovarioles [26] display nonlinear associations with latitude in eastern Australia, which illustrates the fact that not all differentiation occurs in parallel among continents. Finally, recent work has shown a strong cline for the length of time that flies sleep: high-latitude populations of *D. melanogaster* in North America sleep for shorter bouts of time and this pattern is reflected in higher nighttime locomotor activity in these populations [11].

Genetic variation

For decades researchers used single markers to elucidate clinal differentiation and spatial variation in allele frequencies. This approach revealed multiple markers with variation that tracked the clines, including some with the same allele at higher frequency at the same latitude in the Northern and Southern hemispheres. Examples include alcohol dehydrogenase (*Adh*), α-glycerol-3-phosphate dehydrogenase (*Gpdh*), glucose-6-phosphate dehydrogenase (*G6pd*), esterase-6 (*Est-6*), octanol dehydrogenase (*Odh*), and 6-phosphogluconate dehydrogenase (*Pgd*) [30–33] (Table 1). Perhaps the most heavily explored locus in *D. melanogaster* has been *Adh*, the first step in the ethanol detoxification pathway. The *Adh-F* allele encodes high catalytic activity of ADH, but this increase in activity trades off with enzyme stability at higher temperatures [34,35]. Unsurprisingly, the *Adh-F* allele is found at a higher frequency in cooler high-latitude populations, and differentiation has occurred in parallel along clines in

North and South America, Europe–Africa, Asia, and Australia [36–38]. A similar pattern of clinal differentiation exists for the acetaldehyde dehydrogenase locus (*Aldh*) – the second step in the ethanol detoxification pathway – with the derived allele segregating at a higher frequency in high-latitude North American populations [39].

Although researchers have identified many instances of clinal variation in allele frequencies (Table 1), this genetic variation has rarely been connected to variation in fitness or to causal clinal selection pressures. A notable exception is the gene *couch potato* (*cpo*), which has been shown to underlie adaptation to seasonality in *D. melanogaster* from North America [40]. Geographic variation in the incidence of diapause, or reproductive quiescence, has been demonstrated to vary predictably with latitude: female flies sampled at higher latitudes exhibit diapause at higher frequency than females sampled at lower latitudes [25]. Using quantitative trait locus (QTL) mapping and genetic complementation, *cpo* was identified as the causative locus underlying diapause incidence; furthermore, the frequencies of SNPs in *cpo* were strongly correlated with latitude [40,41] (Figure 1). Clinal patterns in *cpo* suggest that increased incidence of diapause in high-latitude flies is strongly linked to clinal selection pressures along the North American cline. Diapause has been directly connected to fitness: in *D. melanogaster* population cages, the frequency of flies expressing the diapause phenotype increased over time after exposure to both starvation and cold stress [42].

This clear picture of clinal adaptation of *cpo* – and its relationship to the diapause phenotype in North America – becomes cloudy when evaluated along the eastern Australian cline. The cline in *cpo* allele frequencies in Australia resembles that found in North America (Figure 1). However, when placed under conditions found to induce diapause in previous studies (i.e., 12 °C and short day length [43]), Australian flies display reduced – but not arrested – egg maturation at pre-vitellogenic stages [44]. Delays in egg maturation were also nonlinear in Australia, with ovarian dormancy increasing toward both ends of the cline. This is in contrast to the North American cline, where high-latitude populations have the highest and low-latitude populations have the lowest incidence of ovarian dormancy under similar conditions [25]. Thus, the clear association between *cpo* genotype and diapause phenotype in North America does not exist in Australia. This highlights a key point: even some of the strongest patterns of clinal adaptation observed in nature may not be repeatable. The factors that lead to such discrepancies provide fruitful avenues for future research. These may be biological in nature – for example, interactions with genetic background – or an artifact of sampling. Because tropical populations below 25°N in the Americas have been underexplored, we simply do not know whether inclusion of these populations would strengthen or lessen support for parallel adaptation among clines.

In addition to single markers, clinal variation has also been detected in larger genomic regions. Due to dramatically reduced recombination in heterokaryotypic individuals (those carrying one copy of each arrangement), inverted regions can contain many variants that all segregate together. Several of the major cosmopolitan inversions, including *In(2L)t*, *In(2R)NS*, *In(3L)Payne*, *In(3R)Payne*, and *In(3R)Mo*, vary predictably with latitude [45–48] and may house genetic factors involved in climatic adaptation [10]. Recent analysis has shown strong differentiation between tropical and temperate populations of *D. melanogaster*: populations in northern Australia carry the inverted arrangement of *In(3R)Payne* at high frequencies while those in the south do not [49]. Because of the very low levels of recombination in inversions, implicating any single variant within them as the causal one can be extremely difficult. Patterns of differentiation across chromosome 3R combined with gene ontology analysis suggest that large areas of the third chromosome – rather than any single variant – are under climatic selection and implicate entire gene families such as developmental and stimulus-response-related genes in clinal adaptation [49].

Genomic variation

Whole-genome analyses of *D. melanogaster* populations have made it possible to identify both large- and fine-scale clinal genomic patterns beyond those identified by candidate gene studies [50–54]. Low-latitude populations on multiple continents display greater sequence diversity, more negative values of Tajima's *D*, and a lower ratio of X-to-autosome variation [50,52]. Whole-genome data continue to support inferences made using smaller datasets; for example, chromosome 3R is the most strongly differentiated region of the genome due to *In(3R)Payne* [50]. This pattern is recapitulated in populations of *D. melanogaster* from Australia, indicating that clinal adaptation of this region has occurred in parallel on the two continents [51].

To identify signatures of adaptation, regions of the genome that are strongly differentiated between the samples are identified, and differentiated regions that overlap among multiple clines – both within and among species – provide evidence of parallel adaptation (Figure 2). For example, in northern and southern populations of *D. melanogaster* from the eastern USA and eastern Australia, the most highly differentiated sites in the genome (approximately the top 60% of F_{ST} values) cluster by environment rather than by continent: at these differentiated sites, populations from Maine more closely resemble populations from Tasmania than they do their lower-latitude American conspecifics [52]. Importantly, parallelism is found even among less-differentiated alleles in North American and Australian populations of *D. melanogaster*, suggesting that many polymorphic sites are targets of clinal selection [52] (Figure 2). One cautionary note is that migrants from Europe or Africa founded the North American and Australian populations, probably bringing along both the high- and low-latitude-adapted alleles [55]. Therefore, any neutral variants linked to the causal variants in the migrant population could also show patterns of clinal selection simply because of their proximity to recently selected sites. Differentiation at both *cpo* and at key circadian rhythm genes also supports parallel adaptation in North American [50] and Australian [51] populations of *D. melanogaster*. Strikingly, clinal variation in circadian genes has previously been identified in salmonids [56,57], passerine birds [58], and plants [59,60] in addition to *D. melanogaster* [11,61–63] (but see [64]).

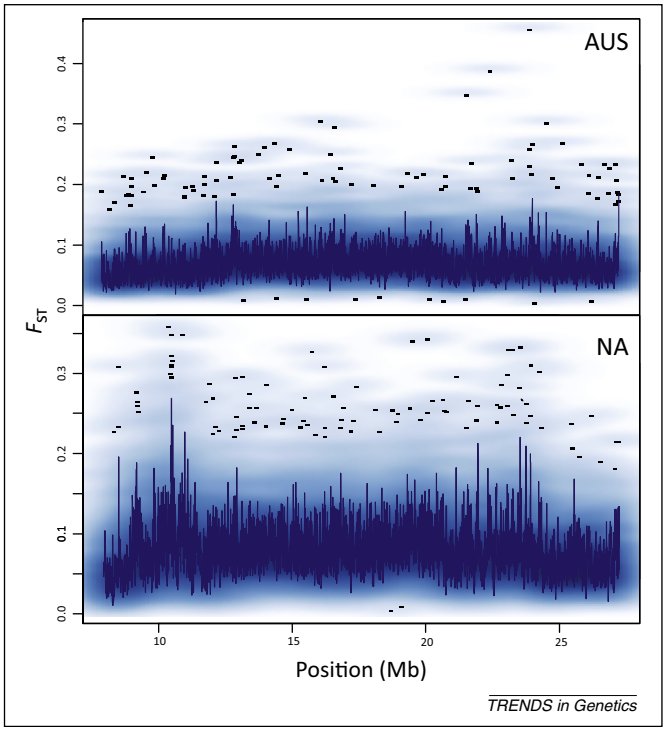

Figure 2. Genome-wide SNP data from the end points of the Australian (top) and North American (bottom) cline. Patterns of differentiation along chromosome arm 3R highlight exceptionally differentiated SNPs – potential targets of clinal selection. The dark blue line represents median estimates of F_{ST}, the density at a position is indicated by the intensity of the blue cloud, and black dots represent outliers. Adapted from [52].

Many genes are differentially expressed between high- and low-latitude populations of both *D. melanogaster* and *D. simulans* in North America [12]. Of the genes that are differentially expressed in both species, most differ in the same direction; that is, highly expressed genes at high latitudes in *D. melanogaster* are also highly expressed in high-latitude populations of *D. simulans* [12]. Although gene expression is a plastic trait that can be affected by the temperature at which flies are raised, genes that are differentially expressed between high- and low-latitude North American populations at specific temperatures also tend to be differentially expressed between Australian populations exposed to similar temperatures [12,65]. Additional studies are needed to strengthen the evidence for parallel adaptation of gene expression between clines within and between species. Regardless, exploring parallel patterns of gene expression will be a fruitful enterprise for discovering novel clines and for identifying the causative alleles underlying quantitative trait variation and local adaptation.

Copy number variants (CNVs) are an important source of functional genomic variation [66] and have been shown to vary with respect to latitude in populations of *D. melanogaster* from both North America [67] and Australia [54]. Analysis of genes within differentiated CNVs suggests that CNVs can respond to spatially varying selection pressures such as pesticides [54,67]. Likewise, the movement of transposable elements (TEs) represents another important source of genetic variation: TEs have been implicated in resistance to viral infection [68] and insecticides in *D. melanogaster* [69] and in resistance to insecticides in the mosquito *Culex pipiens* [70]. Members of many TE families exhibit clinal variation in their relative frequencies with respect to latitude in *D. melanogaster* [71] and recently clines on multiple continents were identified in a TE from the invader4 family, which is associated with shorter development time in *D. melanogaster* [72]. Parallel clines were found in both North America and Australia and similar differences are seen across the Europe–Africa cline, although not within Europe [72]. The flanking regions of this element show signatures of recent positive selection suggesting selection for shorter development times at higher latitudes [72]. These early results, assessing only a limited number of TE families, suggest that clinal analysis of TEs across the genome are needed.

Although not all of the genes involved in clinal adaptation are known, we can speculate on their identity by combining phenotypic and genomic data, and specifically by identifying regions of the genome exhibiting parallel differentiation among continents. Among populations of *D. melanogaster*, recent studies found strong enrichment of many important biological functions including genes involved in embryonic development, larval development, transcriptional regulation, eye development, signaling, and immunity [50–52]. Parallel differentiation among continents has been identified in genes associated with *D. melanogaster* wing morphogenesis [52], suggesting a connection to well-characterized clines in wing size [13,21,73]. More generally, 31% of the genes that are differentiated between end-point populations in North America [50] are also differentiated in Australia [51]. By contrast, some functions – for example, genes associated with pigmentation during development – that are enriched in North America are not enriched in Australia [52]. Unlike pigmentation during development, pigmentation of the thoracic trident has been shown to strongly correlate with latitude in populations of *D. melanogaster* from Australia [74]. Moreover, abdominal pigmentation is correlated with latitude in India [75] and with altitude in Sub-Saharan African populations of *D. melanogaster* [76]. Connections between genomic and phenotypic pigmentation clines within Australian *D. melanogaster* suggest that parallelism among continents is not a necessary requirement to glean key inferences about the targets of clinal selection. Regardless, future concordance among studies in the functional categories of genes that show signatures of parallel adaptation will help provide candidates for future experimentation and analysis [50–52].

The current state of clinal genomics outside *D. melanogaster*

Despite innovations in whole-genome sequencing technologies, there currently exist relatively few studies investigating the impact of clinal selection on genomic variation. There are, for example, currently no published clinal genomic studies for other *Drosophila* species despite much work describing clines in multiple phenotypes and genetic markers. Many of these patterns resemble those seen in *D. melanogaster*, including clinal variation in body size traits in *D. subobscura* [77], *D. simulans* [78], and *D. serrata* [79], temperature tolerance in *D. simulans* [80] and *D. serrata*

[79], pigmentation in *D. yakuba* [81] and *D. simulans* [82], and inversions in *D. subobscura* [83–85]. There are, of course, also many examples of traits that are not clinal in these other species or where the pattern of variation is different from that found in *D. melanogaster*. Still, the parallelism observed between *D. melanogaster* and *D. simulans* in the clinality of gene expression hints at the utility of species comparisons when attempting to identify the genes that are generally important for clinal adaptation [12].

It is perhaps unsurprising that genome-wide patterns of clinal variation in humans and *Arabidopsis thaliana* are among the best-studied cases outside *Drosophila*, given the wealth of geographically stratified genomic data available for both. Although data for these two species and others have not always been collected with clinal variation in mind, their results nevertheless represent pertinent examples of how genomic data can inform our understanding of environmental adaptation. In humans, clines in body size [86–88], skull and brain morphology [89], *HLA* allele frequency [90], skin pigmentation [91], salt sensitivity [93], and susceptibility to hypertension [94] have all been described. Instead of identifying the causal variants underlying many of these well-characterized phenotypic clines, studies utilizing genome-wide SNPs in humans have primarily focused on characterizing population structure across continents, where genomic variation is strongly correlated with geography [95–98]. While migration may explain much of the observed population structure in humans [95], adaptation to local environmental conditions is also recognized as an important factor influencing human genomic differentiation [99]. SNP frequencies correlate with gradients in precipitation, temperature, and solar radiation across multiple geographic regions, as do SNPs associated with thermal tolerance, pigmentation, disease resistance, and life at high altitude [100–103]. It is clear that identifying the genomic basis of many clinal patterns in humans is both attainable and of importance.

In *A. thaliana*, multiple fitness-related life history traits – such as growth rate, flowering time, and seed dormancy – correlate with latitude [104]. Moreover, genome-wide SNP frequencies are associated with both geography and a host of important climatic gradients such as temperature, precipitation, isothermality, aridity, and day length [105–107]. Evidence indicates local adaptation shaping much of this genomic variation; for example, variation in climate data explains more variation in nonsynonymous SNPs than predicted by chance [107]. Moreover, relative to synonymous SNPs, nonsynonymous SNPs were enriched among the loci most strongly associated with precipitation, humidity, temperature, and day length [106]. Finally, alleles associated with high fitness also tend to be locally abundant alleles that covary with climatic factors [105].

In addition to *D. melanogaster* and other model systems, recent studies exploring genomic patterns of local adaptation have surveyed several non-model organisms. Studies of the malaria vector *Anopheles gambiae* have revealed extensive clinal variation along an aridity gradient in Cameroon [108]. In *A. gambiae*, the frequency of a large polymorphic inversion on chromosome 2L covaries with both latitude and aridity (Figure 3A). Moreover, SNPs within the inversion are strongly differentiated between northern and southern populations, whereas SNPs in collinear regions of the genome appear nearly panmictic (Figure 3A). Interestingly, a parallel cline has been observed in a distantly related mosquito, *Anopheles funestus*, that exhibits clinal variation in the frequency of an inversion on chromosome 3R along the same latitudinal transect in Cameroon (Figure 3B). This degree of parallelism provides an excellent opportunity to study the link between inversion polymorphisms and adaptation to the environment. Genome-wide clinal variation has also been identified in *Medicago truncatula*, an annual legume, sampled throughout the Mediterranean [109]. SNPs were correlated with gradients in several abiotic factors such as mean annual temperature, precipitation in the wettest month, and isothermality. Moreover, patterns of nucleotide diversity surrounding clinal SNPs suggest a history of positive selection shaping the evolution of some of these loci [109]. Genome-wide SNPs from Atlantic salmon (*Salmo salar*) sampled along a cline in eastern Canada are correlated with temperature, river properties, geological variables, and longitude [110]. Similarly, strongly differentiated SNPs from Atlantic herring (*Clupea harangus*) sampled around the Baltic Sea were identified along a salinity gradient [111,112]. Finally, SNPs are correlated with temperature and precipitation in populations of the black cottonwood (*Populus trichocarpa*) sampled along a latitudinal transect in North America [113]. These examples of genome-wide clinal variation demonstrate the diversity of climate factors influencing genomic differentiation.

The future of clinal genomics

In *D. melanogaster* and other model systems, whole-genome data from clines will make it possible to identify individual regions of the genome that are differentiated along with phenotypic traits. However, the most impressive genomic patterns of clinal differentiation in *D. melanogaster* come from studies limited to sampling the end points of clines – two geographic locations that often differ dramatically in many environmental factors. Sampling only the end points of a cline potentially limits the power of these studies to link the targets of selection with any particular environmental factor. Moreover, correlations between axes of environmental variation – such as the correlation between day length and temperature – can obscure both the true targets and the agents of selection. Statistical methods that can appropriately handle correlated environmental factors have recently been developed and promise to illuminate many loci responding to selection via a complex environment [114–116]. However, there remains a need for novel population genetic theory that extends classic models of clines with two fitness optima to models incorporating shifting optima over a continuous landscape [145] – the biological reality of environmental gradients in nature (Box 1).

Fine-scale sampling from multiple geographic locations along climatic gradients – and the development of statistical approaches for dealing with such data – will help to parse genomic patterns of adaptation that may be eclipsed by differentiation at larger geographic scales. Likewise,

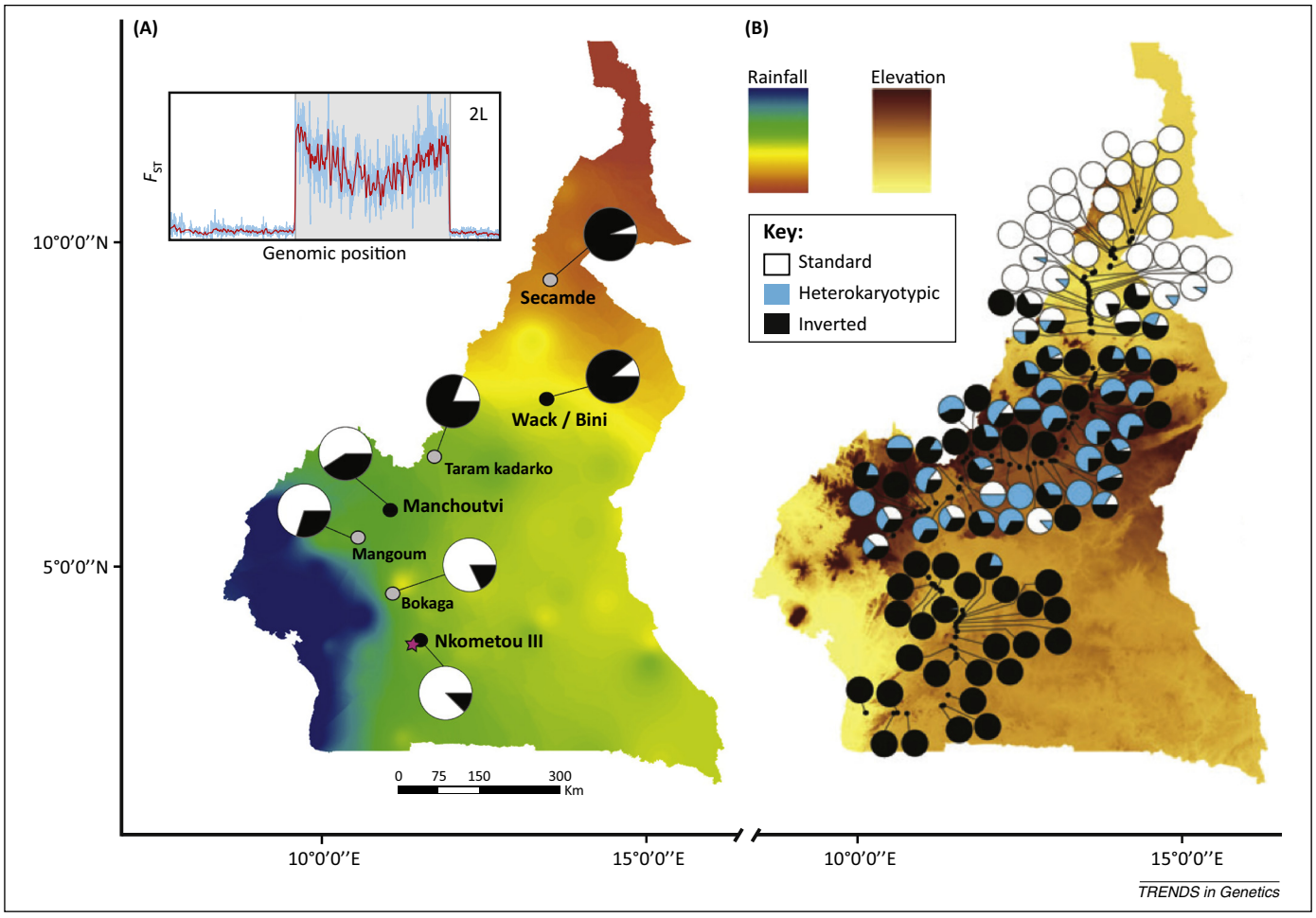

Figure 3. Parallel clines in two distantly related mosquitoes along a latitudinal transect in Cameroon. **(A)** Pies show the frequency of the standard (*2L+*^{a}, white) and inverted (*2La*, black) arrangement of inversion *2La* in *Anopheles gambiae*. The inset displays genomic differentiation surrounding the *2La* inversion (shaded area) as F_{ST} – plotted over 200-kb windows (red) and 20-kb windows (blue) – between high- and low-latitude populations. Adapted from [108]. **(B)** The karyotype frequency of the standard homozygote (white), heterozygote (blue), and inverted homozygote (black) arrangement of inversion *3Ra* in *Anopheles funestus*. Reproduced, with permission, from [144].

fine-scale sampling may also be more effective at revealing the unique environmental variables associated with a particular pattern of genomic variation (e.g., frequency of a SNP, TE, or inversion), as patterns of variation might be expected to become increasingly distinct when sampled at smaller spatial scales. Future studies should reap the benefits of joint approaches that identify connections between both outlier loci and their environmental correlates.

In addition to sampling fine-scale spatial genomic variation, future studies should also consider variation along a temporal scale. Researchers have recently shown that changes in allele frequencies can occur across seasons within populations of *D. melanogaster* [117]. Determining the extent to which the loci under seasonal selection align with those under spatially varying selection will provide an additional avenue for identifying the targets of climatic selection and patterns of parallel adaptation.

Perhaps the most promising avenue for future clinal genomic studies involves harnessing known phenotypic clines, both to characterize their genetic determinants and to discover novel clines correlated with similar geographic axes. Related approaches have been successfully applied to understand the genetic basis of both skin pigmentation and adaptation to life at high altitudes in humans. Skin pigmentation is one of the most noticeable human phenotypes and is strongly correlated with latitude and UV intensity. Both candidate-gene approaches and genome-wide scans have yielded numerous loci associated with variation in pigmentation [118–121]. Likewise, the genetic basis of adaptation to life at high altitudes has been well characterized in populations of Tibetans displaying high-altitude phenotypes [100,102,103,122]. Still, these studies have primarily contrasted highland Tibetans with closely related lowland Han Chinese populations, similar to sampling only the end points of a cline. Future studies that take advantage of gradients in altitude promise to identify novel clines associated with this important geographic axis.

Finally, more data are clearly needed in non-model systems, and once these are available the same approaches that have been useful in model systems can be employed with relative ease. For example, clinal patterns of phenotypic differentiation have recently been characterized in monkeyflowers [123], ivyleaf morning glories [124], Sakhalin firs [125], *Anolis* lizards [126], and Japanese sika deer [127]. Connecting these recent examples of phenotypic variation to patterns of genomic variation will clearly be a fruitful avenue for future exploration. Furthermore, the availability of well-assembled genomes in systems that have previously been found to have phenotypic or

single-marker clines will be one of the most promising developments in the near term. As the province of genomics expands and matures, clinal genomics is poised to deliver one of the most valuable avenues to understand adaptation to spatially varying environments.

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