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NEWS AND VIEWS

PERSPECTIVE

Genomics of clinal variation in *Drosophila*: disentangling the interactions of selection and demography

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Clines in phenotypes and genotype frequencies across environmental gradients are commonly taken as evidence for spatially varying selection. Classical examples include the latitudinal clines in various species of *Drosophila*, which often occur in parallel fashion on multiple continents. Today, genomewide analysis of such clinal systems provides a fantastic opportunity for unravelling the genetics of adaptation, yet major challenges remain. A well-known but often neglected problem is that demographic processes can also generate clinality, independent of or coincident with selection. A closely related issue is how to identify true genic targets of clinal selection. In this issue of *Molecular Ecology*, three studies illustrate these challenges and how they might be met. Bergland *et al.* report evidence suggesting that the well-known parallel latitudinal clines in North American and Australian *D. melanogaster* are confounded by admixture from Africa and Europe, highlighting the importance of distinguishing demographic from adaptive clines. In a companion study, Machado *et al.* provide the first genomic comparison of latitudinal differentiation in *D. melanogaster* and its sister species *D. simulans*. While *D. simulans* is less clinal than *D. melanogaster*, a significant fraction of clinal genes is shared between both species, suggesting the existence of convergent adaptation to clinaly varying selection pressures. Finally, by drawing on several independent sources of evidence, Božičević *et al.* identify a functional network of eight clinal genes that are likely involved in cold adaptation. Together, these studies remind us that clinality does not necessarily imply selection and that separating adaptive signal from demographic noise requires great effort and care.

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Clines as a test bed for the genetics of adaptation

Spatially varying selection along environmental gradients often leads to systematic, gradual changes in phenotypes and genotype frequencies, so-called 'clines' (Huxley 1938). Investigating such clines is a classical method for studying local adaptation (Endler 1977). For example, many decades of work have established fruit flies (*Drosophila*) as an excellent model for studying clinal adaptation (De Jong & Bochdanovits 2003; Hoffmann & Weeks 2007): multiple species of this genus exhibit strong, geographically replicated patterns of latitudinal differentiation (presumably driven by gradients in climate and seasonality) in fitness-relevant traits such as body size and cold tolerance, and these phenotypic gradients are often associated with clines in genetic markers (Fig. 1).

The collinearity of environment, genetic markers and ecologically relevant traits makes clines a powerful system for identifying genic targets of spatially varying selection (Savolainen *et al.* 2013). For a long time, studies of clinal variants were limited to examining single markers, for instance microsatellites, allozymes or inversion polymorphisms (Vasemägi 2006; Adrion *et al.* 2015). Despite low resolution, this approach has led to the identification of many important genotypic clines, the perhaps best-known examples being the *Alcohol dehydrogenase* (*Adh*) locus and the cosmopolitan inversion *In(3R)Payne* in *D. melanogaster* (Adrion *et al.* 2015). Today, lack of resolution is no longer an issue: researchers can now analyse clinal patterns on a genomic scale, at millions of single nucleotide polymorphism (SNP) positions, as has recently been done for latitudinal differentiation in Australian and North American *D. melanogaster* (Turner *et al.* 2008; Kolaczkowski *et al.* 2011; Fabian *et al.* 2012; Bergland *et al.* 2014; Kapun *et al.* 2014; Reinhardt *et al.* 2014). This body of work has identified hundreds of strongly clinal genetic variants, many of which show parallel frequency gradients in Australia and North America, in support of clinal selection. Thus, these are exciting times for those who study adaptation using clinal genomics (Adrion *et al.* 2015).

Despite the power and resolution of genomewide approaches, however, identifying the true genic targets of spatially varying selection remains a considerable challenge. Three new papers in *Molecular Ecology* make important advances towards addressing different aspects of this problem.

Clinal differentiation: selection, demography or both?

Genetic clines, especially when associated with phenotypic clines, and when replicated across broad geographic areas (as is the case in several *Drosophila* species), are commonly

Fig. 1 Clinal adaptation to different habitats in *Drosophila*. Along the North American east coast, *D. melanogaster* and *D. simulans* occur in a range of different habitats and exhibit major clinal differentiation across latitude, both at the phenotypic and genotypic levels. Northern and southern habitats approximating the endpoints of the cline differ in many environmental aspects: climate (e.g. temperature, rainfall), seasonality and photoperiod, species composition and phenology of fruits that serve as nutrition and egg-laying substrates for the flies, and so forth. Top: an apple orchard in Maine (Clark's Cove Orchard, Walpole, ME), illustrating a temperate, seasonal habitat with harsh winters. Bottom: a subtropical habitat in southern Florida (Fruit and Spice Park, Homestead, FL), with many tropical fruits (e.g. bananas, mangoes). Photo credit: Paul Schmidt (University of Pennsylvania).

taken as *prima facie* evidence for the action of spatially varying selection. Indeed, for several fitness-related traits (e.g. body size) and many genetic variants, parallel clines have been observed between Australia and North America (Fabian *et al.* 2012; Reinhardt *et al.* 2014). However, a well-known but often forgotten fact is that demographic processes such as admixture and isolation by distance (IBD) can also generate pervasive clinality (Endler 1977; Caracristi & Schlötterer 2003; Vasemägi 2006; Duchen *et al.* 2013; Kao *et al.* 2015).

In this issue of *Molecular Ecology*, Bergland *et al.* (2015) present convincing evidence suggesting that the historically recent (~150–200 years old) North American and Australian east coastal populations were both likely founded by African and European immigrant genotypes. By drawing on new genomic data and extensive population genetic analyses, the authors infer that secondary contact and hybridization must have made a substantial contribution to the patterns of clinal differentiation seen between both continents, although the results seem to be weaker for Australia. While the specific sources of the genetic influx remain to be identified, the existence of such 'ancestry clines' has several major implications. The first is that there is much more to clinality than selection: demographic processes can contribute to clinality in major ways and thus complicate the adaptive interpretation of clines. Second, parallelism of clinal patterns across broad geographic regions does not automatically imply parallel selection as parallelism can also arise from demographic processes independent of selection. Third, demographic clines and adaptive clines are not mutually exclusive: not only can both types of clines coexist, they can also be superimposed on each other, with colonizing immigrant lineages providing important raw material for ecological niche sorting and for clinal selection to act upon. Perhaps in line with this idea, Bergland *et al.* (2015) find that – remarkably – the proportion of African ancestry in North American and Australian populations is negatively correlated with latitude, whereas the proportion of European ancestry is positively correlated with latitude. This might be consistent with the notion that African genotypes might be 'pre-adapted' and selectively favoured in subtropical and tropical locales, whereas European genotypes might be predominantly favoured in temperate, seasonal habitats.

The strong collinearity of ancestry and selective clines clearly makes differentiating true adaptive from demographic signals an important and nontrivial challenge; future studies will thus need to refine their analyses to separate these signals. This will be especially difficult in cases where demography (e.g. ancestry) and selection causally interact to shape patterns of genetic differentiation.

Comparative genomics: convergent clines between species indicate shared selection

The second study, by Machado *et al.* (2015) capitalizes on the power of comparative genomics to better understand clinal differentiation. For the first time, the authors compare clinal genomic patterns in *D. melanogaster* to those seen its sister species, *D. simulans*. This is a particularly promising approach as both species exhibit a similar ecology, distribution and evolutionary history, including sub-Saharan African origin, parallel out-of-Africa migration, cosmopolitan range expansion, and adaptation to temperate, seasonal habitats.

Consistent with previous work showing stronger phenotypic clinality in *D. melanogaster* than in *D. simulans*, Machado *et al.* (2015) find that *D. melanogaster* harbours a significantly larger proportion of clinal variants (3.7%) than *D. simulans* (2.5%), with the difference being even larger when only the autosomes are considered (4.3% vs. 2.1%). For *D. melanogaster*, the authors observe that clinal variants

are strongly enriched (9%) on chromosomal arm *3R*, a pattern that has been noticed before and which might be explained by the presence of a large, strongly clinal inversion polymorphism, *In(3R)Payne*, in this genomic region (Fabian *et al.* 2012; Kapun *et al.* 2014). This is an interesting finding in view of the fact that *D. simulans* is largely inversion-free; yet, even outside inversions, *D. melanogaster* still has a greater proportion of clinal variation than *D. simulans*. As Machado *et al.* (2015) argue convincingly, the difference in clinality between the two species is most likely explained by the observation that in *D. simulans* clinal variants are much less stable over time, probably due to strong population bottlenecks in winter (note that *D. simulans* is less cold-tolerant than *D. melanogaster*) and that IBD is weaker than in *D. melanogaster* (where IBD is highly significant but overall still weak), presumably due to annual migration and local recolonization in *D. simulans*.

Most strikingly, Machado *et al.* (2015) present compelling evidence for convergent evolution of clinal variants in both species, presumably due to similar clinal selection pressures. Although the authors fail to find significant enrichment for shared clinal SNPs, they detect a significant, 24% enrichment of shared clinal genes (observed proportion: 56%, expected: 45%; ratio: 1.24). Interestingly, when considering 12 genes with substantial literature support for harbouring strongly clinal alleles, the authors find 10 of them to be significantly clinal in both species, including *Adh*, *couch potato* (*cpo*; involved in regulating reproductive diapause), *Insulin-like Receptor* (*InR*; a gene with pleiotropic life history effects; see Paaby *et al.* 2014) and *period* (*per*; a clock gene). This represents additional support for the major role these genes are thought to play in clinal adaptation.

Cold adaptation: a network of clinally varying genes

The third study by Božičević *et al.* (2015) investigates footprints of polygenic cold adaptation between temperate European and tropical African populations of *D. melanogaster* and illustrates the inferential power that can be harnessed by analysing several independent sources of information. To identify adaptive SNPs, the authors use a meta-analysis pipeline, including demographic simulations to assess null distributions and forward simulations of quantitative traits to derive estimates of the power of distinguishing between adaptation and neutrality. Although the geographic sampling of populations in this study is limited, the authors present four lines of strong evidence that several candidate genes (and SNPs located in them) play a major role in temperature adaptation. First, the authors find that SNPs in genes already known from genomewide association (GWAS) studies to be involved in cold adaptation covary significantly with environmental gradients among derived, temperate European populations from the Netherlands and France and in African populations from Rwanda and Zambia. Second, they show that clinally (altitudinally or latitudinally) differentiated SNPs in Europe and Africa overlap with SNPs already known to be latitudinally varying in North America (Fabian *et al.* 2012). Third, the top candidate genes identified by Božičević *et al.* (2015) are enriched for gene ontology (GO) terms related to cold tolerance. Fourth, GO terms enriched for clinal genes in North America exhibit a significant overlap with GO terms for the European and African populations. Based on these synergistic analyses, Božičević *et al.* (2015) examine their top 8 candidate genes in more detail and discover that – intriguingly – they form a functional network that is likely under strong selection and presumably of central importance for cold adaptation. This candidate network thus provides rich material for future functional tests of climatic and clinal adaptation at the genic and SNP level.

Prospects and conclusions

Several important lessons for future work can be learned from the three studies discussed here.

First, clinal patterns for putatively adaptive candidate variants must be contrasted with patterns expected under admixture and IBD. This is currently still rarely being done in analyses of clinal differentiation. At least in principle, it is possible to formally compare the clinality of candidate signals against the clinal demographic 'background' (e.g. IBD, admixture), for example by comparing clinal patterns of candidates with those of likely neutral SNPs (e.g. in short introns). For example, an important future objective will be to examine the effects of admixture on adaptive inference using simulations.

Second, clinal parallelism remains a strong argument for convergent patterns of clinal selection, especially when phenotypic differentiation goes hand in hand with genic differentiation, but can be severely confounded by demography. A potentially powerful approach is to compare clinal patterns between closely related species, as done by Machado *et al.* (2015) for *D. melanogaster* and *D. simulans*. Finding shared clinal genes across sister species that occur along similar environmental (climatic) gradients considerably strengthens the case for clinal selection.

Third, to build a convincing case for clinal adaptation, it is important – whenever possible – to draw on several independent sources of evidence (e.g. sibling species, multiple populations or geographic regions, environmental correlations that account for population structure, GWAS, GO analyses, etc.). For example, the powerful meta-analysis approach of Božičević *et al.* (2015) (or modifications thereof) might be used to decompose the potential superposition of demography and adaptation.

Finally, as pointed out by Bergland *et al.* (2015) and Božičević *et al.* (2015) the perhaps most important future objective of clinal studies will be to perform functional tests of candidate targets of clinal selection. This might be achieved using synthetic recombinant inbred populations to isolate and compare alleles of interest, quantitative complementation tests or reciprocal hemizygosity tests (see Paaby *et al.* 2014 for an example). Ultimately, the 'gold standard' for establishing causal effects of natural alleles is their homologous replacement into a common genetic background (Turner 2014): this can now be achieved in

many systems via CRISPR/Cas-9, a powerful genome editing method that is rapidly improving. While successful applications of this method to SNPs have not yet been published for intraspecific population differentiation in *Drosophila*, there can be no doubt that functional testing of clinal candidates will significantly advance our understanding of local adaptation.

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