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NEWS AND VIEWS

PERSPECTIVE

Connecting genetic variation to phenotypic clines

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From early allozyme work to recent genome-wide scans, many studies have reported associations between molecular markers and latitude. These geographic patterns are tantalizing because they hint at the possibility of identifying specific mutations responsible for climatic adaptation. Unfortunately, few studies have done so because these exciting first glances often prove extremely challenging to follow up. Many difficulties can hinder connecting genetic and phenotypic variation in this context, and without such links, distinguishing the action of spatially varying selection from the other evolutionary processes capable of generating these patterns can be quite thorny. Nevertheless, two papers in this issue report excellent progress in overcoming these obstacles and provide persuasive evidence supporting the involvement of specific natural variants in clinal adaptation of *Drosophila melanogaster* populations (Fig. 1). In the first paper, Paaby *et al.* (2010) describe replicated allele frequency clines for a coding polymorphism in the *Insulin-like Receptor* (*InR*) gene on two continents, findings that strongly point to selection acting at this locus and that likely reflect life history adaptation. McKechnie *et al.* (2010) report compelling functional evidence that *cis*-regulatory variation in the *Dca* (drosophila cold acclimation) gene contributes to an adaptive cline in wing size. Notably, these papers employ largely alternative and complementary approaches, and together they exemplify how diverse strategies may be interwoven to draw convincing connections between genotype, phenotype, and evolutionary process.

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Discovering genetic changes important for adaptation along environmental gradients is a critical goal. These genes can teach us about the size, number, and types of genetic changes involved in historical adaptive shifts as well as the importance of gene function and genetic network structure in constraining evolutionary responses. From these parameters, we can gain predictive power for assessing how species may respond to global change. Both sets of authors chose candidate genes predicted to affect a clinally varying trait as a point of departure. McKechnie *et al.* focused on *Dca* because a previous study found a strong association between this gene and wing size variation in a single population (Rako *et al.* 2007). *InR* was chosen by Paaby *et al.* because, like high-latitude populations compared to low-latitude populations, mutants in *InR* and other insulin signalling pathway genes have longer life spans, increased stress tolerance, and decreased reproductive success compared to wild-type flies (Schmidt *et al.* 2005; Tatar *et al.* 2001). For both *InR* and *Dca*, reciprocal clines in allele frequency for common alleles were found in Australia. Paaby *et al.* also found strikingly similar trends for *InR* alleles in US populations.

While these correlations implicate *InR* and *Dca* in clinal adaptation, correlations can arise for a multitude of reasons and several questions must be addressed to develop the full evolutionary story. First, how finely can the latitude-associated gene region be delimited, allowing spurious associations caused by population structure or selection at linked loci to be excluded? Second, does the allelic variation directly cause changes in a clinally varying phenotype? Finally, is there evidence of selection on either the gene or its associated phenotype by a spatially varying environmental condition?

With respect to the first question, both studies benefit from *D. melanogaster*'s dispersal abilities. Unlike many species, population structure is largely absent even at continental scales, and the vast majority of loci show no association with latitude (Turner *et al.* 2008). Selection at

Fig. 1 *Drosophila melanogaster* mating in the field. Credit: Annalise Paaby.

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linked loci is a more pressing concern. Both *InR* and *Dca* are tightly linked to a chromosomal inversion polymorphism and thus additional genes and markers whose allele frequencies also vary with latitude (Weeks *et al.* 2002). Each study demonstrates their variants are independent of this inversion; their genes still exhibit allele frequency clines when flies with or without the inversion are separately considered.

Paaby *et al*. cleverly took advantage of geographic replication and model system genetics to further delimit their latitude-associated gene region to a candidate nucleotide change and define its function. The authors were able to show through extensive sequencing and genotyping studies that only an indel variant in the first exon exhibits robust allele frequency clines on both continents. Linkage disequilibrium rapidly declines to either side of this variant, further supporting the inference that this is an independent site. Observation of replicate clines of the same variant on two continents is compelling evidence for natural selection on *InR* since other evolutionary processes like genetic drift or migration are highly unlikely to reproduce the same pattern.

To test the functional relevance of the *InR* variation, Paaby *et al*. isolated chromosomes containing the two clinally varying alleles in a common genetic background. After several generations of recombination pared away associations with other loci, flies carrying different *InR* alleles exhibited significant differences for several phenotypes in directions consistent with geographical patterns. Females with the allele at high frequency in high-latitude populations lived longer, recovered faster from cold stress, and laid fewer eggs than females carrying the allele at high frequency in low-latitude populations. If, as the authors propose, the indel contributes to all these phenotypic differences, then that would be a very interesting result because it would provide a concrete mechanistic basis for tradeoffs between correlated life history traits. However, as the authors note, the crossing scheme did not completely dissociate SNPs within *InR* and nearby genes from the indel, and these results cannot decisively address this matter. Moreover, different nucleotides within another pleiotropic *Drosophila* gene independently affect natural variation in different life-history traits (Carbone *et al.* 2006). Nevertheless, the authors have laid solid groundwork toward confirming their hypothesis that this single coding mutation has pleiotropic effects contributing to clinal adaptation.

McKechnie *et al*. followed an alternative experimental path toward defining a role for their gene, *Dca*, in clinal variation of their trait of interest, wing size, because no previous molecular work had demonstrated that *Dca* directly controls wing size. Like a previous study that found an association between variation in the *Dca* promoter and wing size (Rako *et al.* 2007), the authors used a complex crossing scheme that preserved a considerable amount of natural variation to obtain the same result in a second Australian population. Notably, this work helped disentangle *Dca*'s effects from those of the linked chromosomal inversion. While variation in *Dca* was not associated with thorax size, the inversion was associated with changes in both wing and thorax size, indicating that changes in *Dca* may specifically affect wing size and alter the wing : thorax size ratio. McKechnie *et al*. then took advantage of *Drosophila*'s genetic toolkit and overexpressed *Dca* in transgenic flies. *Dca* overexpression reduced wing size but not thorax size, confirming *Dca* specifically functions in wing size regulation.

Based on these results, the authors predicted that if *cis*-regulatory variation at *Dca* contributes to the cline in wing size, then the *Dca* allele associated with smaller wing size should have higher expression. Indeed, expression analysis showed that *Dca* expression increases as the frequency of the allele associated with smaller wing size increases. Thus, the combination of functional and expression studies performed by the authors yielded strong correlative evidence consistent with a causative role for these regulatory mutations in clinal variation. While McKechnie *et al*. provide no population genetic evidence for selection, wing size is known to affect dispersal performance in field release trials (Hoffman *et al*. 2007). Flies with large wings relative to their thorax disperse farther than flies with relatively small wings, and selection on dispersal caused by latitudinal differences in resource distribution or other factors could drive the observed clines in wing size.

Both papers make substantial progress in bridging individual genetic variants, clinally varying phenotypes, and natural selection, but further work is required to fully develop the evolutionary picture in either case. From a molecular perspective, additional transgenic or complementation studies are necessary to directly prove that the *InR* coding change or any of the several regulatory polymorphisms in *Dca* cause changes in life history traits or wing size. From an ecological viewpoint, the changing relationship between these phenotypes and fitness at different latitudes needs further experimental study to link specific selective pressures to organismal variation. The results from these current works will make such studies more feasible as clever genetic manipulation of *Dca* or *InR* could generate flies well suited for such tests.

While generalizations about the genetics of clinal adaptation will require identification of more genes, it is tempting to draw inferences based on the authors' findings. For instance, clinal adaptation appears to involve a plurality of mutation types; these studies and others have implicated both coding and regulatory changes (Caicedo *et al.* 2004; Hoekstra *et al.* 2004; Collinge *et al.* 2008; Fry *et al.* 2008; Mullen & Hoekstra 2008; Schmidt *et al.* 2008).

Both sets of authors speculate that only certain genes will harbour variation capable of effectively responding to selection along environmental gradients, and Paaby *et al.* provide some data consistent with this hypothesis. Other genes in the insulin signalling pathway show similar mutant phenotypes as *InR*, including *chico* (Clancy *et al.* 2001); however, unlike *InR*, the authors found that *chico* lacks clinal variation and exhibits no evidence of long term selection. Only the *chico* coding region was surveyed, however, and given the short distance over which linkage dis-

equilibrium breaks down, a potential role for *cis*-regulatory change cannot be excluded. While the different patterns of amino acid evolution in *InR* and *chico* over phylogenetic time may be attributable to different functional constraints, different clinal variation patterns could also be a consequence of differences in the sampling of ancestral genetic variation. The US and Australian clines have evolved over only the past few hundred years and the same indel mutation is associated with clinal adaptation on both continents, suggesting evolution occurred by selection on standing variation acquired from the source population. Thus, differences in ancestral variation at *InR* and *chico* or the sampling of this variation in founding populations could also yield divergent evolutionary outcomes for the two genes. Further population genetics studies of *InR*, *chico*, and other insulin pathway genes in African populations will help resolve these possibilities.

The sophisticated crossing scheme employed for the association study by McKechnie *et al*. allowed the authors to determine how much of the total variation in wing size in a population is controlled by promoter variation in *Dca*. They estimate that *Dca* controls a large amount (>20%) of the heritable variation in wing size, suggesting that mutations of large effect do play roles in clinal adaptation. From an empirical perspective, this is a cause for optimism. This finding indicates that clines are not solely governed by small changes in allele frequency in many genes with vanishingly small effects, and that additional genes involved in clinal adaptation are likely identifiable. Future studies that creatively integrate diverse experimental strategies like these two papers will undoubtedly be successful at doing so.

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