

Murillo Fernando Rodrigues

**Clinas neutras ou adaptativas? Integrando
variação genômica clinal e sazonal para inferir
seleção natural em *Drosophila melanogaster***

Adaptive or neutral clines? Integrating genome-wide
clinal and seasonal variation to infer natural selection in
Drosophila melanogaster

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Dissertação apresentada ao Instituto de
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Orientador: Prof. Dr. Rodrigo Cogni

Co-orientadora: Prof. Dra. Maria
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Comissão Julgadora

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Resumo

Variação espacial e temporal são ubíquas. Caracteres são chamados clinais quando variam ao longo de um gradiente ambiental, e isso é interpretado como resultado de seleção estruturada no espaço. *Drosophila melanogaster* apresenta clinas em diversos caracteres fenotípicos e genotípicos, as quais são replicadas em diferentes regiões do mundo. Estudos recentes sugeriram que grande parte da variação clinal pode ser atribuída a processos neutros. Como o ambiente varia de maneira similar com a latitude e ao longo do ano, e essa variação sazonal é ortogonal à demografia, uma abordagem possível para inferir seleção natural é integrar variação clinal e sazonal. Neste trabalho, nós testamos se há uma relação entre variação clinal e sazonal ao longo do genoma de *D. melanogaster*. Também, investigamos a proporção de variantes que deveriam estar sob seleção espacial e sazonal para explicar o padrão encontrado. Estimamos a frequência alélica a partir de amostras de *pools* de moscas coletadas em diferentes oito localidades ao longo da costa leste dos Estados Unidos e em diferentes estações do ano na Pensilvânia, EUA. Nós encontramos um padrão genômico de variação clinal refletindo variação sazonal. Esse padrão é mais forte para variantes em regiões exônicas do que intergênicas, consistente com a ação de seleção. A relação entre variação clinal e sazonal encontrada pode ser explicada se 6,6% dos polimorfismos estiverem sob seleção espacial e sazonal. Nossos resultados são consistentes com a hipótese adaptativa de variação clinal e, junto com outras observações, revelam que o papel da demografia na manutenção de clinas em *D. melanogaster* é limitado.

Abstract

Spatial and temporal variation in the environment are ubiquitous. Traits are called clinal when they vary along an environmental gradient, and this is often interpreted as the result of spatially varying selection. *Drosophila melanogaster* is known to have many phenotypic and genotypic clines, replicated in many regions of the world. Recent studies have suggested that most clinal variation could be attributed to neutral, demographic processes. Because the environment varies in similar ways with latitude and across seasons, and seasonal variation is orthogonal to demography, one promising approach is to integrate clinal and seasonal variation to infer selection. Here, we test whether there is a genome-wide relationship between clinal and seasonal variation, and whether the pattern is consistent with selection. Also, we investigate the proportion of the variants that should be under latitudinal and seasonal selection to explain the pattern we uncovered. We estimate allele frequency from pooled samples of flies from eight different locations along the east coast of the US, and 13 samples collected in the spring and in the fall in Pennsylvania. We show that there is a genome-wide pattern of clinal variation mirroring seasonal variation. This pattern is stronger for exonic when compared to intergenic regions, consistent with natural selection. We find that the genome-wide relationship between clinal and seasonal variation could be explained by about 6.6% of our SNPs being under latitudinal and seasonal selection. Our results are consistent with the adaptive hypothesis of clinal variation and, together with other observations, leave little room for the role of demography in maintaining clines in *D. melanogaster*.

Introduction

All species are subject to environmental variation structured through space and time. Different environments can impose various selective regimes on populations, resulting in adaptive differentiation. With spatially varying selection, one allele can be beneficial in one environment and disadvantageous in another (Levene 1953). Similarly, selective pressures can cycle through time, and one allele may be alternatively favored and disfavored (Gillespie 1973). Thus, both spatially and temporally varying selection can result in different forms of local adaptation (Ewing 1979).

Spatial heterogeneity generates a pattern of geographical variation in traits that affect fitness (Kawecki and Ebert 2004). Some species can be found along gradually changing environments, and traits that vary with the environment are called clinal. Examples of clinal variation are abundant in many different taxa, such as thale cress (Zuther et al. 2012), monkeyflowers (Kooyers et al. 2015), ivyleaf morning glories (Campitelli and Stinchcombe 2013), Atlantic salmon (Dionne et al. 2007), vervet monkeys (Cardini et al. 2007) and even humans (Hancock et al. 2008).

Drosophila melanogaster has been used as a model to understand latitudinal variation because, besides being a genetic model, it is a sub-Saharan fly species that has recently invaded most of the world (David and Capy 1988). These flies migrated into Eurasia approximately 15,000 years ago (Li and Stephan 2006), but the colonization of the Americas and Australia likely happened in a single event within the last several hundred years (Bock and Parsons 1981; Keller 2007). The establishment of populations in dramatically different environments (*e.g.*, temperate regions), is thought to have resulted in several climatic adaptations (David and Capy 1988).

Clinal variation has been documented for this species in many characters both at the phenotypic and genetic level. For instance, at the phenotypic level, flies from higher latitudes are darker (David et al. 1985), bigger (Arthur et al. 2008) and show higher incidence of reproductive diapause (Schmidt et al. 2005). At the genetic level, latitudinal clines have been identified for allozymes, chromosome inversions and single nucleotide polymorphisms (Mettler et al. 1977; Knibb 1982; Oakeshott et al. 1982; Schmidt et al. 2000; de Jong and Bochdanovits 2003; Sezgin et al. 2004; Fabian et al. 2012; Kapun et al. 2016).

These clinal traits in *D. melanogaster* are usually thought to be a result of spatially varying selection (Endler 1977). Although a covariation between an environmental variable and a trait is suggestive of natural selection, non-adaptive processes such as isolation by distance, range expansion and admixture can produce similar patterns of spatial change (Wright 1943; Vasemägi 2006; Excoffier et al. 2009; Duchon et al. 2013; Bergland et al. 2016). Because latitudinal clines are often repeated (*e.g.*, across continents), a classical approach to discern between adaptive and neutral differentiation has been to look at parallel clinal variation (Hoffmann et al. 2002; Hoffmann and Weeks 2007; Turner et al. 2008; Paaby et al. 2010; Reinhardt et al. 2014; Schrider et al. 2016). If a trait varies clinally in two or more continents, a simple, plausible scenario is latitudinally varying selection acting on ancestral variation (Endler 1977; Barton 1983; Barton 1999).

A recent study showed that parallel clinal variation between continents could be a result of secondary contact among previously diverged populations of *D. melanogaster* (Bergland et al. 2016). It appears that part of the clinality observed in flies from the east coast of North America and Australia can be attributed to migration of European flies to the high latitude end of the cline and migration of African flies to low latitude locations.

As a consequence, it may be impossible to distinguish adaptive from neutral clines just by looking at patterns of clinal variation.

To reject some of these non-adaptive hypothesis, one would need access to population parameters that are hard to measure or estimate, such as the distribution of fitness effects, landscape of recombination rate, rates of migration, population sizes, changes in population size and historical founding of the populations. Alternatively, one approach to disentangle adaptive from non-adaptive processes is by exploring signatures of parallel variation between latitude and seasons (Cogni et al. 2015).

Drosophila has also been widely used in the study of seasonal adaptation. One of the earliest examples of seasonal variation was observed in chromosomal inversions in *D. pseudoobscura* (Dobzhansky 1943). In *D. melanogaster*, it has been shown that flies collected in the spring are more tolerant to stress (Behrman et al. 2015), show higher diapause inducibility (Schmidt and Conde 2006), have increased immune function (Behrman et al. 2018) and have different cuticular hydrocarbon profiles than those collected in the fall (Rajpurohit et al. 2017). These studies were either conducted in a common laboratory condition or as a field based mesocosm experiment. Furthermore, genome-wide analysis have identified polymorphisms that oscillate in seasonal timescales in several localities in the United States and Europe (Bergland et al. 2014; Machado et al. 2018).

Many environmental variables that vary along a latitudinal transect vary in a similar fashion through seasons. Processes that can generate clinal patterns like isolation by distance and secondary contact from diverged populations are independent to variation across seasons. Therefore, traits that vary both with latitude and through seasons are likely under natural selection. For instance, it has been observed in *D. melanogaster* that both the prevalence of reproductive diapause and the frequency of a

variant in the couch potato gene associated with diapause inducibility vary latitudinally and seasonally (Schmidt et al. 2005; Cogni et al. 2014). The frequency of this diapause-inducing variant drops in the summer and is positively correlated with latitude.

Very few studies have tried to identify parallel clinal and seasonal variation in *D. melanogaster* (Bergland et al. 2014; Cogni et al. 2015; Machado et al. 2018). The association between clinal and seasonal change has been identified in central metabolic genes, which is likely caused by parallel climatic factors driving adaptation in these genes (Cogni et al. 2015). This study focused on a limited number of polymorphisms in metabolic genes. Bergland et al. (2014) found an indicative that clinal variants are more likely to be seasonal. A very recent study observed that seasonal changes mirrors latitudinal changes in *D. melanogaster* with the intent of validating seasonal outliers (Machado et al. 2018), using few seasonal samples from many different locations.

Here, we aim to answer whether the clinal patterns observed in *D. melanogaster* are consistent with the action of natural selection. We do so by looking at parallel clinal and seasonal variation across the genome. Because many environmental variables such as temperature, UV radiation and resource availability vary similarly across latitude and through seasons in a temperate environment, we hypothesize there is a relationship between clinal and seasonal variation. Flies collected in the north should be more similar to flies collected in early spring, whereas southern flies should be more like fall flies. We also tested whether the pattern of clinal mirroring seasonal variation is consistent with selection. Finally, by looking at the relationship between clinal and seasonal variation, we asked how much of the genome is under spatial and seasonal selection. Integrating these two independent sources of evidence can help us disentangle adaptive from non-adaptive processes that contribute to the pervasive and long standing patterns of clinal variation in *D. melanogaster*.

Conclusion

Many species occur along spatially structured environments, and show clinal variation in many traits, so a question that remains open is whether these patterns are the result of natural selection or neutral demographic processes. Temporal variation is also ubiquitous, especially in temperate environments, so seasonal adaptation could be an important feature of organisms that have multiple generations each year (also called multivoltine) (Behrman et al. 2015). Here, we demonstrate that by integrating clinal and seasonal variation, it is possible to discern adaptive and non-adaptive hypothesis for clinal variation. This approach could potentially be applied to other multivoltine species that occur along environmental gradients. Notably, this approach can be applied in invasive species, which are known to often have short-generation times and to reproduce quickly (Sakai et al. 2001). Our data is consistent with the adaptive hypothesis for clinal variation in *D. melanogaster*, and our results could hold for many of the other species which show clinal variation in natural populations.

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