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Clinal variation and selection of MDH allozymes in honey bee populations

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Abstract. Latitudinal clines of malate dehydrogenase-1 (MDH-1) allozymes occur within honey bee populations on three continents: Europe, North America and South America. The North and South American populations are introduced and demonstrate that Mdh allelic clines were established within the last 150 years. The frequency of the 'medium' electrophoretic allele increases in frequency with increasing latitude while the 'fast' allele decreases with latitude on all the three continents. The clines are best explained by the average daily high temperature for July on all continents. These parallel clines provide evidence for selection on Mdh alleles in honey bees.
Key words. Clinal selection; allozymes; malate dehydrogenase; honey bee; *Apis mellifera.*

Natural selection on allozymes has long been a subject of considerable debate. Most of the few convincing cases of such selection have come from the demonstration of parallel latitudinal clines in allele frequencies on different continents. In this paper we show that the allozymes of MDH in honey bees exhibit parallel clines on three continents.

Latitudinal clines have been studied in native and introduced *Drosophila* populations. In *Drosophila melanogaster*, alcohol dehydrogenase (ADH) and glycerol-3-phosphate dehydrogenase (GPDH) show similar allelic clines on different continents[1]. Selection is presumed to be the cause of these clines, although environmental correlates corresponding to allelic variation in these natural populations have been elusive[2]. Genetically isolated species occupying the same range and possessing similar allozymic patterns also suggest that selection maintains their allele frequencies. Such clines have been demonstrated for the EST-6 enzymes of *Drosophila melanogaster* and *Drosophila simulans*[3]. However, like the clines for ADH and GPDH, environmental correlates have not been found. Selection has also been suggested for the occurrence of parallel clines of chromosomal inversion frequencies in *Drosophila subobscura* on three different continents, two of which resulted from introductions from the Old World[4].

European honey bees are an example of another species that has been introduced to several continents, providing population research opportunities similar to those in *Drosophila*. Honey bees were introduced into eastern North America in the 17th century, and after several unsuccessful attempts at importation, were eventually established in California in 1853[5]. The first bees introduced into California were *Apis mellifera mellifera*, a subspecies from northern Europe, followed shortly thereafter by introductions of *Apis mellifera ligustica*, originally from Italy. Similarly, *A. m. mellifera* was originally introduced into northern and southern Brazil in 1845, followed by the introduction of *A. m. ligustica* in 1860 (W. Kerr, pers. commun.). European bees did not thrive in the tropics, and feral population densities have remained low[6]. In 1956, honey bees from Africa (*Apis mellifera scutellata*) were imported into Brazil. These bees were well suited to the tropics and have since spread throughout South, Central, and parts of North America, largely displacing the genotypes of previously established populations[7]. The separate introduction of European and African bees into both New World continents, and the subsequent displacement of European bees by African bees, provide independent tests of clinal selection of allozymes.

We examined honey bee malate dehydrogenase allozyme frequencies over latitudinal transects of Europe, South America, and western North America (fig. 1). Our results demonstrate the presence of strong latitudinal clines for allozymes of Mdh-1 on all three continents (fig. 1, table). The medium electrophoretic allele consistently increases in frequency with increasing latitude, while the fast allele decreases. Allele frequencies are strongly correlated with temperature extremes on all three continents.

Materials and methods

In 1990, we sampled worker honey bees from 202 feral colonies along a 1060 km north-south transect of California. Mdh-1 allozyme frequencies were determined for 8 workers from each colony using cellulose acetate gel electrophoresis[8]. The frequencies from these 8 workers were combined to determine a single colony frequency for each of the three alleles. MDH-1 has three common allelic forms designated slow (65), medium (80) and fast (100), based on their electrophoretic mobilities[9]. Other alleles have been observed although these are very rare. We also determined allele frequencies for 56 workers from Santa Cruz Island, one of the Channel Islands off

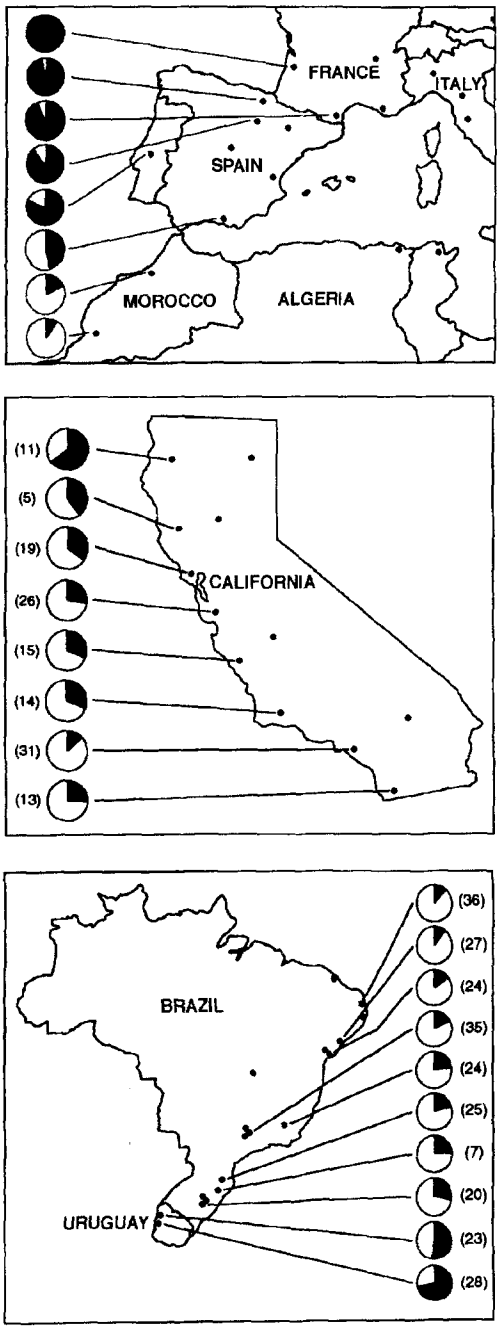

Figure 1. Pie charts show Mdh-1 medium (80) allele frequencies in black with slow and fast combined in white. Points on the maps of Europe and South America represent specific locations where mutliple hives were sampled. Points on the California map are centroids of regions in which bees were sampled from feral colonies. The number of colonies samples from each area is shown in parentheses. We were unable to obtain sample numbers for areas in Europe. Representative subsets of pie charts for all three continents are shown, however data from all points were used for the statistical calculations shown in the table.

the coast of southern California. Santa Cruz Island has a population of feral bees, but no commercial beekeeping. These workers were collected while foraging in several areas of the island. Data for allele frequencies in Brazil and Uruguay are from Lobo et al.[10] and Del Lama et al.[11] and cover a 3240 km north-south transect along the eastern coast of South America. European allele frequency data are from Cornuet[12], who demonstrated that Mdh-1 allele frequencies form a cline in European honey bee (*Apis mellifera L.*) populations.

Because Mdh-1 alleles vary in frequency with latitude, we correlated normalized (arcsin of the square root) allele frequencies with January and July T_{min} and T_{max} temperature data. Partial correlations of allele frequency with latitude were examined to control for the effects of T_{max}, while those with T_{max} control for the effect of latitude. T_{max} is defined as the average of daily high temperatures for a given month over a number of years, similarly T_{min} is the average of daily low temperatures[1]. For our comparisons, January and July T_{min} and T_{max} values were five year averages for Brazil[13], ten year averages for California[14] and ten year or longer averages from Europe and Uruguay[15]. Mdh-1 allele frequency data from colonies surrounding the closest available weather station, for which we found reported data, were averaged to yield a single value. This value was then correlated with the latitude and temperature data from that station. Each colony contributed only one observation for calculating the area average. Data from Santa Cruz Island was not included in the correlations for California.

We also examined the mitochondrial origin of California feral bee colonies that were used to determine Mdh-1 allele frequencies. Site specific primers for PCR were constructed to amplify a 1 kilobase region of the large ribosomal subunit gene in the honey bee mitochondrial genome. The 22 base primer sequences are as follows: 5'GTACCTTTTGTATCAGGGTTGA 3' and 3'CTACAAACGCTGGAGCTACAAC5'. The region amplified with these primers contains an EcoR1 restriction endonuclease site which is present in Eastern European honey bees (*A. m. ligustica, carnica* and *caucasica*) and absent in Western European honey bees (*A. m. mellifera*)[16]. Using this method, the mitochondrial origin was determined for the California feral colonies analyzed for Mdh-1 allele frequencies. Colonies were labeled north or south according to their location in California relative to 37.5° latitude and χ^2 test was used to see if northern and southern California differed from each other in the relative abundance of the two mitochondrial types.

Results

A latitudinal cline was found for the medium and fast alleles of MDH-1 in the feral honey bees of California. Subsequent analyses of previously collected data revealed similar clines for these alleles in commercial honey bees in Europe and South America (fig. 1). The slow allele does not exhibit clinal variation on any of these three continents. However, in Europe, the slow allele is apparently found only in the collection sites in

Correlation, partial correlation and multiple regression coefficients of Mdh-1 allele frequencies with latitude and temperature.

Mdh Allele		Number of colonies	Number of stations	r					Partial r		Multiple R^2
				Latitude	Jan T_{max}	Jan T_{min}	Jul T_{max}	Jul T_{min}	Latitude	July T_{max}	
Fast											
	California	202	60	−0.267*	0.213	0.245	0.259*	0.371**	−0.200	0.189	0.105
	South America	478	13	−0.754**	−0.636*	0.575	0.857***	0.804**	0.878***	0.924***	0.939
	Europe	NA	18	−0.822***	0.460	0.265	0.746***	0.452	−0.642**	0.444	0.740
Medium											
	California	202	60	0.299*	−0.217	−0.023	−0.543***	−0.418***	0.155	−0.494***	0.311
	South America	478	13	0.762**	0.606*	−0.569	−0.860***	−0.797**	−0.864**	−0.916***	0.934
	Europe	NA	18	0.361	0.031	0.115	−0.607**	−0.489*	−0.093	−0.530*	0.374
Slow											
	California	202	60	−0.049	0.021	−0.142	0.276*	0.053	0.045	0.276	0.078
	South America	478	13	0.187	0.504	−0.238	−0.274	−0.333	−0.287	−0.329	0.151
	Europe	NA	18	0.388	0.576*	0.481*	0.077	0.233	0.606	0.510	0.371

MDH-1 allozyme frequency data from California, Brazil and Europe were correlated with latitude and T_{min} and T_{max} for January and July. T_{max} is defined as the average of daily high temperatures for a given month over a number of years, similarly T_{min} is the average of daily low temperatures (Oakeshott et al.[1]).
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
NA: data not available.

Italy, where the medium allele is rare or absent[12]. Excluding Italy from our analyses of Europe, the correlation of the medium allele with latitude increases to $r = 0.909$ ($p < 0.001$), as opposed to $r = 0.361$ ($p > 0.05$) when Italy is included. Allelic analysis of 56 workers from Santa Cruz Island yielded 110 medium alleles and 2 slow alleles.

Latitudinal variation for the medium allele was best explained on the basis of July T_{max} for all three continents (table). Negative correlation coefficients for medium allele frequencies with July T_{min} and July T_{max} are consistently stronger, for all three continents, than correlations with latitude. Partial correlation and multiple regression coefficients are also stronger for July T_{max} than for latitude. Fast allele frequencies correlated positively with increasing July T_{max}, also for these same regions. Significant correlations were found for medium and fast allele frequencies with July T_{min}. This probably is a consequence of positive correlation between T_{min} and T_{max}. Similarly, a positive correlation was observed for medium allele frequency and January T_{max} in South America. This resulted from negative correlation between January T_{max} and July T_{max} ($r = -0.943$, $n = 6$, $p < 0.005$) throughout regions of this continent where the medium allele frequency varied (table).

A contingency table for colony location in California vs mitochondrial type did not yield significant differences. This suggests that both eastern and western European mitochondrial types are equally distributed with respect to northern and southern California.

Discussion

We demonstrate the existence of parallel Mdh-1 allozymic clines on three continents. The existence of clines in the New World demonstrates the rapid formation of latitudinal clines following the introduction of honey bees into California and Brazil. A single cline could be explained by stochastic events, such as genetic drift, or the collision of two populations[17]. However, the presence of three parallel clines on three separate continents strongly suggests that selection is acting on this locus. The clines are closely associated with temperature clines that are negatively correlated with latitude. T_{max} values for July were better predictors of the frequency of the medium allele on all three continents than latitude.

This consistent correlation was not expected because Brazil and Uruguay are located in the southern hemisphere where July is a winter month. We instead expected the cline in Brazil to be more closely correlated with January T_{max}. This inconsistency may be reconciled, however, because the sampling latitudes in the southern hemisphere range from the equator to 30° S, while the range in the northern hemisphere is from 33° to 47° N. Bees swarm during similar seasons at the collection sites in the two hemispheres (early spring to summer in California and Europe[18] and from late winter to spring in Brazil (W. E. Kerr. pers. commun.)). The shift toward earlier swarming in Brazil is probably attributed to warmer conditions closer to the equator and/or a difference in photoperiod. Due to these conditions, July is included in the swarm season in both hemispheres. This demonstrates that seasonal colony development is similar on all three continents during the month of July. July also yields the greatest amount of day to night temperature variation for the collection sites on all three continents[13–15].

Further evidence in support of the selection hypothesis is the near fixation of the medium alelle on Santa Cruz Island. This small island is located off the southern coast of California and supports a large number of feral honey bee colonies. Workers sampled from several foraging areas and directly from feral hives on the island

exhibited a very high frequency of the medium allele. The temperature variation of the island more closely matches that of coastal northwestern California than the variation found at comparable latitudes in southern California. Thus these data support the prediction that medium allele frequency should be greater on the island than inland areas at the same latitude.

There are alternative explanations for the occurrence of the observed clines. Initial importations of bees into California and Brazil were primarily German brown bees (*A. m. mellifera*) and Italian bees (*A. m. ligustica*). German brown bees have a relatively high frequency of the medium allele, while Italian bees have higher frequencies of the slow and the fast. A cline could have been produced in California if primarily German brown bees were imported into the northern parts of California and Italians in the south. However, there is no historical evidence for such differential introductions. Unrestricted importations of honey bees from Europe continued until prohibitive legislation was passed in 1922[19]. We tested the differential introduction hypothesis by examining the distribution of mitochondrial types in California. Maternal ancestry of German brown bees can be distinguished from that of Italian honey bees by the presence of a restriction endonuclease site present only in the Italian honey bee populations[16]. This EcoR1 restriction site resides in the mitochondrial gene coding for the large ribosomal subunit. If area-specific introductions had occurred, then we would expect a higher frequency of *A. m. mellifera* type mitochondria in northern California, and a higher frequency of *A. m. ligustica* type mitochondria in southern California, due to the differences in allele frequencies in these regions of Europe. However, both types are evenly distributed throughout California, suggesting that historical selective introductions cannot explain the cline (fig. 2). Further evidence against such area specific introductions in California is provided by Daly et al.[20] who found a north-south body size cline in feral bees, suggesting that the effects of natural selection on body size follow Bergman's law. In addition, Daly et al.[20] found no evidence for the hypothesis that northern California feral bees were more similar to bees from northern Europe, based on detailed morphometric analysis. Instead, feral honey bees throughout California represent a mixture of European races that have undergone local differentiation.

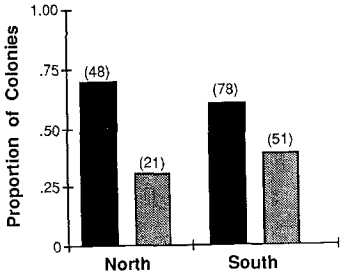

Figure 2. Solid and stippled bars represent the number of colonies with eastern and western European mitochondrial types, respectively, for northern and southern regions of California. These two types are fairly equally distributed relative to the two sections of California, suggesting that introduction of honey bees from eastern and western areas of Europe was fairly uniform in the two regions of the state.

The observed latitudinal cline in Brazil could be the result of the recent 'collision' of two differentiated populations. Honey bees were originally introduced into Brazil from northern Europe, presumably with a high frequency of medium Mdh-1 alleles (*A. m. mellifera*). African bees were introduced into southern Brazil in 1956, presumably with a high frequency of the fast allele[21]. Following its introduction, the African bee (*A. m. scutellata*) swept through much of Brazil. As the African population spread and interbred with European populations possessing other allele frequencies, a cline could result. However, at the time of sample collection, honey bee populations were undergoing a morphological transition from European to African characteristics, suggesting that African bees were mating with and/or displacing previously European populations[10]. The allele frequencies of these two populations would be expected to differ assuming different origins of the populations. Yet the data show the allele frequencies in Uruguay, at that time the current front of the African bees expansion, fit those expected by the latitudinal cline, exhibiting a relatively even increase in the frequency of the medium allele as latitude increases through this front. Thus even on the front of the 'Africanized' bees' advancement, where distinct populations were mixing, Mdh-1 allele frequencies appeared to be highly constrained by selection. Additionally, the cline throughout Brazil is imbedded in a population that is almost completely African, based on morphometric and mitochondrial DNA analysis[22–24].

The population collision hypothesis is also not supported by data shown in the distribution of hexokinase (HK) alleles in the honey bee populations of Brazil. The introduced African subspecies (*A. m. scutellata*) has a relative high frequency of the electrophoretically slow HK allele, with European bees possessing exclusively the fast allele. *A. m. scutellata* are nearly fixed for the fast Mdh-1 allele while European honey bees have a relatively low frequency of this allele. If the introduction of African bees results in the formation of the Mdh-1 cline, then one would expect a coincidental cline in HK alleles as well, with the slow HK allele and fast Mdh-1 allele having higher frequencies at lower latitudes. This, however, is not the case. The frequency of the slow HK allele is highest near the area of introduction and declines with latitude to the north, as if it has diffused from the source of introduction. (A regression of latitude against the transformed frequency of the

slow HK allele is significant ($r = 0.812$, $n = 6$, $p < 0.05$)). However, the frequency of the fast Mdh-1 allele is lowest at the source of introduction and increases with latitude. Thus the two enzymes demonstrate allele frequencies that result in two clines. However, these clines are in opposite orientation relative to each other when considering those alleles that are associated with African populations *A. m. scutellata*.

It is also possible that the cline in Europe may be due to secondary contact between two distinct populations of bees. Spain may be a region where two populations of bees with distinct mitochondrial types and different allelic frequencies converge. Our data do not refute this hypothesis. However, if this convergence is occurring, it does not rule out the possibility of the medium and fast Mdh-1 alleles evolving in response to differential selection associated with thermal variance in the environment.

MDH-1 is a metabolic enzyme that converts malate to oxaloacetate in the energy producing citric acid cycle. Drone and worker honey bees vary in their levels of O_2 consumption according to the Mdh-1 alleles they possess[25]. The amount of CO_2 drones and workers respire during flight across a wide range of temperatures also varies with Mdh-1 genotype (J. Harrison, D. Nielsen and R. Page, unpubl. data). These two findings suggest that Mdh-1 alleles differentially affect the rate of honey bee metabolism. Mdh-1 allelelic variation has been shown to vary according to environmental temperature in a wide range of organisms, including cattails and largemouth bass (see Hoffman and Parsons[26] for a review). The consistent variation of Mdh-1 alleles with the temperature extremes present in the environments associated with these organisms suggests a possible mechanism of selection for Mdh-1 alleles.

Our results suggest that Mdh-1 alleles possessed by honey bees differentially affect the bees' survival in thermally variable environments. Support for this hypothesis comes from the presence of clines on three different continents (after two recent introductions into the New World), differences in metabolic rates that vary according to allozyme genotype, and the presence of an environmental correlate (temperature extreme) that also varies with genotype. There are many similar examples of other organisms demonstrating allelic clines that vary spatially across their ranges. However, examples of parallel allelic clines, at the present time, represent the most convincing evidence to date supporting within population selection on allelic variation.

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