SHORT REPORT
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Malate Dehydrogenase allele frequencies in the commercial honey bee (*Apis mellifera*) population in Thailand reflect those in source populations

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ABSTRACT: In the western honey bee (*Apis mellifera*) populations, cytosolic malate dehydrogenase (cMDH) allele frequencies are correlated with the environmental temperatures. The Slow and Fast alleles predominate in hot climates; the Medium allele predominates in cold climates. We wondered whether natural selection has had anything to do with the Medium allele in the imported exotic *A. mellifera* population in Thailand. We genotyped workers (n = 1032) from 86 commercial colonies from three regions. Three alleles: Fast, Medium, and Slow, were detected. Over 96% of the Thai *A. mellifera* are either homozygous for the Slow or heterozygous for the Fast allele; and the Medium allele has the lowest frequency (c.a. 10%) in all sampled population. This might be indicative of selection against the Medium allele. However, as the cMDH allele frequencies in the Thai *A. mellifera* are similar to those seen in the source populations in the United States and Northern Italy, it might also be that the observed frequencies are reflective of frequent imports from the United States, and that natural selection is unable to overcome the effects of migration.

KEYWORDS: *Apis mellifera*, cytosolic malate dehydrogenase, honey bee import, temperature clines

INTRODUCTION

Cytosolic malate dehydrogenase (hereafter, cMDH) is a metabolic enzyme that catalyzes the interconversion of malate and oxaloacetate [1]. It plays a central role in several metabolic pathways including amino acid synthesis and gluconeogenesis [1, 2]. In many organisms, cMDH has different forms (allozymes) due to polymorphisms in its amino acid sequences. Because of its reliable polymorphism, cMDH was an important contributor to early population genetic studies based on allozyme electrophoresis.

Interestingly, different cMDH allozymes often have different thermostabilities across a range of species [3–5], and this can lead to clines in which the frequency of cMDH alleles is correlated with average environmental temperatures [6–8]. For example, in a serendipitous 'natural' experiment, discharge of cooling water from a power plant into a lake changed the average cMDH allele frequencies in the largemouth bass (*Micropterus salmoides*) population of the lake [9, 10]. The cMDH-B^2 allele predominated in the area near the powerplant discharge point, where the water temperature remains warm throughout the year, but remained rare elsewhere in the lake [7].

cMDH of the honey bee, *Apis mellifera*, has three main allozymes designated Fast, Medium, and Slow based on their movements under electrophoresis [11, 12]. *In vitro* studies have shown that the three alleles have different thermostabilities [5], and that these differences are due to differing numbers of cation and hydrogen bonds between the amino acid residues of the molecule [13]. The Medium allele has the lowest thermostability and the lowest catalytic capacity at high temperatures, but it likely has the highest catalysis at low temperatures [13]. The Fast and, particularly, the Slow alleles are more resistant to heat denaturation and have higher catalytic activity at high temperatures (>50 °C) than the Medium allele. These differences in thermostability and catalytic activity are often manifest at the population level. Clines in honey bee cMDH allele frequencies have been documented on four continents [8, 14, 15] with the Medium allele at the highest frequency in cooler areas. Indeed, the Medium allele is fixed or nearly so in Alpine France [14] and Norway [16]. This is a strong piece of evidence that the honey bee cMDH locus is under natural selection in the wild [17].

Apis mellifera was first introduced to Thailand in 1940, and again in 1953, for research purposes [18]. Since 1970, large numbers of *A. mellifera* have been regularly imported from the United States, Australia, and Taiwan for commercial purposes [19]. Today, *A. mellifera* is widespread, particularly in the Northern Provinces of Chiang Mai and Chiang Rai which have 79 810 and 31 740 colonies, respectively (data obtained from Agricultural Technology Promotion center (economic insects) Chiang Mai Province), and there are more than 300 000 colonies country-wide [19].

Thailand is a tropical country, suggesting that the

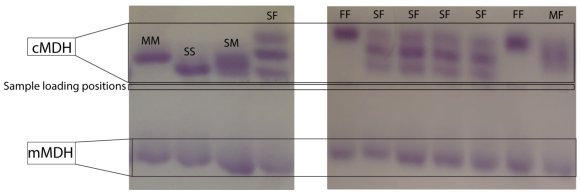

Fig. 1 Cellulose acetate gel electrophoresis of cytosolic malate dehydrogenase (cMDH) from *A. mellifera* after staining with cMDH staining mixtures; mMDH = mitochondrial MDH.

cMDH of the heat-sensitive Medium allele would be at a selective disadvantage relative to the Fast and the Slow alleles and, consequently, would now be at low frequency. To test this hypothesis, we assessed the distribution of cMDH alleles from different areas in Thailand.

MATERIALS AND METHODS

Specimen collection

Adult workers were collected from 86 colonies in 11 provinces in three regions of Thailand: the North, the North-east, and the South, during March 2017–January 2018. Details of the collection sites and number of colonies sampled per site are provided in Fig. S1 and Table S1. The samples were obtained from areas ranging from 8°46′42.2″ N to 19°10′35.0″ N latitude and 98°48′12.4″ E to 103°00′06.5″ E longitude. Twelve adult workers per colony were used for cMDH genotyping (1032 bees in total). The climatic data (mean monthly minimum and maximum temperatures for January and May) of each region were obtained from the online data of the Thai Meteorological Department. All samples were collected from commercial apiaries at multiple sites per province. Adult bees were randomly sampled from honey combs at least three hives per apiary. Sampled bees were immediately frozen on dry ice, and stored at −80 °C in the laboratory until processing.

Allozyme analysis and population study

The cMDH genotype of individual bees was determined using cellulose-acetate gel electrophoresis at 200 V for 20 min in the electrophoresis tank (Helena Laboratories, Texas, USA). The electrophoresis buffers and the histological stains used for the detection of protein bands were according to those previously described [13, 20]. Fig. 1 shows the typical banding patterns of the selected genotypes. The staining procedure unambiguously identifies the cMDH protein, and no other proteins are stained.

Allele and genotype frequencies were determined for the individual regions and the overall area. Allele-frequency based population genetic statistics, including observed (Ho) and expected (He) (assuming random mating) heterozygosities, F_{IS} within regions, and F_{ST} between regions, were calculated using GenAlEx 6.5 [21]. F_{ST} is the measure of subpopulation genic differentiation between regions [22]; F_{IS} is the proportion of the variance in the subpopulation contained within regions [22].

RESULTS AND DISCUSSION

The electrophoretic patterns of the cMDH locus in *A. mellifera* from Thailand are in keeping with honey bees from various countries that the cMDH locus is controlled by the three alleles: Fast, Medium, and Slow [5, 11, 15]. Homozygous bees contain a single major band and heterozygous bees three major bands. The middle band in heterozygous bees results from the dimeric nature of cMDH (Fig. 1).

The observed (Ho) and the expected (He) heterozygosities were similar, suggesting a selectively neutral locus and limited population structuring as indicated by the low FST between regions (Table 2). The Medium allele had the lowest frequency in all three regions (Tables 1 and 2), potentially an indicative of selection against the Medium allele. However, the cMDH allele frequencies of the Thai *A. mellifera* are similar to those observed in the queen breeding population in the United States [23] and Northern Italy [14] (see Table 2).

Although, temperature clines in honey bee cMDH allele frequencies in many countries provide strong evidence that cMDH experiences natural selection in the wild [8, 17]. However, it is likely that the queen trades, the colony migrations, and possibly the new introductions also have an impact on allele frequencies. In Guatemala and Mexico, commercial beekeepers and queen breeders introduced bees with the Fast allele resulting high frequencies of the Fast allele [24]. The *A. mellifera* in Brazil shifted strongly toward the Fast allele as a result of the introduction of African subspecies in the 1950s [11].

The distribution of cMDH allele frequencies of *A. mellifera* in Thailand are similar to those found in the source populations in the United States [23] and Northern Italy [14]. Therefore, we have no evidence that natural selection is selecting against the Medium allele over time. Perhaps, there has been insufficient time for the Medium allele to decline in frequency, but we suggest that frequent imports from the United States probably counter the effects of selection. However, the frequency of homozygous MM bees is less than 2%, suggesting natural selection because a colony is little disadvantaged by having a small number of bees of this genotype.

cMDH allele and genotype frequencies were normalized using the arcsine square root transformation [25]. There was no significant correlation between the allele or genotype frequencies and the average minimum or maximum temperatures across the

Table 1 The number of *A. mellifera* cytosolic malate dehydrogenase (cMDH) genotypes samples in the three regions of Thailand. The numbers in parentheses are the genotypes frequencies.

Region	n	Genotype*					
		SS	SM	SF	MM	MF	FF
Northern	348	171 (0.52)	45 (0.16)	97 (0.24)	13 (0.01)	12 (0.04)	10 (0.03)
North-eastern	360	165 (0.52)	63 (0.19)	105 (0.22)	6 (0.01)	13 (0.04)	8 (0.02)
Southern	324	151 (0.66)	83 (0.14)	65 (0.16)	13 (0.01)	9 (0.02)	3 (0.01)
Total	1032	487 (0.53)	191 (0.19)	267 (0.20)	32 (0.02)	34 (0.04)	21 (0.02)

* Genotype refers to the homozygous and heterozygous cMDH gene: S, M, and F refer to Slow, Medium, and Fast alleles, respectively; SS = homozygous Slow genotype; SM = heterozygous Slow Medium genotype; etc.

Table 2 cMDH allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), and the subpopulation fixation index (F_{IS}) in the honey bee populations in Thailand. The allele frequencies in the queen breeding population in the United States (Shiff and Sheppard 1996) are given for references. Numbers in parentheses represent the standard errors.

Locality	cMDH allele frequencies			Ho	He	F_{IS}	F_{ST}
	S	M	F				
Northern region (n =348)	0.72	0.11	0.17	0.483	0.390	−0.238	
North-eastern region (n = 360)	0.72	0.13	0.15	0.567	0.515	−0.100	
Southern region (n = 324)	0.81	0.09	0.10	0.370	0.393	0.058	
Total (n = 1032)	0.73	0.13	0.14	0.473 (0.057)	0.433 (0.041)	−0.094 (0.085)	0.010
United States queen breeding population (n = 356)	0.70	0.08	0.22				

Table 3 Pearson correlation coefficients (probability in brackets) for cMDH allele and genotype frequencies, minimum and maximum temperatures in January and May with *A. mellifera* cMDH allele frequency for the eleven provinces of collection sites in Thailand (Table S1).

Allele/	January		May	
Genotype	min	max	min	max
F allele	−0.383 (0.246)	−0.260 (0.440)	0.204 (0.547)	0.325 (0.330)
M allele	0.343 (0.302)	0.477 (0.138)	0.286 (0.394)	−0.032 (0.925)
S allele	0.193 (0.569)	−0.318 (0.341)	−0.556 (0.076)	−0.495 (0.121)
FF genotype	−0.254 (0.452)	−0.236 (0.484)	0.276 (0.412)	0.321 (0.336)
MM genotype	0.210 (0.535)	0.577 (0.063)	0.391 (0.234)	0.183 (0.590)
SS genotype	0.104 (0.762)	−0.317 (0.343)	−0.501 (0.116)	−0.377 (0.253)
SM genotype	0.430 (0.186)	0.423 (0.195)	0.169 (0.619)	−0.170 (0.618)
SF genotype	−0.327 (0.326)	−0.296 (0.378)	0.093 (0.785)	0.170 (0.618)
MF genotype	0.047 (0.890)	0.074 (0.829)	0.116 (0.735)	−0.126 (0.712)

Correlation is significant at the 0.05 level (2-tailed).

11 provinces where samples were collected (Table 3). This is probably due to the quite similar temperatures across the sampled areas.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2022.015.

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Appendix A. Supplementary data

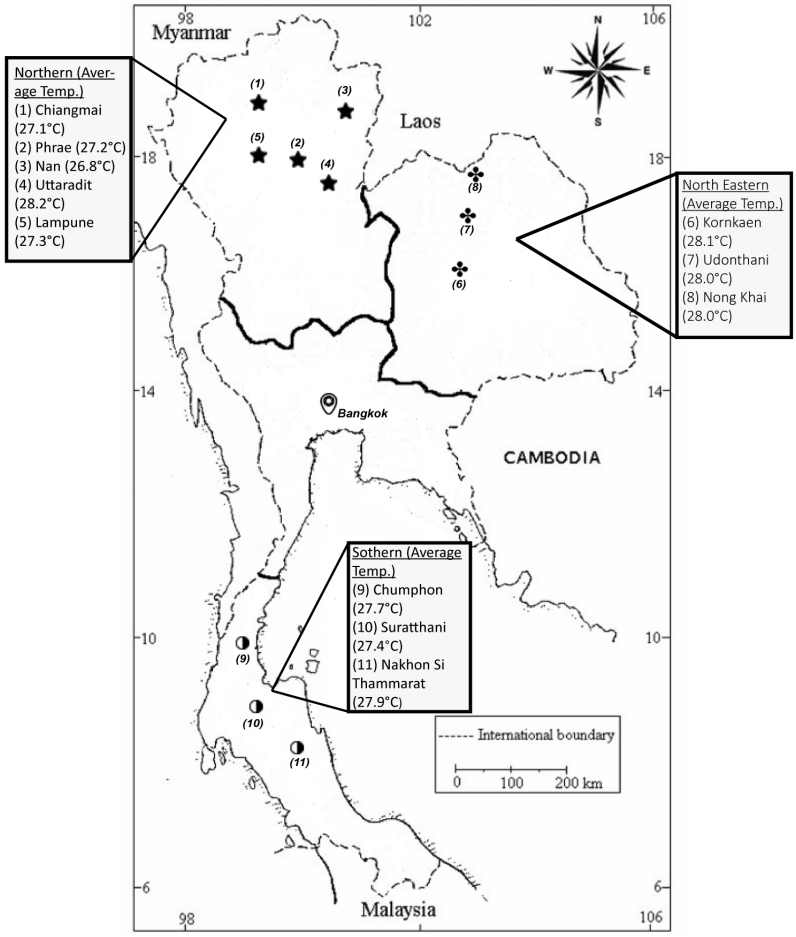

Fig. S1 Sampling sites for *A. mellifera* in Thailand. The numbers indicate collection sites. The boxes provide the names of provinces and the average temperature through the year in each region.

Table S1 Collection sites.

Region	Province	No. colonies	Coordinates	
			Longitude	Latitude
Northern	(1) Chiang Mai	11	18°34′24.1″ N–18°44′20.2″ N	98°48′12.4″ E–98°55′22.3″ E
	(2) Phrae	3	18°06′50.5″ N	100°10′30.3″ E
	(3) Nan	3	19°10′35.0″ N	100°55′14.3″ E
	(4) Uttaradit	3	17°41′25.4″ N	100°09′01.0″ E
	(5) Lamphun	9	18°27′22.7″ N–18°30′57.4″ N	98°54′56.0″ E–98°57′38.0″ E
North-eastern	(6) Kornkaen	10	16°27′05.0″ N–16°45′47.6″ N	102°43′21.7″ E–103°00′06.5″ E
	(7) Udonthani	10	17°27′09.8″ N–17°28′27.8″ N	102°49′52.5″ E–102°50′03.7″ E
	(8) Nong Khai	10	17°52′27.8″ N–17°54′14.8″ N	102°30′37.7″ E–102°35′00.8″ E
Southern	(9) Chumphon	12	9°55′13.0″ N–10°14′35.2″ N	99°01′05.2″ E–99°04′33.1″ E
	(10) Suratthani	10	8°55′32.8″ N–9°03′05.5″ N	99°20′07.0″ E–99°24′45.6″ E
	(11) Nakhon Si Thammarat	5	8°46′42.2″ N–8°46′45.2″ N	99°54′26.4″ E–99°54′28.4″ E