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Mitochondrial DNA polymorphisms in honey bee subspecies from Kenya

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Abstract – Thirty-nine samples of *Apis mellifera monticola* and *A. m. scutellata* from three different regions of Kenya were analyzed for mitochondrial DNA (mtDNA) variation using 6-base and 4-base restriction enzymes. Restriction with *Hpa*II and *Alu*I resulted in distinct patterns that together produced three different haplotypes. While haplotypes 2 and 3 were restricted to samples from the mountain forest, haplotype 1 was found in *A. m. scutellata* and in all samples from the Ngong Hills. No introgression of *A. m. scutellata* mtDNA was detected in bees collected in mountain environments, but a few samples from the savanna had *A. m. monticola* morphology, or mtDNA, or both. These results support the hypothesis that *A. m. monticola* is a distinct subspecies and not an ecotype of *A. m. scutellata*. The polymorphic restriction sites were mapped. Ten samples of *A. m. litorea* from the coast of Kenya were analyzed using polymerase chain reaction (PCR) amplification and subsequent restriction analysis. All samples of *A. m. litorea* shared the *A. m. scutellata* haplotype.

***Apis mellifera monticola* / *A. m. scutellata* / *A. m. litorea* / mtDNA polymorphism / Kenya**

1. INTRODUCTION

Although the taxonomic description of honey bees from the African continent dates back to 1804 when the French entomologist Latreille named a bee from Senegal *Apis adansonii* [12], the study of honey bees from this continent remained superficial and incomplete until far into this century. For more than 150 years, *A. m. adansonii* was used as the only name for all the bees of sub-Saharan Africa [3, 11], although it became

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known later that substantial geographic variability exists among honey bee populations of this vast region [17, 19]. Ruttner [17] recognized 11 subspecies of *A. mellifera* within Africa, mainly on the basis of morphometric statistical analyses, but included aspects of their biology and behavior as well as geological and climatic factors of their distribution in making his classification. Even today, there are suggestions to retain a single name and regard all geographic variability south of the Sahara as an expression of ecotypes [10].

After the description of three different subspecies of honey bees in East Africa [25], the idea of an entirely ecological separation of these races between the coast and the inland mountains prevailed [10, 18, 20, 25]. Obviously, no physical barriers exist between the habitats of these populations, and therefore it seemed justified to assume the existence of an ecocline between the coast and the mountains [20], and to address *A. m. monticola* from the mountain forests and *A. m. litorea* from the coast as ecotypes of the East African subspecies *A. m. scutellata* [10]. More recently, this view was reiterated by Hepburn and Radloff [8] and Hepburn et al. [9], based on statistical analysis of 10 morphological characters, factors of flight physiology and analysis of the COI-COII region of the mitochondrial DNA (mtDNA) of African mountain bees.

However, based on results of detailed morphological analysis of honey bee samples from Tanzania and Kenya, Meixner et al. [13, 14] found a clear separation between mountain and savanna bees and therefore suggested that the disjunct populations of *A. m. monticola* be regarded as remnants of a large and continuous Pleistocene population of forest bees. These results were supported by allozyme analysis that revealed greater similarity between these disjunct populations than to the respective neighboring savanna population [14]. Analysis of mtDNA variation [24] found that samples which were morphometrically identified as *A. m. monticola* had a unique haplotype, in addition to sharing other haplotypes with *A. m. scutellata*, thus lending additional support to the 'refugial hypothesis'. This situation was also found in the subspecies *A. m. sahariensis* whose disjunct, island-like distribution – analogous to *A. m. monticola* – is thought to be a remnant of a larger range of distribution during the Pleistocene [19].

Morphometric analysis of *A. m. litorea* suggests that it is a distinct subspecies [19], but no molecular data are currently available to better describe its relationship to the neighboring *A. m. scutellata*. We report here results of mitochondrial variation for the three subspecies based on restriction analysis with 4-base recognizing enzymes.

2. MATERIALS AND METHODS

Honey bee samples were obtained and extraction of nucleic acids was carried out as follows: we analyzed a total of 39 samples of *A. m. monticola* and *A. m. scutellata* from three mountainous areas (Mount Elgon, Mount Kenya and the Ngong Hills) and adjacent savanna regions from Kenya that had been characterized morphometrically in a previous study [14], and 10 samples of *A. m. litorea* collected on the Kenyan coast near Mombasa.

Total DNA from one bee per sample was extracted following a phenol-chloroform extraction protocol as described by Sheppard and McPheron [23]. For experiments involving the polymerase chain reaction (PCR), the extraction procedure was modified according to Arias and Sheppard [2].

2.1. RFLP analysis

The samples collected at Mount Elgon, Mount Kenya and from the Ngong area were screened for mtDNA variation using the following restriction enzymes, both 6-base and 4-base cutters: *Acc*I, *Alu*I, *Bcl*I, *Bgl*II, *Eco*RI,

*Hpa*II, *Mbo*I, *Rsa*I, *Xba*I. The restriction reactions were performed following the manufacturer's instructions (Bethesda Research Laboratories, Bethesda, MD, USA). The fragments were electrophoresed on 1.2% agarose gels, stained with ethidium bromide, photographed and subsequently blotted on nitrocellulose membranes. The filters were then prehybridized and hybridized at 50 °C using the procedures described in Sheppard and McPheron [23]. A radioactive probe was produced through ^{32}P nick-translation of purified honey bee mtDNA [23].

2.2. Mapping of polymorphic restriction sites

Polymorphic restriction sites were mapped by performing single and double digestions combining the restriction enzymes *Eco*RV, *Xho*I and *Acc*I with *Alu*I and comparison to the honey bee mitochondrial sequence published by Crozier and Crozier [4] for the polymorphic *Hpa*II site.

2.3. PCR amplification and subsequent RFLP analysis

PCR reactions were performed in 50-µL reaction mixtures containing 0.2 µM of each primer, 0.2 mM of PCR Nucleotide Mix (Boehringer, Mannheim), 1.5 mM $MgCl_2$ (Promega), 1X Reaction Buffer (Promega), 1.25 U *Taq* Polymerase (Promega) and 1 µL of DNA template. The primers used were as follows: AMB3: 5' TTT AAA AAC TAT TAA TCT TC 3'; AMB4: 5' GAA AGT TAG ATT TAC TCC 3' for the fragment containing the *Hpa*II site; AMB7: 5' ATA ATA TAA CAT TAG TTT GT 3'; AMB8: 5' GAG TAT TCA ATT GTT TGA A 3' for the fragment containing the *Alu*I site (Arias et al., unpubl. data). Reaction conditions were as follows: 94 °C for 2 min, followed by 30 cycles of 94 °C for 1 min, 48 °C for 1 min 20 s, and 64 °C for 2 min, and a final extension step for 5 min at 72 °C. After electrophoresis in 1.5% agarose gel and scoring for positive amplifications, the PCR fragments were digested with the restriction enzymes *Alu*I and *Hpa*II as described above, followed by electrophoresis, ethidium bromide staining and polaroid photography under ultraviolet (UV) light. Samples known to possess the restriction sites in question were included on each gel as a control.

3. RESULTS

3.1. *A. m. monticola* and *A. m. scutellata*

The samples of *A. m. monticola* and *A. m. scutellata* we analyzed shared the mitochondrial haplotypes produced by the 6-base cutters *Eco*RI, *Bcl*I, *Bgl*II, *Xba*I and *Acc*I and the 4-base cutters *Rsa*I and *Mbo*II. Restriction with the enzymes *Hpa*II and *Alu*I, however, resulted in distinct patterns for both subspecies. These results are shown in Figure 1. Restriction of our samples with

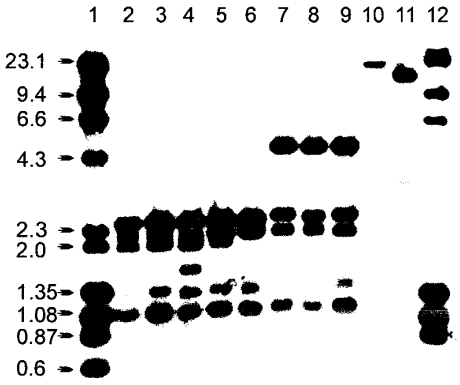

Figure 1. Autoradiograph showing the result of restriction analysis of honey bees from Kenya. Lanes 2–6: *A. m. monticola* cut with *Alu*I; lanes 7–9: *A. m. scutellata* cut with *Alu*I; lanes 10 and 11: samples of *A. m. monticola* cut with *Hpa*II. All samples of *A. m. scutellata* treated with *Hpa*II had the haplotype in lane 10. Lanes 1 and 12: lambda/*Hin*dIII and phiX/*Hae*III molecular weight standard.

Table I. Mitochondrial haplotypes found in honey bee subspecies from Kenya.

Haplotype	*Hpa*II site	*Alu*I site	Typical for
1	Absent	Absent	*A. m. scutellata*
2	Absent	Present	*A. m. monticola*
3	Present	Present	*A. m. monticola*

*Hpa*II produced either one or two fragments, corresponding to the presence of one or two restriction sites. Samples of *A. m. scutellata* always had only one fragment, while the pattern was polymorphic within *A. m. monticola*.

Cutting the mtDNA with *Alu*I resulted in a multitude of small fragments, the largest one being about 4.4 kilobases (kb) in size (Fig. 1). All samples that were morphometrically identified as *A. m. scutellata* showed this fragment, while samples of *A. m. monticola* collected on Mount Elgon and Mount Kenya possessed an additional restriction site which resulted in a loss of this fragment.

The variations observed combined to produce three different haplotypes (Tab. I), resulting in a significant assignment between mitochondrial haplotype and morphological classification of the respective sample. The geographical distribution of the haplotypes among the samples is shown in Figure 2, and followed a characteristic pattern: On Mount Kenya and Mount Elgon, haplotype 1 was found only in samples of *A. m. scutellata* (with a single exception), while haplotypes 2 and 3 were assigned to *A. m. monticola* on these two mountains (with one exception each). In contrast, all the samples collected in the Ngong area showed haplotype 1, irrespective of their morphological classification.

3.2. Mapping of polymorphic restriction sites

Figure 3 shows the position of the polymorphic restriction sites mapped for *Hpa*II and *Alu*I. Digestion of our samples with *Hpa*II produced either one fragment (one site) or two (two sites). In the case of a single fragment, identity of the site with its position in the sequence determined by Crozier and Crozier [4] was confirmed. The second *Hpa*II restriction site, the polymorphic one, was located in the COI gene, about at position 2390.

Restriction with the enzyme *Alu*I produced a multitude of small fragments, and our experiments focused on mapping the single polymorphic site that cleaved the largest observed fragment. We did not attempt to locate any of the other sites. The variable *Alu*I site was mapped by performing double digestions with *Eco*RV and *Xho*I which cut the mtDNA of *A. mellifera* only

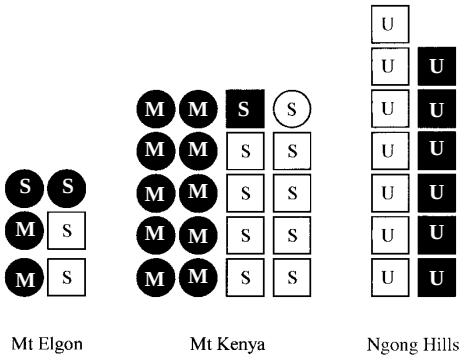

Figure 2. Distribution of mitochondrial haplotypes in Kenya in correlation with morphology and collection environment. Open symbols denote *A. m. scutellata* morphology, closed symbols *A. m. monticola* morphology. Squares stand for *A. m. scutellata* haplotype (haplotype 1), circles describe *A. m. monticola* mtDNA (haplotypes 2 and 3). The letter in each symbol describes the collecting environment; S: savanna; M: mountain forest; U: urbanized environment.

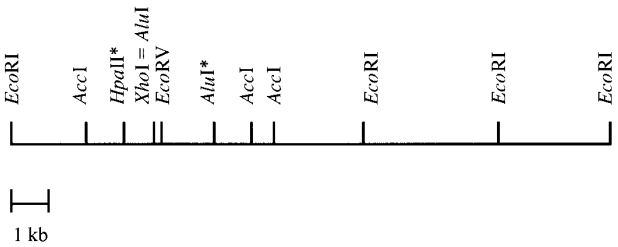

Figure 3. Restriction map of *A. mellifera* mtDNA showing the polymorphic restriction sites for the enzymes *Hpa*II and *Alu*I in the samples studied. The map was linearized at the beginning of the largest *Eco*RI fragment, and the restriction sites for *Eco*RI, *Acc*I, *Eco*RV and *Xho*I were taken from Arias and Nobrega [1]. The polymorphic restriction sites are marked with*. For *Alu*I, solely the restriction site cleaving the largest fragment is shown.

once, and with *Acc*I. The polymorphic site of interest was mapped between base pairs (bp) 4300 and 4400, either in tRNA Asp or in tRNA Lys.

3.3. *A. m. litorea*

The samples of *A. m. litorea* were subjected to PCR amplification and subsequent restriction fragment length polymorphism (RFLP) analysis using the enzymes that produced polymorphisms in our samples of *A. m. monticola* and *A. m. scutellata*. All 10 samples shared the mtDNA haplotypes that were characteristic for *A. m. scutellata*. We did not detect any additional variation within these samples.

4. DISCUSSION

Among the samples studied, we found a strong correlation between mtDNA haplotype and morphology. While haplotypes 2 and 3 were basically restricted to samples with the morphology of *A. m. monticola* collected on Mount Elgon and Mount Kenya, haplotype 1 was observed in *A. m. scutellata* in the vicinity of those mountains and also in all samples collected in the Ngong Hills, a region that is increasingly subjected to the process of urbanization. Thus, the variation in mtDNA corresponds exactly to the pattern observed using morphology and allozymes, that bees from distant mountains are more similar to each other than to their direct savanna neighbors [14]. This is the first observation of diagnostic differences in mtDNA variation patterns in honey bee subspecies of East Africa.

Using the restriction enzyme *Hin*FI, Sheppard et al. [24] found a unique haplotype in *A. m. monticola* in addition to another two that were shared with *A. m. scutellata*. Recently, Hepburn et al. [9] used the *Dra*I restriction analysis of the COI-COII intergenic region [6] in addition to morphological analyses to study the relations between honey bee populations in savanna regions and their mountain neighbors in several regions of Africa. From the absence of distinct polymorphisms that would separate mountain from savanna bees, they concluded that each mountain bee population in Africa should be viewed as an ecotype of the respective surrounding savanna subspecies. This idea may apply to other mountain systems in Africa, but clearly is unsuitable to describe the situation in the mountains of Kenya. Our results present strong evidence for the hypothesis that *A. m. monticola* in Kenya cannot be considered as an ecotype of *A. m. scutellata*, but that it is the unique result of its own evolutionary history. In the light of climatic

changes in East Africa during and after the Pleistocene, as described by several authors [7, 16], the most likely scenario is that *A. m. monticola* populations of today represent disjunct relics of a Pleistocenic population of honey bees now restricted to mountain refugia [13, 14]. This hypothesis is corroborated by the fact that the polymorphisms we describe here also occur further south in honey bee populations from mountainous regions in Malawi (Meixner et al., unpubl. data). Recent sequence analysis of the ND2 gene [2] of samples from Mount Kenya resulted in a sequence divergence rate of 0.76% between specimens identified as *A. m. monticola* and *A. m. scutellata*, suggesting that the two subspecies are probably of Pleistocene origin.

Numerous publications report *A. m. scutellata* to be a highly mobile insect that easily absconds [5, 8, 17, 19, 25–27], often performing seasonal migrations over vast distances [21, 22]. Populations of *A. m. scutellata* have also been quoted to frequently perform vertical migrations between savanna and mountain forest, thus replenishing populations of *A. m. monticola* in the mountains with bees from the savanna [8, 10, 25]. In contrast, virtually no information is available on the absconding and migrating behavior of *A. m. monticola*, but it has been noted that this race shows a less pronounced tendency to abscond [17]. It needs to be stated, however, that reports on vertical migrations of *A. m. scutellata* are predominantly anecdotal, and there is comparatively little information based on extended behavioral studies of resident honey bee populations [21, 22]. Nonetheless, these reports have been used frequently as an argument in favor of the hypothesis of continuous clinal variation between populations of savanna and mountain bees [8–10]. In addition, Kerr [10] and Hepburn and Radloff [8] argue that repeated droughts have largely eliminated the honey bee populations on the mountains in East Africa, which subsequently are said to have been replenished by introgression of *A. m. scutellata* from the savanna. However, there is ample evidence that mountain habitats in East Africa are in general much more humid and thus, because of the buffering capacity of their forests, are less sensitive to prolonged dry seasons than savanna habitats [28]. Nightingale [15] reports that dry areas in Kenya are repopulated on a regular basis by honey bees from higher altitudes.

On the basis of hypothesized large-scale migrations of the savanna bee *A. m. scutellata* between the savanna and the mountains, we would expect to find samples with the morphology and the mtDNA of *A. m. scutellata* that were collected in mountain environments. No such evidence was found within 26 colonies from both Mount Kenya and Mount Elgon, i.e., none of the colonies sampled in the mountains displayed either the morphology or the mtDNA of *A. m. scutellata*. Instead, there was some indication to the contrary. A few colonies that were collected in savanna environments had both the morphology and the mtDNA of *A. m. monticola* (black circles marked with an 'S' in Fig. 2), or the morphology of one race and the mtDNA of the other (open circle with an 'S', or black square with an 'S' in Fig. 2). Only one of the colonies in our study combined the morphology of *A. m. monticola* with the mtDNA of *A. m. scutellata* (black square with an 'S'), and thus gives a record of an introgression of a maternal *scutellata* lineage into *A. m. monticola*. However, this colony was also collected in the savanna, not in the mountains.

In contrast to the pronounced differences between neighboring bee populations on Mount Elgon and Mount Kenya, the bees of the Ngong Hills represent a population with strong hybridization between mountain and savanna bees, as previously reported for allozymes and morphology [14]. All the samples analyzed from this region presented the single haplotype that is otherwise typical for *A. m. scutellata*, irrespective of their morphological classification as *A. m. scutellata* or *A. m. monticola*. We hypothesize that growing deforestation and urbanization

of this region enforces hybridization between the two races, a situation that seems to be atypical for savanna and mountain bees from less disturbed habitats. However, increasing deforestation of mountain regions in East Africa might pose a serious threat to the populations of *A. m. monticola* in its refugia.

All samples of *A. m. litorea* that we analyzed share the mitochondrial haplotype typical of *A. m. scutellata*. This result provides no further resolution to the question of whether *A. m. litorea* should be regarded a distinct subspecies, as proposed by Ruttner [19] and Smith [25] based on morphological analysis, or an ecotype of *A. m. scutellata* as suggested by Kerr [10]. More detailed analyses of *A. m. litorea* mtDNA are needed to better resolve the relationship of the coastal honey bee population to neighboring subspecies.

ACKNOWLEDGMENTS

This paper is dedicated to the memory of Friedrich Ruttner who first encouraged our interest in the honey bees of East Africa. We thank Bruce McPheron for collection assistance with *A. m. litorea* in Mombasa. We gratefully acknowledge the support of Niko Koeniger for discussions and comments on an earlier version of this study.

Résumé – Polymorphisme de l'ADNmt chez les sous-espèces d'abeilles domestiques du Kenya. La variabilité de l'ADNmt a été étudiée sur 39 échantillons d'*Apis mellifera monticola* et d'*A. m. scutellata* identifiés morphologiquement et provenant de trois régions montagneuses du Kenya et des savanes adjacentes. L'ADN total a été digéré par les enzymes *Acc*I, *Alu*I, *Bcl*I, *Bgl*II, *Eco*RI, *Hpa*II, *Mbo*I, *Rsa*I et *Xba*I, reconnaissant des sites de restriction à quatre ou six bases. Les fragments ont été séparés par électrophorèse sur gel d'agarose à 1,2 %, colorés au bromure d'éthidium, photographiés et transférés sur des membranes de nitrocellulose. Ensuite, les membranes ont été préhybridées et hybridées à 50 °C avec une sonde d'ADN radiomarquée produite à partir d'ADNmt purifié d'abeilles. Les sites de restrictions polymorphes ont été cartographiés en effectuant des digestions doubles avec *Eco*RV, *Xho*I et *Acc*I. D'autre part, le polymorphisme de restriction de dix échantillons d'*A. m. litorea* récoltés sur la côte du Kenya a été analysé à l'aide d'amplification par PCR suivie de digestions de restriction. Tous les échantillons de l'étude avaient en commun les profils de restriction produits par les enzymes *Acc*I, *Bcl*I, *Bgl*II, *Eco*RI, *Rsa*I et *Xba*I. La restriction avec les enzymes à quatre bases, *Alu*I, *Hpa*II, a permis de mettre en évidence au sein des deux races *monticola* et *scutellata* (Fig. 1) trois haplotypes différents (Tab. I), qui présentent une répartition géographique caractéristique (Fig. 2). La figure 3 montre les positions des sites de restriction polymorphes cartographiés pour *Alu*I et *Hpa*II.

Tandis que les haplotypes 2 et 3 sont limités aux échantillons de *A. m. monticola* du Mont Elgon et du Mont Kenya, l'haplotype 1 se trouve chez *A. m. scutellata* et dans tous les échantillons des Ngong Hills, région sujette à une urbanisation croissante. C'est la première fois que l'on met en évidence des différences diagnostiques dans la variation des profils d'ADNmt parmi les races d'abeilles mellifères d'Afrique orientale. Nos résultats prouvent clairement la validité de l'hypothèse selon laquelle *A. m. monticola* ne peut être considérée comme l'écotype d'*A. m. scutellata*, mais est le résultat de sa propre histoire évolutive. À la lumière des changements climatiques survenus pendant et après le Pléistocène, les populations actuellement disjointes d'*A. m. monticola* représentent les reliques d'une plus grande population d'abeilles du Pléistocène actuellement limitées à des zones refuges dans les montagnes.

Nous n'avons pas détecté d'introgression chez *A. m. scutellata* parmi les échantillons récoltés dans les régions montagneuses au travers des études de la morphologie et de la

variation de l'ADNmt. Ceci est en contradiction avec l'hypothèse des migrations verticales à grande échelle d'*A. m. scutellata* depuis les savanes vers les montagnes. Au contraire, quelques colonies prélevées dans la savane soit avaient la morphologie et l'ADNmt d'*A. m. monticola*, soit combinaient la morphologie d'une sous-espèce avec l'ADNmt de l'autre (Fig. 2).

Alors que les populations d'abeilles voisines du Mont Elgon et celles voisines du Mont Kenya présentaient des différences prononcées, les abeilles des Ngong Hills n'avaient qu'un seul haplotype typique d'*A.m. scutellata*. Nous émettons l'hypothèse que ceci est le résultat d'une hybridation entre les abeilles de montagne et celles de savane, conséquence d'une déforestation et d'une urbanisation grandissantes de cette région.

Tous les échantillons d'*A. m. litorea* de cette étude partageaient l'haplotype d'ADNmt typique d' *A. m. scutellata*. Ce résultat ne fournit pas de nouvel élément de réponse à la question : *A. m. litorea* doit elle être considérée comme un écotype d' *A. m. scutellata* ou comme une sous-espèce distincte, comme le suggère l'analyse morphométrique ?

***Apis mellifera monticola / A. m. scutellata / A. m. litorea* / polymorphisme ADNmt / Kenya**

Zusammenfassung – Polymorphismen der mitochondrialen DNA bei Unterarten der Honigbiene in Kenia. Die Variabilität der mtDNA wurde an 39 morphometrisch identifizierten Proben von *Apis mellifera monticola* und *A. m. scutellata* aus drei Gebirgsregionen und angrenzenden Savannengebieten von Kenya untersucht. Dabei wurden Restriktionsenzyme mit vier-Basen und mit sechs-Basen-Erkennungssequenz eingesetzt. Die extrahierte DNA wurde mit den Enzymen *Acc*I, *Alu*I, *Bcl*I, *Bgl*II, *Eco*RI, *Hpa*II, *Mbo*I, *Rsa*I und *Xba*I behandelt. Die Fragmente wurden durch Elektrophorese auf 1,2 % Agarosegelen aufgetrennt, mit Ethidiumbromid gefärbt, photographiert und anschließend auf Nitrozellulosefilter geblottet. Anschließend wurden die Filter bei 50 °C mit angereicherter mtDNA von Honigbienen hergestellter und radioaktiv markierter Sonden-DNA hybridisiert. Polymorphe Restriktionsstellen wurden durch Doppelverdau mit *Eco*RV, *Xho*I und *Acc*I kartiert. Zehn Proben von *A. m. litorea* von der Küste Kenias wurden mit Hilfe von PCR Amplifikation und Restriktionsanalyse auf Variabilität an den polymorphen Restriktionsstellen untersucht.

Alle untersuchten Proben hatten Restriktionsmuster für *Acc*I, *Bcl*I, *Bgl*II, *Eco*RI, *Mbo*I, *Rsa*I und *Xba*I gemeinsam. Restriktion mit den vier-Basen-Enzymen *Alu*I und *Hpa*II ergab verschiedene Muster für beide Unterarten (Abb. 1), die sich zu drei verschiedenen Haplotypen zusammensetzten (Tab. I), und eine charakteristische geographische Verteilung aufwiesen (Abb. 2). Abbildung 3 zeigt die Positionen der kartierten polymorphen Restriktionsstellen für *Alu*I und *Hpa*II.

Während die Haplotypen 2 und 3 auf Proben von *A. m. monticola* an Mt. Elgon und Mt. Kenya beschränkt waren, wurde Haplotyp 1 in *A. m. scutellata* und in allen Proben aus den Ngong Hills gefunden, einer Region die in zunehmendem Maße der Verstädterung ausgesetzt ist. Dieses Ergebnis ist der erste Nachweis von diagnostischen Polymorphismen in der mtDNA von Honigbienen in Ostafrika. Unsere Daten unterstützen die Hypothese, daß *A. m. monticola* nicht als Ökotyp von *A. m. scutellata* angesehen werden kann, sondern ihre eigene Evolutionsgeschichte hat. Im Licht der Klimaänderungen während und nach dem Pleistozän erscheint es am wahrscheinlichsten, daß die heutigen disjunkten Populationen von *A. m. monticola* Relikte einer größeren, pleistozänen Bienenpopulation darstellen, die jetzt auf Refugien in den Bergen beschränkt sind. Im Widerspruch zu den Hypothesen über vertikale Wanderungen von *A. m. scutellata* über weite Strecken zwischen Savanne und Gebirgen fanden wir keinen Hinweis auf Introgression von Morphologie oder

mtDNA von *A. m. scutellata* in Bienenproben, die in Gebirgslagen gesammelt wurden. Statt dessen hatten einige in der Savanne gesammelte Völker entweder sowohl die Morphologie als auch die mtDNA von *A. m. monticola*, oder sie wiesen die Morphologie von einer Unterart in Kombination mit der mtDNA der anderen Unterart auf (Abb. 2).

Im Gegensatz zu den ausgeprägten Unterschieden zwischen benachbarten Bienenpopulationen an Mt. Elgon und Mt. Kenya besitzen alle untersuchten Bienen in den Ngong Hills den für *A. m. scutellata* typischen Haplotyp. Wir stellen die Hypothese auf, daß es sich hier um starke Hybridisierung zwischen den Unterarten handelt, die als Folge von zunehmender Entwaldung und Zersiedelung dieser Region auftritt.

Alle untersuchten Proben von *A. m. litorea* besaßen den für *A. m. scutellata* typischen Haplotyp. Die Frage, ob *A. m. litorea* als eine eigene Unterart anzusehen ist, wie aufgrund morphologischer Untersuchungen vorgeschlagen wurde, oder als Ökotyp von *A. m. scutellata* betrachtet werden sollte, kann mit diesem Ergebnis nicht weiter aufgeklärt werden.

***Apis mellifera monticola / A. m. scutellata / A. m. litorea* / mtDNA / Kenia**

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