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Mountain honeybees of Africa

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(Invited paper)

Abstract – The honeybees occurring along transects from low to high altitude were analysed for seven separate mountain systems in Africa using three suites of characters: morphometric characters, flight dimensional measurements and the mitochondrial DNA (mtDNA) restriction length fragments derived from the non-coding region of COI-COII by *Dra*I restriction. Morphocluster definition was consistent with mtDNA cluster membership but not with flight dimensional data. When all three character suites are combined, six different kinds of unrelated mountain bees are obtained. The only commonality among the mountain bees is that they are larger than those of lower altitudes. Because of fundamental differences in the restriction length fragments and other clusters obtained, it is concluded that mountain bees should probably be regarded as ecotypically differentiated populations of the subspecies surrounding each particular mountain.

***Apis mellifera* / Africa / mountain / morphometrics / flight dimension / mtDNA**

1. INTRODUCTION

One of the most intriguing problems related to the delineation of honeybee (*Apis mellifera* L.) populations is that precisely quantitative definitions of subspecific taxa which also have biological meaningfulness have proven extremely elusive [13]. These difficulties are exacerbated by the constraints inherent in the typological Linnean system of nomenclature [3, 4] and the fact that categories have always been defined in terms of phenotypes which may represent direct expression of the genotype(s) or, alternatively, reflect a greater or lesser influence of environmental factors [5, 37, 38] or constitute products of the norm of reaction [6]. The mountain honeybees of Africa supply a rich source of these difficulties [13], and form the basis of this study.

To illustrate these difficulties we shall recapitulate briefly a few examples. In 1961, Smith [35] reported the occurrence of large

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and dark bees at about 2 000 m on the slopes of Mounts Kilimanjaro and Meru in Tanzania, which he named *Apis mellifera monticola*. There were, however, forms of intermediate size between these bees and other smaller bees lower down the mountains which were named *A. m. scutellata*. Ruttner [31, 32], Meixner [18] and Meixner et al. [20] subsequently extended these studies with multivariate techniques and concluded that "the distinct *monticola* areas of today represent the refugia of a former much larger coherent distribution across all of the highlands of East Africa" [19, 31]. This equates to a concept of an archipelago of relictual bees constituting the subspecies *A. m. monticola*. This interpretation was supported by additional analyses of allozymes coupled to morphometric analyses [19, 20]. However, evidence for such an archipelago (as opposed to the Tanzanian case) of *A. m. monticola* type bees using allozymic [21, 33], morphometric and mitochondrial DNA (mtDNA) [8, 10, 33, 34] analyses of honeybees of other mountainous systems in Africa were equivocal in this regard.

Put another way, one can ask the question whether there is a shared genetic heritage among honeybee populations at high altitudes in the different mountain systems of the African continent that would justify considering them more closely related to each other than to their surrounding or nearby lowland neighbours. This is the purpose of the present paper.

2. MATERIALS AND METHODS

2.1. Honeybees

Recently (1997) the morphometric databases on honeybees of the Institut für Bienenkunde (Ruttner Collection at Oberursel, Germany) and of the Apiculture Group at Rhodes University (Hepburn and Radloff Collection, Grahamstown, South Africa) were amalgamated to form a single database for the continent of Africa. These combined data, augmented by more recent measurements of honeybees from Zimbabwe, Malawi, Lesotho and Tanzania, were used to analyse the honeybee populations in two different ways. First, a full morphometric analysis of 14 973 worker bees from 825 colonies at 193 localities in East Africa extending from South Africa to Ethiopia was made [26]. But because of the severe consequences of sample size and sampling distance limitations (see Discussion) in masking small biometric groups [25], a more restricted analysis was made on a transect basis. The localities and transects are shown in Figure 1.

Each of the transects was an average of 1 000 km in length and extended (usually) from near sea level to above 2 000 m altitude in the mountain systems of transect 1 (Ethiopia), transect 2 (Cameroon), transect 3 (Tanzania), transect 4 (Malawi), transect 5 (Zimbabwe/Mozambique), transect 6 (South Africa/Lesotho) and transect 7 (Namibia). Some of the transects simply extend from low to higher altitudes, but others extend from low to higher and then low altitudes again on the opposite side of the mountain system (Tab. I, Fig. 1). All of the worker bees used in this part of the study were sampled from small-scale, fixed-site beekeeping colonies at 31 localities covering all major regions of Africa except the Maghreb in the northwest of the continent. While 'captive' colonies were used, it must be understood that these were simply bees attracted to empty hives from the wild population. In most parts of Africa bees are seldom transported and bee breeding is virtually non-existent. Thus the samples used in this study are authentic samples of unadulterated wild honeybees typical of the areas under consideration.

2.2. Measurements

Three distinct and independent sets of measurements were made: a series of morphometric measurements to determine

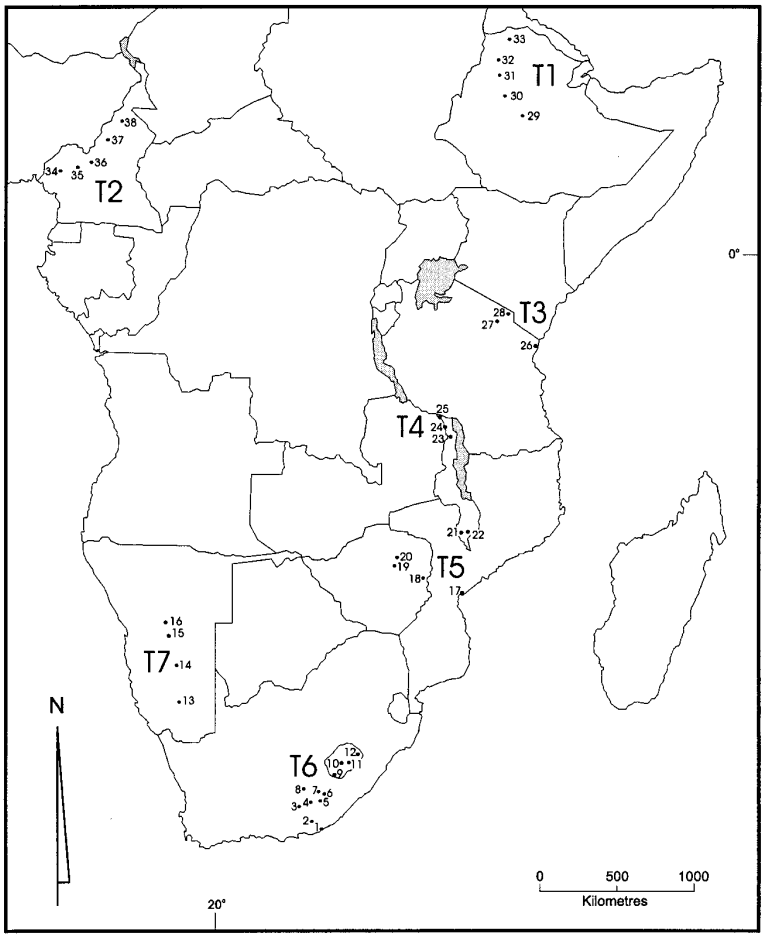

Figure 1. Distribution of the seven transects through different mountainous regions of Africa and the localities in them that were sampled: Localities given by reference numbers are as follows: **Transect 6** (South Africa): 1. Port Alfred, 2. Grahamstown, 3. Hofmeyr, 4. Tarkastad, 5. Queenstown, 6. Dordrecht, 7. Sterkstroom, 8. Burgersdorp; (Lesotho): 9. Quthing, 10. Thaba-Tseka, 11. Semonkong, 12. Mokhotlong; **Transect 7** (Namibia): 13. Keetmanshoop, 14. Mariental, 15. Windhoek, 16. Okahandja; **Transect 5** (Mozambique): 17. Beira; (Zimbabwe): 18. Mutare, 19. Harare, 20. Karoi; **Transect 4** (Malawi): 21. Chikwawa, 22. Thyolo, 23. Rumphi, 24. Chilinda, 25. Chitipa; **Transect 3** (Tanzania): 26. Tanga, 27. Kasungu, 28. Mt. Meru; **Transect 1** (Ethiopia): 29. Holeta, 30. Debre Markos, 31. Bahir Dar, 32. Gonder, 33. Adi Arkay; **Transect 2** (Cameroon): 34. Mamfe, 35. Bamenda, 36. Kumbo, 37. Banyo, 38. Gouna.

morphocluster membership and affinity on a continental and regionally restricted basis, aerodynamic measurements of flight-related parameters which provide a power efficiency index, and analyses of the restriction length fragments for non-coding COI and COII region of cytochrome oxidase in mtDNA.

For the morphometric studies the same nine characters used in previous studies of honeybees in Africa were measured [2, 13,

22]. Their Ruttner numbers [31] are given in brackets as follows: length of cover hair on tergite 5 (1), width of wax plate on sternite 3 (11), transverse length of wax plate on sternite 3 (13), pigmentation of scutellum (35), pigmentation of scutellar plate (36), pigmentation of tergite 2 (32), wing angle B4 (22), wing angle N23 (30) and wing angle O26 (31).

As noted above, Ruttner [31] concluded that as few as 10 characters would be sufficient to morphometrically discriminate African races of honeybees, and this was also borne out by Crewe et al. [2]. Ruttner [31] demonstrated that between 10 and 20 bees would be a sufficient sample size for morphometric statistical analysis. Thus, for each colony, 20 bees served as the standard sample size.

Flight-related parameters from which a power efficiency index was calculated were measured as follows. Worker bees that had been collected in alcohol were subsequently dissected to separate the wings from the thorax and the latter from the other body parts, after which all were weighed on a microbalance to constant dry mass. On dissection, the digestive system was discarded and replaced by a 'clean' dry weight gut measure (details in [14, 15]). The four wings of each bee were slide-projected on a digitizing tablet and scanned to measure total wing surface area. Finally, values for wing surface area (S), whole body mass (M), wing loading ($W = M/S$), thorax mass (m) to M ratio ($r = m/M$) and an excess power index (EPI) were calculated. The EPI for honeybees is a measure of the maximum power available to the bee over that required to maintain equilibrium in steady level flight, and is defined as: $EPI = \sqrt{(r^2/W)}$ and was derived for honeybees from the general theory of flight [17]. Flight dimensional measurements were taken on 12 bees per colony.

2.2.1. mtDNA analysis

Due to a limited availability of specimens, only some transects (South Africa, Zimbabwe, Malawi and Ethiopia) were subjected to DNA analysis.

2.2.2. DNA extraction

Genomic DNA was phenol-extracted from individual worker thoraxes ($n = 5$ per colony) following a modified protocol [7]. Ethanol-preserved workers were initially incubated with agitation in insect Ringer solution (127 mM NaCl, 1.5 mM $CaCl_2$, 5 mM KCl, pH 7.4 with NaOH) for 5, 10 and 15 min at room temperature before extraction. Then, DNA was phenol-extracted from the individual thoraxes. Individual worker DNA was resuspended in 30 µL DDH_2O. To account for unspecific restrictions, DNA was electrophoresed on standard 1% agarose gels. The individual DNA samples were then pooled for each colony.

2.2.3. PCR conditions and Dra*I restrictions*

The mtDNA fragments (including the COI-COII intergenic region) were amplified using the previously reported procedures with primers E2 and H2 [9, 10]. Amplification products were electrophoresed on standard 1% agarose gels to seize the fragments. Then, the amplification products were restricted with *Dra*I following routine protocols [9, 10]. Restriction fragments were separated in 5 and 10% acrylamide gels and UV-visualised after staining in ethidium bromide. Restriction fragment patterns were classed based on the size of the fragments. Fragments smaller than 100 base pairs (bp) were not considered.

2.3. Data processing

The colony means of the morphometric and flight dimensional data were analysed using factor analysis and discriminant analysis procedures. Wilks' lambda statistic was used to test for significant differences between the vector of means of the

characters entered into the discriminant functions [17]. The intercolonial variances within transects were tested for heterogeneity by means of Levene's *F*-statistic. Analysis of variance (ANOVA) and Kruskal-Wallis procedures followed by Scheffé's multiple comparison tests were used to test for significant differences in the means of the flight dimensional measurements between localities.

Chi-square tests using Greenacre's method were used to test for significant heterogeneity in the frequency distributions of the restriction length fragments [12]. This method tests for heterogeneity between clusters using the frequency distributions of the fragment types in a two-way contingency table with *r* rows (transects) and *c* columns (patterns of restriction length fragments). The cut-off point for significant clustering is found from the largest eigenvalue of a Wishart matrix variate $W_k(s)$ where the order $k = \min\{r\text{-}1, c\text{-}1\}$ and the degrees of freedom $s = \max\{r\text{-}1, c\text{-}1\}$ [12].

3. RESULTS

3.1. Morphometric analysis

Morphometric analyses of mountain honeybees present some special problems with respect to sampling distance and size of the area considered. For example, in a previous study of honeybees collected along a transect through Cameroon, a very distinct form was collected at Bamenda in the Adamaoua mountains which was described as '*monticola*-like' [23]. Likewise, in previous morphometric analyses of honeybees from East Africa (including Burundi, Rwanda, Uganda, Kenya, Tanzania, Malawi, Zambia and northern Mozambique) the factor and discriminant analyses revealed yet other '*monticola*-like' morphoclusters, one consisting of large black mountain bees in Kenya and Tanzania, the other of large yellowish bees at Chilinda on the Nyika plateau of Malawi [13]. However, no '*monticola*-like' bees emerged in earlier studies of Ethiopia, Namibia and Zimbabwe/Zambia transects [24, 28, 29].

Because smaller biometric groups are often swamped in large regional analyses and because additional material has become available from transect 3 (Tanzania), transect 4 (Malawi) and transect 6 (Lesotho), new morphometric analyses were performed on the honeybees of these new transects. That from sea level in South Africa to the high mountains of the Drakensberg in Lesotho (transect 6) yielded two quite distinct morphoclusters, with a 100% correct classification for the bees of Lesotho on the one hand and those within South Africa on the other [27]. In the case of Malawi (transect 4), two morphoclusters were found, and the mountain form at Chilinda yielded a correct classification of 92.0% and the bees from neighbouring lower altitudes with 91.3%. Here we present results of the analyses of morphometric data from all seven mountain transects thus far examined.

A factor analysis using the colony means of the nine morphometric characters of worker honeybees from 193 colonies from the localities shown in Figure 1 and in Table I isolated four factors with eigenvalues greater than 1: factor 1, hair length on tergite 5, transverse length of wax plate on sternite 3, wing angle N23 and pigmentation of tergite 2; factor 2, width of wax plate on sternite 3, pigmentation of scutellum and scutellar plate; factor 3, wing angle O26; factor 4, wing angle B4. These four factors accounted for 72% of the variance in the data. The loading for each character had an absolute value greater than 0.6. The factor scores graph revealed three morphoclusters, those colonies from transect 1 (Ethiopia) forming one cluster, those colonies from transects 2 (Cameroon), 3 (Tanzania), 4 (Malawi), 5 (Zimbabwe/Mozambique), 6 (South Africa) and 7 (Namibia) forming a second cluster, and those from transect 6 (Lesotho) forming a third cluster (Fig. 2).

A discriminant analysis confirmed the three morphoclusters with 93.8%

Table I. Means and standard deviations (sd) of whole body mass *M*, wing surface area *S*, wing loading factor *W*, thorax/whole body mass ratio *r*, excess power index and morphometric variances (*Var.*) for honeybees sampled at differing altitudes in seven transects through mountainous areas of Africa[#]. Reference numbers to localities are shown in Figure 1.

Transect No., country and locality	Map ref. No.	Altitude (m)	Sample size	*M* (mg)		*S* (mm^2)		*W*		*r*		*EPI*		*Var.*
				Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	
1 Ethiopia														
Adi Arkay	33	950	5	15.68^{a}	1.03	47.54^{a}	2.13	0.33^{a}	0.01	0.54^{a}	0.02	0.93^{a}	0.02	58.4^{b}
Gonder	32	2 121	6	17.16^{c}	0.99	50.44^{c}	1.77	0.34^{a}	0.01	0.55^{a}	0.01	0.94^{a}	0.02	33.0
Bahir Dar	31	2 400	5	17.46^{c}	1.24	47.59^{a}	0.79	0.37^{c}	0.03^{w}	0.54^{a}	0.01	0.89^{a}	0.05^{w}	21.3
			$F_{2,13}$	3.9	0.3	5.5	0.7	5.7	4.2	0.9	1.6	3.4	4.1	2.1
			P	0.047	ns	0.018	ns	0.017	0.040	ns	ns	ns	0.041	ns
			r	0.61*		0.25		0.55*		0.22		0.34		
2 Cameroon														
Mamfe	34	150	3	17.24^{a}	0.18	46.37^{a}	1.03	0.37^{a}	0.01	0.53^{a}	0.02	0.87^{a}	0.03	28.2
Bamenda	35	2 500	4	19.67^{c}	2.07	49.33^{c}	0.91	0.39^{a}	0.04	0.53^{a}	0.03	0.86^{a}	0.09^{b}	26.6
Kumbo	36	2 100	2	19.07^{c}	0.94	50.66^{c}	1.91	0.38^{a}	0.00	0.56^{a}	0.01	0.91^{a}	0.02	18.4
Banyo	37	1050	2	16.31^{a}	0.78	48.74^{a}	0.11	0.34^{a}	0.02	0.55^{a}	0.00	0.96^{a}	0.02	47.7
Gouna	38	400	4	16.34^{a}	0.67	47.07^{a}	0.54	0.35^{a}	0.01	0.55^{a}	0.00	0.93^{a}	0.02	23.5
			$F_{4,10}$	3.6	2.2	9.0	3.4	2.5	1.7	0.9	2.0	1.7	1.9	4.2
			P	0.044	ns	0.002	ns	ns	ns	ns	ns	ns	ns	ns
			r	0.48		0.85**		0.19		0.26		0.03		
3 Tanzania														
Tanga	26	0	2	14.58^{a}	0.32	50.16^{a}	2.31	0.29^{a}	0.01	0.52^{a}	0.03	0.98^{a}	0.04	39.5
Arusha	28	1 390	3	15.27^{a}	1.53	49.42^{a}	1.80	0.31^{a}	0.02	0.51^{a}	0.02	0.92^{a}	0.05	10.6
Mt. Meru	28	3 000	4	15.91^{a}	0.60	52.67^{a}	1.61	0.30^{a}	0.02	0.55^{a}	0.01	0.99^{a}	0.03	-
			$F_{2,6}$	1.2	2.9	3.1	0.3	0.6	0.7	3.8	2.4	3.6	1.3	1.3
			P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
			r	0.53*		0.63*		0.68*		0.57		0.40		
4 Malawi														
Chikwawa	21	100	6	17.40^{a}	1.04	50.07^{a}	0.93	0.35^{a}	0.02	0.51^{a}	0.03	0.86^{a}	0.05	51.5
Thyolo	22	900	6	18.59^{a}	0.86	52.29^{a}	1.31	0.36^{a}	0.02	0.52^{a}	0.01	0.88^{a}	0.04	46.5
Rumphi	23	1 050	6	18.82^{a}	2.14	54.19^{c}	1.15	0.35^{a}	0.04	0.50^{a}	0.01	0.86^{a}	0.04	49.6
Chitipa	25	1 300	6	16.15^{a}	1.19	50.82^{a}	0.95	0.32^{a}	0.02	0.55^{c}	0.02	0.98^{c}	0.05	54.8
Chilinda	24	2 600	6	20.28^{c}	3.31^{b}	54.52^{c}	1.31	0.37^{a}	0.06^{b}	0.49^{a}	0.04^{w}	0.81^{d}	0.14^{wb}	83.8^{wb}
			$F_{4,25}$	3.9	2.1	17.9	0.7	1.9	1.8	6.0	4.9	4.4	4.7	5.4
			P	0.014	ns	< 0.001	ns	ns	ns	0.002	0.005	0.008	0.006	< 0.001
			r	0.37*		0.61**		0.19		0.19		0.16		

Table I. (continued).

5	**Zimbabwe/ Mozambique**													
	Beira	17	0	**3**	17.54^{a}	2.14	46.65^{a}	1.14	0.38^{a}	0.04	0.53^{a}	0.01	0.87^{a}	0.05	17.4
	Mutare	18	338	**5**	23.37^{c}	1.36	50.16^{c}	0.85	0.47^{c}	0.03	0.50^{a}	0.01	0.74^{c}	0.04	26.1
	Harare	19	1 478	**4**	26.93d	3.84b	51.38^{d}	0.51	0.53^{d}	0.08b	0.50^{a}	0.01	0.70^{c}	0.06	25.7
	Karoi	20	1 251	**5**	23.22^{c}	4.08b	49.95^{c}	1.64w	0.46^{c}	0.07b	0.51^{a}	0.02	0.76^{c}	0.08	42.8
				$F_{3,13}$	5.2	1.2	10.3	3.5	3.8	1.1	3.3	2.7	5.0	0.6	3.2
				P	0.014	ns	< 0.001	0.048	0.038	ns	ns	ns	0.016	ns	ns
				r	0.58*		0.62**		0.54*		0.31		0.49		
6	**Lesotho/ South Africa**													
	Port Alfred	1	0	**5**	19.88^{a}	3.47b	45.58^{a}	0.25	0.43^{a}	0.08^{b}	0.52^{a}	0.05w^{b}	0.80^{a}	0.12^{b}	13.2
	Grahamstown	2	525	**5**	19.89^{a}	1.18	45.45^{a}	0.84	0.44^{a}	0.03	0.51^{a}	0.01	0.78^{a}	0.03	69.9w^{b}
	Queenstown	5	1 077	**5**	21.58^{a}	1.94	46.95^{a}	0.47	0.46^{a}	0.04	0.51^{a}	0.03	0.76^{a}	0.07	34.4
	Quthing	9	1 578	**5**	21.59^{a}	2.17	50.36^{c}	0.91	0.43^{a}	0.04	0.54^{a}	0.02	0.83^{c}	0.06	23.4
	Mokhotlong	12	2 133	**5**	19.87^{a}	0.61	51.06^{c}	0.81	0.39^{a}	0.01	0.55^{a}	0.01	0.88^{c}	0.02	54.6
	Semonkong	11	2 200	**5**	20.91^{a}	1.81	51.06^{c}	1.07	0.41^{a}	0.03	0.55^{a}	0.01	0.86^{c}	0.03	54.8
	Thaba Tseka	10	2 286	**5**	18.71^{a}	0.27	49.99^{c}	1.77w	0.37^{a}	0.01	0.55^{a}	0.03	0.89^{c}	0.06	26.0
				$F_{6,25}$	1.0	1.8	44.4	3.6	2.0	2.1	2.3	2.7	2.7	2.2	6.4
				P	ns	ns	< 0.001	0.011	ns	ns	ns	0.039	0.037	ns	0.026
				r	0.04		0.90**		0.40*		0.51**		0.49**		
7	**Namibia**													
	Keetmanshoop	13	1 773	**4**	18.74^{a}	1.18	49.28^{a}	0.97	0.38^{a}	0.02	0.55^{a}	0.01	0.90^{a}	0.03	28.6
	Mariental	14	1 180	**5**	18.69^{a}	1.48	49.29^{a}	1.66	0.38^{a}	0.02	0.54^{a}	0.00	0.88^{a}	0.02	47.1
	Windhoek	15	1 779	**5**	19.30^{a}	1.37	49.46^{a}	1.02	0.39^{a}	0.03	0.52^{c}	0.01	0.84^{c}	0.03	30.8
	Okahandja	16	1 439	**3**	18.53^{a}	1.58	47.30^{a}	0.54	0.39^{a}	0.03	0.50^{c}	0.01	0.81^{c}	0.05	32.2
				$F_{3,13}$	0.3	0.1	2.4	2.1	0.3	0.4	32.6	2.2	5.4	0.9	0.7
				P	ns	ns	ns	ns	ns	ns	< 0.001	ns	0.013	ns	ns
				r	0.15		0.13		0.12		0.07		0.01		
All transects combined				**$F_{30,105}$**	7.93	1.76	17.43	1.48	9.36	1.92	4.29	3.11	6.94	2.47	3.67
				P	< 0.001	0.019	< 0.001	ns	< 0.001	0.008	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[#] $\mathbf{F}_{df}$: *F*-statistic, **P**: *P*-value, **r**: *r* correlation coefficient; ns: non significant.
* ($P < 0.05$); ** ($P < 0.01$); b: between transects ($P < 0.05$); w: within transects ($P < 0.05$); acd: means with the same do not differ ($P > 0.05$).

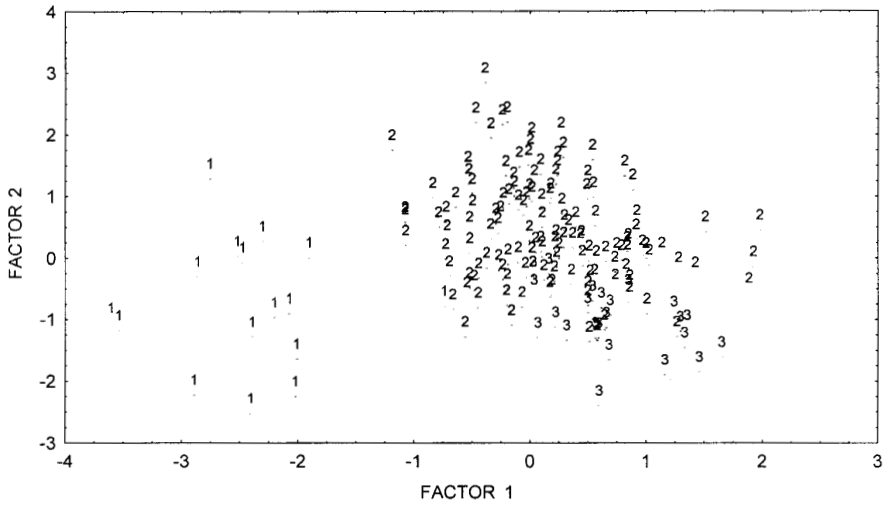

Figure 2. Factor analysis plot using morphometric characters: cluster 1 comprises colonies from transect 1 (Ethiopia), cluster 2 comprises colonies from transects 2, 3, 4, 5, 6 and 7 (Cameroon, Tanzania, Malawi, Zimbabwe/Mozambique, South Africa and Namibia respectively) and cluster 3 comprises colonies from transect 6 (Lesotho).

(1 misclassified) correct classification of the colonies from transect 1 (Ethiopia), with a posteriori probabilities $P = 1.0$ for 14 colonies and $P = 0.98$ for the remaining 1 colony; 97.8% (3 misclassified) correct classification of the colonies from transects 2 (Cameroon), 3 (Tanzania), 4 (Malawi), 5 (Zimbabwe/Mozambique), 6 (South Africa) and 7 (Namibia) with a posteriori probabilities $P = 1.00$ for 97 colonies, $0.90 \leq P \leq 0.99$ for 25 colonies and $0.62 \leq P \leq 0.89$ for the remaining nine colonies; 95.7% (1 misclassified) correct classification of the colonies from transect 6 (Lesotho only) with a posteriori probabilities $P = 1.00$ for 20 colonies and $0.72 \leq P \leq 0.83$ for the remaining two colonies. The jackknife procedure gave the same classification results, except one more colony from Lesotho was classified incorrectly into the second morphocluster.

Considering high and low altitude influences on the morphometric characters, no significant correlations were found between the scores of factors 1 to 4 and altitude when using the data from all three clusters. When, however, the analysis was restricted to the scores from clusters 1 and 3, a significant correlation was found between factor 1 scores and altitude ($r = 0.45$, $P < 0.0001$; Fig. 3).

The variance characteristics of the morphometric data are given on a transect basis in Table I. Significantly high domains of morphometric variance occur in transects 1, 4 and 6 at Adi Arkay (Ethiopia), Chilinda (Malawi) and Grahamstown (South Africa) respectively. Otherwise the bees of transects 2, 3, 5 and 7 (Cameroon, Tanzania, Zimbabwe/Mozambique and Namibia respectively) are morphometrically on the homogeneous side.

3.2. Flight dimensional analysis

In a factor analysis using the colony means of the mass-related characters and the total wing surface area of worker honeybees from 136 colonies, two factors with

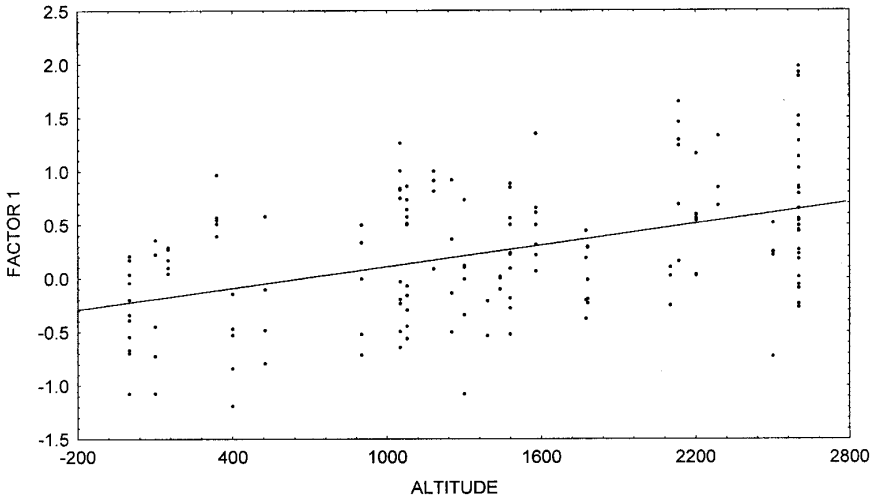

Figure 3. Relationship between the factor 1 scores of clusters 2 and 3 (Fig. 2) using morphometric characters and altitude.

eigenvalues greater than 1 were isolated: factor 1, head, thorax, abdomen and wing mass; factor 2, total wing surface area. These two factors accounted for 80% of the variance in the data. The loading for each character had an absolute value greater than 0.7. The graph of the factor scores showed three clusters: colonies from transects 3 and 4 (Tanzania and Malawi) forming a cluster, colonies from transects 1, 2 and 6 (Ethiopia, Cameroon and Lesotho respectively) forming a second cluster and colonies from transects 6, 5 and 7 (South Africa, Zimbabwe/Mozambique and Namibia respectively) forming a third cluster (Fig. 4). The bees of the first and third clusters consist of groups from more or less neighbouring countries; however, the bees of the second cluster consist of populations in countries that are at a 3 000 to 4 000 km distance from one another.

A discriminant analysis confirmed the separation of the three clusters and correctly classified 94.9% (2 misclassified) of the colonies from transects 3 and 4 (Tanzania and Malawi) with a posteriori probabilities $P = 1.00$ for 30 colonies, $0.90 \leq P \leq 0.99$ for 4 colonies and $0.76 \leq P \leq 0.89$ for the remaining 3 colonies; 93.7% (4 misclassified) of the colonies from transects 1, 2 and 6 (Ethiopia, Cameroon and Lesotho respectively) with a posteriori probabilities $P = 1.00$ for 11 colonies, $0.90 \leq P \leq 0.99$ for 38 colonies and $0.59 \leq P \leq 0.89$ for the remaining 10 colonies; 79.4% (7 misclassified) of the colonies from transects 6, 5 and 7 (South Africa, Zimbabwe/Mozambique and Namibia respectively) with a posteriori probabilities $P = 1.00$ for 11 colonies, $0.90 \leq P \leq 0.99$ for 6 colonies and $0.50 \leq P \leq 0.89$ for the remaining 10 colonies.

The jackknife procedure gave the same classification results, except one more colony from cluster 2 was misclassified into cluster 3. A significant difference was found between the means of the three clusters ($\Delta = 0.1175$ with (4, 2, 133) df; $F = 62.3$ with (8, 260) df, $P < 0.0001$). Considering high and low altitude influences on the flight dimensional characters, a significant correlation was found between factor 2 scores (relating to total wing surface area) and altitude ($r = 0.37$, $P < 0.0001$; Fig. 5).

The means and standard deviations for thorax mass, whole body mass, wing surface

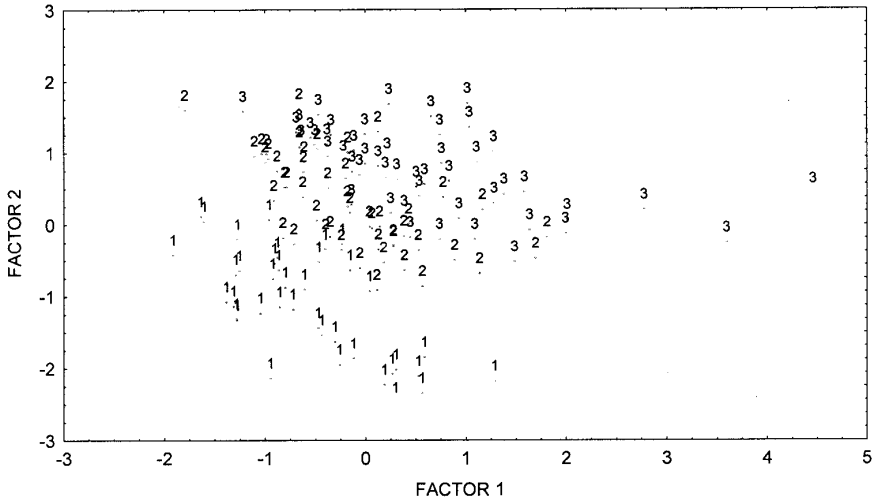

Figure 4. Factor analysis plot using flight dimensional characters: cluster 1 comprises colonies from transects 3 and 4 (Tanzania and Malawi), cluster 2 comprises colonies from transects 1, 2 and 6 (Ethiopia, Cameroon and Lesotho respectively) and cluster 3 comprises colonies from transects 5, 6 and 7 (Zimbabwe/Mozambique, South Africa and Namibia respectively).

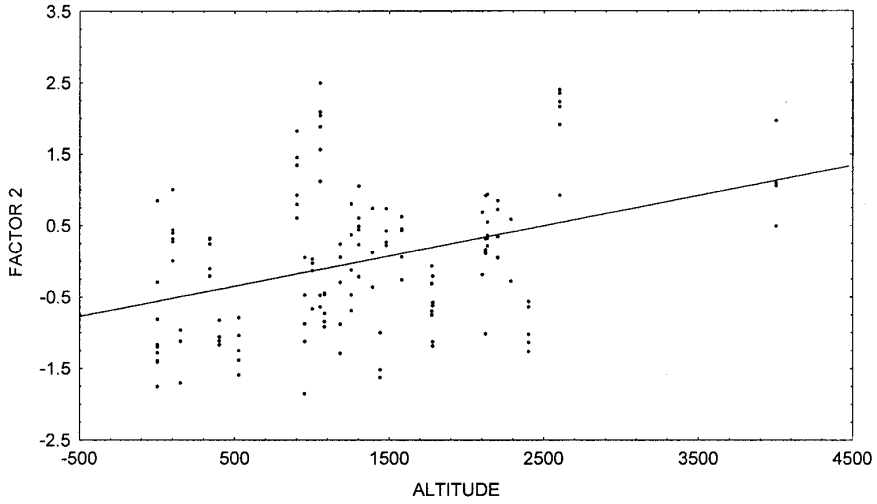

Figure 5. Relationship between the factor 2 scores using flight dimensional characters and altitude.

area, wing loading, thorax/whole body mass ratio and excess power index for worker honeybees at each locality are given in Table I. ANOVA and nonparametric Kruskal-Wallis procedures used to test for significant differences in means between localities revealed significant differences for all the flight dimensional measurements (Tab. I).

The correlation coefficients and corresponding levels of significance between flight dimensional properties and altitude are given in Table I. In several transects the results clearly show that there are significant positive correlations between certain flight properties and altitude which indicate that larger honeybees are found at higher altitudes.

The variance characteristics for the five flight dimensional properties of the honeybees are indicated in Table I. Tests for the equality of the variances showed that there are significantly higher variances at localities at higher altitudes for certain properties, i.e., significantly higher variations in transects 4 at Chilinda (Malawi), 1 at Bahir Dar (Ethiopia), 2 at Bamenda (Cameroon), 5 at Karoi (Zimbabwe) and 6 at Thaba Tseka (Lesotho). Not surprisingly, transect 7 (Namibia) is fairly homogeneous in this regard, but the homogeneity in the case of transect 3 (Tanzania) was unexpected.

3.3. mtDNA analysis

A total of eight different *Dra*I restriction fragment patterns were present in the samples analysed from transects 1, 4, 5, and 6 (Ethiopia, Malawi, Zimbabwe/Mozambique and Lesotho/South Africa respectively). Four of these patterns were restricted to transect 1 (Ethiopia) alone (Tab. II). Another *Dra*I pattern was common to the samples analysed from transects 4, 5 and 6 (Malawi, Zimbabwe/Mozambique and Lesotho/South Africa respectively). The remaining three patterns were distributed among the samples from transects 4 and 5 (Malawi and Zimbabwe). Three significantly different mtDNA clusters were found using Greenacre's chi-square method [12] (χ^2 = 99.6, 4 df, $P < 0.0001$); 1) Ethiopia; 2) Malawi and Zimbabwe (χ^2 for difference = 0.180, ns); 3) Lesotho and South Africa (χ^2 for difference = 0.001, ns). The mtDNA cluster of Ethiopia and the mtDNA cluster of Lesotho/South Africa matched the two morphoclusters obtained in the morphometric analysis.

Considering each mtDNA cluster separately, we see that within the mtDNA cluster of Ethiopia in transect 1, that Holeta, Gonder and Bahir Dar have a common *Dra*I pattern. Different patterns are found at Adi Arkay and Debre Markos. Similarly, within the mtDNA cluster of transects 4 and 5 (Malawi and Zimbabwe/Mozambique) different *Dra*I patterns are found at Chilinda and Mutare respectively, and finally within the mtDNA cluster of transect 6 (Lesotho/South Africa) only one *Dra*I pattern was present at all localities. The single colony from Chilinda may be an incomplete digest of the more typical pattern, given the 469 bp size increase of the amplified fragment over the standard size total found in Table II.

4. DISCUSSION

The morphoclusters obtained in the discriminant analysis of the honeybees of all seven transects considered jointly yielded groups which partially accord with other recently published assessments. That the honeybees of transects 6 and 1 (Lesotho and Ethiopia) are quite distinct from other lower altitude bees surrounding them supports recent interpretations [13, 26, 27]. However, in this present analysis the largest cluster, comprised of bees from transects 4, 3, 5 and 7 (Malawi, Tanzania, Zimbabwe/Mozambique and Namibia respectively) on the one hand and of transect 2 (Cameroon) on the other, is at odds with recent views [13]. The former have previously been classified as *A. m. scutellata* and the latter as *A. m. adansonii*.

Other discrepancies concern the high altitude mountain bees themselves, especially those regarded as *A. m. monticola* [1, 19, 20, 30, 31]. The first point is that those high altitude bees of Tanzania and Kenya were undetectable as a distinct group in other

Table II. Frequency Distribution of eight different mitochondrial DNA restriction length patterns obtained from the non-coding region of COI-COII by a *Dra*I restriction. Reference numbers to localities are shown in Figure 1.

Country and localities	Map ref. No.	No. of colonies	Restriction patterns				
South Africa							
Queenstown	5	4	550	209	100		
Port Alfred	1	2	550	209	100		
Dordrecht	6	3	550	209	100		
Hofmeyr	3	3	550	209	100		
Tarkastad	4	3	550	209	100		
Sterkstroom	7	2	550	209	100		
Burgersdorp	8	3	550	209	100		
Lesotho							
Quthing	9	6	550	209	100		
Mokhotlong	12	6	550	209	100		
Semonkong	11	6	550	209	100		
Thaba Tseka	10	2	550	209	100		
Zimbabwe							
Harare	19	5	550	209	100		
Mutare	18	4	550	209	100		
		1	550	100			
		1	550	191	209		
Malawi							
Chitipa	25	5	550	209	100		
		1	550	100			
Rumphi	23	4	550	209	100		
		2	550	100			
Chilinda	24	5	550	209	100		
		1	550	240	229	209	100
Ethiopia							
Adi Arkay	33	3	550	138	100		
		1	468	132	115		
Holeta	29	1	550	138	100		
Gonder	32	5	550	138	100		
Bahir Dar	31	1	550	138	100		
Debre Markos	30	1	550	138	100		
		2	468	132	115		
		3	550	138			
		2	550	216	100		

geographically large-scale morphometric analyses, and this was the result of sampling distance resolution [25]. This is because the greater the distance between samples, the more distinct the morphoclusters and, when between-group variation is considerably larger than within-group variation, small biometric groups may be obscured entirely. On the other hand, the high mountain bees of both Lesotho and Ethiopia (at the opposite end of the geographical spectrum considered) remain intact as distinct populations and are fundamentally different from the other high altitude mountain bees. So on

morphometric grounds alone, the idea of an archipelago of one subspecies or morphocluster of mountain bees designated as '*A. m. monticola*' is not supported.

These results do not put into question the findings that the high altitude bees of Kenya and Tanzania (the original *A. m. monticola*) can be morphometrically and allozymically distinguished from their lower altitude neighbours in very fine space scale studies [18-20]. Rather, the general case is simply that the magnitude of difference between the high and low altitude bees of transects 1 and 6 (Ethiopia and Lesotho) happen to be significantly greater than is the case in Tanzania or Kenya. Indeed Mounts Kenya, Elgon, Meru and Kilimanjaro shared palaeoclimatic conditions during the Quaternary that were vastly different from all other African mountain regions [11, 16, 36]. Thus, while the *monticola*/*scutellata* separation in Tanzania and Kenya may well be relictual as previously suggested [18–20], the morphometric, flight dimensional and mtDNA restriction length pattern data (cf. below) unequivocally exclude the possibility of an archipelago of related, high altitude bees throughout the mountain systems of the continent.

Specific details in which there is general agreement in the data for high altitude bees is simply that they tend to be larger than bees of lower altitudes. That is the only commonality that holds for the honeybees of all seven mountain systems. Pigmentation is interesting but also problematical, because the high altitude bees of Tanzania and Kenya are more darkly pigmented than the bees immediately surrounding them at lower altitudes, which is precisely the obverse of what occurs in all of the other transects. This also poses physiological questions as to the significance the pigmentation may hold for thermoregulation. In any event, the mountain bees of Lesotho and Ethiopia are far more distinct morphometrically than are any of the high altitude mountain bees of the other transect countries.

ANOVA for the whole data set performed independently of transect groupings show that there are significant differences in all means of the flight-related variables. Only one locality (Chilinda, Malawi) exhibits high variance values for flight dimensions as well as morphometrically. Continuing the comparisons, high interlocality variances for flight are independent of high variance domains for morphometric characters for several localities (Bahir Dar, Ethiopia; Bamenda, Cameroon; Port Alfred, South Africa) which exhibit low intercolonial morphometric variance. Although flight-related variables were shown to lack subspecific discriminatory power [15], they are highly effective in delineating altitudinal clusters [14]. There is no correspondence between those clusters derived from flight dimensions and those defined by traditional morphometric methods or by mtDNA cluster membership in conjunction with the flight clusters.

Turning to the results of the mtDNA analysis, three distinct clusters were formed, each of which closely corresponds with the respective morphoclusters for the same localities (Tab. III). However, when all three cluster sets (morphometric, flight and mtDNA) are jointly considered, it is quite apparent that the mountain honeybees investigated here consist of at least six different populations (Tab. III). Put another way, this simply means that there are at least six entirely different kinds of mountain honeybee populations within Africa. In the absence of relevant mtDNA information, it is not possible to comment on the mountain bees of Kenya.

The only mountain bees for which there is a one-to-one correspondence for the morphoclusters, flight clusters and mtDNA clusters are those of the Lesotho/South Africa transect. While these are the most homogeneous mountain bees of the continent, those of Ethiopia are the most heterogeneous. Also, the latter exhibit the highest degree of intracolonial and intercolonial high

Table III. Variations in cluster formation in discriminant analyses of morphometric, flight dimensional and mitochondrial DNA characters for the mountain honeybees of Africa. The flight dimensional clusters are given different numbers because they are not concordant with the morphometric clusters but form entirely new groups. Transect numbers shown in map of Figure 1.

Country	Transect No.	Morphoclusters	Flight clusters	mtDNA clusters
Ethiopia	1	1	4	1
Malawi	4	2	5	2
Tanzania	3	2	5	–
Cameroon	2	2	4	–
Namibia	7	2	6	–
Zimbabwe	5	2	6	2
Lesotho	6	3	4	3
South Africa	6	2	6	3

variance for morphometric domains, and are also the most variable with respect to mtDNA restriction length patterns. It can be noted in passing that when the bees of transect 1 (Ethiopia) are considered without reference to other countries, the honeybees can be differentiated into three quite distinct populations [13, 24].

There is no commonality of high altitude restriction length fragments of mtDNA that could support the idea of an archipelago of the same subspecies of honeybees. It is an interesting possibility to consider whether the mountain bees within coherent mountain systems are more related to each other than to bees in intrasystem comparisons. That this is actually the case is shown in comparisons of the total set of discriminant analysis clusters for all the mountain systems. The most parsimonious interpretation would be that each of the high altitude groups of mountain bees are nothing more or less than local adaptations of the predominant lower altitude bees surrounding a particular mountain. As such, all of these mountain bees would best be regarded as ecotypes of the prevailing subspecies in the area of the mountain under consideration.

The mtDNA analysis yielded significant heterogeneity among the three mtDNA clusters that were formed (Tab. II). When all of the data are combined the evidence is unequivocal that there are at least six different recognisable kinds of 'mountain' bees (Tab. III) that are more closely related to the local, lower altitude bees surrounding them than to each other on very distant mountain systems. We conclude that each of these kinds of mountain bees is an ecotypically adapted subpopulation of the subspecies prevailing in the area where they are found.

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Résumé – Les abeilles de montagne d'Afrique. Il est un problème particulièrement difficile dans la taxonomie et la biogéographie des abeilles domestiques (*Apis mellifera* L.): définir des taxons infraspécifiques qui soient quantitativement précis et recouvrent aussi une réalité biologique. Les abeilles de montagne d'Afrique illustrent très bien cette difficulté. Plusieurs formes différentes « de montagne » ont été identifiées ces dernières années par l'analyse multivariée des caractères morphométriques, étayée parfois par l'analyse des allozymes ou de l'ADNmt. Nous avons prélevé des abeilles le long de sept transects différents à

travers l'Afrique et les avons analysées à l'aide d'un ensemble de techniques : morphométrie, mesures des caractéristiques liées au vol (telles que surface des ailes, poids corporel, etc.) et analyse de l'ADNmt dans la région non codante de COI-COII. Le tableau I et la figure 1 indiquent les chaînes de montagne et les localités où les abeilles ont été prélevées.

L'analyse factorielle a fourni trois morphogroupes qui ont été ensuite confirmés par l'analyse discriminante et les procédures de « jackknife ». Le premier groupe comprend les abeilles du transect 1 (Ethiopie), le second groupe celles des transects 4, 3, 2, 7, 6 et 5 (Malawi, Tanzanie, Cameroun, Namibie, Afrique du Sud, et Zimbabwe/Mozambique respectivement) et le troisième groupe les abeilles du transect 6 (Lesotho) (Fig. 2).

L'analyse factorielle des caractéristiques liées au vol suivie d'une analyse discriminante et d'une procédure de jackknife a fourni trois groupes distincts : le premier comprend les abeilles des transects 4 et 3 (Malawi et Tanzanie), le second les abeilles des transects 1, 2 et 6 (Ethiopie, Cameroun et Lesotho, respectivement) et le troisième les abeilles des transects 6, 5 et 7 (Afrique du Sud, Zimbabwe/Mozambique et Namibie, respectivement) (Fig. 4). Dans plusieurs transects il y avait des corrélations positives entre certaines caractéristiques liées au vol et l'altitude, qui montraient que les plus grosses abeilles se trouvaient aux altitudes les plus élevées. Les abeilles de haute altitude présentaient aussi des variances significativement plus élevées que celles des altitudes plus basses.

L'analyse de l'ADNmt a montré une diversité significative parmi les trois groupes d'ADNmt formés (Tab. II). La combinaison de toutes les données prouvent sans ambigüité qu'il existe au moins six types reconnaissables d'abeilles « de montagne » (Tab. III). Chaque type se rapproche plus des abeilles locales et de faible altitude qui l'entourent que des abeilles des autres chaînes de montagne très éloignées. Nous concluons que chacun de ces types d'abeilles de montagne est une sous population écologiquement adaptée de la sous-espèce qui prédomine dans la région où ils ont été trouvés.

***Apis mellifera* / Afrique / biogéographie / morphométrie / caractéristique liée au vol / ADNmt / montagne**

Zusammenfassung – Bergbienen von Afrika. Es ist ein überaus schwieriges Problem in der Taxonomie und Biogeographie der Honigbienen, quantitative exakte Definitionen von innerartlichen Einheiten (Taxa) zu erstellen, denen gleichzeitig eine biologische Bedeutung zukommen. Bei den Berghonigbienen von Afrika zeigt sich diese Schwierigkeit besonders deutlich. In den letzten Jahren wurden anhand multivariater Analysen morphometrischer Eigenschaften mehrere verschiedene "Berg" formen identifiziert, die manchmal durch Ergebnisse mit Allozymen oder mtDNA gestützt wurden. Wir haben Honigbienen entlang sieben verschiedener Schnittlinien durch Afrika analysiert, und zwar mit einer Reihe von Techniken: Morphometrie, Daten über Flugeigenschaften und der Analyse von mtDNA in der nicht kodierenden Region von COI-COII. Die Bergsysteme und Orte, von denen die Bienen gesammelt wurden sind in Tabelle I und Abbildung 1 dargestellt.

Eine Faktorenanalyse ergab drei Morphocluster (Punktwolken von Proben), die anschlieβend durch eine Diskriminanzanalyse und dem "jackknife" Verfahren bestätigt wurden. Die Bienen von der Schnittlinie 1 (Äthiopien) bilden eine Gruppe, die der Schnittlinien 4, 3, 2, 7, 6 und 5 (die jeweils Malawi, Tansania, Kamerun, Namibia, Südafrika und Simbabwe / Mosambik entsprechen) die 2. Gruppe und eine 3. Gruppe liegt entlang der Schnittlinie 6 (Lesotho) (Abb. 2). Nach einer Faktorenanalyse der Flugeigenschaften gefolgt von Diskriminanzanalyse und dem "jackknife" Verfahren ergaben sich wieder drei Cluster, die aber verschieden

zu den vorherigen sind: die Bienen von der Schnittlinie 4 und 3 (Malawi und Tansania) bilden eine Gruppe, eine 2. Gruppe umfassen die Bienen der Schnittlinien 1, 2 und 6 (Äthiopien, Kamerun und Lesotho) und die 3. Gruppe bilden die Bienen der Schnittlinien 6, 5 und 7 (Südafrika, Simbabwe / Mosambik und Namibia; Abb. 4). In verschiedenen Schnittlinien ergaben sich positive Korrelationen zwischen einigen Flugeigenschaften und der Höhe, die das Vorkommen von größeren Bienen in größeren Höhen anzeigen. Außerdem weisen Bergbienen auch signifikant höhere Varianzen auf als Bienen von geringeren Höhen. Die mtDNA Analysen ergaben eine signifikante Verschiedenartigkeit zwischen den drei mtDNA Clustern, die in Tabelle II dargestellt sind. Nach der Kombination aller Daten läßt sich eindeutig feststellen, dass es mindestens sechs als verschieden anzusehende Arten von Bergbienen gibt (Tab. III), die näher mit den angrenzenden Flachlandbienen verwandt sind als mit den Bienen der weit entfernten anderen Bergmassive. Wir schließen daraus, dass jede dieser Arten von Bergbienen ökologisch angepasste Subpopulationen der Unterarten sind, die in dem Gebiet vorherrschen, in dem sie gefunden wurden.

***Apis mellifera* / Afrika / Berg / Morphometrie / Flugeigenschaft / mtDNA**

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