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The limitations of biometric control on pure race breeding in *Apis mellifera*

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SUMMARY

German bee breeders have tried to replace the autochthonous honey bee population *Apis mellifera mellifera* with *A. m. carnica*, a SE European race of honey bee, for more than 40 years. The latter race is believed to be more adapted to bee management. In a biometrical study of the honey bee population in lower Bavaria, samples of at least 20 workers per colony were taken from 91 apiaries in autumn 1990. The wing venation patterns of the bees were analysed using multivariate discriminant analysis, and the results were compared with those for 6 samples of *A. m. carnica* used for commercial breeding from Kirchhain and 7 samples of preserved *A. m. mellifera* from Erlangen and Kulmbach dating back to 1911. The data indicate that, in spite of tremendous breeding efforts, the bees of this area form a hybrid type between *A. m. carnica* and *A. m. mellifera*. Although land-based mating stations have thus failed to produce pure racial types in this area, it is considered that such stations are of value to practical honey bee breeding if used appropriately in open population selection schemes.

Keywords: *Apis mellifera mellifera*, *Apis mellifera carnica*, selection programme, breeding, biometry, mating stations, Bavaria

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INTRODUCTION

In bee breeding certain racial types are often believed to show superior performance under certain conditions to other races. This is clearly so in the Americas with the Africanized honey bee problem. There are several programmes attempting to replace the Africanized type with more desirable stock. This is nothing new in bee breeding. In the 1940s and particularly after 1950 German beekeepers, mainly guided by Goetze (1940, 1949, 1964), decided that the locally abundant 'black' honey bee, *Apis mellifera mellifera*, was less suitable for beekeeping than *A. m. carnica*, which has an autochthonous distribution in Austria, Yugoslavia and Hungary (Ruttner, 1988). It was believed that *A. m. carnica* bees were less aggressive and better adapted to early spring honey flows. Nationwide, beekeepers supported very strict breeding regulations in order to replace *A. m. mellifera* with *A. m. carnica* honey bees and the breeding efforts were substantially supplemented by state funds.

Since both races readily interbreed, the problem of hybridization was large. For this reason, several biometrical characteristics that separated *mellifera* well from *carnica* (Ruttner, 1983) were used to try and identify those hybrids that did occur. In particular, the length of the abdominal body hairs, the width of the tomentum, and the so-called cubital index of the cubital cell in the wing venation are strikingly different between the two races (Ruttner, 1969).

Isolated mating apiaries were established to try to reduce hybridization between the *carnica* stock and the autochthonous population. However, many difficulties arose because of the large mating range of queens and drones (Ruttner & Ruttner, 1972). Strict mating control was achieved on North Sea islands, but mating stations on the mainland had substantial problems. Although feral honey bees rarely interfered with efforts to obtain 'pure' matings, uncontrolled hybridization with non-selected managed bee colonies frequently occurred. One attempt to solve this problem, was the creation of pure bred *carnica* areas around the actual mating apiary (Böttcher, 1989). For Bavarian mating stations this protective belt had a diameter of 15 km. *A. m. carnica* queens were distributed free of charge among the beekeepers in this area. These queens were to produce the drones for the mating apiary. Additional 'father' colonies with a large number of *carnica* drones were placed in the centre of the mating apiary, but, in contrast to island mating stations, were expected to have only a minor impact on the mating population of drones. Virgin *carnica* queens in large numbers were placed in the mating apiary during the mating season. Several thousands of queens were mated at these stations yearly, which made many beekeepers believe that in fact the currently most abundant race would be *carnica* instead of *mellifera*.

In order to justify the extra work-load in honey bee breeding due to biometrical analysis it seemed appropriate to study whether the objective of race replacement was actually being achieved.

MATERIALS AND METHODS

Samples

Ninety-one apiaries were sampled in autumn 1990 in lower Bavaria. Each sample was comprised of at least 20 workers per colony. Ten samples were collected within the protective belt of each of two mating apiaries (Schellenberg and Bramandlberg). Two other mating apiaries (Racheldiensthütte and Königswald) provided nine samples each. Ten colonies each were sampled in areas adjacent to the protective area, and 13 samples came from colonies not in the vicinity of any mating apiary (more than 20 km distance).

In order to evaluate to which degree the racial status of the bees had changed during the last 40 years, it seemed appropriate to compare them with *carnica* stock that had been introduced to replace the original honey bee population rather than with autochthonous *carnica* populations of central Europe. Six samples (each of a different breeding family) were obtained from the Hessische Landesanstalt für Tierzucht, Department of Bee Breeding in Kirchhain, Federal Republic of Germany (FRG), which maintains several *carnica* lines from Austria and Slovenia through instrumental insemination. This material includes most strains that have been and are currently in use in German *carnica* breeding schemes.

Seven samples were taken from preserved specimens in the collection of the Bayerische Landesanstalt für Bienenzucht, FRG. They comprised 20 worker bees for each of the colonies sampled in Erlangen, FRG ($n = 6$) and Kulmbach, FRG ($n = 1$) in 1911 and 1912 respectively. This is well before any *carnica* breeding programme was implemented in Bavaria and I will refer to these bees as *mellifera* although they may vary from autochthonous '*mellifera*' found today in other localities.

Biometry

The right forewing of each worker was placed on a slide (35 x 35 mm) and projected on a graphical tablet using a 10-fold magnification. The wing venation pattern was digitized based on 18 intersection points according to DuPraw (1965a, 1965b) and the data was processed directly online. Thirteen angles, length, width, and the cubital index were measured. The length and width were proportionally determined from the venation pattern (DuPraw, 1965b) and do not represent the actual wing dimensions as

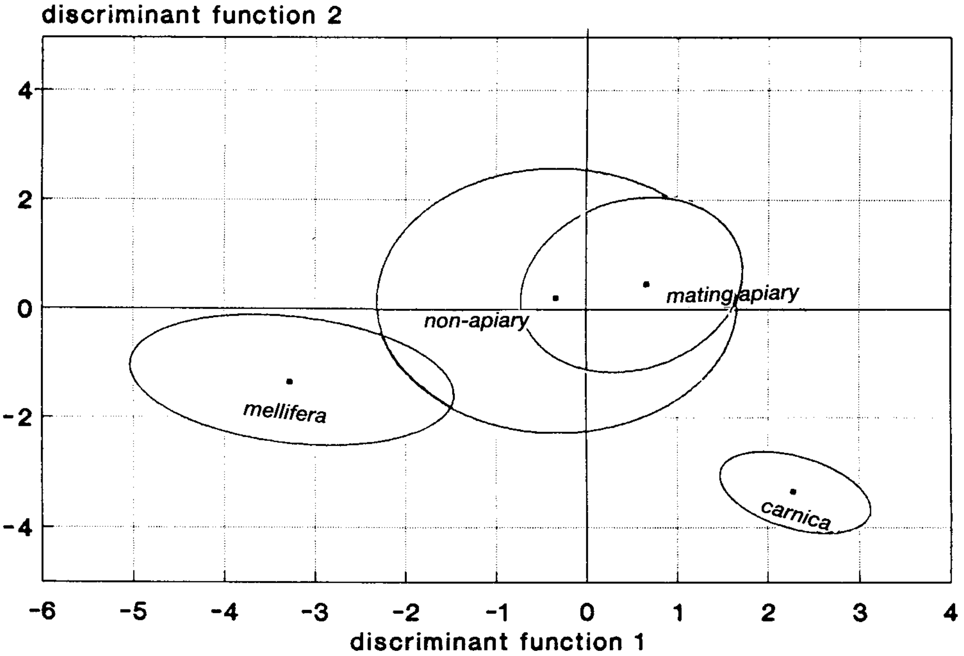

FIG. 1. Centroids of the 75% confidence ellipses (method of Cornuet, 1982) using the two significant discriminant functions 1 (x-axis) and 2 (y-axis). Mating apiary = samples from mating apiary ; *carnica* = bees from contemporary commercial *carnica* stock (Kirchhain); *mellifera* = preserved specimens from northern Bavaria of 1911 and 1912 before the introduction of *carnica* bees; non-apiary = non-mating apiary samples.

used by Alpatov (1928) or Ruttner (1988). The cubital index was determined by dividing distance BC by AC (see DuPraw for point nomenclature). The means of each sample were analysed with a multivariate discriminant analysis using the Statgraphics package (this procedure assumes equal covariance matrices). The biometrical analysis was restricted to the wing venation pattern to facilitate both simplicity and speed in data acquisition, specimen preparation and storage.

RESULTS

Figure 1 shows the graphical presentation of the discriminant analysis using all samples. *A. m. carnica* as well as *A. m. mellifera* form clear non-overlapping clusters. The Bavarian bees sampled from mating apiaries and those sampled in other areas overlap substantially. Only one of the non-mating apiary samples was misclassified into the *mellifera* group (table 1). However, it should be noted that the data might be biased towards correct classification due to the small number of colonies in some of the samples.

The means of all measured characters using the nomenclature of DuPraw (1965b) are given in table 2. The cubital index of the Bavarian honey bees (both mating apiary and non-mating apiary) is similar to the index of pure-bred *A. m. carnica* bees and significantly larger than that of *A. m. mellifera*. The

TABLE 1. Classification results of discriminant analysis using three discriminant functions (the first two functions are highly significant; $P < 0.00005$)

Actual group	Predicted group			
	Mating-apiary	Non-mating apiary	*mellifera*	*carnica*
Mating apiary	26 (68.4%)	12(31.6%)	0	0
Non-mating apiary	15 (28.3%)	37(69.8%)	1 (1.9%)	0
mellifera	0	0	7 (100%)	0
carnica	0	0	0	6 (100%)

TABLE 2. Group means of the 16 characters tested.

Character	Mating apiary	Non-mating apiary	*mellifera*	*carnica*
Angle A1	27±3°	26±4°	20±1°	29±2°
Angle A4	30±2°	30±2°	32±1°	29±2°
Angle B4	109±4°	109±4°	105±3°	112±2°
Angle G7	23±1°	23±1°	22±1°	24±2°
Angle E9	24±2°	23±1°	20±1°	25±2°
Angle J10	50±3°	49±3°	48±2°	53±2°
Angle H12	20±2°	19±2°	20±0.5°	19±0.5°
Angle J16	87±3°	86±3°	81±2°	80±2°
Angle M17	38±3°	38±3°	35±2°	32±2°
Angle G18	92±3°	92±3°	97±2°	92±2°
Angle Q21	36±2°	37±2°	36±0.5°	37±2°
Angle N23	87±3°	86±3°	83±2°	82±2°
Angle O26	40±3°	40±3°	40±3°	40±2°
Distance LG	2.15±0.05mm	1.99±0.04mm	1.94±0.03mm	2.06±0.03mm
Distance AO	4.37±0.03mm	4.36±0.05mm	4.37±0.05mm	4.46±0.04mm
Cubital index	2.72±0.29	2.57±0.39	1.86±0.16	2.94±0.34

intracolonial distribution of the cubital index was in most cases unimodal and only 14 colonies (3 in mating apiaries, 11 in other areas) revealed bimodal distributions. However, in no case was there a statistically significant deviation from unimodality. Bimodality is usually interpreted as a mixed mating of *carnica* queens with both *mellifera* and *carnica* drones (Ruttner, 1983). In spite of this similarity to *carnica*, the wings of the Bavarian samples were small in overall size (length and width) and similar to those of the *mellifera* samples. Most characters are intermediate between the two pure-bred races. However, for two characters of the Bavarian honey bees (angles J16 and N23) the distance to both the *carnica* and the *mellifera* samples is larger than between *carnica* and *mellifera*. Also angle M17 is clearly not intermediate. Angles H12, Q21, and O26 show only little variation among the four groups.

DISCUSSION

In contrast to the belief of many bee breeders, the commonly kept honey bee in lower Bavaria is not pure *A. m. carnica* as it is currently maintained in commercial breeding stock, but actually a synthesis of introduced and autochthonous races. Similar results were also found in Lower Saxony by Reinsch *et al.* (1991) although their 'Landrasse' samples more closely resembled the *carnica* type. Discriminant function 2 indicates that the Bavarian bee clusters are not intermediate to *mellifera* and *carnica*. Therefore, the input of other yet unknown genetic material into the Bavarian gene pool cannot be excluded. However, genetic drift, linkage, or simply the small sample size of the reference *carnica* and *mellifera* samples may also account for this phenomenon.

This evidence is certainly disappointing from the view of the original breeding concept (replacement of *mellifera* with *carnica*). It also has far reaching consequences for bee breeding in general. In spite of a huge breeding effort with extensive requeening operations with *carnica* queens combined with a strict morphometric control of all selected colonies, it was obviously not possible to replace one race with another one. There are several potential reasons for this failure of the breeding concept.

Mating apiary management and mating range

The controlled mating apiaries apparently allowed too many mismatings. The density of bee colonies and beekeepers is high in the study area and the number of colonies ranged from 400 to more than 1 000 within the protective belt of the mating apiaries. In the light of these figures and irregular requeening intervals of more than three years (Kauhausen, 1991), it becomes obvious that mating is not sufficiently controlled to guarantee mating within the same race. Considering that the mating distance of honey bees can exceed 16 km under experimental conditions (Peer, 1975), and the vast mating range of drones (about 78 km^2, Ruttner & Ruttner, 1975) the bio-geographical requirements for mating control become clear. The maintenance of a pure-bred protective belt grows into a herculean task, which may surmount the limits of logistics and manpower available to the few beekeepers in charge of each mating station. It is clearly unrealistic to use lowland mating stations to maintain pure-bred honey bee stock under the conditions found in the study area.

Biometrical analysis

Bee breeders were well aware of the flaws of the land-based mating apiaries. To overcome the problems of hybridization they used biometrical tools in their selection work based on the work of Ruttner (1969). The idea was to identify the racial types by three biometric characters that clearly separated the autochthonous *carnica* and *mellifera* populations. Yet, apparently this was not sufficient to eliminate hybrid colonies in the breeding stock. This is not necessarily surprising because of the extreme selection intensity for these three characters. If a colony did not fulfill the racial requirements it was eliminated, irrespective of its honey production or any other behavioural characteristics. Unavoidably, this leads to a honey bee population that matches the three characters in question. Yet, although the three characters fit the *carnica* standard, the resulting phenotype may be very different from the autochthonous *carnica* race in many other characters. This is exactly in agreement with the present data set. The cubital index (one of the three *carnica* typical characters) of the Bavarian population matches the selection criteria reasonably well. Other characters that were not selected for, indicate extensive hybridization between the racial types.

Consequences for practical bee breeding in Bavaria

The concept of land-based mating stations seems to be inappropriate to conduct any breeding scheme requiring controlled matings. As more than 40 years have not been sufficient to replace the autochthonous *mellifera* race with *carnica* this procedure has clearly failed. In the long run, a limited set of biometrical tools cannot verify the racial status of a honey bee colony if hybridization occurs. Currently it is useless for the bee breeders in lower Bavaria to determine the cubital index because this character no longer discriminates the hybrids from pure commercial *carnica* stock. Realistic alternatives are: (1) discard biometry completely and establish a recurrent selection scheme (for examples see Page & Laidlaw, 1982; Moritz, 1984; Chevalet & Cornuet, 1982; Cornuet & Chevalet, 1987) within the local available honey bee population or (2) use other biometric characters for the discrimination between *carnica* and the hybrid population. However, solution (2) again can only be of temporary value, because of the high probability of repeating the failure of the last four decades of *carnica* breeding work. In the course of such an attempt it will be increasingly difficult to identify the hybrids and finally a result similar to the one presented in this paper will be obtained. In such a scenario, biometry would actually hinder genetic progress for characters, like honey production or defensive behaviour, that are of interest to the beekeeper, if productive colonies are discarded because they do not fit a certain morphometric ideal.

Consequences for bee breeding in general

The conclusions of the present study are not restricted to the population in lower Bavaria. They are valid whenever one race of honey bees is planned to be replaced with another one via land-based mating stations. Currently this may be germane to the Africanized bee problem, since drone control has been suggested as a means to slow down the progress of Africanization (Rinderer *et al.*, 1985). The experience in Bavaria shows that even under radical breeding strategies, in a population with an extremely small number of feral colonies, it is virtually impossible to replace an autochthonous population with another one by artificial selection in a time scale of more than four decades. Both racial types in Bavaria are well adapted to the temperate climate and natural selection is unlikely to form a major differential selective force between the two races. How difficult will it be to maintain a population of European honey bees in an environment where natural selection favours a large feral Africanized population?

Maintenance of pure racial types requires rigorously bee-controlled areas (islands) or instrumental insemination. Land-based mating stations, like those studied in this paper, can only be used for breeding work not in connection with racial types. Nevertheless, the mating stations certainly are of value to practical beekeeping. In fact this study shows that breeding for selectable characters (e.g. cubital index) is possible on land mating stations. Cornuet & Chevalet (1987) and Cornuet & Moritz (1989) presented various strategies for their use in bee breeding. In spite of the limited mating control, substantial genetic progress can be expected, although less than under a strict controlled mating regime.

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