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Genetic characterization of the bees of Kangaroo Island, South Australia[1]

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SUMMARY

On Kangaroo Island, South Australia, an introduced population of *Apis mellifera ligustica* has reputedly not hybridized with other *A. mellifera* subspecies due to isolation. Bees were collected from each of 24 Kangaroo Island colonies to confirm their subspecies. Electromorph relative frequencies for the Kangaroo Island population were: malate dehydrogenase enzyme system, $Mdh^{65} = 0.13$, $Mdh^{80} = 0.11$ and $Mdh^{100} = 0.76$; esterase system, $Est^{100} = 0.95$, and $Est^{130} = 0.05$. Such frequencies are similar to *A. m. ligustica* populations from southern Italy, but dissimilar to populations from northern Italy. Morphology of Kangaroo Island bees is similar to *A. m. ligustica* reference specimens. Mitochondrial DNA restriction mapping suggested that the Kangaroo Island population is of *A. m. mellifera* origin. It is concluded that available reference material is inadequate to conclusively determine the racial origin of the Kangaroo Island population, but that recent hybridization has not occurred.

Keywords: *Apis mellifera ligustica*, *Apis mellifera mellifera*, honey bees, allozymes, esterases, malate dehydrogenase, mitochondrial DNA, restriction mapping, population genetics, hybridization, Australia

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INTRODUCTION

On Kangaroo Island, South Australia, an introduced population of honey bees has been protected from hybridization with other bees by the Ligurian Bee Act of 1886 and a 20 km stretch of ocean. The bees are reputedly 'pure' Italian *Apis mellifera ligustica*. There are several accounts as to how the bees came to the island. Eckert (1958) stated that Italian bees from Queensland were taken to the island in 1884 and that subsequent importations were made directly from Naples shortly afterwards. Hopkins (1886) reported that the bees were initially imported from Italy and established at Brisbane in Queensland during the period 1880–1883. A colony of these bees was taken to Kangaroo Island in 1883. Both reports concur on the approximate date of importation and the fact that the bees were not imported directly from Italy but spent some time at least on the mainland where *A. m. mellifera* had been established some 60 years previously in 1822 (Hopkins, 1886). However, Woodward (1993) suggests that some 'black bees' (presumably *A. m. mellifera*) were present on the island before introduction of the bees from Italy, and that some of these were still present on the island up until the 1960s.

Reports of their fine apicultural characteristics and the doubtful but intriguing possibility that these bees are the only 'pure' random mating population of *A. m. ligustica* left in the world has stimulated considerable scientific and apicultural interest (Woodward, 1993). Various private individuals and the South Australian Department of Agriculture have propagated the bees from time to time and sold them throughout Australia. Eckert (1958) visited the island and was sufficiently impressed with the bees to attempt to import some into the United States. Ruttner (1976) examined the morphology of specimens from three Kangaroo Island colonies and compared them with specimens collected in Italy, concluding that the Kangaroo Island bees showed the "typical morphological characters of *ligustica* bees from Italy". Woyke (1976) examined the population genetics of sex determination in this population that had been isolated for over 100 years, noting reduced brood viability due to a low number of sex alleles.

Whether or not these bees are indeed *A. m. ligustica* or a synthetic that includes some *A. m. mellifera* ancestry from the 19th century or from more recent illegal imports from mainland Australia is of considerable interest. If the bees are a direct lineage from Italy, then they represent an important reference population for studies of racial variation in honey bees. In this study we address this problem by comparing morphological, mitochondrial DNA (mt DNA) and enzyme polymorphism data from Kangaroo Island bees with reference populations reported from Europe.

MATERIALS AND METHODS

Kangaroo Island lies at 137°E, 36°S, south of Adelaide, Australia. It is 145 km long with an area of 4 405 km^2. Feral and domestic honey bees are abundant. Bees from 24 colonies evenly distributed across the island were collected in liquid nitrogen and alcohol. Bees were collected from domestic colonies, many of which were abandoned (19 colonies), from recently hived swarms (five colonies) and one feral colony. Samples were shipped by air to Baton Rouge, Louisiana, USA, where the frozen samples were then stored at –70°C.

Total nucleic acid was extracted from three degastered bees per sample with a protocol similar to Sheppard and McPheron (1991: 92). Each sample of three bees was ground with a pestle on ice in 1 ml of solution A (10 mM tris-HCl [pH 8], 60 mM NaCl, 10 mM EDTA, 5% sucrose) in a 15 ml Corex tube. Then 1 ml of continuously stirred solution B (300 mM tris-HCl [pH 8], 20 mM EDTA, 1.25% SDS, 5% sucrose + 16 µl diethyl pyrocarbonate) was added and the preparation left on ice for 15 minutes followed by one phenol extraction, one phenol/chloroform extraction and one chloroform extraction. An equal volume of TE (10 mM tris-HCl, 0.1 mM EDTA, pH 8) was then added to the aqueous layer. DNA was then twice precipitated on ice for 30 minutes with 0.05 volumes 3 M sodium acetate and 2.5 volumes of cold absolute ethanol, and pelleted by centrifugation at 12 000 rpm for 30 minutes at 4°C. The final pellet was rinsed with 200 µl of cold 80% ethanol, air-dried, and resuspended in 150 µl of TE.

Aliquots (16 µl) of DNA were digested with restriction enzymes using buffer and incubation conditions specified by the manufacturers (Sigma Chemical Co, St Louis, MO, USA; Bethesda Research Laboratories, Gaithersburg, MD, USA). Fragments were separated on 1% agarose gels run at 25–30 V overnight.

All 24 colonies were surveyed with *Eco*R I. Mt DNA fragments were visualized and photographed under ultraviolet light with ethidium bromide staining (Maniatis *et al.*, 1982: 162). Since there is an excess of same-sized mt DNA fragments compared with other DNAs, mitochondrial bands show up clearly with this procedure.

One sample was chosen to create a restriction map for comparison with those previously published (Smith & Brown, 1990; Cornuet & Garnery, 1991). The 15 restriction enzymes (*Acc* I, *Ava* I, *Bcl*, I *Bgl* II, *Eco*O 109, *Eco*R I, *Eco*R V, *Hinc* II, *Hind* III, *Nde* I, *Pst* I, *Pvu* II, *Spe* I, *Xba* I and *Xho* I) used by Smith and Brown (1990) were used to digest the DNA both singly and in all possible combinations. Digests were run with a 1 kb ladder (Bethesda Research Laboratories, Gaithersburg, MD) λ/*Hind* III and ϕX174/*Hae* II as size standards. Gels were vacuum blotted onto Biosbrane nylon membranes according

TABLE 1. MDH allozyme frequencies in populations of European honey bees

Race or description	Locality	Number of colonies	Allele frequencies Mdh^{65}	$Mdh^{80/87}$	Mdh^{100}	Reference
ligustica	Italy	412	0.8	0.03	0.2	[1]
ligustica	Italy	5	0.6	0.1	0.3	[2]
ligustica	Australia	4	0.7	0.1	0.2	[3]
ligustica	USA	24	0.7	0.1	0.2	[4]
ligustica	Brazil	34	0.7	0.2	0.1	[5]
ligustica	Italy	8	0.7	0.0	0.3	[6]
mellifera	Norway	6	0.04	0.8	0.1	[7]
mellifera	France	14	0.0	1.0	0.0	[6]
mellifera	France	–	0.0	1.0	0.0	[1]
Commercial	USA	25	0.6	0.2	0.2	[8]
Feral	Australia	4	0.2	0.5	0.3	[3]
Feral	USA	7	0.3	0.6	0.1	[4]
sicula	W Sicily	–	0.01	0.03	0.9	[9]
?	Kangaroo Is	23	0.1	0.1	0.8	this study

[1]Badino *et al.* (1983) (number of colonies computed by Sylvester, 1986)
[2]Sheppard and Berlocher (1985)
[3]Gartside (1980) (frequencies computed by Sylvester, 1986)
[4]Sylvester (1976)
[5]Contell *et al.* (1977)
[6]Cornuet and Louveaux (1981)
[7]Sheppard and Berlocher (1984)
[8]Sheppard (1988)
[9]Badino *et al.* (1985)

These frequencies are all based on worker allele frequencies. Where colony sample size is small these estimates are heavily biased by queen genotype (Sylvester 1986: 196).

TABLE 2. Pairwise squared Mahalanobis distances among group centroids. Groups are *A. m. mellifera* and *A. m. ligustica* reference populations and the Kangaroo Island population.

From population	Squared distance to: Kangaroo Island	*A. m. ligustica*	*A. m. mellifera*
Kangaroo Island	0	31.81	58.10
A. m. ligustica	43.47	0	73.32
A. m. mellifera	67.43	54.7	0

to the manufacturer's (Stratagene, La Jolla, CA, USA) recommendations and hybridized (50°C, 25% formamide) with ^{32}P-nick-translated probe made from honey bee mt DNA twice purified on a CsCl gradient (Sheppard *et al.*, 1991) to visualize the mtDNA. We were able to resolve fragments as small as 500 kb on most gels. Calculation of map distances was assisted by reference to several known sites from the Smith and Brown (1990) map, and the use of the 'Map' computer program (Intelligenetics, Mountain View, CA, USA).

Population frequency of three electromorphs of the malate dehydrogenase (MDH) and esterase-1 (EST) proteins were estimated using starch gel electrophoresis (Sheppard, 1988). Ten bees were sampled from each of 23 (one colony sample was not available) colonies for MDH, and five bees per colony for EST.

Reference data sets for *A. m. ligustica* and *A. m. mellifera* morphologies were obtained from N Koeniger, Institut für Bienenkunde, Oberursel, Germany. The characters used in this study are given in figure 1. Data were provided representing 20 bees from each of five colonies from both races. From these reference data sets, a discriminant function was computed, which was then used to classify the bees of Kangaroo Island. Ten Kangaroo Island bees were measured from each of the 24 colonies. The Kangaroo Island morphologies were also compared with those of three Kangaroo Island colonies published by Ruttner (1976).

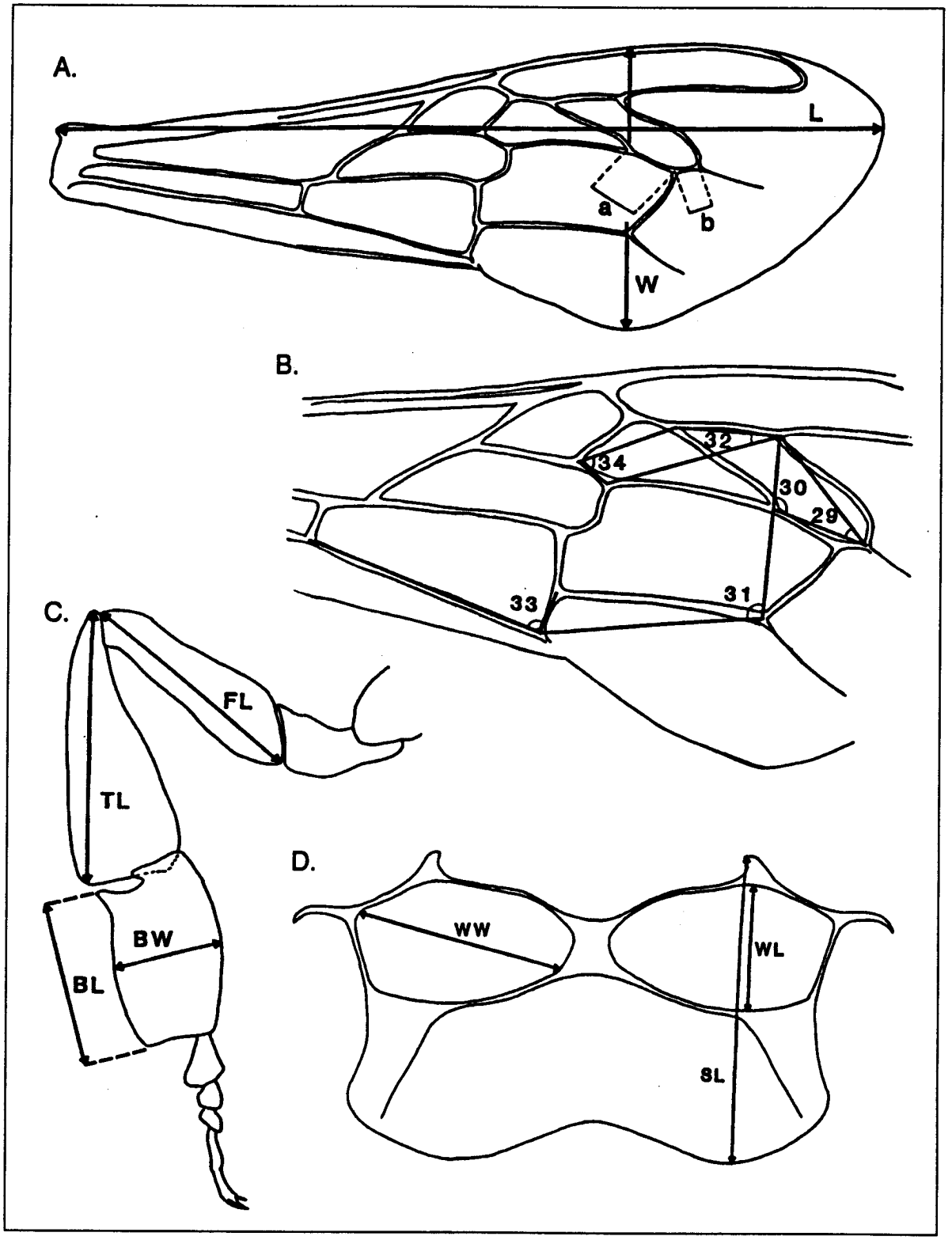

FIG. 1. Morphological measures used in this study.

A. Forewing lengths L = length; W = width; a = length of the cubital vein; b= length of the cubital vein. **B.** Forewing angles 29–34 = angles formed by veins of the forewing. **C.** Hindleg lengths FL = femur length; TL = tibia length; BW = basitarsus width; BL = basitarsis length. **D.** Fourth sternite WW = wax mirror width; WL = wax mirror length; SL = sternite length.

TABLE 3. Morphological measurements (mean ± s.d.) (mm for lengths, degrees for angles) of *A. m. mellifera* and *A. m. ligustica* from reference collections, bees collected from Kangaroo Island in 1991 (this study), and from Kangaroo Island in 1974 (data of Ruttner, 1976). Characters are defined in figure 1.

Character	Reference collections		Kangaroo Island	
	A.m.mellifera	*A.m.ligustica*	1991	1974
	n = 95	*n* = 100	*n* = 236	*n* = 60
	N = 5	N = 5	N = 24	N = 3
Femur length	2.71 ± 0.05	2.66 ± 0.05	2.61 ± 0.06	
Tibia length	3.31 ± 0.07	3.23 ± 0.06	3.11 ± 0.09	
Trochanter length	2.10 ± 0.06	2.08 ± 0.05	2.01 ± 0.05	
Trochanter width	1.15 ± 0.05	1.15 ± 0.04	1.10 ± 0.05	
Sternite length	2.87 ± 0.08	2.76 ± 0.06	2.77 ± 0.07	
Wax mirror length	1.48 ± 0.05	1.33 ± 0.06	1.34 ± 0.06	
Wax mirror width	2.54 ± 0.07	2.36 ± 0.07	2.33 ± 0.07	
Forewing length	9.34 ± 0.13	9.20 ± 0.13	9.09 ± 0.17	9.08 ± 0.11
Forewing width	3.10 ± 0.06	3.20 ± 0.06	3.13 ± 0.07	3.08 ± 0.07
Cubital vein A	0.46 ± 0.05	0.52 ± 0.04	0.53 ± 0.05	0.53 ± 0.04
Cubital vein B	0.25 ± 0.02	0.21 ± 0.02	0.24 ± 0.02	0.23 ± 0.02
Angle 29	32.36 ± 2.59	30.48 ± 1.98	31.24 ± 1.75	31.57 ± 2.55
Angle 30	103.91 ± 4.93	107.63 ± 5.32	107.58 ± 5.26	105.45 ± 6.35
Angle 31	103.70 ± 3.23	98.25 ± 3.17	100.51 ± 3.39	100.17 ± 3.91
Angle 32	18.26 ± 1.00	23.40 ± 1.73	22.00 ± 1.34	21.83 ± 1.22
Angle 33	99.15 ± 3.21	93.41 ± 2.37	94.06 ± 2.65	94.52 ± 2.45
Angle 34	46.30 ± 3.04	52.25 ± 2.90	52.72 ± 2.96	52.05 ± 3.41
Number of hamuli	19.94 ± 2.95	21.26 ± 1.35	21.80 ± 1.45	

N = number of colonies; *n* = number of individuals

RESULTS

Mitochondrial DNA

All colonies surveyed with the *Eco*R I restriction enzyme displayed the mt DNA banding pattern associated with *A. m. mellifera* (Sheppard *et al.*, 1991). The restriction map of the mitochondria of colony 22 is very similar to maps derived from European *A. m. mellifera* (Smith & Brown, 1990; Cornuet & Garnery, 1991; Garnery *et al.*, 1992). Differences include an additional *Hind* III site at 6.5 kb, the absence of one of the *Bcl* I sites at approximately 7.5 kb and some variance in the position of the *Nde* I sites (figure 2). However the map of colony 22 is much closer to published *A. m. mellifera* maps than to *A. m. ligustica* maps, indicating a clear *A. m. mellifera* mitochondrial lineage for the bees of Kangaroo Island.

Allozyme variation

The three common malate dehydrogenase electromorphs (designated Mdh^{65}, Mdh^{80} and Mdh^{100}; Sheppard & Berlocher, 1984) are present at frequencies of 0.13, 0.11 and 0.76 respectively in the Kangaroo Island population of bees. For comparison, MDH electromorph frequencies from previous studies are summarized in table 1. Those reports show that high frequencies of the Mdh^{80} allele are normally associated with *A. m. mellifera* populations and high frequency of the Mdh^{65} allele with *A. m. ligustica* populations.

Only two esterase electromorphs were detected in the Kangaroo Island population: the Est^{100} allele (Sheppard & Berlocher, 1985; *medium* allele of Badino *et al.*, 1985) is present at a frequency of 0.95, while the Est^{130} (Sheppard & Berlocher, 1985; *fast* allele of Badino *et al.*, 1985) is at a frequency of 0.05.

Morphology

A two-group (*A. m. mellifera* and *A. m. ligustica*) discriminant function was computed from the reference population morphology data. Equal variance/covariance matrices were assumed. A cross validation study was conducted on the reference data in which each individual bee was held out while an independent discriminant function was computed. No reference bees were incorrectly classified by any of the 192 discriminant functions produced in this manner. All posterior probabilities were 1.0 for each correctly classified bee. Thus, the function unambiguously separated the reference populations.

When the Fisher discriminant functions were applied to the available 236 Kangaroo Island bees, 232

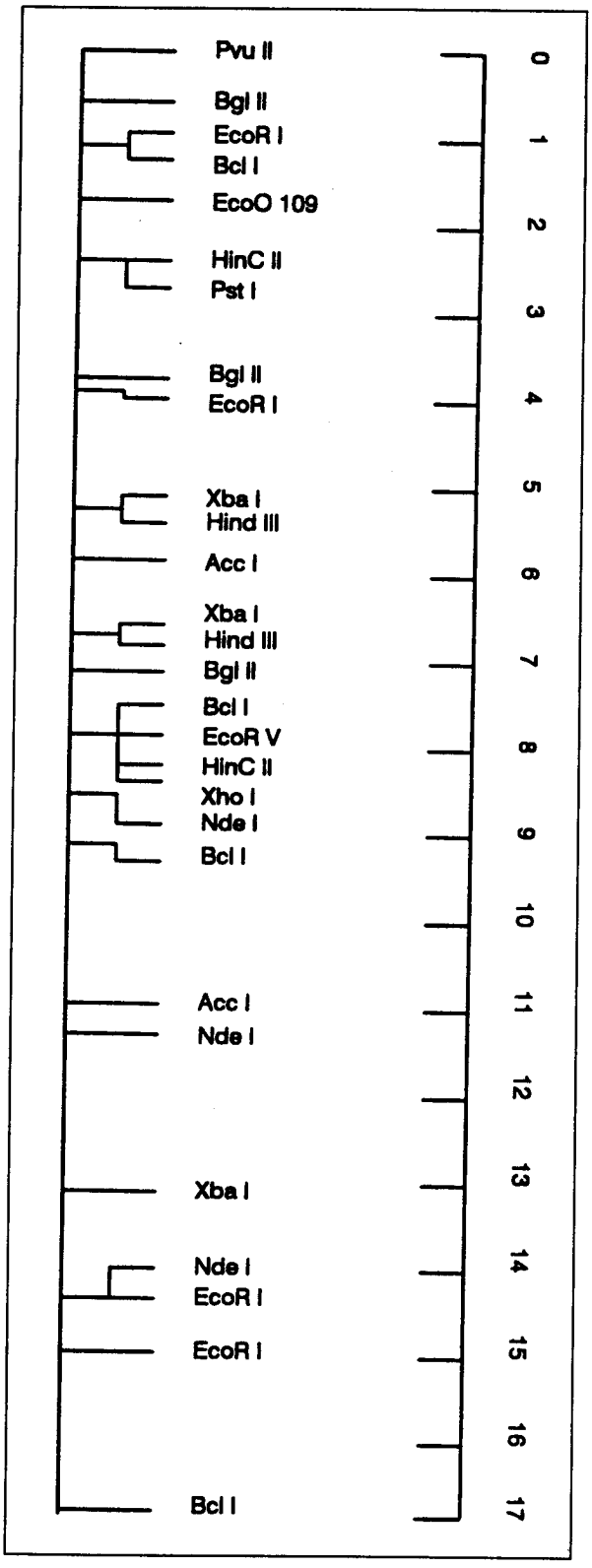

FIG. 2. Restriction map of the mitochondrial genome prepared from Kangaroo Island bees (colony 22 of this study). The *Pvu* I site is the arbitrary starting point used by Smith and Brown (1990), and Cornuet and Garnery (1991).

(98.3 %) were more similar to the *A. m. ligustica* reference specimens than to the *A. m. mellifera* reference specimens. These results were confirmed by the Mahalanobis distances among the group centroids. The Mahalanobis distance from the Kangaroo Island group centroid to the *A. m. ligustica* centroid was approximately half that of the distance to the *A. m. mellifera* centroid (table 2). The reference populations were very different from each other for many of the characters studied (table 3), and the Kangaroo Island population was more similar to *A. m. ligustica* than to *A. m. mellifera* for all characters studied except forewing width, length of cubital vein b and angle 29 (table 3). Further, the bees are yellow, pigmentation generally associated with *A. m. ligustica* and not *A. m. mellifera*.

The Kangaroo Island specimens examined by Ruttner (1976) appear to be very similar to the present collection (table 3).

DISCUSSION

After reference to previously published reports our data suggest that the bees of Kangaroo Island: (1) have mitochondria of *A. m. mellifera* origin (Smith & Brown, 1990; Cornuet & Garnery, 1991; Garnery *et al.*, 1992); (2) have MDH electromorph relative frequencies typical of African subspecies (Sylvester, 1982; Nunamaker 1984), *A. m. sicula* from Sicily (Badino *et al.*, 1984, 1985) or *A. m. ligustica* from southern Italy (Badino *et al.*, 1984); (3) have EST electromorph relative frequencies typical of *A. m. ligustica*, but dissimilar to those of *A. m. sicula* (Badino *et al.*, 1984, 1985); (4) have morphology more similar to *A. m. ligustica* than to *A. m. mellifera*, but not inconsistent with *A. m. sicula* (Ruttner 1988: 249).

Several explanations for these apparently contradictory results can be hypothesized. First, intra-racial variation in mitotypes present in *A. m. ligustica* may be greater than previously thought, and the mitotype usually associated with *A. m. mellifera* may also be present in some *A. m. ligustica* populations which have not yet been surveyed in Italy. Second, genetic drift or selection may have caused genetic changes in the Kangaroo Island population away from the ancestral type. Third, the population may have been established from *A. m. mellifera*/*A. m. ligustica* hybrids in the first place. Four, contemporary populations of *A. m. ligustica* may now be so hybridized (Badino *et al.*, 1984, 1985) that they bear little resemblance to the ancestral type such as that found on Kangaroo Island. Five, the historical record may be inaccurate, and the bees may have been brought from a region other than Italy. Six, the sanctuary may have been contaminated by illegal imports.

The historical record suggests that the bees introduced to the island were not imported directly from Italy but spent at least some time on the mainland.

At that time, the majority of the Australian honey bee population was probably *A. m. mellifera* (Hopkins 1886; Goodacre, 1935) presumably carrying *A. m. mellifera* mitochondria. If the imported queens were replaced by supersedure or reproductive swarming while on the mainland, then the bees of Kangaroo Island would be expected to be a synthetic of *A. m. mellifera/A. m. ligustica* origin. Since honey bee mitochondria appear to be exclusively maternally inherited (Meusel & Moritz, 1990), the likely outcome of such hybridization would be the establishment of an *A. m. ligustica* mitotype on the island. Thus such ancestral hybridization seems an unlikely explanation of these observations. Note, however, that under certain conditions of natural selection, rare mitotypes can rapidly spread through a population (Moritz & Meusel, 1992). This may have occurred with the *A. m. mellifera* mitotype if it was introduced along with a genotype favoured by natural selection.

The uniformity of mitotypes across the island suggests that recent illegal importations to the island (if these have occurred) have not substantially affected the population gene pool. Further, although his sample size was small, the good agreement of Ruttner's (1976) data and that of our study suggest that recent hybridization has not occurred.

Genetic bottlenecks, in which effective population size is reduced to a very low level, can lead to a loss of heterozygosity and genetic drift, processes that can radically alter population gene frequencies (reviewed in Falconer, 1981). The population of honey bee colonies on Kangaroo Island was certainly small upon establishment, and it is possible that this bottleneck may have caused the high frequency of the MDH^{100} allele. However, there is reason to doubt that the genetic bottleneck was very severe for the Kangaroo Island population. In experimental and simulation studies, Leberg (1992) demonstrated that bottlenecks reduce allelic diversity more than heterozygosity, and that if $N_e > 6$ during the bottleneck radical changes in gene frequency are unlikely. The presence of at least three electromorphs of MDH, and two electromorphs of EST suggests that an extreme bottleneck has not occurred in the Kangaroo Island population. Further, the historical record suggests repeated introductions of bees to the island. Thus genetic drift seems an unlikely cause of the dissociation between mitotypes and electromorph relative frequencies. Note, however, that Woyke (1976) could identify only six sex alleles in the island's honey bee population, suggesting that some restriction of population size has occurred.

The morphological data suggest an *A. m. ligustica* origin of the Kangaroo Island bees. Although the Kangaroo Island bees were much more similar to *A. m. ligustica* (nearly all specimens fell within the range of the reference population) than to *A. m. mellifera*, discriminant function analysis was able to separate Kangaroo Island specimens from *A. m. ligustica* reference specimens (table 3). The limited size of the reference collections means that the range of intra-racial variability was not well represented. In the absence of a more extensive reference collection, it seems reasonable to conclude that the bees are morphologically *A. m. ligustica*.

The origin of the bees on Kangaroo Island must unfortunately remain equivocal. Contemporary surveys of presumptive *A. m. ligustica* honey bee populations in Italy and elsewhere have not revealed the presence of a mitotype associated with *A. m. mellifera* (Smith & Brown, 1990; Cornuet & Garnery, 1991; Garnery *et al.*, 1992) that we found on Kangaroo Island. An extensive contemporary survey of bees from Italy might reveal a regional population with mitotypes, MDH frequencies and morphologies similar to the Kangaroo Island population. On the other hand, extensive importation of bees into Italy from the United States and elsewhere means that the results of contemporary surveys need to be treated with caution. However, our study does suggest that intra-racial variance in honey bee mitochondrial DNA restriction maps and MDH allele frequencies may be larger than previously thought. Therefore each alone cannot usually be regarded as completely diagnostic of racial origin without additional corroborating evidence.

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REFERENCES

The numbers given at the end of references denote entries in *Apicultural Abstracts*.

BADINO, G; CELEBRANO, G; MANINO, A (1983) Population structure and *Mdh*-1 locus variation in *Apis mellifera ligustica*. *Journal of Heredity* 74: 443–446. 883/85

BADINO, G; CELEBRANO, G; MANINO, A (1984) Population genetics of Italian honeybee (*Apis mellifera ligustica* Spin.) and its relationships with neighbouring subspecies. *Bollettino del Museo Regionale di Scienze Naturali di Torino* 2(2): 571-584. 889/86

BADINO, G; CELEBRANO, G; MANINO, A; LONGO, S (1985) Enzyme polymorphism in the Sicilian honeybee. *Experientia* 41(6): 752-754. 888/87

CONTEL, E P B; MESTRINER, M A; MARTINS, E (1977) Genetic control and developmental expression of malate dehydrogenase in *Apis mellifera*. *Biochemical Genetics* 15(9/10): 859–876. 1175/78

CORNUET, J-M; GARNERY, L (1991) Genetic diversity in *Apis mellifera*. *In* Smith, D R (ed) *Diversity in the genus* Apis. Westview Press; Boulder, USA; pp 103–115.

CORNUET, J-M; LOUVEAUX, J (1981) Aspects of genetic variability in *Apis mellifera* L. *In* Howse, P E; Clement, J L (eds) *Biosystematics of social insects*. Academic Press; New York, USA; pp 85–94. 1252/82

ECKERT, J E (1958) The Kangaroo Island Ligurian bees. *Gleanings in Bee Culture* 86: 660–663, 722–725. 271/59

FALCONER, D S (1981) *Introduction to quantitative genetics*. Longman; Essex, UK (2nd edition).

GARNERY, L; CORNUET, J-M; SOLIGNAC, M (1992) Evolutionary history of the honey bee inferred from mitochondrial DNA analysis. *Molecular Ecology* 1: 145–154.

GARTSIDE, D F (1980) Similar allozyme polymorphism in honeybees (*Apis mellifera*) from different continents. *Experientia* 36(6): 649–650. 471/82

GOODACRE, W A (1935) *The beginner in bee culture*. New South Wales Department of Agriculture; Sydney, Australia; Farmer's bulletin No. 129.

HOPKINS, I (1886) *Illustrated Australasian bee manual*. I Hopkins; Auckland, New Zealand; pp 13–16 (3rd edition).

LEBERG, P L (1992) Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. *Evolution* 46: 477–494.

MANIATIS, T; FRITSCH, E F; SAMBROOK, J (1982) *Molecular cloning: a laboratory manual*. Coldspring Harbor Laboratory; Cold Spring Harbor, NY, USA.

MEUSEL, M; MORITZ, R F A (1990) Transfer of paternal mitochondrial DNA in fertilization of honeybee (*Apis mellifera* L.) eggs. *In* Veeresh, G K; Mallik, B; Viratamath, C A (eds) *Social insects and the environment*. Oxford and IBH Publishing Co; New Delhi, India; p 135.

MORITZ, R F A; MEUSEL M S (1992) Mitochondrial DNA polymorphism in Africanized honeybees *Apis mellifera* L.: theoretical model and empirical evidence. *Journal of Evolutionary Biology* 5: 71–81.

NUNAMAKER, R A; WILSON, W T; HALEY, B E (1984) Electrophoretic detection of Africanized honey bees (*Apis mellifera scutellata*) in Guatemala and Mexico based on malate dehydrogenase allozyme patterns. *Journal of the Kansas Entomological Society* 57(4): 622–631. 568/86

RUTTNER, F (1976) Isolated populations of honeybees in Australia. *Journal of Apicultural Research* 15(3/4): 97–104. 923/77

RUTTNER, F (1988) *Biogeography and taxonomy of honeybees*. Springer-Verlag; Berlin, Germany; 284 pp. 1155/88

SHEPPARD, W S (1988) Comparative study of enzyme polymorphism in United States and European honey bee (Hymenoptera: Apidae) populations. *Annals of the Entomological Society of America* 81(6): 886–889. 495/90

SHEPPARD, W S; BERLOCHER, S H (1984) Enzyme polymorphism in *Apis mellifera* from Norway. *Journal of Apicultural Research* 23(2): 64–69. 474/85

SHEPPARD, W S; BERLOCHER S H (1985) New allozyme variability in Italian honey bees. *Journal of Heredity* 76: 45–48. 566/86

SHEPPARD, W S; MCPHERON, B (1991) Ribosomal DNA diversity in Apidae. *In* Smith, D R (ed) *Diversity in the genus* Apis. Westview Press; Boulder, USA; pp 89–102.

SHEPPARD, W S; RINDERER, T E; MAZZOLI, J A; STELZER, J A; SHIMANUKI, H (1991) Gene flow between African- and European-derived honey bee populations in Argentina. *Nature* 349: 782–784. 503/92

SMITH, D R; BROWN, W M (1990) Restriction endonuclease cleavage site and length polymorphisms in mitochondrial DNA of *Apis mellifera mellifera* and *A. m. carnica* (Hymenoptera: Apidae). *Annals of the Entomological Society of America* 83(1): 81-88.

SYLVESTER, H A (1976) *Allozyme variation in honeybees* (Apis mellifera *L.*). PhD thesis; University of California at Davis, USA; 60 pp.

SYLVESTER, H A (1982) Electrophoretic identification of Africanized honeybees. *Journal of Apicultural Research* 21(2): 93–97. 513/83

SYLVESTER, H A (1986) Biochemical genetics. *In* Rinderer, T E (ed) *Bee genetics and breeding*. Academic Press; Orlando, USA; pp 177-203. 99/88

WOODWARD, D (1993) Ligurian bees. *American Bee Journal* 133: 124–125.

WOYKE, J (1976) Population genetic studies on sex alleles in the honeybee using the example of the Kangaroo Island bee sanctuary. *Journal of Apicultural Research* 15(3/4): 105–123. 924/77