

temperatures of the continent. On this view, for the continental populations, where high temperatures are sometimes reached, the possession of the most thermostable electromorph (Est-1<sup>0.90</sup>) may be advantageous, whereas the presence of more thermolabile electromorphs may be disadvantageous. Alternatively, in the island localities such high temperatures may not be reached and, therefore, the most thermolabile electromorph (Est-1<sup>0.80</sup>) may reach high frequencies without decreasing the adaptation of these populations.

These differences in heat-sensitivity among the electromorphs of the same enzymatic system would have a great importance for the success of a polyploid complex, such as *U. maritima*, in colonizing very different habitats. Effectively, if, in addition to the enzyme multiplicity characterizing the polyploids, the distinct allelic forms of the same enzyme have different biochemical properties, as is revealed by their differential heat-sensitivity, the range of environments in which normal development can take place may be significantly extended. This type of mechanism may explain the adaptation of *U. maritima* to very different ecological conditions.

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### Similar allozyme polymorphism in honeybees (*Apis mellifera*) from different continents<sup>1</sup>

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**Summary.** Honeybees (*Apis mellifera*) from Australia are polymorphic for 3 enzymes previously reported polymorphic in honeybees from South America: esterase, malate dehydrogenase and alcohol dehydrogenase. However, no genetically determined polymorphism is detected in the 4th protein system found to be variable in South America.

Many workers have predicted that the haplodiploid sex determining system found in the hymenoptera should result in low levels of genetic variability<sup>3-6</sup>. Although the honeybee is a commonly studied hymenopteran, there are conflicting reports about levels of genetic variability detected by electrophoresis of proteins. One study found no variation in enzymes encoded by 3 loci which have been reported polymorphic in other species<sup>7</sup>. Another study

reported 1 locus polymorphic out of 39<sup>8</sup>. Other workers reported 4 enzyme and protein loci polymorphic in honeybees from South America<sup>9-11</sup>. The object of the present investigation was to determine whether the 4 enzyme and protein systems found to be polymorphic in South America were also polymorphic in honeybees from Australia, and whether the loci provided genetic markers for different honeybee races.

### Allozyme polymorphism in *Apis mellifera* from Australia

	Hive	Malate dehydrogenase			Alcohol dehydrogenase		Esterase	
		F	M	S	F	S	F	S
Carniolan stock, Meningie, South Australia	1	0.29	0.42	0.29	0.50	0.50		1.00
	2	0.33	0.63	0.04	0.50	0.50		1.00
	3	0.42	—	0.58	0.38	0.62		1.00
	4	0.50	0.12	0.38	0.17	0.83	0.29	0.71
	5	0.67	0.25	0.08	0.75	0.25	0.04	0.96
	6	0.17	0.66	0.17	0.58	0.42		1.00
	7	0.22	0.22	0.56	0.96	0.04	0.08	0.92
	8	0.25	0.50	0.25	0.88	0.12		1.00
	9	0.50	0.08	0.42	0.29	0.71		1.00
Caucasian stock, Glenrowan, Victoria	10	0.04	0.15	0.81	0.86	0.14		1.00
	11	0.36	0.04	0.60	0.68	0.32		1.00
	12*	0.07	—	0.93	0.89	0.11		1.00
Italian stock, Melbourne, Victoria	13	0.25	0.29	0.46	0.96	0.04		1.00
	14	—	—	1.00	0.79	0.21		1.00
	15	0.07	0.14	0.79	0.50	0.50		1.00
	16	0.46	—	0.54	0.50	0.50		1.00
'Feral' swarms, Southeastern South Australia	17	—	1.00	—	0.58	0.42		1.00
	18	0.71	0.29	—	0.65	0.35		1.00
	19	0.38	0.33	0.29	0.67	0.33		1.00
	20	0.10	0.25	0.65	0.71	0.29	0.08	0.92

The racial origin of each stock was determined by the owner of the hives, and the morphology of worker bees appeared consistent with these classifications. \* Imported queen, natural-mated in Canada. F, fast; M, medium; S, slow.

Individual worker larvae were collected (before sealing) and homogenized in 0.2 ml of 0.25 M sucrose, with 3–5 mg of nicotinamide adenine dinucleotide (NAD) added to each homogenate. Homogenates were centrifuged at  $1500 \times g$  for 10 min, then 11  $\mu$ l of supernatant was inoculated into preformed slots in gels. Electrophoresis was performed on 4.5% acrylamide (AM-9 grout, Cyanamid Australia) gel slabs, 20 cm  $\times$  11 cm  $\times$  0.3 cm, using a constant voltage gradient of 12.5 V/cm along the gel, for an average duration of 3.5 h. Gels were run at  $4 \pm 2^\circ\text{C}$ .

For general proteins, Poulik's discontinuous buffer<sup>12</sup> was used. Gels were stained in 0.5% amidoschwarz 10B in 7% acetic acid. Destaining was carried out by successive washes in 7% acetic acid. Homogenates for general protein staining had to be diluted 1:1 with distilled water before running on gels, to improve staining resolution.

Poulik's discontinuous buffer was also used for esterase. Gels were stained at room temperature in a solution containing 2 mg  $\alpha$ -naphthyl acetate dissolved in 5 ml acetone; 35 ml 0.1 M Tris-HCl buffer, pH 7.0; 35 mg fast blue RR salt.

For malate dehydrogenase, the same sodium borate buffer, pH 8.2 (0.3 M boric acid) was used in the gel and in the electrode chambers, except that the buffer was diluted 1:5 in the gel. The gel buffer contained 0.3 mM NAD. Malate dehydrogenase was stained in the dark at  $35 \pm 3^\circ\text{C}$  in 40 ml of a solution made up in 0.1 M Tris-HCl buffer, pH 8.9, containing 100 mg D,L-malic acid; 15 mg NAD; 5 mg nitroblue tetrazolium and 2 mg phenazine methosulfate.

For alcohol dehydrogenase, a tris borate buffer, pH 8.1 (0.025 M Tris), containing 1.3 mM ethylenediamine-tetraacetic acid (EDTA), and 0.2 mM NAD was used in the gel, and a sodium borate buffer, pH 8.6 (0.13 M boric acid) was used in the electrode chambers. Gels were stained in the dark at  $35 \pm 3^\circ\text{C}$  in 40 ml of a solution made up in 0.1 M Tris-HCl buffer, pH 8.9, containing 0.35 ml of absolute ethanol; 15 mg NAD; 5 mg nitroblue tetrazolium and 2 mg phenazine methosulfate.

The table shows gene frequencies for each hive calculated from the phenotypes of 12 individual worker larvae.

Phenotypes on gels and artificial insemination data (unpublished) are consistent with each of these enzymes being encoded by codominant alleles at single loci. Polymorphism could be detected at only 3 of the 4 enzyme and protein loci found to be variable in Brazilian honeybees. No genetically determined variation was detected in general proteins. This may be due to differences in technique, or to monomorphism in Australian stocks at all of the loci determining general proteins. In addition, only 2 alleles for alcohol dehydrogenase were segregating in Australian honeybees, compared with 3 alleles in the South American stocks. However, as in the South American honeybees, the esterase locus has a predominance of 'slow' alleles.

It is clear that while there may be differences in allelic frequencies between Australian stocks, only esterase appears to show a qualitative difference – in this case presence or absence of a rare allele. Hence, while these polymorphic enzyme loci may serve as quantitative genetic markers for population and commercial studies, they will not be absolutely diagnostic of different commercial stocks.

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## Ten-fold enhancement of 2,4-D effect on water hyacinth by addition of gibberellic acid

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**Summary.** Under greenhouse conditions the effective concentration of 2,4-D (amine salt) for killing water hyacinths could be decreased 10 times if 2,4-D was applied in combination with extremely low concentrations of gibberellic acid (6 g/ha or higher). This implies that in practice the risk of harming nearby vegetation is considerably reduced, and the cost of spraying programmes might be decreased.

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms., is generally considered the world's most troublesome aquatic weed<sup>2,3</sup>. The growth potential of this free floating plant is enormous and it frequently forms dense, impenetrable mats on the surface of water bodies in tropical and sub-tropical areas. If such vegetation blocks navigable rivers, irrigation canals, inlets of hydro-electric installations or other economically important stretches of water the consequences to local communities can be disastrous. Removal of vast water hyacinth masses by manual or mechanical means is often a Herculean task and as a consequence in many cases the application of herbicides cannot be avoided. In practice such chemical control programmes are carried out with only 1 herbicide, 2,4-dichlorophenoxy-

acetic acid (2,4-D), which is relatively cheap and effective against water hyacinths at concentrations of 2–5 kg/ha<sup>3,4</sup>. In 1976 it was reported that the addition of extremely low concentrations of gibberellic acid (GA) to the water in which water hyacinths grow (0.03 mg/l) caused an inhibition of vegetative growth and completely counteracted the formation of float leaves, i.e. leaves characterized by a bulbous swelling of the petiole which provides buoyancy<sup>5</sup>. Subsequently it was observed that an aqueous solution of GA brings about the same inhibiting effects on vegetative growth and float formation when sprayed upon the plants at 15 g/ha and higher concentrations<sup>6</sup>. It has been suggested that GA could perhaps be used to keep water hyacinths under control in certain areas and it was emphasized that