
Biochemical Genetics

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I. INTRODUCTION

The most striking characteristic of the biochemical genetics of honey bees and other Hymenoptera is the lack of variation which is found when their allozymes are analyzed. Allozyme variability in most organisms studied has yielded heterozygosity (H) estimates ranging from about 0.05 to about 0.20 with a median of 0.11 (Lewontin, 1974). In large outbreeding populations of invertebrates, the H is about 0.15 (Selander and Kaufman, 1973). Marine organisms studied at many loci have H of about 0.01 (Schopf and Murphy, 1973) to 0.22 (Ayala *et al.*, 1973). The work of Mestriner (1969) and Mestriner and Contel (1972) with honey bees, Contel and Mestriner (1974) with *Melipona*, and Crozier (1973), Johnson *et al.* (1969), and Tomaszewski *et al.* (1973) with ants indicated that the haplo-diploid Hymenoptera would conform to the high-variability pattern. However, these latter reports dealt with only two or three loci. In more extensive surveys, very low levels of variability have been reported in most of the Hymenoptera.

To save space, only the abbreviations for the commoner enzymes and corresponding loci are used in this chapter. The full names are listed as a footnote to Table 1. "Systems" means "enzyme staining systems," which may be controlled by one or more loci. Unless shown otherwise, each different stained band position revealed in a system is assumed to be controlled by a different locus. In discussing both the locus coding for a particular enzyme and the enzyme itself, it can become unclear whether the locus

TABLE 1. Enzyme Systems Which Have Been Assayed^a in Electrophoretic Surveys of the Hymenoptera

Enzyme ^c	Organism and reference(s) ^b											
	<i>Apis mellifera</i>					<i>Bombus</i>	Other	<i>Polistes</i>	Parasitic	Other	Ants:	<i>Pont.</i>
	1	2	3	4	5	spp.: 2,6,7	bees: 2,6,8,9	spp.: 8,10	wasps: 8,11	wasps: 2,9	12,13	(saw 2
Gp						+	+	+			+	
Acph	+	-	+			+	+		+	-		+
Acon	+		+		+					-		-
Ak		+	+		+	+	+					
Adh			+							+	+	-
Ao	+	+			+	+	+			+	+	
Aldo	+		+		+							
Aph	+		+						+		+	
Amy												
Cat			+									
Est	+	+	+	+	+	+	+	+	+	+	+	+
F1-6dp			+						+		+	
Fum			+		+							
Galdh	+		+							+	+	
G6pdh	+		+	+		+	+	+	+	+	+	
Gdh	+	-	+		+	+	+			+		-
Got			+			-	+		+		+	
Gr	+											
Gapdh	+	+	+		+	+	+	+		+	+	-
αGpdh	+	+	+		+	+	+	+	+	+	+	+
Hk	+	+	+		+	-	+	+				
H6pdh										+		+
Hbdh	+	-			+	+	+	+		+		
Idh	+	+	+	+	+	+	+	+	+	+	+	
Idhn	+											
Ldh	+		+	+	+	-	+	+	+	+	+	
Lap	+	+			+	+	+	+	+	+	+	+
Mdh	+	+	+	+	+	+	+	+	+	+	+	-
Me	+	+		+	+	+	+		+	+	+	-
M6pi					+							
Pep	+						+	+		+		-
6Pgdh	+	-	+			+	+	+	+	+		-
Pgi	+	-		+	+	+	+	+	+	+	+	-
Pgm	+	-	+	+	+	+	+	+	+	+	+	-
Pk			+									
Rdh	+		-									
Rn			+									
Sodh	+	-	+		+	+	-		+	-		
Sudh			+									
Sdh						-	+		+	+	+	
To		-				-	+	+	+		+	
Tre	+		+									
Tpi	+		-		+							
Ty	+									-	+	
Xdh	+	+	+			+	+					

or the enzyme is being discussed at a particular point. A useful convention to avoid this problem is to write both the name of the locus and its abbreviation in italics (e.g., *malate dehydrogenase* or *Mdh*) and write the enzyme name in standard type (malate dehydrogenase or Mdh). Mean heterozygosity is abbreviated as *H* in all cases in this chapter, regardless of the abbreviation used by the authors cited. These authors may not always be referring to exactly the same quantity.

II. VARIABILITY IN HYMENOPTERA

A. Results in *Apis*

For *Apis mellifera*, Sylvester (1976) reported one polymorphic locus (*Mdh*) and an *H* of 0.01 for 39 loci studied using horizontal starch gel electrophoresis (Table 1). Pamilo *et al.* (1978) reported *H* = 0.012 for 16 loci in 11 systems (Table 1). In *A. mellifera*, Nunamaker (1980) found only *Mdh* polymorphic in an analysis of 30 systems using isoelectric focusing (Table 1). Nunamaker and Wilson (1980) reported 28 to 30 tested systems exhibited isozymes, but did not discuss variation (Table 1). Badino *et al.* (1983) reported only *Mdh-1* polymorphic in an analysis of 8 systems (Table 1) in honey bees of the Piedmont of Italy. Sheppard and Berlocher (1985) assayed Italian honey bees for 21 systems (number of loci not reported) and found 3 polymorphic loci, *Mdh*, *Me*, and an *Est*, and a new *Mdh* allele (Table 1).

Tanabe *et al.* (1970) found that *A. mellifera* and *A. cerana* *Est* were differ-

* +, Staining activity detected at one or more loci in one or more species; -, no staining activity detected at one or more loci in one or more species; *, genetic variation detected at one or more loci in one or more species.

* Reference key: 1, Sylvester (1976); 2, Pamilo *et al.* (1978); 3, Nunamaker (1980), Nunamaker and Wilson (1980); 4, Badino *et al.* (1983); 5, Sheppard and Berlocher (1985); 6, Snyder (1974); 7, Pekkarinen (1979); 8, Lester (1975), Lester and Selander (1979); 9, Metcalf *et al.* (1975); 10, Metcalf *et al.* (1984); 11, Shaumar *et al.* (1978); 12, Pamilo *et al.* (1975); 13, Ward (1980a).

* Gp, general protein; Acph, acid phosphatase; Acon, aconitase; Ak, adenylate kinase; Adh, alcohol dehydrogenase; Ao, aldehyde oxidase; Aldo, aldolase; Aph, alkaline phosphatase; Amy, amylase; Cat, catalase; Est, esterase; F1-6dp, fructose-1,6-diphosphatase; Fum, fumarase; Galdh, galactose dehydrogenase; G6pdh, glucose-6-phosphate dehydrogenase; Gdh, glutamate dehydrogenase; Got, glutamate oxaloacetate transaminase; Gr, glutathione reductase; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; α Gpdh, α -glycerophosphate dehydrogenase; Hk, hexokinase; H6pdh, hexose-6-phosphate dehydrogenase; H1bdh, 3-hydroxybutyrate dehydrogenase; Idh, isocitrate dehydrogenase; Idhn, isocitrate dehydrogenase (NADP cofactor); Ldh, lactate dehydrogenase; Lap, leucine aminopeptidase; Mdh, malate dehydrogenase; Me, malic enzyme; M6pi, mannose-6-phosphate isomerase; Pep, peptidase; 6Pgdh, 6-phosphogluconate dehydrogenase; Pgi, phosphoglucose isomerase; Pgm, phosphoglucomutase; Pk, pyruvate kinase; Rdh, retinol dehydrogenase; Rn, ribonuclease; Sodh, sorbitol dehydrogenase; Sudh, succinate dehydrogenase; Sdh, ?, unspecified by author (Snyder, 1974); To, tetrazolium oxidase (Sod); Tre, trehalase; Tpi, triose phosphate isomerase; Ty, tyrosinase; Xdh, xanthine dehydrogenase.

ent but did not find differences between *A. cerana* "flocks" from three locations. Nunamaker *et al.* (1984) detected *Mdh* and *Est* in *A. florea*, *A. dorsata*, and *A. cerana*, from Pakistan. *Est* could be used to distinguish the examined populations of all three species, while *Mdh* could not be used to distinguish *A. dorsata* from *A. cerana*. None of the populations examined exhibited intraspecific genetic variability.

B. Results in Other Hymenoptera

Pamilo *et al.* (1978) reported estimates as follows: $H = 0.017$, 0.005 , and 0.007 for 18, 15, and 15 loci in 12, 11, and 11 systems for 3 populations of *Bombus lucorum*; $H = 0.037$ for 15 loci in 10 systems for *B. terrestris*; $H = 0.048$ for 12 loci in 8 systems for *B. hypnorum*; $H = 0.007$ for 16 loci in 10 systems for *B. lapidarius*; $H = 0.003$, 0 and 0 for 14, 8 and 9 loci in 10, 7, and 7 systems for 3 populations of *B. pascuorum*; $H = 0$ for 11 loci in 7 systems in *B. hortorum*; $H = 0.033$ for 10 loci in 8 systems in *Macropis labiata*; $H = 0.064$ for 8 loci in 7 systems in *Colletes succincta*; $H = 0.037$ for 15 loci in 11 systems in *Andrena clarkella*; $H = 0.007$ for 10 loci in 10 systems in *A. lapponica*; $H = 0$ for 9 loci in 7 systems in *A. vaga* (all bees); $H = 0$ for 13 loci in 11 systems in *Vesputula vulgaris* (wasp); $H = 0$ for 10 loci in 6 systems in *Mimesa equestris* (wasp), and $H = 0.021$ for 18 loci in 13 systems for *Pontania vesicator* (sawfly)(Table 1).

Gorske and Sell (1976) reported four polymorphic loci ($\alpha Gpdh$, *Lap*, *Pgm*, and *Mdh*) in purslane sawflies (*Schizocerella pilicornis* Holmgren). They did not report how many other systems were assayed nor how many were monomorphic, so no estimate of H can be made. However, the presence of this many polymorphic loci is suggestive of a higher level of heterozygosity than that reported for other Hymenoptera.

Snyder (1974) reported no variation for 24 loci in *Lasioglossum zephyrum*, 13 loci in *Augochlora pura*, and 12 loci in *Bombus americanorum* (Table 1). *Lasioglossum zephyrum* may have a female-limited *Est*.

Pekkarinen (1979) reported estimates of H of $0.00-0.14$ for 23 populations in 8 species of *Bombus*, with the mean for up to 12 loci (in 9 systems) over all species of 0.03 (Table 1). "The total number of loci studied was 24, but information on many of these loci was so scant that they have been excluded."

Obrecht and Scholl (1981) found sporadic polymorphisms for 3 loci (*Pgi*, *Acon*, *Est*) out of 10 studied in 12 species of *Bombus* and 4 species of *Psithyrus*, plus more frequent polymorphism for *Pgm* in *Bombus lucorum*. However they did not report allele frequencies, etc.

Lester (1975; Lester and Selander, 1979) reported estimates of mean heterozygosity as follows: $H = 0.043$ for 13 loci in 7 enzyme systems in

Opius juglandis; $H = 0.033$ for 19 loci in 12 systems in *Megachile rotundata* (*M. pacifica*); $H = 0.066$ for 13 loci in 11 systems in *Nomia melanderi*; $H = 0.057$ for 15 loci in 11 systems in *Polistes annularis*; $H = 0.122$ for 13 loci in 9 systems in *P. apachus*; $H = 0.071$ for 13 loci in 9 systems in *P. bellicosus*; $H = 0.061$ for 16 loci in 10 systems in *P. exclamans* (Table 1).

Metcalf *et al.* (1975) reported estimates as follows: $H = 0.056$ for 17 loci in 12 systems in *Stictia carolina*; $H = 0.073$ for 16 loci in 9 systems in *Chalybion californicum*; $H = 0.078$ for 12 loci in 8 systems in *Sceliphron caementarium*; $H = 0.051$ for 15 loci in 8 systems in *Scolia dubia dubia*; $H = 0.059$ for 19 loci in 11 systems in *Trypargilum politum* (all wasps); $H = 0.070$ for 15 loci in 10 systems in *Nomia heteropoda*; and $H = 0.038$ for 16 loci in 11 systems in *Savastra obliqua* (bees) (Table 1). Metcalf *et al.* (1984) reported $H = 0.065$ for 20 loci in 10 systems in *Polistes metricus*, and $H = 0.073$ for 20 loci in 10 systems in *P. variatus*. Fewer wasps were analyzed for *P. apachus*, *P. rubiginosis*, and *P. exclamans*, especially the latter two, so H was not calculated; however, 5 out of 17 loci in 7 systems, 0 out of 19 loci in 9 systems, and 0 out of 13 loci in 5 systems, respectively, were found to vary (Table 1).

In a study of genetic relatedness and social organization of *Polistes* wasp colonies, Lester and Selander (1981) found polymorphic *Lap*, *Pgm*, *Pep*, *Est*, and *Idh*.

Shaumar *et al.* (1978) reported 6 polymorphic loci (*Est-3*, *Acp-2*, *Ldh-1*, *Ldh-2*, *Sodh*, *Pgi*) out of 22 analyzed in 14 systems in the parasitic hymenopteran *Diadromus pulchellus* WSM (Table 1). However, the most common allele at *Pgi* had an allele frequency of 0.992 in females and 0.974 in males in a laboratory population, so it does not meet the usual requirements to be termed polymorphic. Nevertheless, this is a high level of polymorphism for the Hymenoptera. They also found a monomorphic *Est-4* whose expression was limited to males.

Crozier (1977) reported on variation for *Est*, *Mdh*, and *Amy* between populations of the ant *Aphaenogaster rudis*.

Pamilo *et al.* (1975) studied up to 10 loci in 6 systems in 10 species of *Formica* ants and found only one polymorphic *Mdh* in each of only 2 species (Table 1). In a study of genetic population structure in polygynous *Formica* ants, Pamilo (1982) reported polymorphisms in *Mdh*, *Est*, and *Pgi*.

Halliday (1981) surveyed 8 color forms of the *Iridomyrmex purpureus* group in Australia. Levels of genetic variation were estimated in 84 populations for the 15 loci surveyed. Variation was found for *Me*, *Est-1*, *Est-2*, and *Amy*, while the other 11 loci did not show any variation. "The average level of heterozygosity was 3.8% and an average of 11.8% of loci was polymorphic in each population" (Halliday, 1981).

Ward (1980a) reported estimates of H per population of 0.000–0.072 (mean 0.036) for 35 populations (5 species) of the *Rhytidoponera impressa*

group of ants. This was in an electrophoretic survey of 22 loci in 16 systems (Table 1). Ward and Taylor (1981) reported one polymorphic locus, *Amy*, among 16 studied with $H = 0.032$ for the Australian ant *Nothomyrmecia macrops* Clark.

C. Discussion

While these results indicate that honey bees as well as most other Hymenoptera are very uniform in their allozymes, researchers in bee genetics and behavior have found a significant amount of variation in characters which have been shown to be genetically controlled. This then leads to some question as to how accurately allozyme variability reflects or assays the variability of an organism's genome.

One possible explanation for this apparent conflict is that these allozymes are part of the honey bee's metabolism, and thus of its basic interaction with the environment. Honey bees live in perennial colonies, even though each worker only lives a few weeks. There is no reasonable mechanism to allow the queen, which usually lives 1 or more years, to produce workers with a genotype correlated with a particular season. Therefore, every worker must be reasonably well adapted genetically to support the colony at any season. The seasonal changes in the environment would then be dealt with through phenotypic plasticity based on an adapted, generalized genotype. This genotype would show very little variation in basic metabolic characteristics, such as most of the enzymes detected by electrophoresis. There are other genes involved with important but more peripheral characteristics, such as disease resistance, body color, and morphology. These characteristics would vary among locations but would probably be more stable over time at any one location. Selection would then be expected to lead to genetically determined variants adapted to a location but differing from populations at different locations. For example, bees from more northerly regions tend to be larger with relatively smaller appendages and darker (Ruttner, 1975). Such adaptations to a colder climate leave unchanged the basic social structure and metabolism. However, Johnson *et al.* (1969) and Tomaszewski *et al.* (1973) did find a correlation between allozyme polymorphism and environmental patterns in ants. This is expectable since some allozymes may be involved in the more peripheral characters (Ayala and Powell, 1972).

Levins (1968) discusses this relationship between genotype and environment in terms of the grain of the environment. A coarse-grained environment will produce selection pressure favoring balanced polymorphisms while a fine-grained environment will produce selection pressure favoring monomorphism, with the challenges of environmental variation being met by phenotypic plasticity. The relative grain of the environment must be

defined as it is perceived by the organism. The biology and behavior of the Hymenoptera in these reports, particularly the eusocial species, are such that they "perceive" the environment as being more fine-grained than it at first appears. This is primarily due to the following set of factors which applies particularly to honey bees and to a varying extent to other Hymenoptera: (1) adult care of immatures, (2) communication in social species, (3) limited food source, (4) modification of the environment by the organism, (5) food storage, (6) limited temperature range for activity, (7) reduced competition in immatures, (8) foraging area, (9) perennial colonies, and (10) mass mating and drone congregating areas (Sylvester, 1976).

While none of these environment-related factors are exclusive to the Hymenoptera, the interacting complex is rare. This phenotypic plasticity enables them to respond genetically as if they lived in a much more uniform, i.e., fine-grained, environment than that to which most other insects and probably most other organisms are exposed.

Ayala *et al.* (1975) propose that large changes in food availability over time lead to habitat and food-use flexibility and low genetic variability. This model is based on marine organisms facing high instability in one or more environmental characteristics. Availability of food exhibits the most obvious trend. Tropical and deep-sea species, which should have the most stable food supply, are highly polymorphic. Species from environments with an unstable food supply are the least polymorphic.

Over most of the Earth, the availability of food for bees varies greatly through the year. This is particularly noticeable in the temperate zone, where no flowers are available in the winter while an abundance is available for a more or less limited period at other times. Dry season - rainy season changes in the tropics produce similar effects. Bees have met this challenge of an unstable food supply with extensive phenotypic or behavioral plasticity but very limited allozyme variability. Bees then perceive their environment as fine-grained.

Selander and Kaufman (1973) argue that increased mobility and homeostatic control lead to decreased genic heterozygosity. Bees forage over a comparatively large area. Bee colonies can have foragers simultaneously over a large area, giving the colony an effective mobility much greater than any individual forager. The set of environment-related factors discussed above has the effect of significantly increasing the degree of homeostatic control exerted by bees. This supports the argument of Selander and Kaufman. However, this argument is not in opposition to that of Ayala and Valentine, nor is it even an alternative, at least in the case of bees. Mobility and homeostatic control are simply two factors involved in an organism's ability to cope with the environment and in particular with variations in availability of food. More mobile and more highly homeostatic organisms

are less affected by food and other instabilities and so perceive the environment as more fine-grained.

Haplo-diploidy is the other obvious explanation for low allozyme variability in these Hymenoptera, since they all have haploid males and diploid females. With the exception of lethal alleles, there have been two schools of thought about the effects of haplo-diploidy on genetic variability. Crozier (1970) stated that "there is no a priori reason to believe haplo-diploidy reduces the likelihood of balanced polymorphism." However, Hartl (1971) stated, "one should expect to find fewer polymorphisms maintained by overdominance in a male haploid population than in a comparable diplo-diploid population." Since their arguments were based on different assumptions, "Which is in fact the better assumption is a matter that can be decided only by experiment" (Hartl, 1971).

The electrophoretic work reported so far in the Hymenoptera does not allow a decision to be made about haplo-diploidy, since its effects can not yet be separated from those of phenotypic plasticity in responding to environmental variability. However, these reports are sufficient to indicate some studies which should be particularly rewarding. These fall into two classes: studies of other haplo-diploid Hymenoptera, and studies of diplo-diploid, social, non-Hymenoptera. The object of these studies would be to assay the relative contributions of various factors to genetic variability.

Haplo-diploid Hymenoptera exhibiting various aspects of the phenotypic plasticity discussed above do exist. By making appropriate choices, it should be possible to separate the effects of haplo-diploidy from those of phenotypic plasticity. The generally plant-feeding Symphyta should be particularly interesting. Of these, the external feeding sawflies would probably be the best choice since they are so similar to Lepidoptera. Pamilo *et al.* (1978) found $H = 0.021$ for the sawfly *Pontania vesicator* (Table 1), which was similar to the other Hymenoptera he studied (average $H = 0.018$). The results of Gorske and Sell (1976) with the purslane sawfly may be similar, but they cannot be compared here since they did not list how many monomorphic loci they discovered. The parasitic Hymenoptera should also be interesting, particularly those with a narrow host range. The report by Shaumar *et al.* (1978) of higher variability in the parasitic hymenopteran *Diadromus* underscores the potential of such research.

The most obvious and possibly only choice for a eusocial, diplo-diploid nonhymenopteran is the Isoptera or termites. The similarity of their biology to that of ants indicates that they should be a good choice for the separation of the effects of haplo-diploidy from those of the set of environment-related factors. The report by Clement (1981) that European *Reticulitermes* species are polymorphic for 13 out of 25 loci supports the influence of haplo-diploidy in causing low variability in the Hymenoptera.

Thus, if the reduction in allozyme variability in bees and ants is due

primarily to haplo-diploidy, other Hymenoptera should have the same reduced variability regardless of their biology. If the reduction in allozyme variability is due primarily to environmental stability effected by the biology (phenotypic plasticity) of these assayed Hymenoptera, then termites, with a similar biology, should have a similar reduction in allozyme variability. If, as is likely, the reduction is due in part to both causes, choosing Hymenoptera with various combinations of environment-related factors should give some insight into the relative importance of the various factors.

Honey bees are reported as being first in North America in 1622 and in California in the 1850s (Oertel, 1980). Because of the difficulties of transporting live honey bees, these introductions certainly involved only small numbers of queens and their attendant workers. Several subspecies were eventually introduced, and there have been many subsequent importations, but again these probably involved fairly small numbers of queens and workers, at least until recently. It might be argued that the bees in Europe are variable, but this variability was lost in the "bottleneck" when a few bees were introduced to the Americas. Since the introduced bees were from various locations and subspecies, were imported to several locations, and yet are still virtually identical electrophoretically, this does not seem likely to have affected common alleles. Rare alleles may very well have been lost. However, Sheppard and Berlocher (1984, 1985) feel that such a bottleneck has occurred.

Another perspective on the low variability in Hymenoptera is that stated by Crozier (1980): "Rather than Hymenoptera having unusually low levels of genic variation, it seems likely that *Drosophila* has unusually high levels and that this has biased previous surveys."

However, Berkelhamer (1983) analyzed mean heterozygosity values for 101 insect species and found 50 hymenopteran species have significantly lower H values than do 51 diplo-diploid nonhymenopterans. This difference remains significant when *Drosophila* are eliminated from the analysis. Graur (1985), Reeve *et al.* (1985), and Owen (1985) point out classification errors within the Hymenoptera which were made by Berkelhamer and which affect her conclusions about variation within the Hymenoptera. Graur also discusses other problems with Berkelhamer's analysis and concludes that haplo-diploidy per se does not reduce genetic variability. However, the data he presents do not support such a strong conclusion.

Simon and Archie (1985) discussed the effect of choice of enzymes sampled on heterozygosity estimates. They showed that small differences in the selection and resolution of loci can cause large differences in H .

Genetic variation in male haploids and in sex-linked loci has been examined through models by Pamilo (1979), Curtsinger (1980), and Pamilo and Crozier (1981), among others.

Moritz *et al.* (1986) applied DNA restriction enzyme technology to

TABLE 2. Electrophoretic Allele Frequencies Reported for *Apis mellifera*

Researcher	Country	Bee ^a type	Number of colonies	Malate dehydrogenase (Mdh-1)			
				0.50 (S,C,65) ^b	0.63 (M,B,80) ^b	87	1.00 (F,A,100) ^b
Sylvester (1976)	United States	I	24	0.70	0.11		0.20
	Brazil	Az	34	0.01	0.16		0.84
	Colombia	E	13	0.39	0.37		0.24
	Trinidad	E	10	0.50	0.13		0.37
Contel <i>et al.</i> (1977)	Brazil	I	34	0.71	0.15		0.14
	Brazil	Az	78	0.03	0.20		0.77
Gartside (1980) ^c	Australia	Cn	9	0.31	0.32		0.37
	Australia	Cc	2	0.7	0.1		0.2
	Australia	I	4	0.7	0.1		0.2
	Australia	F	4	0.2	0.5		0.3
Nunamaker (1980) ^d	Brazil	Az	4	A: 0.1	B: 0.2		C: 0.7
	Brazil	Az	6		0.008		0.992
	South Africa	A	18				1.0
	South Africa	Ac	2 ^e	0.3	0.3		0.4
Nunamaker and Wilson (1981a)	South Africa	A	10				1.0
	Brazil	Az	12	0.04	0.03		0.93
Cornuet and Louveau (1981)	Italy	I	8	0.75			0.25
	France	M	14		1.00		
Badino <i>et al.</i> (1983)	Italy	I	412	0.77	0.03		0.21
	France	M	7 ^e		1.00		
Martins <i>et al.</i> (1977)	Brazil	I	28				
	Brazil	Az	78				
Mestriner and Contel (1972)	Brazil	I	7				
	Brazil	Az	68				
Bitondi and Mestriner ^f (1983)	Brazil	Az	183				
Sheppard and Berlocher (1984)	Norway	M	6	0.04	0.85		0.1
Sheppard and Berlocher ^g (1985)	Italy	I	5	0.64		0.06	0.3

^a A, African (*A. m. scutellata*); Ac, Cape (*A. m. capensis*); Az, Africanized; Cc, Caucasian (*A. m. caucasica*); Carniolan (*A. m. carnica*); E, European; F, "feral swarms;" I, Italian (*A. m. ligustica*); M, *A. m. mellifera*.

^b Allele designations: Some authors have used designations different from those first published. In these cases the later designations are given in parentheses below the original designation.

^c Means calculated by present author.

^d Mdh-1 allele frequencies for small numbers of colonies from several other locations around the world are presented in this thesis.

^e For 68 bees from several colonies in one apiary.

Alcohol dehydrogenase (Adh-1)			Esterase				Malic enzyme (Me)			General protein (P-3)	
			(Est-1)		(Est-3)						
3	2	1	S	F	S	F	79	100	106	S	F
	(S) ^b	(F) ^b			(100) ^b	(130) ^b					

0.44	0.56	0.95	0.05
0.2	0.8	1.00	
0.3	0.7	1.00	
0.3	0.7	0.98	0.02

0.05	0.10	0.90
	0.70	0.25

		0.91	0.09	0.53	0.47
		0.98	0.02	0.995	0.005
0.02	0.98	0.98	0.02		

0.14 0.86

0.94	0.06	0.94	0.06
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honey-bee mitochondrial DNA and found polymorphism, but at a low level relative to other organisms. Since mitochondrial DNA is maternally inherited, its low level of variability would not be due to haplo-diploidy. However, selection for a generalized genotype with little variation in basic metabolic characteristics would account for this low variability. These results must be regarded with caution since only three determinations were made.

III. KNOWN VARIABLE LOCI IN *APIS*

A. Malate Dehydrogenase (*Mdh*)

Sylvester (1976) reported the presence of 3 alleles at this polymorphic locus in adult worker bees. Allele frequencies were presented for Italian bees from California, Africanized bees from Venezuela, and European bees from Trinidad and Colombia (Table 2). He reported that workers display either one or three bands while drones display only one band, which can be explained if it is assumed that the *Mdh* enzyme is a dimer composed of any two monomers.

Contel *et al.* (1977) reported the Mendelian inheritance pattern of the three alleles at the *Mdh-1* locus and that *Mdh-1* and *Adh-1* are not linked. They also observed an extra band in pupae, compared to larvae and adults. They reported allele frequencies for Africanized and Italian bees in Brazil (Table 2).

Pamilo *et al.* (1978) list 2 polymorphic loci, *Mdh-1* and *Mdh-2*, in a table but do not discuss them further.

Cornuet (1979) found a preponderance of the mid allele (0.63) on Guadeloupe, with the fast (1.00) and slow (0.50) alleles found only where queens have been recently imported and where imported genes would be most likely to diffuse. This indicates the naturalized population was monomorphic until recent importations from Europe introduced the other two alleles. Cornuet and Louveaux (1981) reported that 8 colonies of *Apis mellifera ligustica* (origin unspecified) showed only *Mdh*^A (1.00) and *Mdh*^C (0.50) alleles, with frequencies of 0.25 and 0.75, respectively. Only the *Mdh*^B (0.63) allele was present in 14 colonies of *A. m. mellifera* from France.

Gartside (1980) reported *Mdh* allele frequencies for bee stocks in Australia (Table 2), based on worker larvae.

Nunamaker (1980) described allele frequencies from small numbers of colonies for several races from many countries around the world. A few of these values are presented in Table 2. Nunamaker and Wilson (1981a) reported that African bees (*A. m. adansonii*) from 10 colonies from two locations in South Africa were all homozygous for the fast (1.00) *Mdh* allele. Africanized bees from 12 colonies from Brazil had an *Mdh-1*^{1.00} allele fre-

quency of 0.93, with 8 of the 12 colonies displaying only 1.00/1.00 genotypes (Table 2).

Badino *et al.* (1982, 1983) reported that *A. m. ligustica* in a wide area of the Piedmont of Italy shows a homogenous *Mdh-1* allelic frequency distribution (Table 2). *Apis m. mellifera* from one apiary in France is homozygous for the mid allele, and in two areas where these varieties hybridize the hybrid populations show intermediate frequencies. They also found a new fourth allele which they designated "S₁," which is slower than the 3 previously reported alleles and was rare and present in few samples (Badino *et al.*, 1983).

Sheppard and Berlocher (1984) reported that Norwegian *A. m. mellifera* are polymorphic (see Section IV,A.). They (1985) also reported that *A. m. ligustica* from Italy are polymorphic and have a new *Mdh* allele (Table 2).

Snyder *et al.* (1979) developed a system for the isolation of cytoplasmic *Mdh* from honey-bee larvae. They showed the enzyme is a dimer with a molecular weight of 34,000.

B. Alcohol Dehydrogenase (*Adh*)

Martins *et al.* (1977) found a polymorphic system with 3 *Adh* alleles in drone and worker pupae. The allele frequencies were different in Africanized and European bees (Table 2). *Adh* activity was not detected in young larvae, increased to a maximum in prepupae and white-eyed pupae, and declined to total absence in emerging bees.

Gartside (1980) reported *Adh* allele frequencies for bee stocks in Australia (Table 2), based on analyses of worker larvae.

C. Esterase (*Est*)

Mestriner (1969) determined the inheritance of an *Est* polymorphism with 2 alleles, based on analyses of worker and drone pupae. The esterase patterns are constant during the entire cycle of development. Mestriner and Contel (1972) determined the fast allele of *Est* has an allele frequency of 0.02 in Africanized and 0.09 in Italian bees in Brazil (Table 2). *Est* and *P-3* are not linked.

Tanabe *et al.* (1970) reported that *Apis mellifera* esterase is an acetylcholine esterase and that activity was much higher in female tissue than in male tissue, based on agar gel electrophoresis. They did not find any variation within *A. mellifera* but found differences among 7 species of bees and wasps.

Gartside (1980) assayed *Est* allele frequencies for bee stocks in Australia (Table 2), based on analyses of worker larvae.

Bitondi and Mestriner (1983) detected six esterases that differ in electro-

phoretic mobility, substrate specificity, inhibition properties, and profiles during ontogenetic development. *Est-1*, *Est-3*, *Est-5*, and *Est-6* showed variation, with *Est-3* being the same as discussed above (Table 2). *Est-6* variants had migratory rates which were so similar that they were not separable, while *Est-5^F* was detected only in workers from a single colony. Also, the commonest alleles for the other 2 loci both had allele frequencies of 0.98 over the 4 populations analyzed, so none of these loci meet the conservative criterion, of the commonest allele < 0.95 , to be counted as polymorphic (Ayala *et al.*, 1973).

Sheppard and Berlocher (1985) assayed adults from *A. m. ligustica* colonies from Italy and reported finding *Est¹³⁰* and *Est¹⁰⁰* in one colony while the other 4 were monomorphic for *Est¹⁰⁰*. *Est¹³⁰* did not occur in Norwegian samples of *A. m. mellifera* but does occur in United States *A. mellifera*.

D. Malic Enzyme (*Me*)

Sheppard and Berlocher (1984) discovered two allozymes in 6 Norwegian colonies of *A. m. mellifera* with allele frequencies of 0.14 for *Me 79* and 0.86 for *Me 100* (Table 2). Sheppard and Berlocher (1985) also reported that *A. m. ligustica* from Italy are polymorphic with allele frequencies of 0.94 for *ME¹⁰⁰* and 0.06 for *ME¹⁰⁶*, for the 5 colonies assayed (Table 2).

Variation in *Me* was not detected by Sylvester (1976), Pamilo *et al.* (1978), or Badino *et al.* (1982, 1983).

E. Peptidase

Del Lama and Mestriner (1984) reported a *Pep* polymorphism with a null allele ($f = 0.84$) but did not provide details in their publication.

F. General Protein (*P-3*)

Mestriner (1969) determined the inheritance of a *P-3* polymorphism with 2 alleles which appears only during the pupal stage. Workers show only a single band for either heterozygotes or homozygotes. Mestriner and Contel (1972) reported the *P-3^F* allele had an allele frequency of 0.47 in Italian bees and 0.005 in Africanized bees in Brazil (Table 2). *P-3* and *Est* are not linked.

Gartside (1980) was unable to detect *P-3* in Australian stocks. This is not surprising since this study analyzed larvae and not pupae.

IV. USES OF VARIATION

A. Taxonomy

One of the promising uses for the identifiable biochemical variation in honey bees is as an additional tool for the taxonomist. Sylvester (1982) used the published data for three loci (*Mdh-1*, *Adh-1*, and *P-3*) and the method of Ayala and Powell (1972; Ayala, 1983) to demonstrate an efficient method for separating Africanized bees of Brazil from the sampled European bees of Brazil. This method combines the analysis of these three loci to yield a probability of more than 99% of correct identification of an individual worker bee. Since separately these three loci yield probabilities of 93, 87, and 85%, respectively, the combined analysis is clearly more reliable.

Nunamaker and Wilson (1981a) reported that samples of *A. m. adansonii* from Africa were homozygous for the C (*Mdh-1*^{1.00}) allele, while Africanized bees from Brazil had a C allele frequency of 0.86. This suggested that the high level of the C allele in Africanized bees is from their African ancestors.

Gartside (1980), however, found that *Mdh*, *Adh*, and *Est* were not absolutely diagnostic of different commercial stocks present in Australia.

Cornuet (1979) found that most bees on the islands of Guadeloupe had only or predominantly one *Mdh-1* allele. The presence of the other two alleles was correlated with recent importations of queens. Cornuet and Louveaux (1981) reported that *Mdh* seems to discriminate between *A. m. mellifera* and *A. m. iberica* versus *A. m. caucasica*, *A. m. ligustica*, and *A. m. carnica*, with *mellifera* and *iberica* showing only the B (0.63) allele while *lingustica* showed only the A (1.00) and C (0.50) alleles. Badino *et al.* (1982, 1983) reported *A. m. mellifera* French populations are monomorphic for the M (0.63) allele while *A. m. ligustica* populations show homogeneous allelic frequency distributions with the M allele absent or at very low frequencies. Hybrid populations in very limited alpine areas show intermediate M frequencies. Nunamaker (1980) reported that bees he sampled of *A. m. mellifera* populations showed high frequencies of the B (0.63) allele. Sheppard and Berlocher (1984) reported that 6 colonies of Norwegian *A. m. mellifera* had *Mdh* allele frequencies of 0.04 (*Mdh*⁶⁵ = 0.50), 0.85 (*Mdh*⁸⁰ = 0.63), and 0.11 (*Mdh*¹⁰⁰ = 1.00), while *A. m. ligustica* from Italy (Sheppard and Berlocher, 1985) had frequencies of 0.64 (*Mdh*⁶⁵), 0.06 (*Mdh*⁸⁷), and 0.30 (*Mdh*¹⁰⁰).

This shows that biochemical variation can be useful in separating at least some populations within *A. mellifera*. Discovery of other variable loci, particularly those detectable in adult bees, should significantly increase the discriminatory power of this method.

Biochemical variation would be even more useful in taxonomy if sample collection problems could be solved. The present requirement for live or very-low-temperature frozen samples presents difficulties in obtaining samples from remote areas, particularly in areas where frozen carbon dioxide is not readily available for sample maintenance. Possible solutions include the analysis of different sources of variation, such as more stable proteins or improved methods of storing samples to maintain the enzymes without degradation.

Pekkarinen *et al.* (1979), Obrecht and Scholl (1981), and Pamilo *et al.* (1981) demonstrated the use of enzyme gene variation in studies of phylogenetics in Vespidae, *Bombus*, and *Psithyrus*. Metcalf *et al.* (1984) demonstrated the use of isozyme data in analyzing speciation within the *Polistes fuscatus* species complex. Ward (1980b) used electrophoretic (allozyme) differences in addition to morphological differences in a systematic revision of the *Rhytidoponera impressa* group of ants. Crozier (1981) further discussed the use of allozymes in ant systematics.

B. Genetic Markers

Another promising use for the identified biochemical variation in honey bees is as genetic markers in research. As far as is known, this identified variation has no effects on any characteristic of the bees, including their general fitness. In contrast, most of the other known genetic markers do have other effects on the bees. The eye-color mutants affect vision when the phenotype is displayed, and the wing mutants prevent flight (*short*, *truncate*, and *wrinkled*) or significantly affect flight (*diminutive*). The body-color mutants, *major factor black* and *cordovan*, may affect body temperature or affect general fitness. Therefore allozymes (allelic forms of isozymes) offer the unique advantages of being reasonably easily identified while still causing little or no change in the bees. The allozymes which are likely to be most useful are those which are rare or absent at a particular location.

V. OTHER KNOWN ENZYME SYSTEMS

Several researchers have studied honey-bee enzymes and proteins using electrophoresis or other techniques. While those studies had different aims than assessing population variability, a brief discussion of their results is nevertheless appropriate as a guide to other enzymes or proteins which might be included in a future survey or as further background on those which have already been surveyed.

Galuszka and Kubicz (1968), using paper electrophoresis, reported dif-

ferences between the protein patterns of seminal plasma and drone hemolymph, while spermathecal fluid showed the same pattern as queen hemolymph. Differences were found between the hemolymph protein patterns of queens, drones, and workers.

Tanabe *et al.* (1970) compared esterases in adults of seven species of bees, including *A. mellifera* and *A. cerana*, and wasps using electrophoresis.

Giebel *et al.* (1971) studied endopeptidases from the midgut of adult worker honey bees.

Marquardt and Brosemer (1966) purified and crystallized α Gpdh. Brosemer and Marquardt (1966) reported the enzymic properties and amino acid composition of α Gpdh. Using cellulose acetate electrophoresis, Brosemer *et al.* (1967) studied α Gpdh in honey bees and five other species of Hymenoptera, and reported that honey-bee thoracic extracts showed one major and one very minor band. Tomimatsu and Brosemer (1972) reported that honey-bee α Gpdh is a dimer. Fink *et al.* (1970), using cellulose acetate electrophoresis, found that the honey bee has only one major α Gpdh band while all bumble-bee species show several constant bands. Storey and Hochachka (1975) studied the kinetic requirements of α Gpdh from flight muscle.

In a spectrophotometric survey of oxidases in 79 animal species, Wurzing and Hartenstein (1974) reported that honey bees did not display peroxidase or aldehyde oxidase (Ao) activity. Nevertheless they may still be correct in a sense since their assay used vanillin as the substrate and honey-bee Ao may not react with vanillin. Sylvester (1976) noted that bees displayed two bands for Ao.

Huber and Thompson (1973) reported transglucolytic activity and unusual kinetics for honey-bee sucrase (invertase).

Using a spectrophotometric method, Alumot *et al.* (1969) demonstrated the presence of trehalase (Tre) in honey bees. Lefebvre and Huber (1970) studied the solubilization, purification, and properties of honey-bee Tre. Talbot *et al.* (1975) reported the purification and properties of a free and a bound Tre from thoraces. Talbot and Huber (1976) reported the electrophoretic and pH characteristics of a thoracic and an abdominal Tre. Brandt and Huber (1979) found that thoracic Tre is totally mitochondrial. They also studied the distribution of activities of cytochrome C oxidase, adenyl kinase, and Mdh after dispersion treatments. Brandt *et al.* (1979) reported the kinetic parameters of Tre.

Kubicz and Galuszka (1971) reported on polyacrylamide gel electrophoresis of proteins and acid phosphatase of hemolymph from queens, drones, and workers, and found differences between sexes and castes but did not discuss genetic polymorphisms.

Plantevin and Nardon (1972) used the method "Auxotab" to detect

Acph, Aph, Est, lipase, aminopeptidase, protease, and β -glycosidase activities in the gut of honey bees.

Metcalf and March (1950) and Metcalf *et al.* (1955) reported properties of an acetylcholinesterase. Metcalf *et al.* (1956) separated a specific cholinesterase, an aliphatic esterase, and an aromatic esterase based on reactions with selective inhibitors. Substrate specificities are also noted. Kunkee and Zweig (1963) reported the purification and substrate specificity of acetylcholinesterase.

Marquardt *et al.* (1968) studied the crystallization, quantitative immunochemistry, and electrophoresis of Gapdh from thoraxes.

Gilliam and Jackson (1972a) detected Est, Mdh, Ldh, and α Gpdh activity in polyacrylamide gel disc electrophoresis of adult worker honey-bee hemolymph but did not detect any electrophoretic differences (polymorphisms).

Arnold and Delage-Darchen (1978) reported on a survey to detect the presence of various enzymes in the different salivary glands of honey bees. They tested for activity of the following enzymes: Aph, Est (C4), Est-lipase (C8), lipase (C14) (none), aminopeptidase, trypsin and chymotrypsin (none), Acph, phosphoamidase, α -galactosidase, β -galactosidase, β -glucuronidase (none), α -glucosidase, β -glucosidase, β -glucosaminidase, α -mannosidase, and α -fucosidase.

Gilbert and Wilkinson (1974) reported on epoxidase, hydroxylase, and *o*-demethylase activities in larval and adult workers and drones. Huber and Thompson (1973) reported unusual kinetics for a sucrase (invertase) isolated from whole honey bees. Huber (1975) reported the purification and properties of a honey-bee abdominal sucrase. Huber and Mathison (1976) reported the properties of a different sucrase found mainly in the head.

Blum and Taber (1965) reported on the activities of 13 dehydrogenases in washed honey-bee spermatozoa; NADH₂ dh, NADPH₂ dh, Sudh, Mdh, NADP-Idh, NAD-Idh, α Gpdh, Ldh, Adh, α Gpdh flavoprotein, Gdh, Hbdh (none), and G6pdh.

Metcalf *et al.* (1966) reported that no soluble Ty activity could be detected in homogenates of the honey bee.

In a study of the effect of inbreeding on drones, Moritz (1982) reported on the volume-activities of the enzymes cholinesterase, Acph, Aph, Got, Gpt, Idh, Mdh, and G6pdh.

Martin (1965) reported on an electrophoretic study of hemolymph albumins.

Barker *et al.* (1966) reported the separation of hyaluronidase and phospholipase from venom and their interactions with human serum. Allalouf *et al.* (1975) reported characteristics of a testicular hyaluronidase. Owen (1979) reported on the hyaluronidase activity in the venom of queens and workers.

Lensky (1971a) separated and characterized the hemolymph proteins of worker larvae using several techniques.

Baars and Driessen (1984) reported on aryl hydrocarbon hydroxylase and glutathione S-transferase activity in honey bees and *Varroa* mites.

Del Lama and Mestriner (1984) described and compared zones of exopeptidase activity after starch gel electrophoresis of 14 species of bees (including *A. mellifera*).

Turner *et al.* (1979) reported on protease, hexosaminidase, hyaluronidase, and aryl sulfatases A and B from seminal plasma or sperm extracts.

VI. DEVELOPMENTAL VARIATION IN ENZYMES AND PROTEINS IN *APIS*

Tripathi and Dixon (1968) studied hemolymph Est patterns at eight ages of queen and worker larvae, by starch-gel electrophoresis. They found quantitative and qualitative differences in the patterns with age within and between castes.

Bitondi and Mestriner (1983) reported that three of six esterases studied vary during ontogenetic development.

Tripathi and Dixon (1969) studied hemolymph dehydrogenase isozymes at seven ages of queen and worker larvae. By starch-gel electrophoresis, they found differences within a caste and between castes for G6pdh, 6Pgdh, Mdh, Ldh, α Gpdh, and Gapdh. By spectrophotometric assays, they found quantitative differences over time within a caste and between castes for G6pdh, 6Pgdh, and Mdh.

Liu and Dixon (1965) studied the patterns of hemolymph proteins during the larval life of queen and worker larvae. They found the total protein concentration drops to its lowest level in both castes during the third day of larval life, with queens having the lower concentration. Starch-gel electropherograms showed differences over time for both castes as well as differences between castes.

Using disc, double diffusion, and immunoelectrophoresis, Lensky and Alumot (1969) reported female specific proteins in hemolymph. Lensky (1971b) studied worker hemolymph proteins during development. He found only slight changes during the larval stage and found three main patterns of hemolymph proteins—larval, adult, and common to all stages.

Gilliam and Jackson (1972b) reported on the changes during development in the fluid proteins of workers, as shown by disc electrophoresis. They did not observe any differences in the patterns of mature adult workers. Gilliam and Valentine (1973) reported they were unable to separate adult worker hemolymph proteins on cellulose acetate membranes.

Using isoelectric focusing, Nunamaker and Wilson (1981b) found that the

number of Mdh and Est isoenzymes in eggs increased as development proceeded. Nunamaker and Wilson (1982) found multiple molecular forms of Mdh, α Gpdh, Adh, 6Pgdh, Xdh, G6pdh, Acph, and Est in larval hemolymph. In general, activity declined during the later stages of larval life.

VII. SAMPLING, ANALYSIS, AND INTERPRETATION

Assessing biochemical variation through electrophoresis or other methods is simply a way of measuring a phenotype in order to study the genetics of an organism. Therefore the usual rules of genetic analysis apply. In particular, statements made about populations or subspecies of bees must be based on proper sampling and interpretation. Because the workers in a colony of bees are generally only descended from one queen, sampling a large number of workers from one colony is not the same as sampling the same number of individuals in a population of a non-colonial species. The queen carries her own two haploid genomes plus the genomes of the drones with which she mated. Since a queen is usually estimated to mate with an average of 10 drones (Koeniger, Chapter 10), a colony of bees represents, on average, 12 genomes. Furthermore, each succeeding bee sampled is not independent and has a decreasing probability of sampling even those 12 genomes. It is clear that there is a very rapidly decreasing probability that each subsequent worker or drone sampled will contribute new information about the queen's two genomes. Similarly, but more slowly, each subsequent worker sampled will have a lower probability of sampling an unsampled drone genome. Also, the 10 drones will probably not produce equal numbers of progeny (Moritz, 1983), increasing the error. Therefore, at least with regard to population genetics, the usefulness of repeated samples from one colony is greatly reduced after only a very few workers have been sampled: probably about three or four, but the calculations have not yet been made. Thus exhaustive sampling of a few colonies, regardless of the number of bees sampled, only provides information about a few genomes. It is much more informative to sample one or a few workers from each of several colonies in as many apiaries and locations as possible, rather than to exhaustively sample a few colonies. Unfortunately some authors have chosen the latter approach and, in so doing, greatly reduced the value of their studies in answering questions about biochemical variation in bees.

Statements about the variation present in a population or subspecies of bees are best supported by allocating resources to extensive sampling of as many locations as possible. Next in priority is to sample as many apiaries per location as possible. Colonies should be regarded as families, not as populations, and sampled accordingly, i.e., very few bees per colony.

to estimate genetic correlations can be made from analyses of covariance. Some examples are presented in Table 5. However, these are very seldom precise. One can estimate correlations from responses to selection in a manner similar to that for realized heritability or, a more useful process, one can predict the change in character Y following selection on character X if one knows the genetic correlation and the heritabilities for the two characters. A discussion of the calculations involved for these estimates is presented by Falconer (1981).

We can also make use of correlations between characters in a process called indirect selection. In indirect selection one selects for a character of secondary importance in order to improve a correlated character of major importance. This can be done if the secondary character has a higher h^2 than the primary character, and if the genetic correlation is high. A possible example involves sex- or caste-limited traits which are correlated to a character expressed in both sexes or castes. On the whole, however, indirect selection is not as effective as simultaneous selection for the two characters, a topic discussed more thoroughly in the next chapter.

ACKNOWLEDGMENT

The chapter was prepared in cooperation with the Louisiana Agricultural Experiment Station.

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populations for which no baseline data are available. It may very well be capable of doing so, but this must be determined for each population before claims of the value of the method are made for such populations or types of bees.

ACKNOWLEDGMENTS

This chapter was prepared in cooperation with Louisiana Agricultural Experiment Station. R. H. Crozier, R. F. A. Moritz, T. E. Rinderer, and H. Shimanuki made useful suggestions for improvements on earlier manuscript drafts.

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