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Variable Enzyme Systems in the Hymenoptera

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Abstract—Hymenoptera typically have low levels of allozyme variation yet genetic markers are required for studies of relatedness. Results of a survey of allozyme variation in the order are presented. Data were analysed both in terms of the proportion of times variation has been found with particular enzyme-staining systems and the average heterozygosity detected with each system. Some of the most frequently variable enzymes have not been routinely surveyed. Some possible reasons for this are discussed and some technical improvements are suggested.

Introduction

Allozyme variation can be used to calculate coefficients of relatedness among interactants in natural populations (for a review of methods for estimating relatedness see [1]). Such studies are particularly crucial to the understanding of the evolution of sociality in the Hymenoptera because of the high coefficients of relatedness among sisters that may result under conditions of haplodiploidy and monoandry [2, 3]. The demonstration of high relatedness among nestmates would support the genetic relatedness hypothesis for the evolution of a worker caste. Unfortunately, comparatively few investigations of relatedness among nestmates in hymenopteran societies have been performed, particularly for primitively eusocial species [4, 5]. One reason for this has been the comparative paucity of suitable allozyme variation in the Hymenoptera as a whole [6–8].

It is well known that there is substantial variation in heterozygosity among enzymes [9, 10]. However, there have been no published surveys of the frequency with which different enzyme systems have been found to exhibit variation in the Hymenoptera. This paper serves to fill this gap. We find that some of the potentially most useful (i.e. most frequently variable) enzymes are not routinely surveyed.

Two measures of genetic variation were used: P_v—the proportion of loci for a particular enzyme staining system that exhibited variation and H_e—the average expected heterozygosity. Both variables are averaged across taxa for each of the 49 enzyme staining systems for which sufficient data were available. Wherever the number of loci (N_l) is referred to, all isozyme loci for an enzyme are included. Where the number of studies (N_s) is referred to, an enzyme is included for each species once, irrespective of the number of isozyme loci reported.

Results

Data were available for a total of 172 species, some of which have been studied more than once to give a total of 194 species/populations surveyed for allozyme variation. Of these, 27 (20) were sawflies (figures in brackets refer to the number of species surveyed, those before brackets include a species once for each separate study), 22 (21) parasitica, 46 (44) ants, 22 (20) vespid wasps, 71 (61) bees, five (five) sphecid

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wasps and one (one) a scoliid. The number of loci surveyed per species ranged from seven to 51 (average 18.3±10.3) and the entire data set represents over 3,700 hymenopteran loci. The mean number of haploid genomes was 80.7±81.5 for species sampled as free-living individuals (as opposed to colonies). For social species with results presented as number of nests sampled rather than the number of individuals, the mean number of colonies surveyed was 19.1±17.8.

The enzymes surveyed, their EC numbers (from [11]) and estimates of P_v and, where calculable, H_e are listed in Table 1. The data are broken down into various taxonomic subgroups. G tests (with Williams' correction) were performed for intertaxon comparisons where 15 or more loci had been studied per subgroup. Twenty-two comparisons were made, thereby making it likely that some significant results were obtained through type 1 error. However, only two comparisons were significant at the 0.05 level (Lap and Mdh), the remainder were more highly significant as follows: Amy is less variable in sawflies that in ants ($P<0.001$), Fum and Iddh are less variable in sawflies than in bees ($P<0.01$ in both cases) and Pgm appears less variable in ants than in other Hymenoptera ($P<0.005$).

Spearman's rank correlation coefficient was calculated between N_s and both P_v and H_e across the 49 enzymes. Neither relationship was significant ($r_s=0.204$ and 0.182 between N_s and P_v and H_e, respectively). This suggests that not all of the more variable enzymes are routinely surveyed in studies of Hymenoptera.

Discussion

The most highly variable enzyme systems can be seen at a glance in Fig. 1. Of the most variable systems, only four are routinely surveyed (Est, Gpi, 6Pgd and Pgm with 89, 118, 64 and 128 studies, respectively). The other most variable enzyme systems have been surveyed an average of only 23 times (these are Aat, Aha, Ala, Amy, Cat, Diap, Bgal and Gda). Why are these systems less frequently investigated?

It is not easy to obtain clear staining results for some of these variable enzymes. Aha and Bgal are more active in larvae or pupae [12] than in the adult stage normally used in electrophoresis. Nonetheless, we have successfully used Aha on recently eclosed bees and have obtained faint, but scorable, results from one-year-old queen sweat bees (unpublished). We have not yet obtained clear results for Bgal. We have had great difficulty obtaining results with Ala despite having tried a wide variety of recipes and buffer systems. Most recipes for Amy cannot be used on starch gels (for exceptions see [13, 14]) and we have not detected activity for this enzyme using cellulose acetate. Polyacrylamide may give better results. We find results with Cat recipes are blurry and often ephemeral and difficult to score. Gda can be stained clearly in Halictidae when the recipe (see appendix) is followed carefully but seems more difficult to visualize in other bee families (unpublished) and perhaps other hymenopteran groups. NADPH-dependent diaphorase is not often surveyed perhaps because of the great expense of the substrate (NADPH). For a recipe that uses less of this chemical see Ref. 15.

Interlaboratory variation in the detection of genetic variation is well known [16]. Not many laboratories have tested a wide range of hymenopteran taxa for a wide range of enzymes. The six significant differences in individual enzyme P_v among taxa may result from variation in methods used among laboratories rather than reflecting real differences among taxa. Nonetheless, there is one comparison for which a speculative explanation for the significant difference may be justified: that between levels of Amy variation in sawflies and ants. All sawflies surveyed are herbivorous, whereas the ants are predominantly animal-eating. Starch digestion is more important to sawflies, perhaps resulting in greater constraints upon amylase variation than in ants. This could be tested by comparing amylase variation in parasitoid sawflies (the family Orussidae) and ants with much starchy food in their diet (e.g. *Pogonomyrmex*).

The results presented herein suggest that some enzyme systems are more likely to detect genetic variation useful for relatedness estimation than others. However, it should be stressed that some of the less frequently variable systems do provide useful genetic markers in some instances. It is recommended that 30 or more individuals be tested for as many enzymes as possible. The cost of surveying this number of individuals for around 35 enzymes, resulting in between 45 and 52 scorable loci, in our laboratories approximates U.S.$320 for all chemicals including starch. It should be possible to reduce this by using cellulose acetate gels for the most expensive enzyme stains. In addition to the detection of as many variable loci as possible, such efforts will also produce useful estimates of heterozygosity, themselves badly needed to test the various hypotheses of the causes of low levels of genetic variation in the Hymenoptera.

We would be grateful for summaries of the kind of information presented here from researchers who have surveyed allozyme variation in Hymenoptera not included in our review.

Methodological

We surveyed the literature for studies of hymenopteran allozyme variation. Early reports that presented information on one or a few systems are not included as these were frequently biased towards documenting variation. Similarly, we have not included data from papers that estimated relatedness using allozyme markers without also reporting those loci that proved to be monomorphic.

The entire hymenopteran literature on allozyme variation has been surveyed, not just that for groups which contain social species. This is because there were many enzyme systems surveyed by students of one taxonomic group that had not been used by researchers of other taxa. Efforts to stain enzymes that are variable in one group but unstudied in others should be worthwhile but first we have to know which enzymes these are.

With one exception, a species is included in the survey each time it has been studied. The exception is the honey bee for which repeated verification of the monomorphism or polymorphism of particular loci has been made independently for different races or the same race from different localities. Only those enzymes for which 10 or more loci (i.e. 10 species for a single locus system, five species for a two locus system) have been studied are included in the analysis.

Two measures of variability were considered: P_v, the proportion of loci detected by a particular enzyme staining system that was variable at any frequency at all within species/populations; and H_e, the average expected heterozygosity calculated across taxa. The latter parameter often had to be calculated for individual taxa from published raw data. Some studies listed variable and invariant loci without providing heterozygosity estimates or actually stating gene frequencies. Consequently, sample sizes are smaller for analyses of H_e than for P_v.

Published data were often presented by populations of a species. In such cases, an allozyme locus was scored as variable if one or more populations exhibited any heterozygosity at that locus and heterozygosity was calculated after combining allele counts across populations.

No attempt has been made to homologize different isozyme loci within an enzyme-staining system across taxa because information permitting this was not always presented. For example, some studies differentiated between mitochondrial and soluble forms of enzymes such as Acon and Mdh. However, this was not always done, necessitating the grouping together of all isozymes for a particular enzyme system.

When an aldehyde is used as substrate, most studies have called the enzyme aldehyde oxidase. We have found better results when NAD is added to the enzyme

TABLE 1. NAMES, EC NUMBERS, ABBREVIATION SYMBOLS AND LEVELS OF VARIATION FOUND IN VARIOUS HYMENOPTERAN TAXA. P_v AND H_e ARE EXPRESSED AS PERCENTAGES

Enzyme	EC number	Abbreviation	Symphyta				Parasitica				Formicidae				Vespidae				Apoidea				Total			
			NI*	P_v	NI*	H_e	NI*	P_v	NI*	H_e	NI*	P_v	NI*	H_e	NI*	P_v	NI*	H_e	NI*	P_v	NI*	H_e	NI*	P_v	NI*	H_e
Acetylhexosaminidase	3.2.1.52	Aha	0	—	0	—	0	—	0	—	1	0.0	0	—	0	—	0	—	17	41.2	16	8.7	18	38.9	16	8.7
Acid phosphatase	3.1.3.2	Acp	1	0.0	1	0.0	6	16.7	0	—	1	0.0	0	—	0	—	0	—	25	20.0	18	9.3	33	18.1	19	8.8
Aconitate hydratase	4.2.1.3	Acon	40	22.5	40	5.5	12	16.7	12	5.1	4	75.0	0	—	0	—	0	—	36	8.3	33	1.2	92	15.2	86	3.8
Adenylate kinase	2.7.4.3	Ak	0	—	0	—	6	0.0	3	0.0	4	25.0	2	0.0	18	0.0	18	0.0	47	10.6	44	1.6	75	8.0	66	1.1
Alanine aminotransferase	2.6.1.2	Ala	14	21.4	14	7.7	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	14	21.4	14	7.7
Alcohol dehydrogenase	1.1.1.1	Adh	9	0.0	9	0.0	0	—	0	—	3	0.0	0	—	0	—	0	—	9	11.1	9	6.2	21	4.8	18	2.9
Aldehyde oxidase	1.2.3.1	Ao	0	—	0	—	8	12.5	2	0.0	52	9.6	34	2.8	6	0.0	6	0	48	18.8	47	1.8	115	13.0	90	1.9
Aldolase	4.1.2.13	Ald	14	14.3	14	1.2	10	0	3	0	4	25.0	4	12.0	0	—	0	—	21	0.0	27	0.0	55	5.5	44	1.4
Alkaline phosphatase	3.1.3.1	Akp	0	—	0	—	6	33.3	0	—	1	0.0	0	—	9	22.2	9	1.3	10	0	10	0	26	11.5	19	0.6
Amylase	3.2.1.1	Amy	18	5.5	18	0.9	0	—	0	—	42	59.5	24	22.7	9	0.0	9	0	0	—	0	—	69	37.7	51	10.7
Arginine kinase	2.7.3.3	Ark	3	0.0	3	0.0	0	—	0	—	1	0.0	0	—	0	—	0	—	35	2.9	34	0.1	39	2.6	38	0.1
Aspartate aminotransferase	2.6.1.1	Aat	16	25.0	16	12.5	15	13.3	12	4.6	25	12.0	8	2.1	0	—	0	—	18	22.2	18	4.2	74	17.6	54	6.4
Catalase	1.11.1.6	Cat	9	66.7	9	24.9	6	0	0	—	1	0.0	0	—	9	55.5	9	14.2	0	—	0	—	25	44.0	18	19.6
Diaphorase (NADH)	1.8.1.*	Dia	0	—	0	—	7	14.3	0	—	4	25.0	3	0.0	18	22.2	18	4.2	50	8.0	40	1.3	79	12.7	60	2.7
Diaphorase (NADPH)	1.6.99.*	Diap	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	36	30.6	35	4.8	36	30.6	35	4.8
Enolase	4.2.1.11	Enol	0	—	0	—	0	—	0	—	1	0.0	0	—	0	—	0	—	17	11.8	16	0.6	18	11.1	16	0.6
Esterase	—	Est	6	16.7	6	11.5	24	25.0	6	9.1	60	25.0	32	8.0	61	39.3	61	10.6	97	30.9	86	6.4	261	31.8	214	8.6
Fructose bisphosphatase	3.1.3.46	Fdp	14	0.0	14	0.0	0	—	0	—	2	0.0	0	—	0	—	0	—	1	0.0	1	0.0	17	0.0	15	0
Fumarate hydratase	4.2.1.2	Fum	15	6.7	15	3.6	3	0.0	2	0.0	7	0.0	5	0.0	0	—	0	—	27	44.4	19	4.2	52	25.0	41	3.3
B-galactosidase	3.2.1.23	Bgal	14	64.3	14	27.3	0	—	0	—	2	0.0	0	—	0	—	0	—	0	—	0	—	16	56.3	14	27.3
General protein	—	Gp	27	0.0	27	0.0	0	—	0	—	35	8.6	29	0.1	2	0.0	2	0.0	6	0.0	6	0.0	62	4.8	56	0.1
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6pd	0	—	0	—	9	0.0	4	0.0	11	0.0	11	0.0	11	0.0	11	0.0	40	17.5	32	3.3	72	11.1	59	1.9
Glucosephosphate isomerase	5.3.1.9	Gpi	27	51.8	27	15.2	20	50.0	11	14.7	5	40.0	4	12.8	22	36.4	22	9.1	56	30.4	54	2.3	132	38.6	120	7.9
a-Glucosidase	3.2.1.20	aGlu	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	30	0.0	29	0.0	30	0.0	29	0.0
Glutamate dehydrogenase	1.4.1.2	Glud	0	—	0	—	0	—	0	—	7	0.0	5	0.0	0	—	0	—	10	0.0	10	0-0	21	0.0	19	0.0
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	Gapd	14	50.0	14	13.1	9	11.1	5	0.1	5	0.0	4	0.0	3	0.0	3	0.0	27	3.7	25	0.7	65	13.8	58	3.5

TABLE 1—*CONTINUED*

Enzyme	EC number	Abbreviation	Symphyta				Parasitica				Formicidae				Vespidae				Apoidea				Total			
			*Nl**	P_v	*Nl**	H_e	*Nl**	P_v	*Nl**	H_e	*Nl**	P_v	*Nl**	H_e	*Nl**	P_v	*Nl**	H_e	*Nl**	P_v	*Nl**	H_e	*Nl**	P_v	*Nl**	H_e
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3pd	6	50.0	6	8.7	30	16.7	22	3.1	41	29.3	38	2.5	26	11.5	26	3.1	118	22.9	106	1.9	234	21.8	212	2.6
Guanine deaminase	3.5.4.3	Gda	0	—	0	—	0	—	0	—	1	0.0	0	—	0	—	0	—	10	60	9	10.9	11	54.5	9	10.9
Guanylate kinase	2.7.4.8	Guk	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	15	0.0	15	0.0	15	0.0	15	0.0
Hexokinase	2.7.1.1	Hk	25	20.0	25	8.5	23	17.4	16	12.1	36	5.6	32	0.4	19	10.5	19	2.2	86	8.1	84	1.0	189	10.6	174	3.1
Hydroxybutyrate dehydrogenase	1.1.1.30	Hbdh	4	25.0	4	15.6	0	—	0	—	1	0.0	0	—	2	0.0	2	0.0	34	23.5	33	2.3	44	25.0	42	4.9
L-iditol dehydrogenase	1.1.1.14	Iddh	17	0.0	17	0.0	4	25.0	3	0.0	6	16.7	3	0.0	0	—	0	—	23	26.1	22	3.9	54	13.0	51	1.8
Isocitric dehydrogenase	1.1.1.42	Idh	27	7.4	27	3.2	21	14.3	16	2.6	35	20.0	15	6.1	25	28.0	25	3.0	62	24.2	60	3.8	181	18.8	154	3.3
Lactic dehydrogenase	1.1.1.27	Ldh	23	13.0	23	4.4	11	27.3	4	7.6	37	2.7	20	1.0	12	16.7	12	4.6	27	11.1	27	0.4	114	12.3	90	2.8
Leucine aminopeptidase	3.4.11.1	Lap	11	36.4	11	6.7	18	11.1	18	1.8	44	11.4	27	2.6	17	47.0	17	9.7	64	15.6	62	3.5	156	18.6	126	4.3
Malate dehydrogenase (NAD)	1.1.1.37	Mdh	51	15.7	51	2.8	40	7.5	27	2.1	66	19.7	49	4.2	42	4.8	42	0.7	115	10.4	104	0.8	326	13.2	285	2.0
Malate dehydrogenase (NADP)	1.1.1.40	Me	36	38.9	36	23.2	19	0.0	10	0.0	43	18.6	25	6.8	15	13.3	15	3.9	64	21.9	55	2.4	178	21.3	142	5.6
Mannose phosphate isomerase	5.3.1.8	Mpi	2	100.0	2	26.2	—	—	—	—	4	25.0	4	3.1	—	—	—	—	6	0.0	6	0.0	12	25.0	12	5.4
Peptidase	3.4.*.*	Pep	18	11.1	18	1.8	0	—	0	—	13	7.7	13	0.3	5	40.0	5	2.0	70	21.4	74	2.0	106	18.9	108	1.8
Phosphoglucomutase	5.4.2.2	Pgm	29	48.3	29	18.0	25	56.0	16	8.6	30	13.3	12	2.0	43	34.9	43	4.6	59	42.4	55	6.1	194	37.6	167	7.3
6-phosphogluconate dehydrogenase	1.1.1.43	6pgd	0	—	0	—	10	50.0	9	20.0	5	20.0	3	1.1	15	20.0	15	5.5	49	20.4	56	5.5	83	27.7	71	7.1
Phosphoglycerate kinase	2.7.2.3	Pgk	0	—	0	—	0	—	0	—	1	0.0	0	—	0	—	0	—	24	0.0	23	0.0	25	0.0	23	0.0
Phosphoglycerate mutase	5.4.2.1	Pgam	0	—	0	—	0	—	0	—	0	2.0	0	—	0	—	0	—	25	4.0	25	0.5	27	3.7	25	0.5
Pyruvate kinase	2.7.1.40	Pk	0	—	0	—	0	—	0	—	2	0.0	0	—	0	—	0	—	24	8.3	24	2.5	26	7.7	24	2.5
Shikimate dehydrogenase	1.1.1.25	Skdh	14	0.0	14	0.0	0	—	0	—	2	0.0	0	—	0	—	0	—	0	—	0	—	16	0.0	14	0.0
Superoxide dismutase	1.15.1.1	Sod	19	10.5	19	1.5	10	10.0	2	11.3	37	5.4	20	0.6	19	0.0	19	0.0	29	0.0	29	0.0	116	4.3	90	0.7
Triosephosphate isomerase	5.3.1.1	Tpi	3	0.0	0	—	0	—	0	—	1	0.0	0	—	0	—	0	—	24	12.5	23	1.8	28	10.7	27	1.5
Uridine monophosphate kinase	2.7.4.*	Umpk	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	22	0.0	16	0.0	22	0.0	16	0.0
Xanthine dehydrogenase	1.1.1.204	Xdh	1	0.0	1	0.0	3	0.0	3	0.0	19	5.3	19	2.0	0	—	0	—	7	0.0	7	0.0	30	3.3	30	1.3
Total			525	21.9	522	6.7	355	18.9	206	5.3	699	16.7	430	4.1	408	21.8	408	4.6	1620	17.4	1474	2.4	3704	17.2	3176	3.5
References			7, 17–20				8, 20–24				25–31				6–8, 32–34				15, 35-42, Gan *et al.*, unpublished; Packer and Owen, unpublished; Owen *et al.*, unpublished.							

**Nl* refers to number of loci upon which P_v or H_e were calculated.

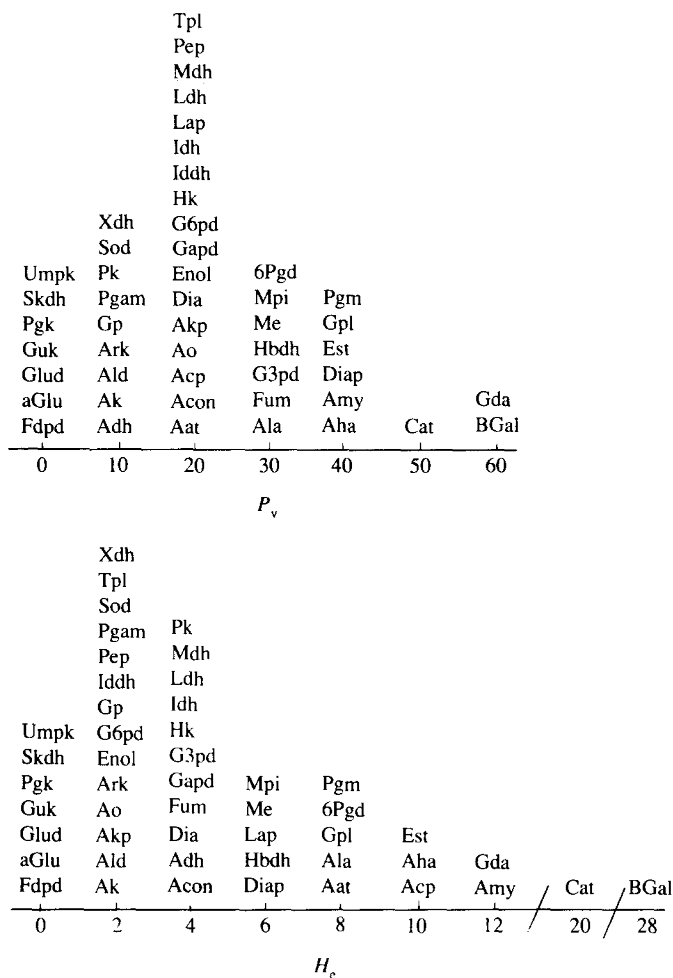

FIG. 1. LEVELS OF VARIATION IN P_v AND H_e FOR 49 ENZYME STAINING SYSTEMS AVERAGED ACROSS ALL HYMENOPTERA SURVEYED. For abbreviations used for the enzymes see Table 1. P_v and H_e are expressed as percentages. Figures along the abcissae represent the maximum values attained by any entry in the column above.

recipe and, consequently, may be staining aldehyde dehydrogenase (EC 1.2.1.3). In our analyses we have combined all records of these two enzymes together.

For some analyses, the entire data set for Hymenoptera was broken down into taxonomic subgroups. The categories chosen reflect the biases of a social insect biologist. Consequently, the aculeate Hymenoptera (which contains all of the eusocial species) are subdivided more finely than the other two major groups. This is justifiable, perhaps, because there are more data for the various subgroups of the aculeata than for the other major divisions of the Hymenoptera.

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Appendix

Recipe for guanine deaminase (modified from Crozier, unpublished).
Guanine stock solution: Guanine, >100 mg; 1M sodium hydroxide, 5 ml; distilled water, to 50 ml; heat gently in water bath at 60°C. Stain recipe: Gda stock, 3 ml; Tris pH 8.0, 20 ml; 1% MTT solution, 1 ml; 1% PMS solution, 100 μl; xanthine oxidase, 3 units. Combine, then add 20 ml of 2% agar in Tris pH 8.0.