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Allozyme Variation in Bumble Bees (Hymenoptera: Apidae)

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Allozyme variation at an average of 37.3 loci was assessed in queens of 16 Bombus *and 2* Psithyrus *bumble bee species from North America. The mean expected heterozygosity* ($\overline{H}$) *for the* Bombus *species was 0.008 ± 0.006 (95% confidence limits) and that for the* Psithyrus *was 0.007 ± 0.007. These levels are significantly lower than found in other Hymenoptera but are comparable to those found in previous studies of bumble bees based on far fewer loci. Neutral mutation and random genetic drift can account for the observed variation, but this implies a very small effective population size for species of bumble bees.*

KEY WORDS: heterozygosity; allozymes; Hymenoptera; *Bombus; Psithyrus.*

INTRODUCTION

Haplodiploid and X-linked gene loci undergoing mutation, drift, and selection are predicted to exhibit less genetic variation than comparable diploid loci (Avery, 1984). Indeed, low levels of allozyme variation are found in the Hymenoptera (Berkelhamer, 1983; Graur, 1985; Owen, 1985) and the haplodiploid Thysanoptera (Crespi, 1991).

Within the Hymenoptera there is considerable variation in heterozygos-

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ity among genera, but this does not appear to be associated with the presence, absence, or level of eusociality (Graur, 1985; Owen, 1985; Crespi, 1991).

In a review of the literature, Owen (1985) noted that bumble bees (*Bombus* species) as a *genus* had a significantly lower mean heterozygosity ($\overline{H} = 0.015 \pm 0.007$) than other Hymenoptera ($\overline{H} = 0.037 \pm 0.005$). However, this conclusion was based on data obtained from only seven *Bombus* species (Snyder, 1974; Pamilo *et al.*, 1978), only three of which were surveyed at 15 or 16 loci; the others were assessed at 10–12 loci. Similar estimates based on 9–12 loci were also obtained by Pamilo *et al.* (1984) for five other species.

Estimates based on fewer than 20 loci are considered unreliable (Graur, 1985) due to the large interlocus variance of heterozygosity (Nei and Roychoudhury, 1974). Therefore, the purpose of this study was to reassess the amount of heterozygosity in bumble bees by examining more species (16) at a larger number of loci (mean, 37.3).

MATERIALS AND METHODS

Bees

Queens of all species were collected in the spring of 1989 and 1990 in southern Alberta at three locations: Ya-Ha Tinda Ranch (51°43′N, 115°30′W), Calgary (51°0′N, 114°10′W), and the Kananaskis River Valley (51°02′N, 115°02′W) (Fig. 1). Altogether queens of 16 *Bombus* species [belonging to five subgenera *sensu* Richards (1968)] and 2 *Psithyrus* species

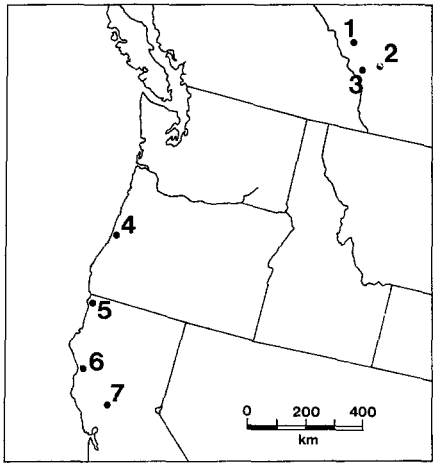

Fig. 1. Collection locations. 1, Ya-Ha Tinda Ranch; 2, Calgary; 3, Kananaskis River Valley; 4, Florence; 5, Crescent City; 6, Westport; 7, Rumsey.

(social parasites of *Bombus*) were collected. In addition, queens of one of these species, *B. melanopygus,* were collected at four locations in Oregon and California (Fig. 1) in 1988. Of these, 79 were assessed at 16 loci, and the remaining were combined with those collected in Alberta (to give a total of 30) and assessed using the full suite of 38 loci (see Tables II and III). All bees were transported live to Calgary, where they were frozen at −70°C prior to electrophoresis.

Electrophoresis

Either head and thoracic muscle or abdominal tissue was used, depending on the enzyme to be stained (Table I). The tissue was weighed and then homogenized on ice using 1:2 w (mg)/v (μl) of double-distilled water. The homogenate was centrifuged at 9000 rpm for 2 min. The supernatant was applied to 5 × 15-mm wicks (Whatman No. 3 filter paper), which were inserted into a slit in the 10-mm-thick horizontal starch gel (Connaught starch; 10.5%, w/v). Most gels were run for 4.5 h. Activity was detected for 37 enzyme systems (Table I), to give a maximum of 40 loci in some species (Table III). Recipes for enzyme stains were modified from Brewer (1970), Shaw and Prasad (1970), Ayala *et al.* (1972), Harris and Hopkinson (1976), Pasteur *et al.* (1988), and Hebert and Beaton (1989) [see Packer and Owen (1989, 1990) for additional details].

RESULTS

Genetic variation was detected at at least one locus in 10 of the 16 *Bombus* and in both of the *Psithyrus* species. Allele frequencies and the expected heterozygosity at each of the variable loci are given in Table II. Overall heterozygosity, averaged across all loci (monomorphic ones included), is very low in most species (Table III). However, some species (e.g., *B. sylvicola*) are considerably more variable than the others. The mean expected heterozygosity ($\overline{H}$) equaled 0.008 ± 0.006 (95% confidence limits) for the *Bombus* species and 0.007 ± 0.007 for the *Psithyrus* species.

B. melanopygus showed minor and nonsignificant allele frequency differences between locations (Table IV).

DISCUSSION

The mean heterozygosity of the *Bombus* and *Psithyrus* species that we examined was very low, though comparable to the levels found in previous surveys of bumble bees (Snyder, 1974; Pamilo *et al.,* 1978, 1984). Moreover, our results corroborate Owen's (1985) observation that *Bombus* species have

Table I. Enzymes Examined: Buffer and Tissue Used

Enzyme	Symbol	EC No.	Number of loci	Tissue[a]	Buffer[b]
Aconitate hydratase	ACON	4.2.1.3	1	A	BI
Adenylate kinase	AK	2.7.4.3	1	A	BI
Alcohol dehydrogenase	ADH	1.1.1.1	1	A	BI + NAD
Aldehyde dehydrogenase	ALDDH	1.2.1.3	1	A	BV
Aldolase	ALD	4.1.2.13	1	A	CAM
Arginine kinase	ARK	2.7.3.3	1	A	BI
Diaphorase (NADH)	DIA	1.8.1	1	A	BV
Diaphorase (NADPH)	DIAP	1.6.99	1	A	BV
Esterase	EST	—	1	H,T	BI
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	1	A	CAM
Glucose-6-phosphate isomerase	GPI	5.3.1.9	1	H,T	BI
Glutamate dehydrogenase	GLUD	1.4.1.2	1	H,T	BI
Glyceraldehyde-3-phosphate dehydrogenase	GAPD	1.2.1.12	1	H,T	BI
Glycerol-3-phosphate dehydrogenase	G3PD	1.1.1.18	2	A	BI
Hexokinase	HK	2.7.1.1	1	H,T	BI
Hydroxybutyrate dehydrogenase	HBDH	1.1.1.30	2	A	BI + NAD
Isocitrate dehydrogenase (NAD)	IDH (NAD)	1.1.1.41	1	H,T	BI
Isocitrate dehydrogenase (NADP)	IDH (NADP)	1.1.1.42	1	H,T	BI
Lactate dehydrogenase	LDH	1.1.1.27	1	A	BI + NAD
Leucine aminopeptidase	LAP	3.4.11.1	1	H,T	BI
Malate dehydrogenase (NAD)	MDH	1.1.1.37	2	H,T	CAM
Malate dehydrogenase (NADP)	ME	1.1.1.40	1	H,T	BI
Octanol dehydrogenase	ODH	1.1.1.73	1	A	BI
Peptidase Leu-Ala	PEP-LA	3.4.11	1	A	RSL
Peptidase Phe-Pro	PEP-PP	3.4.11	1	A	RSL
Peptidase Leu-Leu-Gly	PEP-LLG	3.4.11	1	A	RSL
Phosphoglucomutase	PGM	5.4.22	1	H,T	BI
6-phosphogluconate dehydrogenase	6PGD	1.1.1.43	1	A	CAM
Phosphoglycerate kinase	PGK	2.7.2.3	1	H,T	BI
Phosphoglycerate mutase	PGAM	5.4.2.1	1	H,T	BI
Pyruvate kinase	PK	2.7.1.40	1	A	BI
Sorbitol dehydrogenase	SDH	1.1.1.14	1	A	BV
Superoxide dismutase	SOD	1.15.1.1	1	A	BIorBV
Trehalose	TRE	3.2.1.28	1	H,T	BI
Triosephosphate dehydrogenase	TPI	5.3.1.1	1	A	BI
Uridine monphosphate kinase	UMPK	2.7.4.*	1	A	BI
Xanthine dehydrogenase	XDH	1.1.1.204	1	H,T	BI + NAD

[a]A, abdomen; H,T, head and thorax.
[b]References for buffer recipes: BI and BV, Shaw and Prasad (1970); CAM, Clayton and Tretiak (1972); RSL, Ridgway *et al.* (1970).

considerably less allozyme variation than most other Hymenoptera, the mean H_{exp} for 30 genera of Hymenoptera which have been assessed at 15 or more loci being 0.048 [which is significantly lower than the mean heterozygosity of diploid insects (Crespi, 1991)].

It is interesting that the *Psithyrus* species do not have lower levels of heterozygosity than *Bombus,* since the former, being social parasites, might be expected to have lower population sizes than their hosts. However, in North America it appears that the *Psithyrus* species (of which there are only seven) are weakly host-specific and successfully invade nests of two or three host species (Hobbs, 1966, 1967). Clearly this could lead to larger population sizes than in species that are highly host-specific. Therefore it is not possible to conclude a priori that effective population size in North American *Psithyrus* species will necessarily be smaller than that in *Bombus* species. Nevertheless, this may be the case with some European *Psithyrus,* which tend to be more host-specific than their North American counterparts (Alford, 1975).

It is well-known that X-linked or haplodiploid loci undergoing selection exhibit less variation than comparable diploid loci (Bennett, 1957; Mandel, 1959; Curtsinger, 1980; Pamilo and Crozier, 1981; Owen, 1988). However, since there is no a priori reason to think that selection is extremely severe in bumble bees, it is unlikely that selection accounts for the difference between them and the other Hymenoptera.

Lower heterozygosity at X-linked than autosomal loci is also predicted by the hypothesis of neutral mutation and random genetic drift (for review see Avery, 1984). This hypothesis also does generate testable predictions concerning the expected variance in heterozygosity between species (Avery, 1984). Assuming an infinite allele model Nagylaki (1981) showed that the expected frequency of heterozygotes,

$$E(H) = \frac{4N_{ex}u}{1 + 4N_{ex}u},$$

where u is the mutation rate per generation and N_{ex} is the X-linked effective population size (Wright, 1933). Avery (1984), following Stewart (1976) and Nagylaki (1981), gave an approximation for the variance in heterozygosity at a single neutral X-linked locus,

$$\text{Var } H \simeq \frac{8N_{ex}u}{(1 + 4N_{ex}u)^2(2 + 4N_{ex}u)(3 + 4N_{ex}u)}.$$

The between-species variation in a group (e.g., genus) is then, according to Avery (1984), approximated by

$$\text{Var } (\bar{H}) \simeq (\text{Var } H)/\bar{n},$$

Table II. Allele Frequencies and the Expected Heterozygosity [Nei's (1978) Unbiased Estimate] at Each of the Variable Loci

Species	Locus	Allele[a]	Frequency ± SE	H_{exp} ± SD
B. moderatus	*Pgm*	F	0.933 ± 0.032	0.127 ± 0.057
		S	0.067 ± 0.032	
B. terricola	*Hbdh*	F	0.905 ± 0.045	0.176 ± 0.076
		S	0.095 ± 0.045	
	Mdh-1	F	0.954 ± 0.032	0.090 ± 0.059
		S	0.046 ± 0.032	
B. bifarius	*Lap*	F	0.900 ± 0.039	0.183 ± 0.064
		S	0.100 ± 0.039	
B. centralis	*Me*	F	0.200 ± 0.073	0.331 ± 0.125
		S	0.800 ± 0.073	
	Hk	F	0.933 ± 0.046	0.129 ± 0.082
		S	0.067 ± 0.046	
B. flavifrons	*Mdh-1*	F	0.967 ± 0.023	0.065 ± 0.044
		S	0.033 ± 0.023	
	Pgm	F	0.050 ± 0.028	0.097 ± 0.052
		S	0.950 ± 0.028	
	Tre	F	0.067 ± 0.032	0.127 ± 0.057
		S	0.933 ± 0.032	
B. frigidus	*Me*	F	0.867 ± 0.044	0.234 ± 0.066
		S	0.133 ± 0.044	
	Pk	F	0.033 ± 0.023	0.065 ± 0.044
		S	0.967 ± 0.023	
	Hk	F	0.017 ± 0.041	0.295 ± 0.073
		M	0.833 ± 0.048	
		S	0.050 ± 0.028	
	Gapdh	F	0.033 ± 0.023	0.065 ± 0.044
		S	0.967 ± 0.023	
B. huntii	*Idh* (NAD)	F	0.812 ± 0.056	0.311 ± 0.074
		S	0.188 ± 0.056	
B. melanopygus	*Gpi*[b]	F	0.980 ± 0.011	0.039 ± 0.022
		S	0.020 ± 0.011	
	Idh (NAD)[b]	F	0.257 ± 0.036	0.382 ± 0.035
		S	0.743 ± 0.036	
	Pgm[b]	F	0.038 ± 0.015	0.073 ± 0.028
		S	0.962 ± 0.015	
	Hk[c]	F	0.950 ± 0.028	0.097 ± 0.052
		S	0.050 ± 0.028	
	Tpi[c]	F	0.967 ± 0.023	0.065 ± 0.044
		S	0.033 ± 0.023	
B. sylvicola	*Acon*	F	0.067 ± 0.032	0.127 ± 0.57
		S	0.933 ± 0.032	
	Alddh	F	0.399 ± 0.063	0.675 ± 0.028
		M	0.375 ± 0.064	
		S	0.286 ± 0.060	
	Gapdh	F	0.107 ± 0.041	0.195 ± 0.067
		S	0.893 ± 0.041	
	G6pd	F	0.161 ± 0.049	0.275 ± 0.069
		S	0.839 ± 0.049	
	Sdh	F	0.821 ± 0.051	0.299 ± 0.069
		S	0.179 ± 0.051	

Table II. (continued)

Species	Locus	Allele[a]	Frequency ± SE	H_{exp} ± SD
B. ternarius	*Gapdh*	F	0.067 ± 0.046	0.129 ± 0.082
		S	0.933 ± 0.046	
P. insularis	*Mdh-1*	F	0.917 ± 0.056	0.159 ± 0.099
		S	0.083 ± 0.056	
P. suckleyi	*Lap*	F	0.917 ± 0.056	0.159 ± 0.099
		S	0.083 ± 0.056	
	Mdh-1	F	0.833 ± 0.076	0.290 ± 0.110
		S	0.167 ± 0.076	

[a]F, fast; S, slow.
[b]Oregon and California locations only, combined.
[c]All locations (Fig. 1) combined.

where $\bar{n}$ is the harmonic mean of the number of loci studied for each species in the group. The observed $\overline{H}_{exp}$ is first equated to $E(H)$, allowing Var H and then Var $(\overline{H})$ to be calculated.

Using this method we can compare our results on bumble bees,

Table III. Mean Expected Heterozygosities for Each of the 18 Bumble Bee Species

Genus	Subgenus	Species	Location[a]	N^b	Loci	H_{exp} ± SD
Bombus	*Bombus s.s.*	*moderatus*[c]	1	30	39	0.003 ± 0.003
		occidentalis	3	30	37	0.000
		terricola	2	22	36	0.007 ± 0.005
	Bombias	*nevadensis*	3	24	40	0.000
	Fervidobombus	*californicus*	3	15	40	0.000
	Cullumanobombus	*rufocinctus*	3	15	40	0.000
	Pyrobombus	*bifarius*	3	30	39	0.005 ± 0.005
		centralis	2	15	40	0.012 ± 0.009
		flavifrons	3	30	39	0.007 ± 0.004
		frigidus	3	30	38	0.017 ± 0.010
		huntii	3	24	39	0.008 ± 0.008
		melanopygus	3–7	30	38	0.004 ± 0.003
			4–7	76,79	16	0.031 ± 0.024
		mixtus	3	15	37	0.000
		perplexus	2	15	40	0.000
		sylvicola	3	28	37	0.042 ± 0.021
		ternarius	2	15	40	0.003 ± 0.003
Psithyrus		*insularis*	3	12	40	0.004 ± 0.004
		suckleyi	3	12	40	0.011 ± 0.008

[a]1, Ya-Ha Tinda Ranch; 2, Calgary; 3, Kananaskis River Valley; 4–7, Oregon and California. See Fig. 1.
[b]Sample size: number of queens (diploid females).
[c]Previously *B. lucorum;* see Scholl *et al.* (1990).

Table IV. Allele Frequencies at the Three Variable Loci in *Bombus melanopygus* (Fig. 1)[a]

	Locus								
	Pgm			*Gpi*			*Idh* (NAD)		
Location	*N*	Allele	Frequency ± SE	*N*	Allele	Frequency ± SE	*N*	Allele	Frequency ± SE
Kananaskis	24	S	1.000 − 0.001	24	F	1.000 − 0.001	16	S	0.875 ± 0.058
		F	0.000 + 0.001		S	0.000 + 0.001		F	0.125 ± 0.058
Florence	12	S	0.958 ± 0.041	11	F	0.955 ± 0.044	11	S	0.727 ± 0.095
		F	0.042 ± 0.041		S	0.045 ± 0.044		F	0.273 ± 0.095
Crescent	34	S	0.971 ± 0.020	32	F	0.984 ± 0.016	31	S	0.839 ± 0.047
City		F	0.029 ± 0.020		S	0.016 ± 0.016		F	0.161 ± 0.047
Westport	16	S	0.969 ± 0.031	16	F	0.969 ± 0.031	15	S	0.733 ± 0.081
		F	0.031 ± 0.031		S	0.031 ± 0.031		F	0.267 ± 0.081
Rumsey	17	S	0.941 ± 0.040	17	F	1.000 − 0.001	17	S	0.588 ± 0.084
		F	0.059 ± 0.040		S	0.000 + 0.001		F	0.412 ± 0.084

[a]At each locus there is a nonsignificant difference in allele frequency between adjacent locations (*z* test).

specifically *Bombus,* to results on other Hymenoptera. An important assumption of this method is that the species within the various groups (here genera) have similar effective population sizes (Avery, 1984) [see also Crespi (1991) on the use of genera for comparisons of heterozygosity]. Table V compares our data on *Bombus* with data on other hymenopteran genera

Table V. Comparison of Observed Standard Deviation of Heterozygosity in Six Genera of Hymenoptera with That Predicted on the Basis of Neutral Mutation and Random Genetic Drift[a]

				SD	
Genus[b]	Number of species	Number of loci ($\bar{n}$)[c]	$\bar{H}_{exp}$	Observed	Predicted
Aphidius (1)	6	16.0	0.034	0.040	0.026
Neodiprion (2)	15	19.6	0.069	0.059	0.032
Bombus (3)	16	35.7	0.008	0.012	0.009
Polistes (4)	12	19.1	0.057	0.020	0.030
Lasioglossum (5)	13	42.7	0.021	0.016	0.013
Rhytidoponera (6)	5	22.0	0.035	0.019	0.022

[a]The method of Avery (1984) was used; for details see text.
[b]Source; (1) Unruh *et al.* (1989); (2) Kuenzi and Coppel (1986) and Woods and Guttman (1987); (3) this study; (4) Lester and Selander (1979), Metcalf *et al.* (1984), and Nozawa and Ito (1989); (5) Snyder (1974), Kukuk and May (1985), and Packer and Owen (unpublished); (6) Ward (1980).
[c]Harmonic mean.

(compiled from the literature) for which a reasonable number of species have been analyzed at at least 15 loci. It is clear from Table V that, as a genus, *Bombus* is much less variable than the other hymenopteran genera. Also, for *Bombus* the observed and theoretically predicted standard deviations of heterozygosities are very close. For two of the other five genera, agreement between observed and predicted is also close. The exceptions are *Aphidius* and *Neodiprion,* which show larger observed to predicted standard deviations, and *Polistes,* which shows the reverse. Overall, though, the agreement between observation and theory is quite good given the number of assumptions involved. These data from the Hymenoptera are compatible with the hypothesis that much of the allozyme variation results from mutation and random genetic drift. However, if this is so, then the results imply that *Bombus* in general have very small *species* effective population sizes. Nei and Graur (1984) note that for neutral theory, it is the effective population size for the whole species that is important, not that of a local population. This, of course, is difficult, if not impossible, to quantify in practice.

Little population differentiation was found in the one species, *B. melanopygus,* which was collected in Alberta and at four locations in Oregon and California (Fig. 1). Indirect evidence for gene flow in this species also can be inferred from the existence of a cline in pile color allele frequency down the west coast of North America. *B. melanopygus* exhibits a red/black dimorphism of the pile on abdominal terga 2 and 3, which is controlled by a single diallelic locus with the red (*R*) allele dominant to the black (*r*) (Owen and Plowright, 1980). The red allele is near fixation in Washington state and northern Oregon, but to the south it is progressively replaced by the black allele, which reaches fixation in northern California. The transition from red to black occurs over a distance of nearly 600 km [see Owen and Plowright (1980) and Owen (1986) for diagrams and analysis of the cline]. Clines result from a balance between selection and dispersal of individuals (Haldane, 1948). Therefore the existence of this, albeit shallow, cline provides evidence that gene flow must be occurring between local populations of *B. melanopygus.* Although *B. melanopygus* appears to be relatively homogeneous at the level of enzymes–genes, other species with more patchy distributions may show significant population differentiation. The more variable enzyme loci identified in Table II could be used to investigate this.

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