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A Comparison of Genetic Variation in Two Sibling Species Pairs of Haplodiploid Insects

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*Sibling species pairs of sweat bees (*Halictus confusus *and* H. tumulorum*) and pine sawflies (*Neodiprion pratti *and* N. maurus*) were surveyed for genetic variability using enzyme electrophoresis. Levels of heterozygosity were found to be within the ranges earlier recorded for Hymenoptera. Expected heterozygosities were not significantly higher in the sawflies than in the sweat bees. Estimates of genetic identity between the sibling species were not lower than those generally found for diplodiploid insect species: no evidence was found for an increased rate of evolution in these haplodiploids. Genetic identity data among populations of* H. confusus *and between* Halictus *species were within the range expected for conspecific populations and sibling species, respectively. In* Neodiprion *all genetic distances were low but the two populations of* N. pratti *had similar genetic distances as each did to* N. maurus*, indicating the necessity for further systematic studies of the genus.*

KEY WORDS: bees; sawflies; sibling species; genetic distance; heterozygosity.

INTRODUCTION

Since the early seventies, many workers have reported lower levels of genetic variation in the Hymenoptera compared to other insect orders (Snyder, 1975; Metcalf *et al.*, 1975; Pamilo *et al.*, 1978; Lester and Selander, 1979;

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Berkelhamer, 1983; Crespi, 1991). Several hypotheses have been suggested to account for these results. These are based upon either the sex determining system of the Hymenoptera (arrhenotokous parthenogenesis) or behavioral/ecological characteristics of most of the electrophoretically studied species.

The sex determining system of Hymenoptera, where females are diploid and males develop as haploids from unfertilized eggs, can result in reduced levels of genetic variation through a variety of mechanisms. First, haplodiploidy results in a decreased effective population size (unless the sex ratio is strongly female biased), and lower levels of neutral genetic variation are expected as a result (Crozier, 1970; Hartl, 1971; Pamilo and Crozier, 1981). Selection can also result in reduced levels of variation in haplodiploids for two main reasons. First, slightly deterious mutant alleles are less likely to become established because they must be expressed in hemizygous males (Suomaleinen, 1962; Crozier, 1970). Second, it is more difficult to obtain a stable polymorphism in a haplodiploid genetic system than in a diplodiploid system (Avery, 1984; Owen, 1988).

The above arguments have haplodiploidy as their basis and are thus applicable to all Hymenoptera and other organisms with this sex determining mechanism. The ecological and behavioral explanations for decreased levels of genetic variability in the Hymenoptera are less broadly applicable, and only two are really relevant to the data base available so far: both are ultimately attributable to the social behavior that most of the electrophoretically studied species possess. (i) Eusociality may account for some loss of genetic variation due to a further reduction in effective population size (Berkelhamer, 1983). (ii) As the nest architecture of many eusocial species keeps the inside environment rather stable, it may act as an "environmental buffer" and thereby diminish the importance of environmental variation in maintaining polymorphism (Metcalf *et al.,* 1975; Snyder, 1975). In comparison to the environmental vagaries experienced by most free-living insects, the nest building habits of even the solitary bees and aculeate wasps provide some environmental buffering capacity.

Thus far, the data are insufficient to differentiate among these various hypotheses, although there is a general impression that the free-living sawflies have higher levels of genetic variation than nest-building aculeates (Sheppard and Heydon, 1986). However, there is substantial interlaboratory variation in levels of heterozygosity detected even when the same species are surveyed (Simon and Archie, 1985) and it is not possible to state whether the differences observed between sawflies and aculeate Hymenoptera result from differences in the organisms themselves or variation in methodologies between laboratories conducting the studies. Thus, adequate comparisons

of the two taxa are required under laboratory conditions which are as similar as possible.

In addition to its effects upon heterozygosity, the reduced effective population size that haplodiploidy confers may also increase the rate of evolution (Hartl, 1971; Crozier, 1985). Although there are a few examples of surprisingly large genetic distances among morphologically apparently conspecific populations (Packer and Owen, 1989; Blanchetot and Packer, 1993), this aspect of hymenopteran evolutionary genetics remains to be investigated in detail. To assess whether there may be an elevated rate of evolutionary change in haplodiploid insects, more data on intraspecific genetic distances and levels of genetic differentiation between sibling species pairs are needed.

In this paper we present information on heterozygosity and genetic differentiation for two pairs of sibling species of Hymenoptera: the sweat bees *Halictus confusus* and *H. tumulorum* (suborder Apocrita; family Halictidae) and the pine sawflies *Neodiprion pratti* and *N. maurus* (suborder Symphyta; family Diprionidae). For one of each sibling species pair, two or more geographically disparate populations were available for analysis; for the sawfly *Neodiprion pratti* the two populations represent different subspecies.

METHODS

The Study Species

Halictus confusus has a holarctic distribution and is very common in northern North America, where two subspecies have been described (Moure and Hurd, 1987): *arapahonum* in the West and *confusus* throughout. Three additional subspecies are recorded from the Palaearctic (Ebmer, 1988), but only the nominal subspecies was available for the present study. *Halictus tumulorum* is an Old World species which is common in Europe, parts of the Middle East, and Japan (Sakagami and Ebmer, 1979; Ebmer, 1988). Both species excavate comparatively shallow nests in the ground and are primitively eusocial (Dolphin, 1966 quoted in Michener, 1974; Sakagami and Okazawa, 1985), and although detailed information on the level of social behavior is not available, there appear to be few individuals per nest—fewer than five.

The two pine sawfly species, *Neodiprion pratti* and *N. maurus,* live gregariously as larvae, feeding upon the needles of pines (Knerer and Wilkinson, 1990; Knerer, 1990). *Neodiprion pratti* occurs from Florida to Northern Ontario and shows many local adaptations to climatic conditions (Knerer, 1984; Knerer and Wilkinson, 1990). This species is morphologically

and phenologically very variable and three subspecies have been described—*N. pratti pratti,* predominantly from the southern states; *N. p. banksianus,* from the Great Lakes region; and *N. p. paradoxicus,* from the eastern seaboard (Ross, 1955). The former two subspecies were available for study. *Neodiprion maurus* has a more restricted range, being found North of latitude 46°N in Eastern North America, where it is rather uncommon (Knerer, 1990).

Insect Sampling

Free-flying *Halictus confusus* females were collected from three areas in North America: Calgary, Alberta, in 1988; the Metropolitan Toronto region in 1990; and Sydney, Nova Scotia, in 1991. *Halictus tumulorum* was collected from the Thiais cemetery near Paris, France, in 1988. All sawflies were collected in 1989. Two populations of *Neodiprion pratti* were sampled: one in Virginia (subspecies *pratti*) and the other from Geraldton, Northern Ontario (subspecies *banksianus*). *Neodiprion maurus* was also collected in Geraldton. Each individual sawfly sampled was from a different family collected as larvae or eggs except the Ontario population of *N. pratti* which constituted the F_1 generation from a stock of 10 mated females.

Electrophoretic Methods

Genetic variability was studied by means of horizontal starch gel electrophoresis following the methods outlined by Packer and Owen (1989, 1990; Packer *et al.,* 1992) with the following additional specifications. Bee head and thoraces or abdomens were ground in 45 μl of grinding buffer; whole sawflies were ground in 90 μl. Additional staining recipes are for *Aat* (Cardy *et al.,* 1980), for *Acy* (Qavi and Kit, 1980), and for *B-Gal* and *Fk* (Pasteur *et al.,* 1988). A list of enzymes stained and the buffers used is provided in Table I.

The genetic basis of variability in electromorph patterns was determined taking the gender of the specimen and tertiary structure of the stained enzyme into account and also considering the possible occurrence of interlocus heterodimers and posttranslational modifications at some loci (Packer and Owen, 1989).

The survey provided data on allozyme variation at 36 loci for sweat bees and 45 loci for sawflies. Expected heterozygosities, genetic distances, genetic identities (Nei, 1978), percentage of loci polymorphic, mean number of alleles per locus, and mean sample size per locus were calculated using the BIOSYS computer program (Swofford and Selander, 1989). The program was run three times on the sweat bee data: once comparing only *Halictus*

Table I. Enzyme Names, Abbreviations, EC Numbers, Number of Loci, and Electrode Buffers for Bees and Sawflies

			Bees		Sawflies	
Name	Abbrev.	EC No.	*n* loci	Buffer[a]	*n* loci	Buffer
Aspartate aminotransferase	AAT	2.6.1.1	1	I + NAD	2	III + NAD
Aconitate hydratase	ACO	4.2.1.3			2	CAM
Aminoacylase	ACY	3.5.1.14	1	RSL	1	RSL
β-*N*-Acetylhexosaminidase	AHA	3.2.15.2	2	I	2	I
Adenylate kinase	AK	2.7.4.3	1	CAM	3	I + NADP
Fructose biphosphate aldolase	ALD	4.1.2.13	1	V	1	III + NAD
Arginine kinase	ARK	2.7.3.3	1	I	1	I + NADP
β-Galactosidase	βGAL	3.2.1.23			1	I
β-Glucuronidase	βGLU	3.2.1.31			1	I + NAD
Diaphorase (NADH)	DIA	1.8.1.*	1	V	1	V
Diaphorase (NADPH)	DIAP	1.6.99.*	1	V	1	V
Enolase	ENOL	4.2.1.11	1	I	1	I
Esterase	EST	3.1.1.1	1	RSL	4	RSL
Fructokinase	FK	2.7.1.4	1	CAM		
Fumarate hydratase	FUM	4.2.1.2	1	1 + NAD		
Glyceraldehyde-3-phosphate dehydrogenase	GAPD	1.2.1.12	1	I + NAD	1	I + NAD
α-Glycerophosphate dehydrogenase	αGPD	1.1.1.8	2	I + NAD	2	I + NAD
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	1	CAM	1	CAM
Glucose-6-phosphate isomerase	GPI	5.3.1.9	2	I	1	I + NADP
Hydroxyacylglutathione hydrolase	HAGH	3.1.2.6	1	V + NAD		
β-Hydroxyacid dehydrogenase	HAD	1.1.1.30	1	I + NAD	1	I + NAD
Hexokinase	HK	2.7.1.1	2	I	2	I + NADP
Isocitrate dehydrogenase	IDH	1.1.1.42	1	CAM	1	CAM
L-Lactate dehydrogenase	LDH	1.1.1.27			1	I + NAD
Malate dehydrogenase	MDH	1.1.1.37	2	CAM	2	CAM
Malate dehydrogenase (NADP)	ME	1.1.1.40	1	I		
Peptidase Gly-Leu	PEP_{gl}	3.4.11.*			1	RSL
Peptidase Leu-Ala	PEP_{la}	3.4.11.13	2	RSL	2	RSL
Peptidase Phe-Pro	PEP_{pp}	3.4.13.8	1	RSL	1	RSL
Phosphoglycerate mutase	PGAM	5.4.2.1	1	I	2	I
Phosphogluconate dehydrogenase	6PGD	1.1.1.43	1	CAM	1	CAM
Phosphoglucomutase	PGM	5.4.2.2	2	I		
Phosphoglycerate kinase	PGK	2.7.2.3	1	I		
Pyruvate kinase	PK	2.7.1.40	1	V	2	V
Sorbitol dehydrogenase	SDH	1.1.1.14	1	V	1	V
Superoxide dismutase	SOD	1.15.1.1	2	CAM + NADP	1	I + NADP
Triosephosphate isomerase	TPI	3.5.1.1	1	I + NAD	1	I + NAD

[a] For details for buffer references and running conditions, see Packer and Owen (1989), with the modification that all CAM buffers were run with 50 mg of NADP added to the gel before degassing.

confusus from Ontario with *H. tumulorum* (36 loci), once comparing these two for only those isoenzymes also scored for the sawflies (30 loci), and once comparing all four bee populations with loci sampled for fewer than 10 individuals per population excluded (26 loci). For the sawflies, one run compared the three populations for all 45 loci surveyed, and the second analysis included only the 36 loci for enzymes stained in common with the bees. The analyses including only loci stained for both bees and sawflies were necessary to ensure direct comparability of results for the two groups: it is well-known that some enzyme loci are more often variable than others [see Packer and Owen (1992) for a review of the hymenopteran data on this]. This suggests the importance of using the same suite of loci when interspecific comparisons of heterozygosity are being made.

Statistical comparisons of heterozygosity and genetic distance and identity data were performed after generating variance estimates for single-locus values using the bootstrap method (Efron, 1982). This involved sampling the original set of single-locus values (for heterozygosity or genetic distance and identity) at random and with replacement (i.e., such that any original single-locus estimate may be included once, several times, or not at all in each one of the bootstrap replicates). Two hundred replications were performed, and the 95% confidence intervals calculated from the resulting distribution of values.

At all loci with an expected heterozygosity (H_{exp}) of 0.200 or higher, H_{exp} was compared with the observed number of heterozygotes using the chi-square test. Due mainly to small sample sizes, only five loci satisfied the requirements of the test: one in bees and four in sawflies.

RESULTS

Variable enzymes and their mobilities, expected heterozygosities (H_{exp}), and sample sizes are listed in Tables II and III for bees and sawflies, respectively.

Table IV shows mean sample sizes per locus, mean numbers of alleles per locus, percentage of loci polymorphic, H_{exp}, and Nei's (1978) genetic distance and identity for bees and sawflies for 26 and 45 loci, respectively. Heterozygosity and genetic distances are shown for only the directly comparable loci in Table V (30 and 35 loci for bees and sawflies, respectively). As can be seen from the 95% confidence limits, there are no significant differences in heterozygosity among taxa.

The results of the chi-square test comparing the frequency of observed and expected numbers of heterozygotes are summarized in Table VI. Of the five loci tested, only *Pepla-1* in *Neodiprion pratti* from Ontario had genotype frequencies significantly different from expected Hardy–Weinberg proportions.

Table II. Allele Frequencies, Individual Locus Heterozygosity (Nei's Unbiased H_{exp}), and Sample Sizes for Variable Loci in Bees [Sample Sizes (n) Are in Number of Haploid Genomes]

Locus	Mobility	Conf. Ont.	Conf. Alta	Conf. N.S.	Tum
ACY	20	0.000	0.000	0.000	1.000
	25	0.833	1.000	1.000	0.000
	28	0.167	0.000	0.000	0.000
	H	0.333	0.000	0.000	0.000
	n	6	20	24	6
AK	32	0.000	0.000	0.000	1.000
	35	1.000	0.450	0.000	0.000
	42	0.000	0.550	1.000	0.000
	H	0.000	0.521	0.000	0.000
	n	34	20	24	34
DIA	8	0.939	1.000	0.917	1.000
	10	0.061	0.000	0.083	0.000
	H	0.116	0.000	0.159	0.000
	n	33	20	24	34
EST	30	0.000			0.000
	32	0.029			0.000
	35	0.000			0.029
	38	0.559			0.941
	44	0.412			0.029
	H	0.525			0.116
	n	34			34
FK	12	1.000	1.000	1.000	0.941
	14	0.000	0.000	0.000	0.059
	H	0.000	0.000	0.000	0.114
	n	34	20	24	34
FUM	8	1.000	1.000	0.958	0.971
	20	0.000	0.000	0.000	0.029
	26	0.000	0.000	0.042	0.000
	H	0.000	0.000	0.082	0.059
	n	30	20	24	34
αGPDH-2	38	1.000	1.000	1.000	0.882
	44	0.000	0.000	0.000	0.118
	H	0.000	0.000	0.000	0.214
	n	34	10	24	34
GPI-2	−1	0.029	0.000	0.000	0.000
	11	0.942	1.000	1.000	1.000
	16	0.029	0.000	0.000	0.000
	H	0.116	0.000	0.000	0.000
	n	34	20	24	34
HK-1	8	0.029	0.000	0.000	0.000
	12	0.912	1.000	1.000	1.000
	18	0.059	0.000	0.000	0.000
	H	0.169	0.000	0.000	0.000
	n	34	20	24	34

Table II. (continued)

Locus	Mobility	Conf. Ont.	Conf. Alta	Conf. N.S.	Tum
IDH	16	1.000			0.000
	19	0.000			1.000
	H	0.000			0.000
	n	30			32
MDH-1	30	0.000	0.000	0.000	0.029
	38	1.000	1.000	1.000	0.971
	H	0.000	0.000	0.000	0.057
	n	34	20	24	34
PEP1a-2	36	1.000	1.000	1.000	0.000
	39	0.000	0.000	0.000	1.000
	H	0.000	0.000	0.000	0.000
	n	34	20	24	34
PEPpp	22	1.000	1.000	1.000	0.000
	29	0.000	0.000	0.000	1.000
	H	0.000	0.000	0.000	0.000
	n	6	20	24	6
PGK	19	1.000	0.950	1.000	1.000
	24	0.000	0.050	0.000	0.000
	H	0.000	0.100	0.000	0.000
	n	34	20	24	34
PGM-2	28	0.059	0.000	0.000	0.000
	31	0.000	0.000	0.000	1.000
	37	0.941	1.000	1.000	0.000
	H	0.114	0.000	0.000	0.000
	n	34	20	24	34
PK	6	0.000	0.056	0.042	0.000
	12	1.000	0.944	0.958	1.000
	H	0.000	0.111	0.083	0.000
	n	32	18	24	34
SDH	9	0.000	0.000	0.000	1.000
	11	1.000	0.650	0.000	0.000
	14	0.000	0.350	0.357	0.000
	15	0.000	0.000	0.643	0.000
	H	0.000	0.479	0.507	0.000
	n	34	20	24	34
SOD-1	−8	1.000	1.000	1.000	0.000
	−5	0.000	0.000	0.000	1.000
	H	0.000	0.000	0.000	0.000
	n	34	20	24	28
TPI	20	0.000	0.050	0.000	0.000
	22	0.000	0.000	0.045	0.000
	27	0.970	0.000	0.000	0.000
	33	0.030	0.950	0.955	1.000
	H	0.062	0.100	0.082	0.000
	n	32	20	24	34

Table III. Allele Frequencies, Individual Locus Heterozygosity (Nei's Unbiased H_{exp}), and Sample Sizes in Haploid Genomes for Variable Loci in Sawflies

Locus	Mobility	*pratti pratti*	*pratti banksianus*	*maurus*
AAT-1	−10	0.933	0.000	0.900
	−8	0.000	1.000	0.100
	−6	0.067	0.000	0.000
	H	0.129	0.000	0.185
	n	30	40	40
ACO-1	−15	0.000	0.850	0.000
	−11	1.000	0.150	1.000
	H	0.000	0.268	0.000
	n	35	40	40
ACO-2	14	0.067	1.000	0.000
	16	0.933	0.000	1.000
	H	0.129	0.000	0.000
	n	15	20	20
AHA-2	19	0.000	0.200	0.110
	25	0.000	0.000	0.667
	40	0.000	0.500	0.167
	45	1.000	0.300	0.056
	H	0.000	0.620	0.527
	n	15	10	18
βGAL	38	0.010	0.000	0.000
	58	0.979	1.000	1.000
	76	0.010	0.000	0.000
	H	0.040	0.000	0.000
	n	125	40	40
βGLU	30	0.750	1.000	0.250
	37	0.250	0.000	0.750
	H	0.395	0.000	0.387
	n	20	20	16
DIA	10	0.017	0.000	0.000
	17	0.983	1.000	1.000
	H	0.034	0.000	0.000
	n	60	40	40
ENOL	32	0.762	1.000	1.000
	35	0.238	0.000	0.000
	H	0.366	0.000	0.000
	n	35	40	40
EST-1	11	1.000	1.000	0.650
	15	0.000	0.000	0.350
	H	0.000	0.000	0.467
	n	20	20	20

Table III. (continued)

Locus	Mobility	*pratti pratti*	*pratti banksianus*	*maurus*
EST-2	20	0.050	0.050	0.000
	25	0.950	0.950	0.150
	30	0.000	0.000	0.850
	H	0.100	0.100	0.262
	n	20	20	20
EST-4	45	0.000	0.000	0.050
	47	0.750	1.000	0.950
	49	0.250	0.000	0.000
	H	0.395	0.000	0.097
	n	20	20	20
GAPD	32	0.992	1.000	1.000
	40	0.008	0.000	0.000
	H	0.016	0.000	0.000
	n	125	40	40
αGPD-2	29	0.031	0.100	0.000
	36	0.969	0.900	1.000
	H	0.060	0.185	0.000
	n	65	40	40
HAD	20	0.008	0.000	0.000
	29	0.992	1.000	1.000
	H	0.016	0.000	0.000
	n	125	40	40
IDH	−2	0.000	0.000	0.075
	7	0.985	1.000	0.925
	13	0.015	0.000	0.000
	H	0.031	0.000	0.141
	n	65	40	40
MDH-1	−23	1.000	1.000	0.975
	−13	0.000	0.000	0.025
	H	0.000	0.000	0.049
	n	65	40	40
PEP1a-1	7	0.031	0.000	0.000
	12	0.594	0.710	1.000
	18	0.375	0.290	0.000
	H	0.514	0.422	0.000
	n	32	38	40
6PGD	1.000	0.015	0.000	0.125
	4	0.985	1.000	0.875
	H	0.031	0.000	0.224
	n	65	40	40
TPI	23	0.000	0.725	0.000
	30	1.000	0.000	1.000
	37	0.000	0.275	0.000
	H	0.000	0.409	0.000
	n	63	40	40

Table IV. Heterozygosity, Genetic Distance (Below the Diagonal), and Genetic Identity (Above the Diagonal) Data for Bees and Sawflies Based on the Maximum Number of Loci Scored for an Adequate Sample of Individuals in All Populations (26 for Bees, 45 for Sawflies)

Taxon	Mean n^a size per locus	Mean n alleles per locus	% loci polymorphic	H_{exp}	Distance and Identity			
					confusus			
					Ont.	Alta	N.S.	*tumulorum*
confusus								
Ont.	18.0	1.30 (0.10)[b]	19.2	0.022	—	0.984 (0.95–1.0)[b]	0.931 (0.77–1.0)	0.805 (0.65–0.92)
Alta	9.9	1.20 (0.10)	19.2	0.050	0.016	—	0.978 (0.89–1.0)	0.820 (0.65–0.92)
N.S.	12.9	1.20 (0.10)	19.2	0.035	0.072	0.022	—	0.812 (0.65–0.92)
tumulorum	16.8	1.20 (0.10)	15.4	0.017	0.217	0.198	0.262	—
					pratti pratti	*p. banksianus*	*maurus*	
pratti pratti	41.3	1.30 (0.10)	28.9	0.047	—	0.933 (0.85–0.98)	0.935 (0.85–0.98)	
p. banksianus	17.2	1.20 (0.10)	13.3	0.046	0.070	—	0.882 (0.76–0.95)	
maurus	19.6	1.20 (0.10)	20.0	0.052	0.067	0.125	—	

[a]Given as number of diploid individuals.
[b]Numbers in parentheses are 95% confidence limits for distance data and standard errors for alleles per locus.

Genetic distance data for the bees showed that all populations of *Halictus confusus* were more similar to each other than any of them were to *H. tumulorum.* Eight fixed allelic differences were found between the species (at the loci *Acy, Ak, Idh, Pepla-2, Peppp, Pgm-2, Sdh,* and *Sod-1*). Three loci exhibited marked allele frequency differences among *H. confusus* populations. (i) At *Ak* the Ontario and Nova Scotia populations were fixed for alternative alleles, both of which were found in the Albertan sample. (ii) For *Sdh* the Ontario population was fixed for a slow allele, Albertan bees were polymorphic for slow and medium alleles, and the Nova Scotian sample possessed the medium allele and a fast one. (iii) At *Tpi,* the common allele in Albertan and Nova Scotian bees was rare in the Ontario sample, for which the common allele was not found in the other populations. For 11 loci an allele which was uncommon in one species was not detected in the other.

Based upon 45 loci, the genetic distance between *Neodiprion p. pratti* and *N. maurus* was marginally less than that between the two *N. pratti*

Table V. Heterozygosity and Genetic Distance Data for Bees and Sawflies for a Suite of Loci Directly Comparable Between the Two Groups

	H (95% CI)	Genetic distance (95% CI)	
		tumulorum	
confusus, Ont.	0.033 (0.004–0.087)	0.809 (0.65–0.92)	
tumulorum	0.012 (0.000–0.032)	—	
		p. banksianus	*maurus*
pratti pratti	0.045 (0.013–0.087)	0.954 (0.875–0.999)	0.943 (0.868–0.994)
p. banksianus	0.048 (0.011–0.105)	—	0.923 (0.831–0.995)
maurus	0.052 (0.017–0.095)	—	—

subspecies, with no fixed differences between *N. maurus* and *N. p. pratti. Neodiprion p. banksianus* had one fixed difference in comparison to both *N. maurus* and *N. p. pratti,* at *Tpi* (which was polymorphic for two alleles in *N. p. banksianus* but fixed for a third in the other two samples). *Aconitase-2* exhibited a fixed difference between *N. p. banksianus* and *N. maurus* with *N. p. pratti* harboring both alleles. Only five loci exhibited substantial allele frequency differences among the three sawfly samples, with *N. maurus* having its most common allele not represented in either *N. pratti* population for the loci *Aat-1, Aha-2, Est-2,* and β*Gus,* whereas *N. p. banksianus* had a common allele not present in the other two populations for the locus *Acon-1.* All other differences between sawflies involve less common alleles in one or two populations being absent in the other(s). Genetic differentiation among the sawfly populations is not great.

Table VI. Results of Chi-Square Test on H_{exp} and Counted Numbers of Heterozygotes

Population	Enzyme	Exp. No.	Obs. No.	χ^2	*P*
confusus	AK	5.0	3	0.524	ns
pratti pratti	PEP_{1a-1}	6.7	7	0.498	ns
p. banksianus	PEP_{1a-1}	7.8	3	7.076	<0.05
p. pratti	TPI	7.6	11	2.845	ns
maurus	EST-2	2.6	3	3.197	ns

DISCUSSION

We have compared two sibling species of sawflies and two sibling species of bee in an attempt to obtain comparative data on levels of genetic variation in these insects. Our choice of species also permits some comparison of levels of genetic divergence between hymenopterous sibling species with those available from the literature. In the following discussion we concentrate first upon levels of genetic variation within species and then deal with comparisons of genetic identities between taxa.

Table VII shows expected heterozygosities for various sawflies recently compiled from several sources by Crespi (1991). In his analysis, Crespi used genetic means in order to reduce possible taxonomic artifacts. As Crespi did not include the data on sawflies accumulated by Kuenzi and Coppel (1986), we have recalculated the mean H_{exp} for sawflies, including the data available from all three studies. The value calculated, 0.070, is higher than the mean H_{exp} for pine sawflies found in this study but not significantly so.

As the sawflies are all solitary and their larvae do not live in environmentally buffered nests, their mean H_{exp} was expected to be higher than the sweat bees'. Although this was the pattern observed, the differences are not significant. We have also compared heterozygosities of sawflies to those of other Hymenoptera using data from Crespi (1991), Packer *et al.* (1992), and the present study. Using a one-tailed Mann–Whitney test, the generic means for sawfly heterozygosities ranged marginally significantly higher than those for other Hymenoptera [$U_s = 144$, $t_s = 1.63$ ($t_{0.05} = 1.64$)]. However, when the parasitic Hymenoptera, which also do not live in nests, are removed from the data, the result is clearly not significant ($U_s = 10$, $t_s = 0.45$, $P > 0.3$). Thus, the hypothesis that hymenopteran nests function as environmental buffers and promote a reduced level of genetic variation through the niche variation hypothesis is not supported by the data available at this time.

The difference between the data values presented in Table VII and those reported for diplodiploid insects is in excess of 25%. As suggested by Crespi (1991), if a decrease in effective population size due to haplodiploidy

Table VII. Values of H_{exp} for Sawflies from Various Sources

Source	H_{exp}
Mean for present study	0.048
Pamilo *et al.* (1978)	0.021
Kuenzi and Coppel (1986)	0.074
Woods and Guttman (1987)	0.027
Sheppard and Heydon (1986)	0.144
Mean	0.070

alone is the cause of reduced genetic variation in the Hymenoptera, a reduction in heterozygosity of only 25% may be expected (although this statement makes some untested assumptions about sex ratios). Consequently, some factor other than just haplodiploidy may contribute to reduced levels of genetic variation in Hymenoptera.

The deviation from Hardy–Weinberg proportions found in *Neodiprion p. banksianus* in Ontario is likely due to the fact that the sampled individuals were derived from only ten families. The small number of independent genomes surveyed may also have resulted in a lowered H_{exp}. However, only rarer alleles are likely to have been missed, and these contribute comparatively little to the overall heterozygosity, so our sample is probably fairly representative of genetic variation in the sampled population.

The estimates of genetic identity among the populations of *Halictus confusus* vary between 0.931 and 0.984. This range is within that found by Brussard *et al.* (1985) for local populations of conspecific insect species. The identities between *Halictus tumulorum* and *H. confusus* range from 0.805 to 0.820, which match the level expected for sibling species (Brussard *et al.*, 1985).

A quite surprising pattern of genetic differentiation was detected in the sawflies. The level of genetic differentiation between *N. pratti pratti* and *N. maurus* was similar to that between the two *N. pratti* subspecies, and both values are within Brussard and co-workers' (1985) range for local conspecific populations. The identity between *Neodiprion p. banksianus* and *N. maurus* was also similar to that often found between subspecies. The genetic distance between *Neodiprion maurus* and *N. pratti* was estimated by Kuenzi and Coppel (1986) to be 0.109, fairly close to the distance we found between *N. p. banksianus* and *N. maurus* (0.125), but almost twice as high as that between *N. p. pratti* and *N. maurus* (0.067).

These high identity values should not be taken as evidence that *N. maurus* and *N. pratti* should not be considered as separate species: their ecology, phenology, and color patterns are certainly distinct (Knerer, 1984, 1990), as are their wing vein patterns when subjected to morphometric analysis (Packer and Dennison, in preparation). The results indicate that the populations of *Neodiprion pratti* are sufficiently differentiated to warrant at least subspecies status, as has been suggested (Ross, 1955). These data show the necessity of further systematic studies of *Neodiprion,* in particular, and further analysis of the genetics of speciation in Hymenoptera, in general.

Since the genetic identities we have found average higher, not lower, than those obtained between diplodiploid insects of equal rank by Brussard *et al.* (1985), these data do not support the faster rate of evolution in Hymenoptera predicted by Hartl (1971), at least not as applied to allozyme

evolution. However, another complicating factor is that the estimates reported here are based upon larger numbers of loci than generally used; perhaps genetic identity (like H_{exp}) shows a negative correlation with number of loci surveyed. This possibility remains to be investigated.

In contrast, Packer and Owen (1989) and Blanchetot and Packer (1993) have found very large genetic distances between apprarently conspecific populations for two sweat bee species. Additional data on genetic variation between sibling species and conspecific populations are available in the literature, but interlaboratory variation makes comparisons difficult to interpret.

The data provided in this paper do not differentiate between the effects of eusociality or the nest environment as causes of reduced levels of genetic variation in the bees in comparison to the sawflies. Comparative studies of other solitary and eusocial Hymenoptera and other insects, involving many loci, carried out under identical conditions (i.e., in the same laboratory), are needed to provide useful information on this subject.

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