POPULATION GENETICS OF HAPLODIPLOID INSECTS

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ABSTRACT

Genic variation of seven species of Hymenoptera is described, using electrophoretic techniques. The heterozygosities range from 0.033 to 0.084. An average heterozygosity is calculated for 23 species of haplodiploid insects, and this value is significantly different from the same value for 18 Drosophila species or for 24 diploid insect species (including Drosophila). The niche width-genetic variation hypothesis is rejected as an explanation. A comparison of selection models and neutral models shows that both hypotheses are capable of explaining the data.

FOR over a decade, electrophoretic techniques have been used to measure amounts of genic variation in natural populations of animals and plants. Whether random drift of neutral alleles, mutation-selection equilibrium of slightly deleterious alleles or balancing selection best explains the substantial amount of protein polymorphism that has been uncovered is controversial (reviewed by LEWONTIN 1974; NEI 1975). Although there is an average difference between vertebrates and invertebrates (SELANDER and KAUFMAN 1973b), considerable similarity exists among species in percentage of loci that are polymorphic in populations and heterozygous in individuals. However, organisms that do not have a diploid, outbreeding genetic system may have unusually low or high levels of polymorphism and heterozygosity (SUOMALAINEN and SAURA 1973; SELANDER and KAUFMAN 1973a; PARKER and SELANDER 1976).

The haplodiploid genetic system of Hymenoptera is analogous to the sex-linked loci of most diploid animals. Reproduction involves arrhenotokous parthenogenesis; males develop from unfertilized eggs and are haploid, whereas females develop from fertilized eggs and are diploid. The haploidy of the male has a severe effect on most models that specify conditions leading to stable genetic polymorphisms. Haplodiploidy reduces recombination and dominance effects and causes temporal oscillatory changes in gene frequencies between the sexes. Some authors have predicted that populations with this genetic system will have fewer polymorphic loci because of selection against deleterious recessives in the male (SUOMALAINEN 1950, 1962; WHITE 1945; HARTL 1971), except at loci expressed only in females (KERR 1969, 1976). CROZIER (1977) has recently reviewed both

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empirical and theoretical research on the evolutionary genetics of the Hymenoptera.

This report deals with an analysis of genic variation at structural gene loci in three solitary (nonsocial) and four social species of Hymenoptera. The solitary species are *Opius juglandis* (Braconidae), a native North American parasitoid wasp of the larvae of fruit flies of the genus Rhagoletis; *Megachile pacifica* (Megachilidae), an introduced, trap-nesting, leaf cutter bee that has developed large populations in the western United States; and *Nomia melanderi* (Halictidae), a native burrowing bee nesting in large aggregations throughout the western United States. The social species are the paper wasps, *Polistes exclamans*, *P. annularis*, *P. apachus* and *P. bellicosus*, all native to western North America.

MATERIALS AND METHODS

Samples

Opius juglandis: Samples of adults were obtained after they emerged from Rhagoletis pupae collected at six localities in Arizona and New Mexico. (Table 1). Both *Rhagoletis juglandis* and *R. boycei* were host species at Safford and Oak Creek Canyon, but only *R. juglandis* was present in collections from other localities.

Magachile pacifica: Samples were collected by sweep-net from large populations nesting in artificial trap-nests in alfalfa fields (BOHART 1972) at five localities in Utah and California (Table 1).

Nomia melanderi: Three samples were collected. At Loma, Colorado, individuals were taken by sweeping in an alfalfa field; at Artois and Willows, California, nesting aggregations were located and individuals were netted above the burrows.

Polistes species: Nests were collected and all individuals in residence were taken to the laboratory in plastic bags. Collecting was confined to Texas, and the number of colonies (nests) examined is indicated for the four species in Table 1.

Laboratory Procedures

All specimens were stored at — 76° prior to processing for electrophoresis. Individuals of the solitary species were sexed and then homogenized with one to four drops of deionized water, depending on the size of the individual. Only the head and thorax of the Polistes species were ground except for *P. exclamans*, which was ground whole. Polistes were homogenized in three to six drops of deionized water.

Electrophoresis was carried out with the apparatus and methods described by SELANDER *et al.* (1971). Proteins stained were: glucose-6-phosphate dehydrogenase (*G6pd*), α-glycerophosphate dehydrogenase (*αGpd*), 6-phosphogluconate dehydrogenase (*6Pgd*), isocitrate dehydrogenase (*Idh*), malate dehydrogenase (*Mdh*), hexokinase (*Hk*), phosphoglucose isomerase (*Pgi*), phosphoglucomutase (*Pgm*), glutamic-oxaloacetic transaminase (*Got*), peptidase (*Pep*), leucine aminopeptidase (*Lap*), esterase (*Est*), superoxide dismutase (*Sod*) and nonenzymatic protein (*Pt*). Stain recipes for hexokinase and peptidase were those of SHAW and PRASAD (1970).

RESULTS

Mendelian segregation could not be checked by progeny testing, but allozymes at polymorphic loci occurred in females and males in a way consistent with expectations for Mendelian loci in a haplodiploid organism. Allozyme frequencies derived from genotypes for both sexes were used to compute estimated heterozygosity (in females) from the Hardy-Weinberg equation; 95% confidence limits

TABLE 1

Samples of Hymenoptera collected for studies of genic variation

Species	Locality	Number of* Colonies	Number of* Haploid genomes
Solitary species			
Opius juglandis	10 mi SW Safford, Arizona	—	53
	6 mi W Mt. Lemmon, Arizona	—	49
	3 mi N Carrizo, Arizona	—	64
	10 mi SW Flagstaff, Arizona	—	65
	4 mi SW Prescott, Arizona	—	40
	4 mi NW Reserve, New Mexico	—	70
	TOTAL		341
Megachile pacifica	1.5 mi S Sugarville, Utah	—	81
	Woodrow, Utah	—	81
	8 mi SE Dixon, California	—	73
	2 mi W Willows, California	—	71
	Davis, California	—	56
	TOTAL		362
Nomia melanderi	5 mi SE Orlando, California	—	119
	1.5 mi W Willows, California	—	77
	1 mi N Loma, Colorado	—	54
	TOTAL		250
Social species			
Polistes exclamans	Brenham, Texas	11	72
	Manor, Texas	13	68
	North Austin, Texas	15	86
	South Austin, Texas	18	80
	Brady, Texas	11	46
	Del Rio, Texas	7	28
	Dallas, Texas	5	38
	Garden City, Texas	5	26
	TOTAL	85	444
Polistes annularis	Briarcliff, Texas	16	128
Polistes apachus	Banquete, Texas	5	38
	Kingsville, Texas	1	8
	Lake Somerville, Texas	2	16
	TOTAL	8	62
Polistes bellicosus	Banquete, Texas	1	8
	Kingsville, Texas	3	24
	Padre Island, Texas	5	38
	TOTAL	9	70

* Mean number sampled per locus, but no more than eight haploid genomes (four females) per colony in social species.

were calculated (NEI and ROYCHOUDHURY 1974). In Opius and Nomia, female and male allele frequencies were compared by Fisher's exact test of independence in a 2 × 2 table. Too few males of Megachile were collected to make this test feasible.

Opius juglandis: As a species, this wasp was polymorphic at four of the 13 loci scored, although single populations were polymorphic at only two or three loci

TABLE 2

Allele frequencies at 13 loci in Opius juglandis

Locus*	Allele	Locality: Safford, Arizona	Mt. Lemmon, Arizona	Carrizo, Arizona	Flagstaff, Arizona	Prescott, Arizona	Reserve, New Mexico
αGpd-1	1	0.98(60)†	0.98(56)	1.00(71)	1.00(66)	1.00(44)	1.00(66)
	2	0.02	0.02				
6Pgd	1	0.94(54)	1.00(43)	0.76(55)	1.00(44)	0.98(44)	0.95(76)
	2	0.06		0.24		0.02	0.05
Pgm-2	1	0.09(45)	0.14(43)	0.27(62)	0.34(73)	0.21(44)	0.10(58)
	2	0.91	0.86	0.73	0.66	0.79	0.90
Pgm-3	1	1.00(45)	0.88(43)	0.94(68)	0.99(73)	0.89(44)	0.87(58)
	2		0.12	0.06	0.01	0.11	0.13
Proportion of loci polymorphic		0.15	0.15	0.23	0.07	0.15	0.23
Mean heterozygosity		0.024 ±0.006‡	0.038 ±0.014	0.067 ±0.024	0.047 ±0.022	0.044 ±0.017	0.039 ±0.013

* Invariant loci: *G6pd*; *αGpd-2*, *αGpd-3*; *Mdh-1*, *Mdh-2*, *Mdh-3*; *Pgi*; *Pgm-1*, and *Lap*.
† Number of haploid genomes sampled.
‡ 95% confidence interval.

(Table 2). At least three areas of esterase activity were present on the gels, but no system could be scored. Because esterases are, in general, highly polymorphic enzymes, their exclusion caused an underestimate of the genic variability in this species. The *Pgm-2* locus had the highest levels of heterozygosity. Neither *Pgm-2* nor *Pgm-3* was highly polymorphic, having only two allozymes each; the com-

TABLE 3

Allele frequencies at 19 loci in Megachile pacifica

Locus*	Allele	Locality: Sugarville, Utah	Woodrow, Utah	Dixon, California	Davis, California	Willows, California
6Pgd	1	1.00(70)†	0.97(60)	1.00(68)	1.00(64)	1.00(90)
	2		0.03			
Pgi	1	0.98(114)	0.97(68)	0.96(72)	0.97(72)	0.98(65)
	2	0.02	0.03	0.04	0.03	0.02
Pgm-2	1			0.01		
	2	1.00(92)	1.00(126)	0.99(122)	1.00(67)	1.00(69)
Est-4	1	0.66(92)	0.54(132)	0.51(92)	0.47(58)	0.38(60)
	2	0.34	0.46	0.49	0.53	0.62
Number of loci		18	19	17	15	14
Proportion of loci polymorphic		0.06	0.05	0.06	0.07	0.07
Mean heterozygosity		0.027 ±0.012‡	0.032 ±0.013	0.035 ±0.015	0.037 ±0.018	0.036 ±0.019

* Invariant loci: *G6pd*; *αGpd*; *Idh*; *Mdh-1*, *Mdh-2*, *Mdh-4*; *Hk-1*, *Hk-2*; *Pgm-1*, *Pgm-3*, *Pgm-4*; *Pep-1*, *Pep-2*; *Est-1*; *Sod*; and *Pt-1*.
† Number of haploid genomes sampled.
‡ 95% confidence interval.

mon allozymes have an unweighted mean frequency of 0.81 and 0.93 in *Pgm-2* and *Pgm-3*, respectively. All the low-frequency allozymes apparently are shared, but side-by-side comparisons could not be made because the entire sample was used on a single run. Male and female allozyme frequencies did not differ significantly (at the 5% level) at any of the polymorphic loci.

Estimates of proportion of loci polymorphic per population, mean proportion of loci heterozygous per individual (with 95% confidence limits), and mean number of allozymes per locus are given in Table 2. Over all populations, the mean proportion of polymorphic loci is 0.163, and the mean heterozygosity ($\bar{H}$) is 0.043 (0.039–0.047). These values are lower than those for all other insect species surveyed electrophoretically, except *Drosophila busckii* (PRAKASH 1973b).

Megachile pacifica: Of the 14 to 19 loci scored per population in this species, only one is polymorphic, *Est-4* (Table 3). Variant electromorphs were present at *Pgi*, *6Pgd*, and *Pgm-2*, but never in frequencies greater than 0.04. The Pgi^2 allozyme had a uniformly low frequency in all populations, but $6Pgd^2$ and $Pgm\text{-}2^1$ were unique to the Woodrow and Dixon populations, respectively. Variation among populations is limited to the *Est-4* locus and the rare allozymes at *6Pgd* and *Pgm-2*; the estimates of genic variability emphasize the similarly among the collections (Table 3). This slight differentiation among populations probably is caused by the commercial sale and distribution of this species, but the low genic variability must have other explanations.

Nomia melanderi: All polymorphic loci are listed in Table 4. The *αEst* locus is active only in females and was the most polymorphic locus. Both esterases show rather large frequency differences between Colorado and California. Because *αEst* is active only in the female, and, in that sense, different from the other loci, it was omitted and a second heterozygosity value was computed for the normal haplodiploid loci. This second figure is considerable lower than the first. With the *αEst* locus included, Nomia, on the average, has higher values than the other two solitary species in proportion of loci polymorphic and mean heterozygosity (Tables 2, 3, and 4).

The *βEst* and *Pgi* loci were tested for significant allozyme frequency differences between the sexes. None of the loci tested showed a significant difference, but sample sizes were small. Until very large samples of haplodiploid organisms are tested, we will be uncertain whether to attribute sexual differences to sampling variance or to changes of allozyme frequencies in males or females causing the oscillation between generations and sexes characteristic of the haplodiploid genetic system.

Polistes annularis: Of the 15 loci scored, 12 were monomorphic. For the three polymorphic loci, *Idh*, *Pgi*, and *Est-4*, allozyme frequencies and heterozygosities are given in Table 5. The *Est-4* locus was the most polymorphic and accounted for about one-half of the heterozygosity recorded in this species.

Polistes apachus *and* P. bellicosus: These species will be discussed together because their gene pools are linked by hybridization (LESTER, unpublished data). *P. apachus* and *P. bellicosus* are monomorphic for the same allozymes at eight

TABLE 4

Allele frequencies at 13 loci in Nomia melanderi

Locus*	Allele	Locality: Orlando, California	Locality: Willows, California	Locality: Loma, Colorado
6Pgd-1	1	0.98(48)†	0.95(20)	—
	2	0.02	0.05	
Mdh-2	1	0.98(126)	1.00(96)	1.00(65)
	2	0.02		
Pgi	1	0.06(135)	0.17(96)	0.03(61)
	2	0.94	0.80	0.97
	3		0.03	
Pgm	1	0.02(169)	0.03(98)	0.02(65)
	2	0.98	0.97	0.98
Pep	1	0.99(77)	1.00(50)	1.00(37)
	2	0.01		
βEst	1	0.94(148)	0.99(98)	0.62(65)
	2	0.05	0.01	0.38
	3	0.01		
αEst‡	1	0.59(130)	0.74(78)	0.87(32)
	2	0.41	0.26	0.13
Number of loci		13	13	12
Proportion of loci polymorphic		0.23	0.15	0.17
Heterozygosity (*αEst* included)		0.065 ± 0.022§	0.068 ± 0.022	0.066 ± 0.024
Heterozygosity (*αEst* excluded)		0.030 ± 0.008	0.042 ± 0.018	0.052 ± 0.029

* Invariant loci: *Gpd*; *G6pd*; *Idh*; *Mdh-1*, *Mdh-3*; and *Hk*.
† Number of haploid genomes sampled.
‡ Locus expressed only in females.
§ 95% confidence interval.

TABLE 5

Allele frequencies at 15 loci in P. annularis

Locus*	Allele	Frequency
Idh	1	0.99
	2	0.01
Pgi	1	0.27
	2	0.73
Est-4	1	0.16
	2	0.07
	3	0.77
Proportion of loci polymorphic		0.13
Mean heterozygosity		0.053 ± 0.019†

* Invariant loci: *αGpd*; *6Pgd*; *Mdh-2*; *Hk-1*, *Hk-2*; *Pgm-2*, *Pgm-3*; *Pep*; *Est-1*, *Est-3*; *Sod*; and *Pt-1*.
† 95% confidence interval.

loci: *G6pd*, *Hk-1*, *Hk-2*, *Mdh-1*, *Mdh-2*, *Pep*, *6Pgd*, and *Pgi*. Colonies identified as *P. bellicosus* also were monomorphic at two phosphoglucomutase loci (*Pgm-1* and *Pgm-3*) that were polymorphic in *P. apachus* (Table 6). Three loci were polymorphic in both species: *Pgm-2*, *Est-1*, and *Idh*. Both species had the same common allele at all loci except *Pgm-1* and *Est-1*.

A summary of the results is given in Table 6. The proportion of polymorphic loci and the average heterozygosity per individual are different in *P. bellicosus* (0.15 and 0.071) and *P. apachus* (0.38 and 0.084). In the four hybrid colonies, allozyme frequencies are appropriately intermediate at *Pgm-1*, *Est-1* and *Idh*. However, the frequency of $Pgm\text{-}3^2$ is higher than in *P. apachus*, the only parent possessing it. Another peculiarity is the appearance of an electromorph ($Pgm\text{-}2^3$) that was not detected in either parental form.

Polistes exclamans: This species was the most extensively sampled. Many loci were examined, but only 16 were reliably scorable. Seven loci showed no variation: *αGpd*, *Hk-1*, *Idh*, *Mdh-2*, *Mdh-3*, *Pgi* and *Pt-2*. At the remaining nine loci, variation was recorded at one or more of the localities. Three leucine aminopeptidase loci (*Lap-1*, *-2*, and *-3*) were among these. *Lap-1* had a single allozyme ($Lap\text{-}1^1$) at all localities except Brenham, where it was polymorphic for this allozyme and $Lap1^2$. *Lap-2* was polymorphic in all populations sampled (Table 7). At the following localities *Lap-3* had a fast electromorph ($Lap\text{-}3^1$): South Austin, Brady and Garden City. All three *Pgm* loci were polymorphic (Table 7). *Pep-1*, which was scored from tris-hydrochloric acid gels (buffer system 1, SELANDER *et al.* 1971), had an electromorph of faster mobility in the samples from North Austin ($Pep\text{-}1^1 = 0.02$), South Austin ($Pep\text{-}2^1 = 0.06$) and Garden City ($Pep\text{-}1^1 = 0.04$). A second peptidase locus differed from the first in being dimeric

TABLE 6

Allele frequencies at 13 loci in P. apachus *and* P. bellicosus

Locus*	Allele	*P. apachus*	*P. bellicosus*
Idh	1	0.87	0.96
	2	0.13	0.04
Pgm-1	1	0.92	—
	2	—	1.00
	3	0.08	—
Pgm-2	1	0.79	0.77
	2	0.21	0.23
	3	—	—
Pgm-3	1	0.89	1.00
	2	0.11	—
Est-1	1	—	0.02
	2	0.89	0.41
	3	0.11	0.57
Proportion of loci polymorphic		0.38	0.15
Mean heterozygosity		0.084 ± 0.020†	0.071 ± 0.027

* Invariant loci: *G6pd*; *6Pgd*; *Mdh-1*, *Mdh-2*; *Hk-1*, *Hk-2*; *Pgi*; and *Pep*.
† 95% confidence interval.

TABLE 7

Allele frequencies at 16 loci in P. exclamans

Locus*	Allele	Locality: Brenham	Manor	North Austin	North Austin	Brady	Del Rio	Dallas	Garden City
Pgm-1	1	0.96	0.98	1.00	0.87	0.96	0.88	1.00	1.00
	2	0.04	0.02	—	0.13	0.04	0.12	—	—
Pgm-2	1				0.06				
	2	0.94	0.97	0.97	0.91	0.98	1.00	1.00	1.00
	3	0.06	0.03	0.03	0.03	0.02			
Pgm-3	1	—	0.03		0.03	0.02	—	—	
	2	—	0.93	0.96	0.97	0.92	—	—	1.00
	3	—	0.04	0.04		0.06	—	—	
Pep-1	1			0.02	0.06				0.04
	2	1.00	1.00	0.98	0.94	1.00	1.00	1.00	0.96
Pep-2	1	1.00	1.00	0.92	0.99	1.00	0.79	1.00	1.00
	2			0.08	0.01		0.21		
Lap-1	1	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2	0.06							
Lap-2	1	0.01	0.17	0.21	0.10	0.12	0.21	0.05	0.08
	2	0.99	0.83	0.79	0.90	0.88	0.79	0.95	0.92
Lap-3	1				0.06	0.05			0.08
	2	1.00	1.00	1.00	0.94	0.95	1.00	1.00	0.92
Est-1	1	0.04	0.11					0.13	0.08
	2	0.96	0.89	1.00	1.00	1.00	1.00	0.87	0.92
Proportion of loci polymorphic		0.07	0.20	0.13	0.25	0.13	—	—	—
Mean heterozygosity		0.026 ±0.006†	0.043 ±0.012	0.046 ±0.012	0.048 ±0.011	0.037 ±0.010	—	—	—

* Invariant loci: *αGpd*; *Idh*; *Mdh-2*, *Mdh-3*; *Pgi*; *Hk-1*, and *Pt-2*.
† 95% confidence interval.

and having a slow electromorph that was best scored on phosphate gels (buffer system 7, SELANDER *et al.* 1971). The slow electromorph ($Pep\text{-}2^s$) was detected in samples from North Austin ($Pep\text{-}2^s = 0.08$), South Austin ($Pep\text{-}2^s = 0.01$) and Del Rio ($Pep\text{-}2^s = 0.21$). Only one esterase locus had a strong enough banding pattern to be scored consistently, and it had a low-frequency allozyme present among the colonies from Dallas ($Est\text{-}1^1 = 0.13$), Brenham ($Est\text{-}1^1 = 0.04$), Manor ($Est\text{-}1^1 = 0.11$) and Garden City ($Est\text{-}1^1 = 0.08$). In Table 7, values are given for gene frequency at variable loci, proportion of loci polymorphic and mean heterozygosity with confidence intervals. All populations are heterogeneous in that the samples were obtained from different sites within the localities, except for the collection at Brady where all of the nests were obtained from a single house and its outbuildings. The fact that this population had the lowest heterozygosity may be significant.

DISCUSSION

Three other surveys of genic variability in Hymenoptera species have been

TABLE 8

Estimates of heterozygosity in Hymenoptera

Study	Number of species*	Mean number of loci	Mean of heterozygosity per species
SNYDER (1974)	3	16	0.0
METCALF *et al.* (1975)	7	16	0.061
PAMILO *et al.* (1978)	6	16	0.010
This study	7	14	0.053
Mean	—	15.4	0.037

* Only species scored for 12 or more loci using samples of 50 or more haploid genomes are tabulated.

published. SNYDER (1974) examined collections of three bee species, using electrophoretic techniques, and found no allozymic variability even though he looked at 12 to 24 loci in each species. METCALF, MARLIN and WHITT (1975) studied seven species of solitary Hymenoptera from different genera (five wasps and two bees) and reported mean heterozygosities ranging from 0.038 to 0.078 (Table 8). PAMILO, VARVIO-AHO and PEKKARINEN (1978) analyzed the allozymic variation in 15 hymenopteran species and described heterozygosities ranging from 0.0 to 0.064, based on 8 to 18 loci per species. These papers support the finding of low heterozygosity in the Hymenoptera reported above.

A summary of the genic variation in 23 hymenopteran species for which mean heterozygosity has been computed over 12 or more loci is given in Table 8. A number of other hymenopteran species have been studied at one or a few loci (CONTEL and MESTRINER 1974; CROZIER 1973; JOHNSON *et al.* 1969; MESTRINER and CONTEL 1972; PAMILO, VEPSALAINEN and ROSENGREN 1975; TOMASZEWSKI, SCHAFFER and THOMAS 1973), but these single-locus heterozygosities were omitted because in general they were selected for their polymorphism and would bias this analysis. For comparison, a record of heterozygosity in 18 Drosophila species (AYALA *et al.* 1974; KOJIIMA, GILLESPIE and TOBARI 1970; LAKOVAARA and SAURA 1971a,b; PRAKASH 1969, 1973a,b; PRAKASH, LEWONTIN and HUBBY 1969; SAURA 1974; YANG, WHEELER and BOCK 1972), three Homoptera species (KREPP and SMITH 1974; SAURA, HALKKA and LOKI 1973), two Coleoptera species (SUOMALAINEN and SAURA 1973) and one Orthoptera species (SELANDER and KAUFMAN 1973b) was compiled see (POWELL 1975). The mean heterozygosity for all 24 insect species is 0.155 ($s^2 = 0.00294$), and for the 18 Drosophila species it is 0.135 ($s^2 = 0.00333$). A Student-Newman-Keuls test was used to compare the means and the Hymenoptera mean was significantly different ($p < 0.01$) from both of the above means. Thus, the Hymenoptera have levels of genic variability significantly different from those of other insect species for a reason or reasons yet to be explained.

Before discussing the lower heterozygosity of haplodiploid species as a general phenomenon, it should be mentioned that there are reasons other than the effect of haplodiploidy for expecting reduced heterozygosity in some of the species

studied. As a parasitoid, Opius may experience periodic bottlenecks in population size due to changes in host abundance. The presence of a bottleneck in the recent past of the U.S. population of Megachile is clearly indicated by historical observations. This Eurasian species was first reported in this country in 1937 on the Atlantic Coast (MITCHELL 1937) and rapidly spread westward (BOHART 1972). Unfortunately, there is no information on the number and size of the colonizing events. In contrast, there is no reason to postulate historical constrictions of population size in Nomia. It is a common, native bee species in the west, occurring in large local aggregations that may be relatively stable over time. Polistes species are also widespread and common, but this may be a recent condition encouraged by the appearance of human structures. It appears that, in relation to solitary species, Polistes species are not hampered by low population size and high population viscosity in the maintenance of genic variability. Indeed, the Hymenopteran species examined have a remarkable uniform distribution of values for percent polymorphic loci and heterozygosity.

Haplodiploidy and models of genic variation

Niche-variation hypothesis: SNYDER (1974) used the niche-variation hypothesis to explain the absence of allozymic variation in three bee species. The niche variation model of genotype-environment interaction (VAN VALEN 1965) has a great deal of intuitive appeal, but the analyses of morphological measurements to test the hypothesis have been ambiguous (WILLSON 1969; SOULE and STEWART 1970; ROTHSTEIN 1973), and the studies based on allozyme analysis of natural populations have rejected the hypothesis (SABATH 1974; SOMERO and SOULE 1974). In contrast to the studies of natural populations, POWELL (1971) reported that experimental populations of Drosophila retained more genetic variation when subjected to more variable environmental conditions.

Unfortunately, there are few comparative data on niche width, and we must resort to inferences in analyzing the hypothesis. For instance, the larval stages of most Hymenoptera are insulated from the external environment by the host body, brood cell, plant material, soil or nest structure, and increased buffering from temperature fluctuations might reduce selection, causing allozyme polymorphisms. However, a Hymenopteran larva in a temperate climate probably experiences no less temperature fluctuation that a Drosophila larva in the litter on the floor of a tropical forest; yet, tropical Drosophila are significantly more variable. A similar argument applies to humidity and other physical factors.

If the physical component of the niche is not significantly constricted in the Hymenoptera, then the biotic component should be examined. Host specificity is one aspect of a narrow niche for phytophagous and parasitoid species. However, many nonaculeate Hymenoptera are polyphagous (ASKEW 1971); MUESEBECK, KROMBEIN and TOWNES 1951); also, there is no lack of host specificity in other insect groups, including Drosophila (KIRCHER and HEED 1970). It appears that no strong argument can be made that a narrow niche is a general characteristic of Hymenoptera species, and we concur with METCALF, MARLIN and

WHITT (1975) that the niche-variation argument is too weak to explain the general phenomenon of reduced heterozygosity.

Selection models: METCALF, MARLIN and WHITT (1975) contend that the evidence of low heterozygosity in haplodiploid species is proof of the action of selection on the haploid male. They conclude that their results "are consistent with substantial portions of observed enzyme variation being selectively not neutral." However, these authors did not examine the structure of the neutral theory to determine whether it would, in fact, yield a similar prediction concerning the level of heterozygosity in haplodiploid species. After the discussion of selection and neutral models, the reader will understand why these data cannot be used to argue the case of either selection or neutrality, as these authors have done. Both models predict that haplodiploid populations will contain fewer loci with alleles in intermediate frequencies.

Numerous treatments of the problem of polymorphism at sex-linked loci using this type of model are available. Early papers deal with the conditions for a stable equilibrium under selection (BENNETT 1957; HALDANE 1926; HALDANE and JAYAKAR 1964; LI 1967; MANDEL 1959). The primary complication is that males and females must be treated as separate populations. When comparing the haplodiploid and diploid models under selection, the intuitive response is that polymorphisms will be more difficult to maintain because, in the haplodiploid model, there are five genotypes that must have their fitnesses balanced instead of three. In this model, overdominance in females is not sufficient to maintain a stable equilibrium. However, if the fitness differences between homozygotes and hemizygotes are positively correlated, then heterozygote advantages in the females becomes necessary for an intermediate equilibrium. We have evaluated the equilibrium gene frequency and the conditions of a real, stable stationary state, if one exists, for selection models of overdominance, dominance and negative correlation between the sexes. An overdominance model in which the fitnesses of the homozygotes and hemizygotes are equal provides the greatest buffering of the inherent asymmetry in the haploidiploid fitness model and the closest approach to a simple diploid model of overdominance. For the other selection models, a stable equilibrium is either not possible, or more difficult to obtain in a haplodiploid than in a diploid population.

HARTL (1971, 1972) derives a form of the fundamental theorem of natural selection (FISHER 1930) for haplodiploid or sex-linked loci. Deleterious alleles go to extinction quite rapidly under this model, as HARTL's (1972) computer simulation demonstrates. More importantly, the following relationship holds: $d\,\bar{w}\bar{v}/dt$ ($\bar{w}$ = average female fitness, $\bar{v}$ = average male fitness) in a haplodiploid population equals (4/3) $d\bar{w}/dt$ in a comparable diploid population.

Everyone is familiar with numerous cases of sexual dimorphism, and it is common knowledge that the physiology of the sexes may differ markedly, as in humans. Sex-limited enzyme loci, such as the *αEst* locus in Nomia, indicate that this process of differentiation descends to the level of the genetic regulatory system. To have an equilibrium caused by opposing selection in the sexes may

be more common than its meager representation in the literature attests (LI, 1963).

CROZIER's (1970) computer-simulation study of genetic polymorphism in diploid and haplodiploid populations demonstrated no significant difference in the number of polymorphic loci maintained in his hypothetical populations, although the haplodiploid population had fewer polymorphisms. The reason for his finding is his use of random numbers for the assignment of fitness values. Our analysis shows that, for most models normally used to assign fitness values, haplodiploid populations will maintain fewer polymorphic loci under selection. This finding is in agreement with the conclusion of HARTL (1971), CROZIER (1977) and several earlier studies mentioned above.

Neutral model: The hypothesis that most amino acid substitutions in molecular evolution are neutral has been modified many times since it was first proposed by KIMURA (1968) and KING and JUKES (1969). Some of the most important changes concerning the fluctuation of selection coefficients around zero (OHTA 1972a; OHTA and KIMURA 1972), the relationship between population size and the level of selection considered effectively neutral (OHTA 1972b), and the effect of linkage on the drift of nearly neutral alleles (OHTA 1973; OHTA and KIMURA 1975; MAYNARD SMITH and HAIGH 1974; HAIGH and MAYNARD SMITH 1976).

Most of the analyses of the behavior of neutral alleles are based on the diffusion equation popularized by KIMURA (1962, 1964). We evaluated the probability of fixation of a mutant gene in a diploid and a haplodiploid population using the diffusion equation and WRIGHT's (1969) formulas for the variance effective numbers. If the populations have equal numbers of genes, the values of fixation probability are approximately equal, but if the populations have equal numbers of individuals, the fixation probability in the haplodiploid population may reach a maximum of 1.33 times that in the diploid population.

Unlike the equation for fixation probability, the integral expression describing the average number of generations to fixation cannot be evaluated explicitly (KIMURA and OHTA 1969, 1971). Some things can be deduced about the behavior of this quantity from the form of the equation. It is sensitive to changes in the variance of the between-generation gene frequency change ($V_{\Delta x}$), which has an inverse relationship to the value of the integral. Thus, the slightly larger values of $V_{\Delta x}$ for haplodiploid populations cause a decrease in the time to fixation of neutral alleles. In addition, the presence of fewer possible frequency states in an equivalently sized haplodiploid population speeds the drift to fixation (W. H. LI, personal communication).

A simple equation for the average heterozygosity of electrophoretic alleles (allozymes) was proposed by OHTA and KIMURA (1973). If all the effects of haplodiploidy on effective population size are assumed to be inoperative except the reduction of number of genes, then the relationship N_e (haplodiploid) = 3/4 N_e (diploid) can be used to compute the expected ratio of heterozygosity between diploid and haplodiploid populations of the same size. The equation for the diploid population's heterozygosity is $H_D = 1 - /\sqrt{1+8N_e v}$ and the

equation for the haplodiploid population is $H_{H-D} = 1 - 1/\sqrt{1+6N_e v}$ (v = the mutation rate to electrophoretic alleles). The ratio H_{H-D}/H_D approaches 0.75 as $N_e v$ diminishes and 1.0 as $N_e v$ increases. The ratio of $\bar{H}_{H-D}$ from Table 8 and $\bar{H}$ for the 24 insect species used above is 0.047/0.155, or 0.30. This value suggests that, in addition to the reduced N_e because of haploid males, other factors are operating to lower heterozygosity in haploidiploid populations. Selection, smaller population sizes and effects of haplodiploidy on the sampling of genes could all be operating to increase the difference between the observed heterozygosities over that predicted by the above model. (This analysis was suggested by an anonymous reviewer.)

The neutral argument can be described in terms of nearly neutral alleles, with selection coefficients fluctuating around zero. There are at least two reasons for a change in the effect of selection on a gene. First, the external or internal (genetic) environment can change and alter the intensity or direction of selection on the gene (OHTA 1972a; OHTA and KIMURA 1972). Second, a gene is linked to other loci and selection on linked alleles may bestow an apparent selective value on the neutral allele (OHTA 1973; OHTA and KIMURA 1975; MAYNARD SMITH and HAIGH, 1974). In haplodiploid species, linkage should be increased over the level in diploids because there is no opportunity for crossing over or chromosome segregation in males.

Using OHTA's (1972a) equation for the effect of fluctuating selection intensities, it can be shown that the effects of haplodiploidy on the variance of selection coefficients and the variance of allele frequency changes will result in fewer mutants moving to fixation faster than in a diploid population. The effect of linkage depends on whether the allele is linked to deleterious or advantageous neighbors. Associative overdominance will be absent in males of a haplodiploid population. Also, the strength of this process is related to the load of deleterious alleles, which will be less in a haplodiploid population because of the increased dominance of deleterious mutants.

A more important factor may be the ability of advantageous mutants to carry linked alleles with them as they increase in frequency (*i.e.*, the "hitchhiking effect" of MAYNARD SMITH and HAIGH 1974). When an advantageous mutant moves to fixation, the frequencies of the alleles on the chromosome in which it originally occurred tend to increase (MAYNARD SMITH and HAIGH 1974). Because of the absence of recombination in the male, the length of chromosome over which the originally associated neutral alleles will be fixed is increased in comparison to species with recombination in both sexes. Over an additional portion of the chromosome, loci with neutral alleles will have their heterozygosity reduced by this effect. OHTA and KIMURA (1975) examined the hitchhiking effect using a diffusion model and concluded that this effect is not as important as MAYNARD SMITH and HAIGH (1974) suggested. The diffusion model was used to analyze the effect of certain parameters on the heterozygosity of the neutral mutant. Of particular interest is the effect of the product of effective population size (N_e) and recombination frequency (c). As $N_e \cdot c$ decreases, the hitchhiking effect increases in a geometric fashion. It is clear that the effect

of haploidiploidy will be to reduce both N_e and c, thereby increasing the hitchhiking effect in relation to a similar diploid population.

In summary, selection against recessive deleterious alleles is more effective in haplodiploid populations, and genetic equilibria are more difficult to maintain by selection. Haplodiploid populations have a smaller effective population size and a shorter time to fixation for new mutants than diploid populations of equal size. Associative overdominance is weak in haplodiploids because of the reduced load of deleterious alleles. The hitchhiking effect is stronger because of the lower level of recombination in haplodiploids. All of these and other differences between diploid and haplodiploid populations produce the result that fewer alleles are moving to fixation faster in haplodiploid populations, resulting in lower levels of heterozygosity.

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