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Genetic Differentiation Between Two Host "Races" and Two Species of Cleptoparasitic Bees and Between Their Two Hosts

Laurence Packer,[1,4] Angela Dzinas,[1] Karen Strickler,[2] and Virginia Scott[3]

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In this paper we test the following two hypotheses: (1) that apparently conspecific samples of the cleptoparasitic bee Coelioxys funeraria, *differing markedly in size and reared from different host species, do indeed represent one panmictic population; (2) that bees that nest in holes in wood or twigs have higher levels of genetic variation than those nesting in the ground. Based upon 41 loci, the genetic differences between the two samples of* C. funeraria *could be explained entirely in terms of sampling error. In contrast, the sympatric* C. moesta *showed 16 fixed allelic differences from the* C. funeraria *samples. Similarly, the two hosts of* C. funeraria, Megachile relativa *and* M. inermis, *had 21 fixed allelic differences between them out of 42 presumptive gene loci. Heterozygosities among the wood-nesting bees were not particularly high for Hymenoptera, ranging from 0.045 to 0.054. Comparisons of heterozygosity estimates among bees remain ambiguous as to whether soil nesting confers sufficient environmental buffering effects to reduce possible advantages of heterosis in ground-nesting species.*

KEY WORDS: bees; host races; genetic differentiation; heterozygosity.

INTRODUCTION

Female mass-provisioning bees gather together all the pollen and nectar required for the complete development of one offspring before ovipositing.

[1] Department of Biology, York University, 4700 Keele Street, North York, Ontario M3J 1P3, Canada.
[2] Parma Research and Extension Centre, 29603 University of Idaho Lane, Parma, Idaho 83660.
[3] University of Colorado Museum, Campus Box 218, Boulder, Colorado 80309-0218.
[4] To whom correspondence should be addressed.

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Consequently, the size of the emerging adult is determined primarily by the size of the pollen mass the mother constructs. Cleptoparasitic bees, which make up approximately 15% of the North American bee fauna (Bohart, 1970), lay their eggs on, or near, the pollen mass constructed by their hosts. In these bees offspring size is determined by the size of the provision mass gathered by females of the host species. Some cleptoparasitic species are quite variable in size. In some cases this is because they attack social hosts and parasite size variation reflects host caste size variation (Knerer, 1973). Other examples of cleptoparasite size variation are apparently caused by variation in size among alternative host species. However, in these cases it would be useful to have genetic data to verify that apparent host "races" are not cryptic species.

Coelioxys, a genus of cleptoparasitic bees in the family Megachilidae, conceal eggs in the cells of their hosts, which are usually bees of other genera of Megachilidae although sometimes other groups of solitary, long-tongued bees are attacked (Baker, 1975). The cells parasitized by *Coelioxys* are either under construction or being provisioned by the host female (Graenicher, 1927). Some *Coelioxys* attack more than one host species (Baker, 1975; Hurd, 1979). These hosts may vary in size, and as a result *Coelioxys* populations may contain individuals of several size classes.

In this paper we compare two host "races" of the cleptoparasitic bee *Coelioxys (Schizocoelioxys) funeraria* Smith to see whether samples reared from different hosts do represent one panmictic population. The population surveyed was bimodal in size, with smaller individuals emerging from nests of *Megachile relativa* Cresson and large ones from *M. inermis* Provancher. These two hosts differ markedly in size: Female *M. relativa* range from 9 to 12 mm in length and *M. inermis* females are between 15 and 20 mm long (Mitchell, 1962). Field observations indicate that the larger parasites attack only the larger hosts and that the smaller parasites attack only the smaller host species. Although *C. funeraria* has been recorded attacking five *Megachile* host species (Baker, 1975), only the aforementioned two hosts are utilized in the area surveyed. Samples of both host species were available for electrophoretic comparisons as were individuals of an additional cleptoparasitic species, *C. (Boreocoelioxys) moesta* Cresson. Voucher specimens of all species are housed in the Canadian National Collection and the University of Colorado Museum.

Haplodiploid insects typically have low levels of genetic variation in comparison to diplodiploids (Crespi, 1991; Packer and Owen, 1989, 1990, 1992; Shoemaker *et al.,* 1992). Many hypotheses have been put forward to explain this observation. One suggestion is that the nesting habit minimizes variance in environmental conditions reducing possible advantages of heterosis. Although this hypothesis was initially suggested for highly eusocial

species which maintain homeostatic control of the nest environment (Metcalf *et al.,* 1975; Snyder, 1975), it could also be applied to solitary species which nest in the ground because of the potentially ameliorating influence of the subterranean environment (Rosenmeier and Packer, 1993). In contrast, individuals maturing in a twig or stem nest may be exposed to a wider range of environmental variation (especially in temperature) than are larvae of ground nesting bees or juvenile insects capable of moving from unfavorable conditions. Consequently, one way in which the environmental buffering hypothesis could be tested would be to compare levels of heterozygosity between species that nest in the ground and those that nest in twigs and stems. Here we use genetic variation data from both hosts and parasites to make such a comparison.

METHODS

Bee Samples

Bees used in this study were obtained from trap-nests in Dickinson and Iron counties in Upper Michigan in the summer of 1992. Sample sites were at most 20 km apart, and as the intervening areas were also suitable for the host bees, we assumed that all samples represented one panmictic population and they were pooled for electrophoretic analysis. Traps were placed near the edges of meadows that were surrounded by secondary growth forest. Occupied nests were retrieved from the fields and opened in May 1993. Cells from all nests were removed, labeled, and reared individually in plastic tubes or dishes. They were then monitored daily for emergence of "host" bees or parasites (Strickler and Scriber, 1994). Within 36 hr after emergence, live adult bees were chilled in a refrigerator, identified to species (by V. Scott), transferred to cryotubes, and submerged in liquid nitrogen. When complete, all samples were shipped on dry ice to Toronto for analysis.

A subsample of 38 *Coelioxys funeraria* females used in electrophoresis had their heads removed for size measurements in order to document the discrete size distributions of the two host "races."

Electrophoretic Methods

Details concerning gel running conditions, enzyme staining recipes, and scoring procedures in the senior author's laboratory can be obtained from earlier papers (Packer and Owen, 1989, 1990, 1992). Because of the large variation in size among individuals sampled, bees were homogenized in varying amounts of grinding buffer (1% solution of dithiothreitol in double

Table I. Sample Sizes for Allozyme Loci Used in This Study

				Number of haploid genomes				
				Coelioxys[a]				
				funeraria		*moesta*	*Megachile*	
Enzyme	Locus	EC No.	Buffer	Host A	Host B	Host B	*inermis*	*relativa*
Aspartate aminotransferase	*Aat-1*	2.6.1.1	RSL				21	14
	Aat-2						21	14
Aconitate hydratase	*Acon-1*	4.2.1.3	CAM	23	29	6	31	14
	Acon-2			10	10	4	31	14
Aminoacylase	*Acy*	3.5.1.14	RSL	34	40	8	51	50
β-*N*-Acetylhexosaminidase	*Aha-1*	3.2.15.2	I	36	35	12	51	39
	Aha-2			41	35	4		
Adenylate kinase	*Ak-1*	2.7.4.3	CAM	28	35	12	42	25
	Ak-2			13	20	0	31	14
	Ak-3			28	35	12	31	25
Aldehyde dehydrogenase	*Alddh*	1.2.1.3	V	26	20	8	59	37
Arginine kinase	*Ark*	2.7.3.3	I	15	15	10	24	24
Diaphorase (NADH)	*Dia*	1.8.1*	V	41	35	8	25	20
Diaphorase (NADPH)	*Diap*	1.9.99*	V	9	9	6	8	8
Enolase	*Enol*	4.2.1.11	I	10	10	8	16	16
Esterase	*Est-1*	3.1.1.1	RSL	66	59	12	31	31
	Est-2						44	34
Fructose biphosphate aldolase	*Fba*	4.1.2.13	V				25	20
Fumarate hydratase	*Fum*	4.2.1.2	I	35	34	10	39	32
Glyceraldehyde-3-phosphate dehydrogenase	*Gapdh*	1.2.1.12	I	41	35	10	56	45
Guanine deaminase	*Gda*	3.5.4.3	V	28	25	11	63	40
Glycerol-3-phosphate dehydrogenase	*G3p-1*	1.1.1.8	I	44	44	10	19	41
	G3p-2			35	35	10	19	41
Glucose-6-phosphate isomerase	*Gpi*	5.3.1.9	I	26	18	8	46	31
Glucose-6-phosphate dehydrogenase	*G6pdh*	1.1.1.49	CAM	14	14	8	68	41
β-Hydroxyacid dehydrogenase	*Had*	1.1.1.30	I	36	30	6	62	48
Hydroxyacylglutathione hydrolase	*Hagh*	3.1.2.6	V	28	34	10		
Hexokinase	*Hk-1*	2.7.1.1	I	66	59	8	25	35
	Hk-2			45	40	4		
Isocitrate dehydrogenase	*Idh*	1.1.1.37	I	26	20	0	25	20
L-Iditol dehydrogenase	*Iddth*	1.1.1.15	V	18	25	6	42	18
Leucine aminopeptidase	*Lap*	3.4.11.1	I	36	30	8	41	36
Malate dehydrogenase	*Mdh-1*	1.1.1.37	CAM	63	69	14	51	46
	Mdh-2			63	69	14	72	50
Malate dehydrogenase NADP	*Me*	1.1.1.40	I	61	54	10	39	32

Table I. (continued)

				Number of haploid genomes				
				Coelioyus[a]				
				funeraria		*moesta*	*Megachile*	
Enzyme	Locus	EC No.	Buffer	Host A	Host B	Host B	*inermis*	*relativa*
Peptidase glycine-leucine	*Pep$_{gl}$*	3.4.11*	RSL	21	20	10	26	25
Peptidase leucine-alanine	*Pep$_{la}$*	3.4.11.13	RSL	53	42	2	26	26
Peptidase phenylalanine-proline	*Pep$_{pp}$*	3.4.13.8	RSL	37	35	10	47	40
Phosphogluconate dehydrogenase	*6Pgd*	1.1.1.43	CAM	0	0	0	52	69
Phosphoglycerate mutase	*Pgam*	5.4.2.1	I	27	21	0	25	20
Phosphoglycerate kinase	*Pgk*	2.7.2.3	V	15	15	0		
Phosphoglucomutase	*Pgm*	5.4.2.2	I	15	15	6	23	30
Pyruvate kinase	*Pk*	2.7.1.40	V	24	24	8	39	22
Superoxide dismutase	*Sod*	1.15.1.1	CAM	62	63	10	35	30
Uridine kinase pyrophosphorylase	*Ugp-1*	2.7.7.9	RSL	15	15	10	16	16
	Ugp-2			15	15	10	16	16

[a]Host A, *M. inermis;* Host B, *M. relativa.*

distilled water), ranging from 90 μl for the smallest to 300 μl for the largest individuals. Lists of loci scored, sample sizes (number of haploid genomes), acronyms, and associated enzyme commission numbers are given in Table I.

Although sample sizes for *C. moesta* were very small, estimates of genetic distance and identity are rendered more inaccurate by poor sampling of enzyme loci than small numbers of individuals (Nei, 1978). With a sample size of 36 loci scored for both cleptoparasitic bee species, we are confident that our measures of heterozygosity within and genetic differentiation between these two species are reasonably accurate.

Data Analysis

Heterozygosity and Nei's (1978) measures of genetic identity and distance were obtained using BIOSYS (Swofford and Selander, 1989).

Comparisons of heterozygosity among hymenopteran taxa were made using the mean value for all species surveyed in each genus or subgenus. This protocol precludes the phylogenetic bias that may occur when data from closely related species are included in the same analysis (see Crespi, 1991).

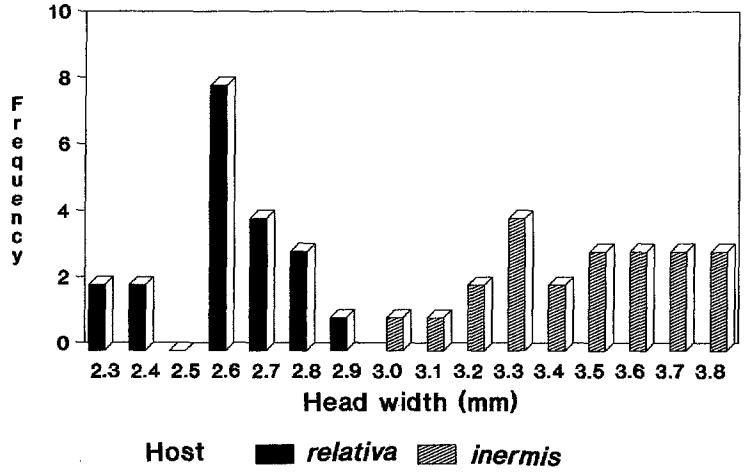

Fig. 1. Size frequency histogram for *Coelioxys funeraria* females reared from two *Megachile* host species.

RESULTS

Size variation among samples of the two host "races" of *C. funeraria* is provided in Fig. 1. Although the sample sizes are not large, there was no overlap in head width between host "races."

Forty-one loci were surveyed for *Coelioxys funeraria* samples, and 36 for *C. moesta* (Table I). Allelic mobilities and frequencies and expected heterozygosities for variable loci in these three samples are shown in Table II. Data for the two *Megachile* species are presented in Table III.

There were 16 fixed differences between the two *Coelioxys* species (at the loci *Acon-1, Acon-2, Aha-1, Alddh, Diap, Enol, Est, Gda, G3pd-1, G3pd-2, Gpi, G6pdh, Hk-2, Mdh-2,* Pep_{gl}, and Pep_{la}). One additional locus (Pep_{pp}) exhibited marked allele frequency differences between the two species. Combined with the monomorphic loci and those with minor allele frequency differences, these data gave a genetic identity estimate of 0.57 and a genetic distance of 0.55, based upon 36 loci.

In contrast, and as can be seen from Table II, there were no fixed differences between host "races" of *C. funeraria,* and all allele frequency differences were consistent with sampling error. Only two loci gave nonzero genetic distance estimates to the second decimal place (*Acy* and *Est*), and when all loci were combined the genetic distance was not detectably different from 0.000 and the genetic identity was unity, based upon all 41 loci.

The two *Megachile* species exhibited 21 fixed differences (at the loci *Aat-1, Acon-1, Acon-2, Acy, Ak-1, Ak-2, Ak-3, Alddh, Enol, Est-2, Fum, Gda,*

Table II. Allele Frequencies for Variable Loci for the Three Samples of Cleptoparasitic Bee Species Surveyed[a]

Parasite: Host:	*C. funeraria* *M. inermis*	*C. funeraria* *M. relativa*	*C. moesta* *M. relativa*
Locus			
Acy			
vs	0.000	0.000	0.375
s	0.029	0.025	0.625
m	0.714	0.575	0.000
f	0.257	0.400	0.000
H	0.43	0.52	0.5
N	34	40	8
Aha			
s	0.951	0.914	–
f	0.049	0.086	–
H	0.09	0.16	
N	41	35	
Est			
vs	0.136	0.169	0.000
s	0.000	0.000	0.875
m	0.773	0.831	0.000
f	0.091	0.000	0.000
vf	0.000	0.000	0.125
H	0.38	0.28	0.23
N	66	59	8
Gpi			
s	0.500	0.611	–
f	0.500	0.389	–
H	0.51	0.51	–
N	26	18	
Hk-2			
s	0.622	0.600	–
f	0.378	0.400	–
H	0.48	0.49	
N	45	40	
Pep_{gl}			
s	–	–	0.500
f	–	–	0.500
H			0.526
N			10
Pep_{la}			
s	0.000	0.024	–
f	1.000	0.976	–
H	0.0	0.06	–
N	53	42	
Pep_{pp}			
s	0.054	0.029	0.375
m	0.000	0.000	0.375
f	0.946	0.971	0.250
H	0.10	0.06	0.70
N	37	35	8
Mean *H*	0.049	0.051	0.054
Alleles/locus	1.195	1.195	1.139
% polymorphic (1%)	14.6	17.1	11.1

[a]Thirteen loci which were not variable within either species had fixed differences between the *C. funeraria* and the *C. moesta* samples; see text. vs, very slow; s, slow; m, medium; f, fast; H, heterozygosity; N, number of haploid genomes.

Table III. Gene Frequencies for Two *Megachile* Species[a]

	M. relativa	*M. inermis*
Acon-2		
s	–	0.903
f	–	0.097
H	–	0.18
N		31
Acy		
s	0.940	–
f	0.060	–
H	0.11	–
N	50	
Aha		
s	0.871	0.961
f	0.129	0.039
H	0.23	0.08
N	39	51
Est-1		
s	0.129	0.000
m	0.839	1.000
f	0.032	0.000
H	0.28	0.0
N	31	33
Est-2		
s	–	0.114
m	–	0.772
f	–	0.114
H	–	0.45
N		44
Fum		
s	0.031	–
f	0.969	–
H	0.06	–
N	32	
Gda		
s	0.950	–
f	0.050	–
H	0.10	–
N	40	
Gpi		
s	0.968	1.000
f	0.032	0.000
H	0.06	0.0
N	31	46
G6pdh		
s	0.073	–
m	0.902	–
f	0.024	–
H	0.18	–
N	41	

Table III. (continued)

	M. relativa	*M. inermis*
Had		
s	0.042	–
m	0.937	–
f	0.021	–
H	0.12	–
N	48	
Hk		
s	0.486	0.680
f	0.514	0.320
H	0.51	0.44
N	35	25
Iddth		
s	0.111	1.000
f	0.889	0.000
H	0.20	0.0
N	18	42
Mdh-1		
s	0.022	0.000
m	0.000	0.039
f	0.978	0.961
H	0.04	0.05
N	46	51
Pep_{gl}		
s	0.000	0.077
m	0.960	0.923
f	0.040	0.000
H	0.08	0.14
N	25	26
6Pgd		
s	0.192	0.000
m	0.808	0.058
f	0.000	0.942
H	0.13	0.11
N	52	69
Mean *H*	0.05	0.045
Alleles/locus	1.38	1.17
% polymorphic (1%)	30.95	16.67

[a]The following loci had fixed differences—with *M. inermis* having the faster allele: *Ak-1, Ak-3, G3p-1, G3p-2, Mdh-2, Me,* and Pep_{pp}. *M. relativa* had the faster mobility for the loci *Aat-1, Acon-1, Ak-2, Alddh, Enol, Pgm,* and *Pk.* For *Acon-2, M. relativa* was fixed for an allele with a mobility intermediate between those found for the polymorphic *M. inermis.* s, slow; m, medium; f, fast; H, heterozygosity; *N*, number of haploid genomes.

G6pdh, G3pdh-1, G3pdh-2, Had, Mdh-2, Me, Pep_{pp}, *Pgm,* and *Pk*) in addition to 2 loci with marked allele frequency differences (*Iddh* and *6Pgd*). These results gave a genetic identity of 0.49 and a genetic distance of 0.71.

The mean number of alleles per locus, proportion of loci that were

Table IV. Heterozygosity Estimates for Bee Subgenera That Nest in the Ground or in Twigs and Stems

Genus	Subgenus	Species	Generic/ subgeneric average H (SD)	No. of loci	Reference[a]
		Ground-nesting species			
Agapostemon	Not subdivided	3	0.031 (0.031)	na[b]	Crespi (1991)
Lasioglossum	*Dialictus*	7	0.037 (0.020)	na[b]	Crespi (1991)
Lasioglossum	*Evylaeus*	2	0.028 (0.009)	27	Packer *et al.* (1993)
Halictus	*Halictus*	*rubicundus*	0.049	33	Packer and Owen (1989)
Halictus	*Seladonia*	2	0.023 (0.008)	32	Rosenmeier and Packer (1993)
Augochlorella	*Augochlorella*	*striata*	0.116	33	Packer and Owen (1990)
Svastra		*obliqua*	0.038	16	Graur (1985)
		Stem- and twig-nesting species			
Megachile	*Megachile*	2	0.059 (0.007)	28	This paper
Megachile	*Eutricharaea*	*rotundata*	0.033	19	Crespi (1991)
Coelioxys	*Schizocoelioxys*	*funeraria*	0.056	28	This paper
Coelioxys	*Boreocoelioxys*	*moesta*	0.055	28	This paper

[a]Data from papers with the senior author as an author were used in the comparison of data from one laboratory only; all data listed above were used in the other comparison (see text).
[b]Data not available.

polymorphic, and expected heterozygosities for all samples are shown in Tables II and III. All heterozygosity values fall between 0.045 and 0.054, not unusually high for hymenopteran heterozygosity but low for insects in general (Graur, 1985; Packer and Owen, 1990).

DISCUSSION

In this paper we test two hypotheses. First, we test the null hypothesis that samples of *C. funeraria* which differed markedly in size and were reared from different host species are not in fact genetically differentiated taxa. Second, we test the hypothesis that comparisons of heterozygosity among bee taxa are detectably influenced by the environmental buffering effects of the nest environment in ground-nesting bees.

Coelioxys funeraria has a wide range of hosts including members of at least four subgenera of the genus *Megachile* (Baker, 1975). In Michigan, trap nest data reveal that two hosts of very different sizes (*M. relativa* and *M.*

inermis) are attacked; no other host *Megachile* species were found in the trap surveys. Genetic data obtained from parasites reared from nests of the two hosts indicate unambiguously that they are the same species: There are no fixed allelic differences, and no significant allele frequency differences between the samples, and the genetic distance between them was zero to the third decimal place. This is despite the fact that field observations suggest that small parasites always attack the small host and large parasites attack only the large host (Scott, unpublished observations). In contrast, there were no fewer than 16 fixed allele differences between *C. funeraria* and *C. moesta:* species which are in different subgenera (Baker, 1975). There were also 21 fixed differences between the two *Megachile* hosts, which are consubgeneric, both being in the nominate subgenus (Hurd, 1979). Genetic identity estimates for both congeneric species pairs are consistent with their being nonsibling species according to the literature survey results of Brussard *et al.* (1985).

Causal analyses of differences in heterozygosity data among taxa are fraught with difficulties, predominant among them being the influence of locus sample size and interlaboratory variation on the estimated level of genetic variability (Simon and Archie, 1985; Singh and Rhomberg, 1987; Rosenmeier and Packer, 1993). Here it was possible for us to compare heterozygosity estimates using the same suite of loci scored in the same laboratory for three subgenera of twig-nesting bees (four species) and four subgenera (five species) of ground-nesting species. Heterozygosity estimates for the suite of loci common to all studied species are listed in Table IV. The variation in total number of loci among taxa results from multiple loci for the same enzyme staining system being recorded in some studies. All three wood-nesting taxa had higher heterozygosities than all soil-nesting taxa except for *Augochlorella striata,* which has the highest heterozygosity recorded for any bee species (Packer and Owen, 1990). A Mann–Whitney U test indicated that there is no significant difference in average heterozygosity between nest site types ($U_s = 9$, $P > 0.1$, one-tailed test). However, *A. striata* nested in thin layers of soil among stones and developing brood and adults experienced extremely high variation in both temperature and humidity as a result of the heat-retaining capacity of the stones (Packer *et al.,* 1989; Packer and Owen, 1990). When the heterozygosity estimate for this species is removed from the comparison, the resulting test is significant ($U_s = 9$, $P = 0.05$). When data from other laboratories are added to ours (Table IV), the conclusions remain the same ($U_s = 21$; $P = 0.1$ or 0.05 when *A. striata* is left in or removed from the data, respectively). However, such jiggery-pokery with a small data set seems difficult to justify and the potential effects of environmental buffering of soil nests upon heterozygosity levels in Hymenoptera must be considered as remaining inadequately tested. Additional

electrophoretic data from wood-nesting halictids and ground-nesting megachilids would be useful as would some allodapine scrunching.

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