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Research article

Genetic differentiation across a behavioural boundary in a primitively eusocial bee, *Halictus poeyi* Lepeletier (Hymenoptera, Halictidae)

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Summary. In Florida, the primitively eusocial bee, *Halictus poeyi* Lepeletier exhibits two different colony cycles: populations in northern and central Florida are univoltine with an annually brooded colony cycle, while populations in southern Florida, including the Florida Keys, are multivoltine with a continuously brooded colony cycle. In this study, we test the hypothesis of reduced gene flow between the colony types by comparing levels of genetic differentiation among populations having similar colony phenologies with that among populations having different colony phenologies. We use allozyme markers to estimate F_{ST}, a robust measure of genetic differentiation. We found that genetic differentiation is not significantly higher between populations having different colony phenologies than between populations having similar colony phenologies. Environmental conditions thus may play an important role in influencing the expression of the alternative colony types of *H. poeyi* populations in Florida.

Key words: Genetic differentiation, gene flow, behavioural plasticity, *Halictus poeyi*, Halictidae.

Introduction

Social behaviour, ranging from communal living and cooperative breeding to fixed castes with a reproductive division of labour between generations (eusociality), has evolved multiple times in several insect orders (reviewed in Crespi and Choe, 1997). Among insects, eusociality evolved most frequently in the order Hymenoptera, and particularly often in the bee family Halictidae (Michener, 1974; Richards, 1994; Danforth and Eickwort, 1997; Packer, 1997; Danforth et al., 1999). There are at least seven genera/subgenera of halictines that contain both solitary and eusocial species (reviewed by Packer, 1997). Not only does social organization vary between closely related species in the Halictidae, it also varies within the same species; there are at least 9 species that are known to be behaviourally polymorphic (i.e. they have different social organization in different populations or even in different nests of the same population) (Packer, 1997; Wcislo and Danforth, 1997). For example, the usually social *Halictus rubicundus* (Christ) has been found to be solitary at high elevations in the Colorado Rockies but eusocial at a lower altitude (Eickwort et al., 1996). This is also true of *Lasioglossum* (*Evylaeus*) *calceatum* (Scopoli) in Japan (Sakagami and Munakata, 1972), and *L. (E.) albipes* (Fabricius) in France (Plateaux-Quénu, 1989;1993).

Environmental conditions seem to play a major role in shaping the social organization of halictine bees (Hogendoorn and Leys, 1997; reviewed by Wcislo, 1997). Yanega (1993; 1997) showed that abiotic factors during brood production can affect the expression of social behaviour in halictine bees by influencing the colony's demography. For example, the production of males in *Halictus rubicundus* (Yanega, 1993) is associated with high temperatures and long photoperiods. Increasing male bias in the first brood is, in turn, associated with decreasing proportions of social colonies (Yanega, 1993). Similarly, Richards and Packer (1995; 1996) and Dunn et al. (1998) have shown that the expression of eusocial colony behaviour, in *H. ligatus* Say and *H. poeyi* Lepeletier respectively, is strongly affected by local environmental conditions, mainly temperature and moisture (precipitation). Thus, variation in environmental conditions, by altering body/brood size and colony demographics/survival, influences the social behaviour of halictine bees.

Genetic factors, although less studied, may also be important in influencing social behaviour, and the extent of its plasticity. For example, Plateaux-Quénu et al. (2000) reared eusocial and non-eusocial populations of *L. (E.) albipes* in the laboratory under different photoperiod and temperature regimes. They discovered that most females from non-eusocial populations retained their behaviour under temperature and photoperiod regimes typical of social populations. Similarly, females from eusocial populations retained their behaviour under regimes typical of non-eusocial popula-

tions. Thus, the expression of social behaviour, must, at some level, have a genetic basis. Nonetheless, it is clear that the actual social behaviour observed depends also upon environmental factors, including the behaviour of nest mates (Wcislo, 1997).

Halictus poeyi is a primitively eusocial bee found in the southeastern USA (Packer, 1999). It is morphologically indistinguishable from its genetically divergent sibling species *H. ligatus* (Carman and Packer, 1996; Danforth et al., 1998), which is found to the north and west of the range of *H. poeyi*. Two alternative colony cycles have been described for *H. poeyi* populations. In northern Florida (Packer and Knerer, 1987), *H. poeyi* (studied as *H. ligatus*) has an annual colony cycle typical of many temperate primitively eusocial sweat bees: queens initiate a nest in the spring, produce one or more worker broods, then a reproductive brood near the end of the colony cycle during late summer and autumn. However, in southern Florida and the Florida Keys, *H. poeyi* has a continuously brooded colony cycle, where nests can be initiated at any time of the year, and the entire colony cycle lasts less than 6 months (Packer and Knerer, 1987). Unlike the univoltine annually brooded colonies in northern Florida, continuously brooded colonies of *H. poeyi* are multivoltine (Packer and Knerer, 1986; 1987). Data from intermediate sites in central Florida were inconclusive as to whether the colonies were annual or continuously brooded (Packer and Knerer, 1987). Phylogenetic analysis of the genus *Halictus* (Danforth et al., 1999) shows that the continuously brooded colony cycle is derived. The sub-tropical climate of southern Florida is suitable for bee activity throughout the entire year, and must be responsible, at some level, for this change in colony phenology (Packer and Knerer, 1986).

Commonly neglected in discussions of evolution and speciation in favor of morphology, behaviour is often the first aspect of the phenotype to evolve in new directions (West-Eberhard, 1989; reviewed by Wcislo, 1989). The apparently abrupt transition (approximately 160 Km) between annually brooded and continuously brooded colony cycles for *H. poeyi*, in Florida, provides an interesting opportunity to test the effects of alternative adaptations (as per West-Eberhard, 1986; 1989) upon the genetic differentiation of populations. The alternative colony cycles of *H. poeyi* in Florida are presumably adaptive strategies induced by the differing climatic regimes (temperate and sub-tropical) in Florida. Assuming that the differences between the two colony cycles are genetically influenced, one might expect reduced gene flow between the two different colony phenologies, due to selection against intermediates. This would eventually lead to increased genetic differentiation between populations with different colony cycles, as genetic drift will not be countered effectively by gene flow across the behavioural boundary. Different selection pressures accompanied by drift and reduced migration should increase the levels of genetic differentiation between populations with different colony phenologies. In this paper, we examine the levels of genetic differentiation between *H. poeyi* populations having similar and different colony phenologies.

Materials and methods

Collection of samples

Halictus poeyi samples were collected from 9 sites in Florida, USA, between May 6th and 21st 2000 (Table 1). All bees were collected while foraging mainly on the composite flower *Bidens pilosa* between 10:00 am and 3:00 pm, the period of peak bee activity. Bees were stored in liquid nitrogen, and later transferred to a −80 °C freezer at York University. Voucher specimens of *Halictus poeyi* collected for this study were deposited in the York University bee collection, and the Royal Ontario Museum insect collection.

Sampling sites 1 to 7 (MIL, POR, AUG, CRO, OCA, GUL, and CHR. Table 1) were classified as annually brooded populations since they all lie north of the behavioural boundary, as discovered by Packer and Knerer (1986). Similarly, sampling site 9, in Key West, was classified as a continuously brooded population as it falls within the continuously brooded region outlined by Packer and Knerer (1986). Site 8 (JUP) was classified as continuously brooded, since a continuously brooded phenology was observed nearby (Packer and Knerer, 1987).

Allozyme electrophoresis

Allozyme electrophoresis on horizontal starch gels was used to examine genetic differentiation in *H. poeyi* samples. Enzyme staining recipes, gel recipes, gel running conditions, and scoring procedures followed Packer and Owen (1989; 1990). Nine loci were found to be polymorphic (Table 2).

Genetic differentiation

Genetic differentiation was measured as the population subdivision statistic, F_{ST} (Wright 1951). Weir and Cockerham's (1984) estimator of F_{ST}

Table 1. *Halictus poeyi* sample sites in Florida, May 2000

Site	Location	Colony Type	Sample Size (N)
1. Milton (MIL)	30.63 °N 87.05 °W	Annually brooded	15
2. Port Saint Joe (POR)	29.82 °N 85.31 °W	Annually brooded	31
3. St. Augustine (AUG)	29.83 °N 81.38 °W	Annually brooded	31
4. Cross City (CRO)	29.64 °N 83.14 °W	Annually brooded	24
5. Ocala (OCA)	29.32 °N 81.97 °W	Annually brooded	21
6. Gulf Hammock (GUL)	29.25 °N 82.72 °W	Annually brooded	30
7. Christmas (CHR)	28.54 °N 81.04 °W	Annually brooded	25
8. Jupiter (JUP)	26.92 °N 80.11 °W	Continuously brooded	31
9. Key West (KEY)	24.57 °N 81.74 °W	Continuously brooded	21

Table 2. Enzyme names, abbreviations, EC numbers and buffer systems used

Enzyme	Acronym	EC number	Buffer System
Adenylate kinase	*Ak-1*	2.7.4.3	CAM
Aldehyde dehydrogenase	*Alddh*	4.1.2.13	AYALA B
Arginine kinase	*Ark*	2.7.3.3	CAM
Diaphorase (NADH)	*Dia*	1.8.1.n	AYALA B
Esterase	*Est-3*	3.1.1.1	RSL
Glycerol-3-phosphate dehydrogenase	*G3pdh*	1.1.1.8	BI
Glucose-6-phosphate dehydrogenase	*G6pdh*	1.1.1.49	CAM
Hexokinase	*Hk*	2.7.1.1	BI
Phosphogluconate dehydrogenase	*6Pgd-1*	1.1.1.43	CAM

was calculated from the allozyme data using the population genetics software package GENEPOP 3.2a (Raymond and Rousset, 1995a). Weir and Cockerham's (1984) estimator of F_{ST} is robust since its formulae do not make assumptions concerning numbers of populations, sample sizes, or heterozygote frequencies. These properties of F_{ST} render it very useful in providing robust estimates of genetic differentiation among populations, and is preferable to indirect measures of gene flow such as *Nm* (Whitlock and McCauley, 1999). Departure from HW equilibrium, for all loci and sites, was examined using an exact HW test, following Weir (1990), as implemented in GENEPOP 3.2a (Raymond and Rousset, 1995a). Loci not at Hardy-Weinberg equilibrium were not included in the analysis.

An exact test for population differentiation was used to corroborate the F_{ST} data. We used Raymond and Rousset's (1995b) exact test for genic differentiation between population pairs, as implemented in GENEPOP 3.2a (Raymond and Rousset, 1995a) to test departures from the null hypothesis of identical allelic distributions across populations. Significant *P*-values indicate genic differentiation.

We should note that that measured F_{ST} is a long term historical variable. Assume a situation in which gene flow had been common until some recent fragmentation event that completely precludes transfer of individuals among populations. F_{ST} estimates now would reflect the historically high gene flow rather than its contemporary absence (Roderick, 1996; Packer and Owen, 2001).

Statistical analysis

Most conventional statistical tests are inappropriate for comparing pairwise estimates of F_{ST} because these are not independent from one another (e.g. Slatkin, 1993). However, we wish to answer a very simple question; is there less genetic differentiation between populations having the same colony cycle than there is between populations having different colony cycles? We separated pairwise F_{ST} values into two groups: within similar colony phenologies (i.e. between pairs of populations that have the same colony cycle), and between different colony phenologies (i.e. between populations with different colony cycles). We used the nonparametric Mann-Whitney U test to test the null hypothesis of increased genetic differentiation between populations with different, versus populations having the same, colony cycle.

Finally, variation in geographic distance between populations can potentially confound the pairwise genetic differentiation results (Peterson and Denno, 1998; Packer and Owen, 2001). Theoretically, the accumulation of local genetic differences between two populations, due to drift, should increase proportionally with pairwise distance (e.g. large pairwise distance will correspond to large F_{ST} estimates, and vice versa). This is referred to as isolation by distance (Slatkin, 1993; Hutchison and Templeton, 1999). If an isolation by distance pattern is evident in the data set, then comparisons of pairwise F_{ST} within similar, and between different, colony phenologies should be conducted only at similar geographical distances. Prior examination of the pairwise matrix of geographic distances revealed that mean pairwise distance within similar colony phenologies (=251.4 km ± 159.9 S.D.), was nearly half of that between different colony phenologies (=517.8 km ± 183.4 S.D.). Both groups are normally distributed, and their variances are not significantly different ($F=0.20$, $p=0.88$). The area of overlap between the normal distribution of pairwise distance from the two groups is approximately the upper half of the distance distribution in the 'within similar colony phenologies' group (≥251.4 km), and the lower half of the 'between different colony phenologies' group (≤517.8 km). If isolation by distance is detected, then comparisons of pairwise F_{ST} will be conducted in this area of overlap. We detect isolation by distance, using the entire dataset, following Rousset (1997) by regressing $F_{ST}/(1-F_{ST})$ against the natural logarithm of distance (ln d). We use a mantel test (Mantel, 1967), with 1000 permutations, to examine the significance of the regression, as implemented in GENEPOP 3.2a (Raymond and Rousset, 1995a).

Results

Isolation by distance, and F_{ST}

Allele frequencies for the nine polymorphic loci used for this study are presented in Table 3. Three loci were not at HW equilibrium for a total of 7 sites: *Alddh* (at OCA, CHR, and KEY), *G6pdh* (at JUP and KEY), and *6-Pgd-1* (at POR, OCA, and KEY) (Table 3).

In our within colony type F_{ST} estimates (Table 4), all but one pair are from annual brooded populations, as only two localities were sampled from the continuously brooded area. Fig. 1 shows that this one continuously brooded estimate falls well within the range of both the F_{ST} and distance variables for the within similar colony phenology estimates. Thus, the pairwise F_{ST} estimate from the two continuously brooded populations was combined with the estimates from the annually brooded populations to form the within similar colony phenologies group.

The regression between $F_{ST}/(1-F_{ST})$ and ln d, for all 36 pairwise comparisons, has a small negative slope (Fig. 1), but this is not significant (Mantel test, one tailed $p=0.5780$), thus isolation by distance is not present. If gene flow is selected against, then pairwise F_{ST} is expected to be higher between than within colony phenologies. In fact, the opposite pattern is found, but this difference is also not significant (F_{ST} within similar colony phenologies: $=0.0283 \pm 0.00881$ S.E., F_{ST} between different colony phenologies: $=0.0196 \pm 0.00683$ S.E; one-tailed Mann-Whitney $U=154.0$, $p=0.50$. Table 4). This result remains the same when the comparison is conducted only over overlapping geographical distances (Fig. 1) (F_{ST} within similar colony phenologies: $=0.0257 \pm 0.0158$ S.E., F_{ST} between different colony phenologies: $=0.00922 \pm 0.0067$

Table 3. Allele frequencies for *Halictus poeyi* samples. Florida, 2000.
*(Relative allele mobility: F – Fast, M – Medium, S – Slow, V – Very. N is the haploid sample size, HW (p) is the significance of the exact HW test, * – significant departure from HW equilibrium. NA – When a sample is monomorphic for a locus, or if a rare allele is represented by only one copy, the exact HW test is not applicable, and thus no p value is presented)*

	MIL	POR	AUG	CRO	OCA	GUL	CHR	JUP	KEY
AK-1									
F	0.100	0.077	0.086	0.000	0.000	0.043	0.125	0.031	0.069
M	0.900	0.923	0.914	1.000	1.000	0.957	0.875	0.906	0.931
S	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000
N	30	26	35	30	29	23	32	32	29
HW (p)	1.00	1.00	1.00	NA	NA	NA	1.00	1.00	1.00
ALDDH									
VF	0.000	0.017	0.000	0.021	0.000	0.000	0.000	0.016	0.000
F	0.367	0.153	0.115	0.271	0.075	0.069	0.180	0.262	0.171
M	0.567	0.763	0.820	0.583	0.825	0.603	0.660	0.607	0.805
S	0.067	0.068	0.066	0.125	0.100	0.328	0.160	0.115	0.024
N	30	59	61	48	40	58	50	61	41
HW (p)	0.39	1.00	0.19	0.31	**0.03***	0.12	**0.04***	**<0.01***	1.00
ARK									
F	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.019	0.073
M	1.000	0.983	1.000	1.000	1.000	1.000	0.929	0.981	0.927
S	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	30	59	61	40	40	58	42	53	41
HW (p)	NA	NA	NA	NA	NA	NA	1.00	NA	1.00
DIA									
F	0.200	0.151	0.156	0.021	0.000	0.058	0.150	0.061	0.065
S	0.800	0.849	0.844	0.979	1.000	0.942	0.850	0.939	0.935
N	30	53	45	48	40	52	40	49	31
HW (p)	1.00	0.08	0.06	NA	NA	1	0.35	1.00	1.00
EST-3									
F	0.067	0.020	0.017	0.000	0.000	0.019	0.000	0.000	0.024
M	0.933	0.980	0.932	1.000	1.000	0.981	1.000	1.000	0.976
S	0.000	0.000	0.051	0.000	0.000	0.000	0.000	0.000	0.000
N	30	51	59	38	40	52	50	61	41
HW (p)	1.00	NA	1.00	NA	NA	NA	NA	NA	NA
G3PDH									
F	0.933	1.000	1.000	1.000	1.000	1.000	0.909	1.000	1.000
S	0.067	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000
N	30	26	25	30	29	23	22	22	19
HW (p)	1.00	NA	NA	NA	NA	NA	1.00	NA	NA
G6PDH									
VF	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000
F	0.067	0.000	0.000	0.000	0.000	0.000	0.071	0.132	0.171
M	0.800	0.911	0.957	0.921	0.950	1.000	0.905	0.736	0.659
S	0.133	0.089	0.043	0.079	0.050	0.000	0.000	0.132	0.171
N	30	45	47	38	40	44	42	53	41
HW (p)	1.00	0.14	1.00	0.08	1.00	NA	1.00	**0.03***	**<0.01***
HK									
F	0.900	0.966	0.967	1.000	1.000	0.962	0.920	0.967	0.854
S	0.100	0.034	0.033	0.000	0.000	0.038	0.080	0.033	0.146
N	30	59	61	48	40	53	50	61	41
HW (p)	1.00	1.00	1.00	NA	NA	1.00	1.00	1.00	1.00
6PGD-1									
F	0.000	0.057	0.000	0.025	0.100	0.000	0.000	0.000	0.146
S	1.000	0.943	1.000	0.975	0.900	1.000	1.000	1.000	0.854
N	30	35	47	40	40	33	32	43	41
HW (p)	NA	**0.03***	NA	NA	**<0.01***	NA	NA	NA	**0.03***

Table 4. F_{ST} and geographic distance (km) matrix for *Halictus poeyi* sites in Florida, 2000

Pairwise genetic differentiation – F_{ST}										
		MIL	POR	AUG	CRO	OCA	GUL	CHR	JUP	KEY
Pairwise geographic distance (km)	MIL		−0.0112	−0.0130	0.1017	0.1124	0.0252	−0.0152	0.0273	0.0084
	POR	189		−0.0228	0.0527	0.0662	−0.0048	−0.0050	−0.0026	0.0121
	AUG	552	379		0.0581	0.0703	0.0022	−0.0013	0.0061	0.0169
	CRO	392	210	171		−0.0050	0.0062	0.0757	0.0224	0.0709
	OCA	511	327	81	119		0.0170	0.0820	0.0311	0.0750
	GUL	444	259	145	60	73		0.0187	−0.0145	0.0117
	CHR	626	439	147	238	126	182		0.0117	−0.0015
	JUP	791	602	345	424	322	363	199		0.0120
	KEY	853	682	585	580	528	529	446	310	

Table 5. Exact tests for genic differentiation between *Halictus poeyi* populations in Florida. *P* is the exact probability for rejecting Ho, which assumes that the allelic distribution is identical across populations. Significant values are bolded

Unbiased *P*-value estimate									
	MIL	POR	AUG	CRO	OCA	GUL	CHR	JUP	KEY
MIL	–	0.918	0.735	**0.014**	**0.008**	0.355	0.842	0.227	0.645
POR		–	0.959	0.344	0.179	0.975	0.524	0.744	0.202
AUG			–	0.102	0.066	0.626	0.311	0.215	0.111
CRO				–	1.000	0.854	**0.020**	0.878	0.082
OCA					–	0.675	**0.015**	0.702	0.139
GUL						–	0.225	0.963	0.394
CHR							–	0.339	0.821
JUP								–	0.345
KEY									–

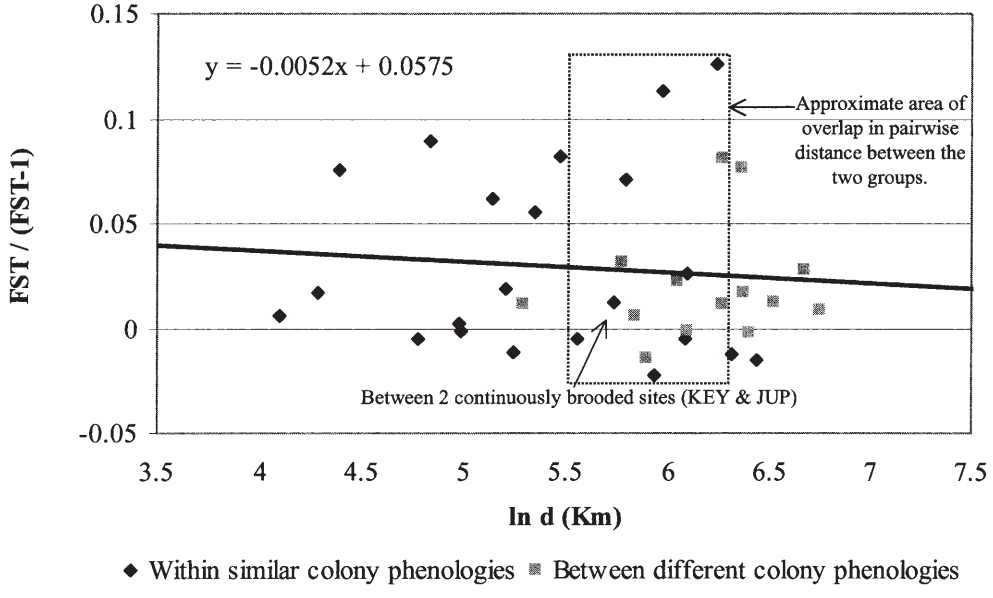

Figure 1. Regression of FST/(FST-1) and ln distance to detect isolation by distance. The regression is not significant, thus no isolation by distance is detected

S.E; one-tailed Mann-Whitney U = 30.0, p = 0.50). This data is supported by the exact tests of genic differentiation (Table 5). Genic differentiation was not evident between the continuously brooded populations and the annually brooded populations. Indeed, the only significant genic differentiation in the matrix occurred between four pairs of annually brooded populations.

Discussion

If the differences in colony phenologies between southern and northern Florida *H. poeyi* populations are genetically influenced, it is expected that gene flow between the two colony phenologies will be reduced in comparison to that within similar colony phenologies. Over time, we might expect reduced gene flow across the behavioural boundary to become insufficient to counteract selection and drift, and this could eventually cause the two gene pools to diverge (e.g. Kruckeberg, 1986; Macnair and Gardner, 1998). However, our data suggest that gene flow between the two colony cycles is actually slightly, but not significantly, higher rather then reduced. It thus seems more likely that differences in the social organization of *H. poeyi* in Florida is influenced primarily by environmental conditions, as has been found in several other halictines (e.g. Yanega, 1993; Richard and Packer, 1995, 1996). West-Eberhard (1986; 1989) argued that alternative phenotypic adaptations can arise in a species without the development of reproductive isolation or genetic differentiation between the two alternative morphs. Our study lends support to the first stage of West-Eberhard's model, as *H. poeyi* populations having different colony cycles are clearly not genetically differentiated. The expression of two alternative colony phenologies in *H. poeyi* populations in Florida may therefore be induced facultatively depending on environmental conditions (West-Eberhard, 1986, 1989; Wcislo and Danforth, 1997).

Our findings, regarding the levels of genetic differentiation, agree with another genetic study conducted on *Halictus poeyi* in Florida. Using allozymes, Carman and Packer (1996) found no fixed differences in *H. poeyi* from annually brooded and continuously brooded colonies. Furthermore, they showed that genetic identity values for annually brooded *H. poeyi* were similar and overlapped with those of continuously brooded *H. poeyi*, indicating no genetic divergence between the two colony phenologies. However, they did not analyze their data in terms of population subdivision, or gene flow. It is worth mentioning, that the observed pattern of genetic differentiation and geographic distance (Fig. 1) could potentially be due to a lack of equilibrium (Slatkin, 1993) between populations in northern and southern Florida. However, this is highly unlikely given Florida's biogeography (Ellsworth et al., 1994, and references within); populations persisting in Florida since the Pleistocene had sufficient time to reach equilibrium.

To facilitate comparisons with other studies, we translated our average F_{ST} estimate into the average number of migrants successfully entering a population per generation, *Nm*, using the following equation: $F_{ST} = 1/(4Nm+1)$ (Wright, 1951). Using *Nm* as an actual estimate of the number of migrants per generation per population is not recommended since the mathematical model underlying Wright's equation makes many biologically unrealistic assumptions (Whitlock and McCauley, 1999). However, these estimates may be informative when comparing large numbers of species (Whitlock and McCauley, 1999). Recently, Packer and Owen (2001) provided a summary of gene flow estimates from 44 species of Hymenoptera, 12 of which were bees. The average estimate of *Nm* for all *H. poeyi* populations in Florida is 9.79 migrants per generation per population. This ranks *H. poeyi* in the top 15% of all bees and all hymenopterans surveyed by Packer and Owen (2001). Nonetheless, despite the apparent high levels of gene flow, *H. poeyi* has unusually high levels of diploid male production, which has been linked to chronically small effective population sizes (Zayed and Packer, 2001).

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