JOURNAL OF THE KANSAS ENTOMOLOGICAL SOCIETY
76(2), 2003, pp. 221–235

Gene Flow and Population Structure in an Oligolectic Desert Bee, *Macrotera* (*Macroteropsis*) *portalis* (Hymenoptera: Andrenidae)

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ABSTRACT: Seven microsatellite loci from 176 female specimens of the oligolectic desert bee, *Macrotera portalis,* were analyzed to detect population subdivision among eight geographically distinct localities (17 to 342 km apart) across southwestern New Mexico and southeastern Arizona. In spite of the absence of obvious geographic barriers to gene flow, genic and genotypic frequencies revealed significant genetic heterogeneity among localities. *F* statistics revealed a high level of inbreeding within populations (F_{is} = 0.3241, $P < 0.001$), as well as substantial differentiation among localities (F_{st} = 0.2037, $P < 0.001$). A significant correlation was detected between distance among populations and the level of gene flow. Various factors may contribute to the low level of gene flow observed among populations, including narrow host-plant specificity, specificity in soil composition, habitat specificity, and the existence of large-headed, flightless males that mate within the nests. Five microsatellites from 192 male and female *Macrotera portalis* (Timberlake) reared from nests were analyzed in order to estimate intra-nest relatedness. Relatedness among female nestmates was significantly greater than zero for two of seven nests. Our results have implications for understanding patterns of bee diversity.

KEY WORDS: Gene flow, population genetics, isolation by distance, speciation

Bee diversity is generally thought to be highest in the arid and semi-arid regions of the world, especially those areas showing Mediterranean climate patterns (Michener, 1979, 2000; Wcislo and Cane, 1996; Blondel and Aronson, 1999; Arianoutsou and Graves, 1994; diCastri and Mooney, 1973). Areas of highest bee diversity are also characterized by an unusually high proportion of species with narrow hostplant preferences ("oligolectic" species), suggesting that hostplant specialization and bee diversity are interrelated (Ayala *et al.* 1993; Griswold *et al.,* 1997). Moldenke (1979), for example, estimated that 62% of the bee species of the Sonoran desert and 60% of the bee species of the Mojave desert were floral specialists (compared to roughly 47% in more mesic parts of California). Numerous authors have commented on the association between bee diversity, aridity, and oligolecty (reviewed in Wcislo and Cane, 1996). All available evidence suggests that speciation in arid and semiarid environments is accelerated in bees, and that the higher rate of speciation is restricted to primarily oligolectic lineages.

What mechanisms could explain the elevated rate of speciation in desert bees? Some authors have hypothesized that the temporal unpredictability of rainfall in arid regions might promote bee diversity (Linsley, 1958; Michener, 1979). Linsley (1958), for example, hypothesized that synchrony between oligolectic bee emergence and host plant bloom coupled with temporal and spatial variation in rainfall could provide a mechanism for disrupting gene flow among solitary bee populations. Linsley (1958) cited empirical studies suggesting that oligolectic bee emergence was temporally synchronized with host plant bloom (e.g., Hurd, 1957). Additional supporting evidence was provided by Rust (1988). The hypothesis of allochronic speciation is supported by the observation that many

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lineages of oligolectic desert bees include clades (monophylectic groups) in which all the species visit the same host plants, suggesting that host switching alone does not explain the numerical predominance of oligolectic bees in arid regions. Indeed, for *Diadasia* (Apidae), a predominantly oligolectic genus of desert bees, Sipes and Wolf (2001) showed that host switching is rare relative to the number of species in the genus. The same pattern is evident in other oligolectic bee genera, including *Andrena, Calliopsis, Macrotera,* and *Perdita* (Andrenidae).

Since Linsley's (1958) review additional empirical evidence has emerged for the role of host-plant synchrony in bee speciation. Several authors have demonstrated that bees closely assess environmental conditions and can remain in diapause for long periods of time when conditions are unfavorable. For example, Houston (1991) reported 10 year larval diapause in *Amegilla dawsoni* Rayment (Apidae), and Rozen (1990) reported that larvae of *Pararhophites orobinus* (Morawitz) (Megachilidae) emerged after a seven year diapause. Additional examples of extraordinarily long diapause in bees are cited in Danforth (1999). Prolonged diapause, coupled with rainfall induced emergence is a prerequisite for synchrony between bees and their host plants. Biogeographic patterns also support Linsley's (1958) hypothesis (Minckley *et al.,* 1999, 2000). Minckley *et al.* (2000) showed that the proportion of oligolectic bees increases as one proceeds from regions of higher and more predictable rainfall (Upper Sonoran desert) to regions of lower and less predictable rainfall (Lower Sonoran desert). This is exactly the prediction one would make if synchrony between oligolectic bees and their host plants allows oligolectic bees to inhabit extremely arid habitats. Furthermore, a long-term study of one oligolectic, desert bee (*Macrotera portalis* (Timberlake)) demonstrated that emergence is induced by high humidity (=rainfall) and that larvae pursue a bet-hedging life history (only approximately 50% of the larvae emerged as adults even under ideal conditions; Danforth, 1999). Both bet hedging and rainfall induced emergence could contribute to genetic differentiation among populations.

One correlate of Linsley's hypothesis is that desert bees should show evidence of genetic divergence among populations, in spite of the absence of obvious geographic barriers to gene flow. While gene flow has been estimated based on allozyme and microsatellite data for many social bees (reviewed in Packer and Owen, 2001), no studies have focused on solitary or communal ground nesting desert bees, which comprise the majority of bees in the areas of highest bee diversity (arid regions). We present below the results of a long-term study of gene flow and nestmate relatedness in a desert bee, *Macrotera portalis* (referred to in previous publications as *Perdita* [*Macroteropsis*] *portalis* Timberlake), based on seven variable microsatellite markers. Our results provide some evidence in favor of Linsley's hypothesis. Populations in *M. portalis* show substantial genetic differentiation even over relatively short distances, consistent with the idea that gene flow is limited even in the absence of geographic barriers, however, other aspects of the biology of *M. portalis* could contribute to low gene flow among populations.

The biology of *M. portalis* has been described elsewhere (Danforth, 1991a, b, 1999; Danforth and Desjardins, 1999). In summary, male and female *M. portalis* overwinter underground as last instar larvae for approximately 11 months. During the late summer rainy season in the Chihuahuan desert adults emerge following heavy rains and females commonly re-use their mothers' nests. Active nests contain up to 30 adult females (and as many large-headed males; see below). All females have fully developed ovaries and there is no reproductive division of labor. Females provision larval cells exclusively with pollen and nectar from the mallow genus *Sphaeralcea* (Malvaceae).

Males of this species occur in two forms: large-headed, flightless males and small-headed flight-capable males (Danforth, 1991b). Large-headed males mate exclusively within the nest. The intra-nest mating in *M. portalis* may have some obvious population genetic effects, such as elevating overall levels of homozygosity through sib mating. We discuss the evidence for this below.

Gene flow and population genetic data provide valuable insights into the long term viability and conservation status of species. In a recent review, Packer and Owen (2001) emphasized the importance of understanding gene flow in pollinating insects and the relevance of population genetic data for assessing the long-term viability of potentially endangered pollinator species. Their review emphasized how little we know about gene flow and population genetics in ground-nesting, oligolectic bees: "one of the major findings of our literature review is that there has not been a single population genetic study of any oligolectic bee species, and almost no large-scale studies of any species . . ." (Packer and Owen, 2001). Studies of gene flow in pollinating insects should also shed light on the conservation biology of pollinators (Buchmann and Nabhan, 1996; Allen-Wardell *et al.,* 1998; Cane and Tepedino, 2001), and microsatellite loci are now the most widely used markers for population genetic studies (Bruford and Wayne, 1993; Estoup and Angers, 1998; Estoup and Cornuet, 1998). As the first study of its kind involving an oligolectic desert bee, this research may provide a glimpse of possible mechanisms underlying speciation in bees inhabiting arid and semi-arid regions.

Materials and Methods

Field work

Six of eight collection sites were located in New Mexico, with two sites located in southeastern Arizona (see Table 1, Fig. 1). Distances between localities ranged from 17 km up to 342 km. Each locality was located in mixed Chihuahuan semidesert-grassland habitat with scattered *Sphaeralcea* plants. This habitat occurs intermingled with true Chihuahuan desert habitat throughout the range of *M. portalis.* Both habitat types are described in detail in Brown (1994).

Individuals for this study were collected in two different ways. First, we collected female bees on *Sphaeralcea* flowers at eight localities using an aerial net. These collections were made over several years in order to get as complete a sample at each population as possible. This sample of 176 females collected on flowers was used to estimate geneflow among populations (we refer to this sample of bees below as the "population sample"). Second, bees were collected by nest excavation in order to obtain an estimate of nestmate relatedness. These nests were excavated following standard protocols and correspond to the nests used in a previous study of emergence dynamics in *Macrotera portalis* (Danforth, 1999). We used 42 male and 150 female bees from seven nests in five localities for the estimate of nestmate relatedness (we refer to this sample of bees below as the "nestmate sample"). After collection, specimens were sorted, identified to species, and stored at –80°C. The overall collection period spanned nine years, including collections in the summers of 1989, 1991, 1992, 1993, 1995, and 1998.

Microsatellite primer development

We have used the following protocols to isolate and characterize microsatellites in solitary (*Macrotera portalis,* Andrenidae) and social (*Lasioglossum umbripenne* (Ellis), (Halictidae) [Wcislo and Danforth, in prep.]) bees. Seven pairs of primers for PCR

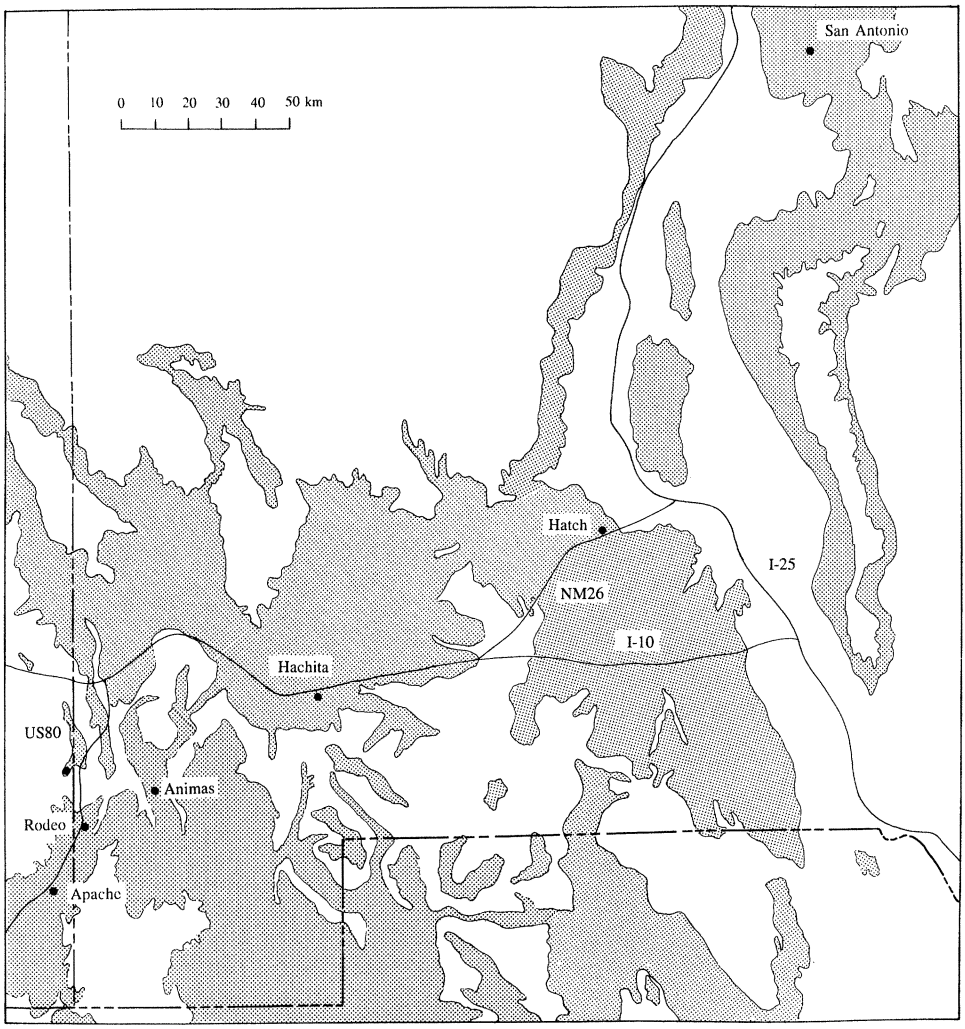

Fig. 1. Map showing distribution of collecting sites (black dots) and Chihuahuan desert/grassland habitat (shaded areas). The map shows the junction of the southeastern corner of Arizona, the southwestern corner of New Mexico, and northern Mexico. Most sites are located in New Mexico. Map redrawn from Brown, 1994. North is towards the top of the figure.

amplification of microsatellite loci were developed for this study according to protocols developed by S. Bogdanowicz (Bogdanowicz *et al.,* 1997).

Genomic DNA was extracted from 10–20 frozen bees following standard protocols (Danforth *et al.,* 1996). Total genomic DNA was then digested to completion with Sau3A (Promega, Madison, WI), and run out on a low-melting point agarose gel. DNA fragments of 500–1200 bp were cut out of the gel and recovered from the gel slices using the Promega Wizard DNA purification system (Promega, Madison, WI). Restriction fragments were then dephosphorylated with calf intestinal alkaline phosphatase. Dephosporylated insert DNA fragments were then ligated to pUC 18 vectors at a molar ratio of 10:1. This molar ratio ensured a recombination rate of greater than 90%. Following transformation into DH5α cells, the genomic DNA library was titred and plated out at a concentration of

Table 1. Summary of localities and dates of female *Macrotera portalis* specimens included in the population level study for each of the 8 collection localities.

Locality	Years collected	Map location	No. specimens
E. Animas	1991–1993	9.3 km E. Animas, Hidalgo Co., NM	37
E. Apache	1993, 1995	2 km E. Apache, Cochise Co., AZ	12
San Antonio	1992	33 km E. San Antonio, Socorro Co., NM	6
N. Hachita	1989, 1993	30 km N. Hachita, Grant Co., NM	41
N. Rodeo	1991–1993	2 km N. Rodeo, Hidalgo Co., NM	31
W. Animas	1992	11 km W. Animas, Hidalgo Co., NM	4
W. Hatch	1992, 1993, 1998	21 km W. Hatch, Luna Co., NM	39
NE Portal	1989	14.5 km NE Portal, Cochise Co., AZ	6
Total			176

approximately 200 colonies/plate (10 cm in diameter). Colonies were then transferred onto Nylon membranes (Micron, Inc.) and the DNA was denatured following standard protocols (Sambrook *et al.,* 1989) and bound to the membrane by baking at 80°C.

We screened the genomic library with all possible combinations of trimeric nucleotide repeats using 20 to 24 bp oligonucleotides. We targeted trimeric repeats because dimeric repeats, while typically showing higher levels of variability than trimers, are difficult to score unambiguously. Oligonucleotides were end-labeled with ^{32}P-ATP and then used to probe the Nylon membranes. Pre-hybridization and hybridization were performed in a Hybaid oven at 55 to 60°C for 24 hr each, following protocols in Aquadro, *et al.* (1992). Following hybridization, membranes were washed twice in 2× wash (0.3M NaCl, 3 mM sodium citrate, 0.1% SDS) at room temperature, once in 2× wash at 55 °C and finally in 0.5× wash at room temperature. We screened approximately 10,000 colonies and obtained from 50 to 100 positive clones (approximately 1% of colonies contain microsatellite repeats). Positive clones were then picked and grown up for plasmid DNA mini-preps following standard protocols (Sambrook *et al.,* 1989). The plasmid DNAs were then used to make a dot-blot and the screening procedure was repeated to identify false positives. Five to 10% of the original putative positives were false positives and were eliminated from further screening.

Once positive clones were identified, we used restriction digests to determine the size of the insert DNA. Inserts of <1000 bp were selected for sequencing. Sequencing of clones was done on an ABI377 automated sequencer using M13 forward and M13 reverse primers. Once the sequences are obtained and edited they were checked for Sau3A sites that indicate the junction between insert and vector DNA as well as Sau3A sites that result from re-annealing of individual restriction fragments of bee DNA (this should be rare, since insert DNA was dephosphorylated). Clones in which the microsatellite repeat region was within 20 bp of vector sequence or within 20 bp of a Sau3A site are excluded at this point because it was impossible to make primers that flank the microsatellite region. From sequences for the remaining microsatellite clones we designed 20 to 26 bp oligonucleotide primers that flank the repeat region. Primer pairs were designed to have similar and high annealing temperatures (~70°C), 50% G/C content, to produce PCR products of from 150 to 250 bp in length, and to be non-complementary.

Preparation of genomic DNA for genotyping

DNA extractions were done according to the procedure used by Danforth *et al.* (1996) for the majority of specimens. The remaining DNA extractions were performed using a

Table 2. Microsatellite loci analyzed. T_m refers to the optimal annealing temperature determined empirically for each primer pair. Note that for SB61 we ran the PCR reactions at two different temperatures in order to assess the effect of T_m on *F*-statistics (54°C and 58°C). The lower temperature is recommended.

Locus	Core sequence	No. alleles	Primer sequence (5′→3′)	T_m (°C)	Gene diversity	Heterozygosity
SB6	$(TCC)_{15}$	8	AATCGGCTTTAGAGGCACCG TCGGGCGATTTTTCAACTGG	64	0.607	0.312
SB13	$(AGG)_8$	5	GCAGCTTTGGTATGCTAACCGG GATCGATTCGACGGATATCGAGC	62	0.451	0.243
SB47	$(CTG)_6$	4	AGTCGCGGTCACGTTCGTC AGTCACTCGCTGACGAGTTGAC	64	0.225	0.229
SB50	$(TCC)_8$	8	TCGGTCGACTTCGCTAGTCG AGTGTCTCATTAAGCCGAGTGC	58	0.586	0.516
SB61	$(AC)_6AT(AC)_3$	7	GATATCCAGAGATGTACAGAATACCCAG GCAGGTTTCGTTGGAGACTTCG	54	0.400	0.359
SB64	$(CGG)_6$	7	TGGAGCCTCGTGACTTTTGC TTGGCAACGAGCTGCATCG	58	0.539	0.187
SB66	$(TCC)_7$	4	AAGCTCCGATAATGACCGGAC TGTAGAAGGTTCGAATGTTCCG	58	0.361	0.265

QIAGEN QIAamp Tissue Kit as per the manufacturer's instructions. DNA yields were approximately 1–3 µg/specimen.

Amplification, electrophoresis, and autoradiography

Table 2 summarizes the number of alleles, repeat motif, primer sequence, T_m, gene diversity, and heterozygosity for each microsatellite locus. One primer from each set was end-labeled with ^{33}P. Each 10 µl PCR amplification included approximately 20 ng of genomic DNA, 4.3 µl dH_20, 1 µl 10× buffer, 1 µl $MgCl_2$ (25 mM), 2 µl dNTPs (1 mM each), 0.15 µl unlabeled primer (16 µM), 0.5 µl end-labeled primer (16 µM), and 0.05 µl Taq DNA polymerase (Promega, 5 units/µl). Amplification was performed in a Hybaid Omni-E thermal cycler with initial denaturation of 94°C for 1 min followed by 35 cycles of 94°C for 45 sec, individual primer melting temperature (summarized in Table 2) for 45 sec, and 72°C for 30 sec. Following amplification samples were electrophoresed on 8% acrylamide gels for approximately 2 hours. Gels were transferred to chromatography paper, dried for 1.5 hours, and exposed to film for one to seven days. Individuals were arranged randomly on gels and scored without knowledge of the locality of the bee.

Data analysis

From 4 to 8 alleles were recorded at each microsatellite locus (Table 2). The complete data set is available from the first author as an Excel spreadsheet. Appendix I summarizes allele frequency data for all loci and all localities. We tested Hardy-Weinberg equilibrium using a Fisher exact test for the alternative hypothesis of heterozygote deficiency (homozygote excess) based on the multisample score test of Raymond and Rousset (1995b) as implemented in the GENEPOP program (http://wbiomed.curtin.edu.au/genepop/). This test provides unbiased estimates of Hardy-Weinberg exact *P*-values based on observed and expected heterozygosities by the Markov chain method. We also tested for significant linkage disequilibrium across loci using GENEPOP.

We also evaluated the level of population differentiation based on genic and genotypic frequencies as implemented in GENEPOP (Raymond and Rousset, 1995b). Genic

differentiation was estimated using a Fisher exact test as described by Raymond and Rousset (1995a), and genotypic differentiation was estimated using a log-likelihood (G) based test as described by Goudet *et al.* (1996). For evaluating the levels of gene flow among and within populations, we used Wright's F statistics (Wright, 1951) based on Weir and Cockerham (1984) as implemented in the GENEPOP package. We estimated hierarchical F statistics, including F_{is} (the excess of homozygotes within individuals relative to a local population, or inbreeding coefficient for a population) and F_{st} (the excess of homozygotes within a local population relative to the total, or genetic differentiation between populations) (Raymond and Rousset, 19956). Resampling with replacement (bootstrapping) was done using FSTAT to determine the significance of the F statistics (Goudet, 1995).

We tested the hypothesis that genetic differentiation was correlated with geographic distance among localities by evaluating the relationship between F_{st} and distance (isolation by distance; see Peterson and Denno, 1998b). To do this, we regressed F_{st} and $F_{st}/(1 - F_{st})$ on log distance and calculated the least squares regression lines for both. We tested the significance of the relationship between distance and gene flow using the isolation by distance test implemented in GENEPOP (Raymond and Rousset, 1995b). Rousset (1997) discusses the interpretation of these regression equations.

We assessed the relatedness within nests using Relatedness v. 5.0 (K. F. Goodnight and D. C. Queller; http://gsoft.smu.edu/GSoft.html). We analyzed relatedness based on larvae collected from seven nests located in five localities. For each nest, we calculated the average and pairwise relatedness values among male and female nestmates with background allele frequencies obtained from the population data (the bees collected randomly on flowers) using methods described in Queller and Goodnight (1989). Relatedness calculates standard errors and 95% confidence intervals based on bootstrap resampling.

Results

Population differentiation

The 7 loci are apparently unlinked; the null hypothesis of loci independence is consistent with the results of the Fisher exact test for genotypic linkage disequilibrium (P-values > 0.43319 for all pairwise comparisons among loci).

We tested the genic and genotypic differentiation among localities using a Fisher exact test and a log-likelihood test for each microsatellite locus. Each locus revealed highly significant genic ($P \leq 0.00001$) and genotypic ($P \leq 0.00001$) differentiation among the collection localities (Table 3). Wright's F statistics (based on Weir and Cockerham, 1984) were used to determine the level of inbreeding within localities (F_{is}) and the level of genetic differentiation among localities (F_{st}). These results, summarized in Table 3, were relatively heterogeneous across loci. All F_{is} values were significantly different from zero except locus SB47. F_{is} based on all loci combined was also significantly different from zero (F_{is} = 0.3241; $P < 0.005$), suggesting a moderate level of inbreeding or possibly an excess of homozygotes due to the existence of null alleles. We detected null alleles at two loci: SB61 and SB64. While the existence of null alleles is likely to give elevated levels of F_{is}, null alleles are not likely to bias the estimate of F_{st} in any way (see below).

F_{st} values for each locus were significantly different from zero, indicating substantial differentiation among localities (Table 3). The overall estimate of F_{st} was 0.2037, suggesting limited migration among localities. Overall, our estimates of gene flow suggest that, based on the sample of populations analyzed, there is a surprisingly high level of inbreeding within populations and clear differentiation among populations. Such differentiation is also evident

Table 3. *F* statistics and estimates of population subdivision based on 7 loci.

Locus	F_{is}	F_{st}	Fit	*P*-value[1]
SB6	0.4338 ($P < 0.005$)	0.1094($P < 0.005$)	0.4958	<0.0001
SB13	0.5191 ($P < 0.005$)	0.2001 ($P < 0.005$)	0.6153	<0.0001
SB47	–0.0105 ($P < 0.46$)	0.0952 ($P < 0.005$)	0.0856	<0.0001
SB50	0.0924 ($P < 0.035$)	0.2684 ($P < 0.005$)	0.3360	<0.0001
SB64	0.6406 ($P < 0.005$)	0.1788 ($P < 0.005$)	0.7049	<0.0001
SB66	0.1965 ($P < 0.005$)	0.3414 ($P < 0.005$)	0.4708	<0.0001
SB61	0.1728 ($P < 0.005$)	0.1804 ($P < 0.005$)	0.3220	<0.0001
All loci:	0.3241 ($P < 0.005$)	0.2037 ($P < 0.005$)	0.4618	<0.0001

[1] *P*-values correspond to the probability of accepting the null hypothesis of no population subdivision based on Fisher exact tests derived either from genic or genotypic frequencies.

when one inspects the allele and genotypic frequencies obtained for each locality. In many cases, common alleles at one locality are rare or absent at another (see Appendix I).

We also tested for genetic differentiation among years using the same data set. We coded individuals according to the year they were collected (see Table 1) and analyzed the data set by the same methods as for population differentiation. Because the bees were collected at different sites on some years the data set confounds variance due to locality and year. Nevertheless, the results indicate that there is substantial year to year variation in gene frequencies based both of F_{st} and on exact tests of population differentiation (Table 4), although the F_{st} estimates based on year to year variation ($F_{st} = 0.0940$; $P < 0.005$) are lower than those based on population differentiation ($F_{st} = 0.2037$; $P < 0.005$).

We tested the hypothesis of isolation by distance using two options in the Genepop program We regressed F_{st} on Ln(distance) and $F_{st}/(1- F_{st})$ on Ln(distance) using 100 permutations. In both cases the regression was highly significant ($P \leq 0.00001$), and the regression equations indicated a significant positive association between either estimate of gene flow and log distance:

$$F_{st} = -0.186 + 0.084 * (\text{Ln distance})$$
$$F_{st}/(1 - F_{st}) = -0.306 + 0.126 * (\text{Ln distance}),$$

indicating that gene flow decreases significantly with distance among localities. Fig. 2 shows the raw data plotted as F_{st} and $F_{st}/(1 - F_{st})$ vs. log distance, and Table 5 presents the pairwise F_{st} estimates.

Table 4. *F* statistics and estimates for the year to year variation in gene frequencies based on 7 loci.

Locus	F_{is}	F_{st}	Fit	*P*-value[1]
SB6	0.4672 ($P < 0.005$)	0.0467 ($P < 0.005$)	0.4920	<0.0001
SB13	0.5877 ($P < 0.005$)	0.0426 ($P < 0.015$)	0.6053	<0.0001
SB47	0.0042 ($P < 0.005$)	0.0856 ($P < 0.395$)	0.0895	<0.0001
SB50	0.2132 ($P < 0.005$)	0.1449 ($P < 0.005$)	0.3272	<0.0001
SB64	0.6758 ($P < 0.005$)	0.0737 ($P < 0.005$)	0.6997	<0.0001
SB66	0.3508 ($P < 0.005$)	0.1668 ($P < 0.005$)	0.4591	<0.0001
SB61	0.2527 ($P < 0.005$)	0.0807 ($P < 0.005$)	0.3129	<0.0001
All loci:	0.3973 ($P < 0.005$)	0.0940 ($P < 0.005$)	0.4539	<0.0001

[1] *P*-values correspond to the probability of accepting the null hypothesis of no subdivision among years based on Fisher exact tests derived either from genic or genotypic frequencies.

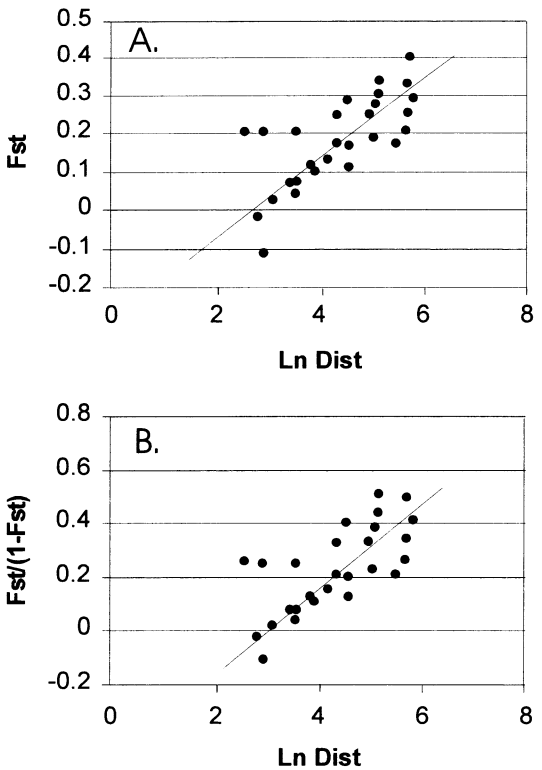

Fig. 2. Relationship between gene flow and geographic distance among sites. (A) F_{st} vs. Ln distance; (B) $F_{st}/(1 - F_{st})$ vs. Ln distance.

Nestmate relatedness

We estimated relatedness among seven nests based on a total of 192 individual bees. The relatedness values were heterogeneous (ranging from less than zero to 0.25). In only two nests (95-202 and 93-102) was relatedness among female nestmates significantly greater than zero (Table 6). Interestingly, in one nest (95-202) average pairwise female relatedness was close to 0.25, the level one would expect if female nestmates are half-sib sisters. Average relatedness among males is difficult to interpret due to small sample sizes, but appears indistinguishable from zero for all nests analyzed. Overall, we conclude that there is little evidence of consistently high levels of nestmate relatedness in this bee, although our sample size is small.

Discussion

The high F_{is} indicates a high level of non-random mating within localities. Biologically, the observed excess of homozygotes within localities is most likely the result of inbreeding within nests due to the presence of large-headed flightless males. Based on data collected over 10 years in Arizona, nests persist for long periods of time with successive generations occupying the same nest (Danforth, 1999). Approximately one-third of the male population must remain in the nests with the female occupants; these nest-bound males are macrocephalic and incapable of flight (Danforth, 1991b, 1999). The inability of large-headed males to disperse means they mate exclusively with female nestmates, some of

Table 5. Pairwise F_{st} and distance values for all populations.

Pop 1	Pop 2	F_{st}	Dist (km)
EAnimas	EApache	0.1163	47
EAnimas	ESanAnt	0.2112	298
EAnimas	NHachita	0.1031	51
EAnimas	NRodeo	0.2049	35
EAnimas	NEPortal	0.044	35
EAnimas	WAnimas	–0.0161	17
EAnimas	WHatch	0.2515	149
EApache	ESanAnt	0.2939	342
EApache	NHachita	0.1701	97
EApache	NRodeo	0.024	23
EApache	NEPortal	0.0719	33
EApache	WAnimas	0.072	35
EApache	WHatch	0.2889	95
ESanAnt	NHachita	0.1766	249
ESanAnt	NRodeo	0.4062	321
ESanAnt	NEPortal	0.3325	312
ESanAnt	WAnimas	0.2572	308
ESanAnt	WHatch	0.1914	160
NHachita	NRodeo	0.2481	79
NHachita	NEPortal	0.176	79
NHachita	WAnimas	0.1329	65
NHachita	WHatch	0.1145	99
NRodeo	NEPortal	0.206	13
NRodeo	WAnimas	0.2039	19
NRodeo	WHatch	0.3391	177
NEPortal	WAnimas	–0.1128	18.5
NEPortal	WHatch	0.3072	177
WAnirnas	WHatch	0.2781	163

whom are sisters (Danforth, 1991b). The combination of nest persistence and intra-nest mating could potentially explain the high levels of inbreeding within populations suggested by the high Fis values.

An alternative explanation for the high Fis values is the existence of a "temporal Wahlund effect" (Tonsor *et al.,* 1993). A temporal Wahlund effect can arise when mating is random within localities but the allele frequencies vary from year to year, such that on any one year a (genetically) non-random sample of the local population emerges and mates.

Table 6. Relatedness values calculated for each nest using Relatedness v. 5.0 with background allele frequencies obtained from the population level analysis. Mean relatedness among groups is shown as mean ± 95% confidence interval (*n*).

Nest	Locality	*R* (among females)	*R* (among males)
91–21	N. Rodeo	0.1904 ± 0.4313 (8)	undefined (0)
95–202	E. Apache	0.2561 ± 0.166 (31)*	1.0 ± 0.0 (2)
91–54	E. Animas	0.0791 ± 0.2168 (20)	0.1068 ± 0.7775 (2)
92–63	E. Animas	0.1206 ± 0.2769 (22)	0.0636 ± 0.4928 (5)
93–100	N. Hachita	0.0141 ± 0.275 (28)	–0.0449 ± 0.1325 (11)
93–102	N. Hachita	0.1154 ± 0.1147 (33)*	–0.0693 ± 0.1214 (19)
93–150	W. Hatch	0.2149 ± 0.3858 (8)	–0.1783 ± 0.694 (3)

We have documented above year to year variation in allele frequencies and previous studies (Danforth, 1999) have demonstrated that only approximately 50% of the overwintering larvae emerge on any one year, making a temporal Wahlund effect at least possible in this species.

Given the high Fis estimates, one might have expected to see high levels of relatedness among nestmates. However, our limited estimates of nestmate relatedness indicate that only two of seven nests show elevated levels of nestmate relatedness. A factor that may confound our analysis of nestmate relatedness is that the regression method described by Queller and Goodnight (1989) assumes that Fis is zero when evaluating nestmate relatedness. As we have shown, Fis is significantly greater than zero, meaning that the regression methods of Queller and Goodnight will tend to *underestimate* relatedness in our data set. We believe that the elevated levels of Fis obtained from the population sample indicates a biologically meaningful elevation in the level of homozygosity within localities. The most likely explanation for this is intra-nest mating due to the presence of large-headed flightless males. Computer simulation studies (Danforth, unpublished) indicate that relatedness among nestmates varies with a number of factors, including the proportion of offspring fathered by large-headed males, the number of female co-foundresses at the origin of a nest, and time since nest founding. Relatedness generally increased in the first five years of a colony's life and then decreased thereafter. When nests were founded by a single female, maximum relatedness values ranged from 0.20 to 0.60, depending on the proportion of offspring fathered by large-headed males within the nest. The variance among nests observed in this study could be related to the age of nests, with those newly founded nests showing higher levels of relatedness among female nestmates than older nests. Studies of other ground-nesting, communal bees have generally found low levels of nestmate relatedness (Danforth *et al.,* 1996; Kukuk and Sage, 1994; Paxton *et al.,* 1996, 2000), but none of these species are known to have dimorphic males and obligate intra-nest mating.

In comparison with a survey of the population genetics of other phytophagous insects (Peterson and Denno, 1998a), *M. portalis* has one of the highest F_{st} values of all species studied. *M. portalis* is in approximately the upper 10 percent of all 92 species surveyed by Peterson and Denno (1998a). One should be cautious about drawing extensive conclusions from these studies as the Peterson and Denno survey included only allozyme data. By comparison, the F_{st} estimate we obtained for *M. portalis* populations corresponds roughly to the average differentiation observed (based on six microsatellite markers) between African and European populations of *Apis mellifera* (Franck *et al.,* 2000).

Low levels of gene flow among populations of *M. portalis* could be due to a number of factors, including Linsley's (1958) hypothesis of population divergence due to allochronic emergence. Indeed, our estimates of between year genetic differentiation (Table 4) support this hypothesis. However, other factors may also contribute to reduced gene flow among populations. First, female *M. portalis* are host-plant specific and the patchy distribution of host plants may serve to isolate populations of *M. portalis* Second, *M. portalis* nests are located in a particular type of soil that is rich in silt and less sandy than typical ground nesting bees (reviewed in Cane, 1991). Such soil types occur primarily at the low points within valleys and are often devoid of vegetation (Danforth, pers. obs.). Third, *M. portalis* nest sites are restricted, as far as we know, to mixed Chihuahuan desert grassland habitats in Arizona and New Mexico (Fig. 1). Such habitat is intermixed with and sometimes surrounded by true Chihuahuan desert habitat, which may also provide a barrier to gene flow. Fourth, female *M. portalis* tend to reuse their mothers' nests, thus limiting gene flow among sites. Finally, with approximately 30% of males in the population flightless (Danforth, 1999), there may be substantially less male-mediated gene flow

among populations than in a "typical" ground nesting bee in which all males are capable of flight. Evaluating the role of allochronic speciation in desert bees will require additional studies of other bee species. Ideally, such studies should include matched comparisons of closely related polylectic and oligolectic species (Minckley and Danforth, in prep.). Only with additional studies of other ground nesting, solitary bees will we be able to assess the general patterns of gene flow in these important pollinators.

Acknowledgments

We are grateful to the following people for reading earlier drafts of this paper: William L. Crepet, C. Drew Harvell, Richard G. Harrison, Malcolm Schug, Corey Freeman-Gallant, Pierre Franck, Robert Minckley, Jay Evans, Arnaud Estoup, Jean-Marie Cornuet, and two anonymous reviewers. We are particularly grateful to Steve Bogdanowicz for providing advice on microsatellite development and screening. Pierre Franck generously provided advice and assistance in the analysis of *F* statistics and nestmate relatedness. Funds for this research were provided by the Hughes Scholars Program (Cornell University), two Theodore Roosevelt Memorial Grants from the American Museum of Natural History, a Pre-doctoral Fellowship from the Smithsonian Institution, a National Science Foundation Postdoctoral Research Fellowship in Environmental Biology (DEB-9201921), and a National Science Foundation Research Grant in Systematic Biology (DEB-9508647). We are grateful to the Southwestern Research Station (American Museum of Natural History) for providing essential facilities. BND is grateful to Jean-Yves Rasplus and Jean-Marie Cornuet (Centre de Biologie et de Gestion des Populations, Montpellier, France) for their help during his sabbatical leave in spring, 2002. Jerry Rozen made this study possible by showing BND nestsites of *Macrotera portalis* in September, 1989.

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Appendix 1. Allele frequency data by locality and summary statistics.

Allele	EAnimas	EApache	ESanAnt	NHachita	NRodeo	NEPortal	WAnimas	WHatch	Means
SB6									
10	0	0.083	0	0.026	0	0	0	0.2	
11	0	0	0	0.026	0	0	0	0	
13	0.014	0	0	0.077	0	0.167	0.250	0.033	
16	0.122	0.25	0	0.051	0.581	0.167	0.125	0.083	
19	0.703	0.292	0.833	0.474	0.371	0.417	0.625	0.45	
22	0.162	0.292	0.167	0.346	0.048	0.25	0	0.167	
25	0	0.083	0	0	0	0	0	0	
28	0	0	0	0	0	0	0	0.067	
Prop. Heterozygotes	0.459	0.417	0	0.333	0.516	0.167	0.5	0.1	0.312
Gene Diversity	0.471	0.786	0.303	0.654	0.531	0.773	0.607	0.729	0.607
SB13									
10	0.365	0.75	0.25	0.768	0.879	0.583	0.5	0.887	
13	0.284	0	0	0.159	0.017	0	0	0.032	
16	0.014	0.125	0.75	0	0.086	0.083	0.125	0.016	
19	0.338	0.125	0	0.049	0.017	0.333	0.375	0.032	
22	0	0	0	0.024	0	0	0	0.032	
Prop. Heterozygotes	0.351	0.167	0.5	0.146	0.069	0.333	0.25	0.129	0.243
Gene Diversity	0.681	0.424	0.409	0.386	0.223	0.591	0.679	0.213	0.451
SB47									
13	0	0	0	0	0	0	0	0.141	
16	0.014	0	0	0	0	0	0	0	
19	0.176	0	0.167	0.125	0	0.083	0.125	0.231	
22	0.811	1	0.833	0.875	1	0.917	0.875	0.628	
Prop. Heterozygotes	0.297	0	0.333	0.25	0	0.167	0.25	0.538	0.229
Gene Diversity	0.316	0	0.303	0.222	0	0.167	0.25	0.539	0.225
SB50									
10	0.324	0.333	0	0.037	0.25	0.75	0.5	0	
11	0	0.042	0	0	0	0	0	0	

Appendix 1. Continued.

Allele	EAnimas	EApache	ESanAnt	NHachita	NRodeo	NEPortal	WAnimas	WHatch	Means
13	0.149	0	0	0.025	0	0	0	0	
16	0.054	0.042	0.417	0.35	0.033	0.167	0.25	0.821	
17	0.041	0	0	0.05	0	0	0	0.026	
19	0.216	0.542	0.583	0.15	0.617	0.083	0.125	0.154	
22	0.216	0.042	0	0.388	0.1	0	0.125	0	
Prop. Heterozygotes	0.865	0.75	0.5	0.525	0.533	0.5	0.25	0.205	0.516
Gene Diversity	0.785	0.616	0.53	0.709	0.555	0.439	0.75	0.306	0.586
SB64									
10	0	0	0	0.029	0	0	0	0	
16	0	0	0	0.015	0	0.167	0	0	
17	0	0	0.2	0	0.069	0	0	0	
19	0	0	0.2	0.059	0.207	0	0	0.538	
22	0.694	0.313	0.2	0.426	0.172	0.833	1	0.064	
25	0.226	0.313	0	0.426	0.397	0	0	0.308	
28	0.081	0.375	0.4	0.044	0.155	0	0	0.09	
Prop. Heterozygotes	0.161	0.25	0	0.265	0.207	0.333	0	0.282	0.187
Gene Diversity	0.469	0.708	0.8	0.639	0.754	0.333	0	0.611	0.539
SB66									
10	0.569	0.273	1	0.762	0.21	0.417	0.375	0.905	
13	0.431	0.727	0	0.138	0.79	0.583	0.625	0.014	
16	0	0	0	0.037	0	0	0	0	
19	0	0	0	0.063	0	0	0	0.081	
Prop. Heterozygotes	0.417	0.182	0	0.4	0.29	0.5	0.25	0.081	0.265
Gene Diversity	0.497	0.416	0	0.399	0.337	0.53	0.536	0.176	0.361
SB61									
11	0.108	0.042	0.5	0.125	0.032	0	0	0.205	
12	0.068	0	0.2	0.2	0	0	0.125	0.192	
14	0.689	0.917	0.3	0.35	0.968	1	0.875	0.346	
16	0.108	0.042	0	0.263	0	0	0	0.077	
17	0.027	0	0	0	0	0	0	0	
22	0	0	0	0.063	0	0	0	0	
24	0	0	0	0	0	0	0	0.179	
Prop. Heterozygotes	0.459	0.083	0.8	0.625	0.0650	0	0.25	0.59	0.359
Gene Diversity	0.503	0.163	0.689	0.759	0.063	0	0.25	0.773	0.4
All Loci									
Mean Allele Number	3.857	3.143	2.286	4.714	3	2.429	2.429	4.143	
Allele Number SD	1.345	1.464	0.951	1.496	1.414	0.976	0.976	1.215	
Heterozygote prop.	0.43	0.264	0.305	0.363	0.24	0.286	0.25	0.275	
Heterozyg. prop. SD	0.219	0.251	0.316	0.167	0.217	0.185	0.144	0.209	
Mean Gene Diversity	0.532	0.445	0.433	0.538	0.352	0.405	0.439	0.478	
Gene Diversity SD	0.154	0.287	0.268	0.202	0.277	0.262	0.276	0.246	