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PHYLOGEOGRAPHY OF THE SOCIALLY POLYMORPHIC SWEAT BEE *HALICTUS RUBICUNDUS* (HYMENOPTERA: HALICTIDAE)

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Abstract.—The evolution of sociality in insects holds a central place in evolutionary theory. By examining the phylogenetic patterns of solitary and social behavior and how they correlate with ecological variables, we may identify factors important in the evolution of sociality. In this study, we investigated historical and biogeographical patterns of sociality in a socially polymorphic bee species (one that demonstrates both social and solitary nesting behavior). This unique system allows for a more powerful examination of evolutionary transitions in sociality than interspecific studies of obligately social and solitary species. We conducted a phylogenetic analysis among populations of the halictine bee *Halictus rubicundus* and then identified relationships among mitochondrial DNA sequence data, sociality, environmental conditions at the nesting site, and geographic location of populations of this species. Within North America, populations of *H. rubicundus* expressing social and solitary behavior belong to different genetic lineages. Sociality is also correlated with at least one environmental variable used in this study. Taken together, the results support the predictions for genetic control of sociality, but they are still consistent with social behavior at some level being determined by the environmental conditions at the nesting site.

Key words.—Hymenoptera, matrix correlation, phenotypic plasticity, phylogeography, social evolution.

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The evolution of social behavior continues to hold a central place in the study of evolutionary biology. Many fascinating questions stem from the patterns of social evolution among insects. For instance, Crespi (1996) presented empirical evidence supporting a link between haplodiploidy and eusocial behavior, because eusocial behavior has evolved multiple times in Hymenoptera, but rarely among other insects. Based on phylogenetic patterns of sociality in bees, it seems sociality is not an evolutionary endpoint, but that solitary behavior may arise from eusocial ancestors (Wcislo and Danforth 1997). Socially polymorphic species, those that demonstrate social and solitary behavior in the same or different populations, provide opportunities to track transitions in sociality within a single species and to understand the relationship of social transitions to intrinsic and extrinsic causal factors.

Within the bee family Halictidae there have been multiple origins of sociality (Michener 1974; Danforth 2002). There is evidence that environmental conditions determine the level of sociality expressed by many halictid species (reviewed in Wcislo 1997; Yanega 1997). The idea that sociality is ultimately linked to climate is supported by existing patterns of species distribution, because many solitary species inhabit cool, temperate, northerly regions, whereas closely related social species live in warmer, more southerly regions (Wcislo 1997). The link between sociality and climate is also supported by phylogenetic evidence; many lineages of halictids have undergone switches and reversals in sociality, perhaps in response to climate changes during the Quaternary period (reviewed by Wcislo and Danforth 1997). In cold regions, the growing season may be too short to support social populations that require sequential worker and reproductive broods (Sakagami and Munakata 1972; Eickwort et al. 1996).

The idea that ecological conditions influence many aspects of social behavior in other hymenopterans is well established (reviewed in Ross and Keller 1995). Environmental variation has been found to affect mode of colony founding, mating structure, caste determination, and brood sex ratio in wasps, ants, and bees (Ross et al. 1996; Pearson and Raybould 1997; Pearson et al. 1997). Variation in these aspects of social behavior may affect population genetic structure and ultimately even cladogenesis. However, very little is known about the role of environmental conditions on the earliest transitions in social evolution—specifically the switch from solitary to social behavior.

Facultatively social species, those that have the ability to switch between social and solitary behavior, offer an unparalleled perspective for studying transitions in sociality, because unlike social species that have been obligately social for more than 80 million years (Michener and Grimaldi 1988), socially polymorphic species presumably still possess the characteristics that permit transitions in sociality. At least nine species in the family Halictidae have been identified as socially polymorphic (Packer 1997), and these may be able to provide information about the factors that facilitate and the patterns that result from social transitions, as obligately social or solitary species cannot. Although several studies have investigated the evolutionary patterns of sociality across halictine species (Richards 1994; Plateaux-Quénu et al. 1997; Packer 1998; Danforth 1999; Danforth et al. 1999), this study is the first to investigate sociality in relation to the evolutionary and biogeographic history of a single species.

In this study, we investigate the evolutionary transitions in sociality for a socially polymorphic halictine bee using phylogenetic methods based on DNA sequence data. We reconstruct the pattern of social transitions among populations

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of this species and compare it to geographical and ecological data using matrix correlation methods. In this way, we attempt to establish the congruence of sociality with varying environmental conditions at the nesting site and with patterns of genetic variation.

Halictus rubicundus, a holarctic halictine bee, demonstrates social and solitary behavior in different parts of its range. Social populations are typically found in areas with longer growing seasons such as New York, Kansas (Yanega 1993), southern Ontario (Knerer 1980), interior regions of the Netherlands (Hogendoorn and Leys 1997), and coastal British Columbia (Packer and Owen 1989). Solitary populations are typical of areas with short growing seasons such as Scotland (Potts and Willmer 1997), Alaska (Armbruster and Guinn 1989), and the mountains of Italy (Bonelli 1967) and Colorado (Eickwort et al. 1996). Social populations of *H. rubicundus* have a colony cycle typical of many primitively eusocial sweat bees (Michener 1990). Mated, overwintered females emerge from hibernacula with the onset of warm weather, initiate nests, and rear a female-biased worker brood (75–100% female offspring) of about five to eight total offspring. The workers then help the gyne rear a second brood of approximately 40% females, with 10 to 15 total individuals (Soucy 2002). Second-brood males and females mate and the latter enter diapause to repeat the cycle the following year. In solitary populations of *H. rubicundus,* gynes begin nesting in late spring or early summer. These females rear a single brood that is approximately 40% female, all of which mate and immediately enter diapause (Eickwort et al. 1996). In marginal environments, so-called social populations of this species may also demonstrate brood divalency, in which some first-brood daughters become early diapausing females (Yanega 1989). This may be a form of bet-hedging (sensu Seger and Brockmann 1987), so that egg-laying females ensure some mated daughters in an unpredictable environment (Soucy 2002).

To test whether sociality is under environmental control in another socially polymorphic halictid species (*Lasioglossum* [*Evylaeus*] *albipes*), bees were reared from social and solitary populations under reversed environmental conditions (Plateaux-Quénu et al. 2000). Even under short-season conditions with long photoperiods, females from social populations produced a brood of workers before producing sexual males and females. Conversely, the majority of females from a solitary population produced no workers when faced with conditions indicative of a long growing season. The authors surmise that sociality in this species, at least in part, is genetically controlled. Although not conclusive, phylogenetic studies suggest that social and solitary populations of this species are genetically distinct (Danforth 1999).

Common-garden experiments, like those conducted by Plateaux-Quénu et al. (2000) on *L.* (*E.*) *albipes*, have not been conducted on *H. rubicundus*. Therefore, it is unclear whether social and solitary behaviors are genetically determined in *H. rubicundus*. Following an intensive six-year study of a population in New York, Yanega (1989) proposed a proximal mechanism by which the environment may determine sociality in *H. rubicundus*. He suggested that gynes emerge from hibernation in response to a temperature cue, so that females in warm climates will emerge earlier than those in cool climates. Yanega (1993) went on to propose that gynes will lay mostly female eggs if the photoperiod is short and a high proportion of male eggs if the photoperiod is long. This hypothesis is consistent with the distribution of social populations in areas with long growing seasons, because the first brood of eggs, laid under early-season conditions of short photoperiod, will be female biased and will constitute a worker caste. Females from colder regions, who emerge from hibernation and begin provisioning closer to the summer solstice, will produce a single mixed male-female reproductive brood. Richards and Packer (1995) theorized that this and other eusocial sweat bees may respond to local environmental conditions by maintaining a variety of reproductive options. Michener (1990) invoked environmental control of sociality to explain the current holarctic distribution of *H. rubicundus*. He theorized that social populations in Europe and Asia switched to solitary behavior as they crossed the Bering Land Bridge during the Pleistocene and then reverted back to social behavior as they dispersed southward in North America.

In this study, we tested several hypotheses regarding the evolution of social behavior and the biogeographic history of *H. rubicundus*, using DNA sequence data and phylogeny reconstruction. First, we indirectly tested the hypothesis that sociality is genetically controlled. In a phylogenetic sense, this hypothesis predicts that social populations will be more closely related to each other than they are to solitary populations and vice versa, regardless of the geographic distance between them. Furthermore, considering that solitary populations inhabit areas of different climate than social populations, a model of genetic control predicts that lineages expressing different behaviors will inhabit regions to which they are best suited. Therefore, according to this hypothesis, genetic relatedness will be correlated both with sociality and environment. An alternative hypothesis is that all populations have the capability of expressing either social or solitary behaviors and they do so in response to environmental conditions at the nesting site. If this is true, sociality will be correlated with environmental variables but not with phylogeny.

We also tested three hypotheses regarding the biogeographic history of North American populations of *H. rubicundus*. The hypotheses used in this analysis rely on changing climatic regimes in North America and the movement of individuals across the Bering Land Bridge during the past 2 million years. Such events have been used to explain the current biogeographic distribution of many plants and animals in recent studies (Avise 1999). One hypothesis tested in this study is that populations at different altitudes represent different genetic lineages. This pattern may result if different lineages are best adapted to particular climatic conditions; therefore, as North American temperatures rose in the past 15,000 years, resident lineages tracked the climatic zones to which they were best suited. Those lineages that colonized the continent first will be found in the coolest localities on the continent (at higher altitudes), whereas more recent colonizers will be found in warmer climates. A second hypothesis is that the Rocky Mountains have acted as a barrier to dispersal for lineages inhabiting low altitudes. In this scenario, three continental zones (west of the Rockies, east of the Rockies, and in the Rocky Mountains themselves) will

TABLE 1. Amplification and sequencing primers used in the present study. Positions are based on the 5′ end of the primer and correspond to the positions reported in Crozier and Crozier (1993) for *Apis mellifera.*

Primer name	Position	Primer sequence
Sense primers		
GW	2049	5′-GGA TCA CCT GAT ATA GCA TTC CC-3′
Jerry	2481	5′-CAA CAT TTA TTT TGA TTT TTT GG-3′
Rick	2730	5′-CCA ACA GGA ATT AAA GTT TTT AGA TG-3′
Antisense primers		
Madeline	2585	5′-TTC TTT TTT [T/A/C]CC [T/A]CT TTC [A/G]TT [A/G]AA-3′
Pat	3382	5′-TCC AAT GCA CTA ATC TGC CAT ATT A-3′
Pat (modified)	3382	5′-TTC ATT [A/G]CA CTA TTC TGC CAT ATT A-3′
Marilyn2	3942	5′-CAT ATC TTC A[G/A]T ATC ATT GAT GTC C-3′

each be inhabited by a distinct lineage. A third hypothesis combines elements of the first two: Not only did populations track the climatic zone to which they were best suited, but unsuitable climatic zones act as a barrier to dispersal if they are located between zones of similar climate. The pattern resulting from this biogeographic history is that populations at high altitudes in the Rocky Mountains are one genetic lineage, those at a slightly lower altitude are a second lineage, and populations on either side of the Rocky Mountains are different from each other and from the two in the mountains. These three hypotheses do not directly imply genetic control of sociality, but they do require that different genetic lineages are best suited to different environmental conditions. Sociality may be linked to genetic make-up directly, if social structure is under genetic control, or indirectly, if social structure is environmentally determined and genetic lineages inhabit different environmental conditions.

MATERIALS AND METHODS

Population Sampling

Females and males of *H. rubicundus* were collected throughout its range in the United States during the summers of 1997 through 1999 and central Europe in the summer of 2000. Determination of the altitude as well as the longitude and latitude coordinates of each site was done with the use of a global positioning system device. Additional specimens were obtained from colleagues (L. Packer, K. Hogendoorn, C. Plateaux-Quénu, C. Skov) and collections (USDA Bee Lab, Logan, UT). For those specimens, localities were typically reported as the town or city in or near where the collection was made. We determined approximate altitude and longitude and latitude coordinates for them using topographic maps of each area. All specimens used for sequencing were preserved in 95% ethyl alcohol, but recently collected dried specimens (<5 years) that yielded good quality, high-molecular weight DNA were also used.

Laboratory Protocols

Abdomens of all specimens were removed before processing tissues for DNA extraction to prevent contamination by gut contents. Total cellular DNA was isolated from the remaining tissues by standard protocols (Sambrook et al. 1989). Specimens were ground in the presence of 2× CTAB extraction buffer and proteinase K, and incubated at 55°C for 2 h. The DNA mixture was then extracted with chloroform-isoamylalcohol, phenol-chloroform-isoamylalcohol, and chloroform-isoamylalcohol, in that order. This was followed by cold ethanol precipitation. DNA was resuspended in Tris-EDTA buffer and diluted to approximately 25 ng/μl.

A region of the mitochondrial genome corresponding to most of the cytochrome oxidase I (COI) gene, the entire leucine tRNA, and a portion of the noncoding AT-rich region that follows was amplified via the polymerase chain reaction (PCR; Saiki et al. 1988) using one or more primer pairs (Table 1). Initially the primers used were standard insect COI- and COII-specific primers (Simon et al. 1994). After generating a few *Halictus* sequences, new primers were developed that specifically amplified several overlapping regions of the mitochondrial genome totaling approximately 1900 nucleotide sites. PCR products were either gel purified in low-melting-point agarose gels or directly purified using Wizard PCR preps DNA purification system (Promega Corporation, Madison, WI) and sequenced in both directions on an ABI 377 automated sequencing machine (Applied Biosystems, Foster City, CA). Sequencing required a combination of amplification primers and internal primers, which varied for each sample depending on the exact combination of primers used for amplification.

Phylogeny Estimation

Sequences were aligned using the software package Sequencher (GeneCodes Corp., Ann Arbor, MI). Reliable sequence for each individual varied in length, from approximately 1500 to 1800 bp, due to differences in the sequencing process and the different primers used. We trimmed the trailing ends so that the sequence for each sample reflected a single homologous region of the genome, 1492 bp in length. Alignment of the sequence was unambiguous, with no insertion/deletion mutations detected within the coding region. Two insertion/deletion regions were detected in the AT-rich region. All members of the ingroup had a 3-bp deletion that was not detected in any of the outgroups, and some outgroups possessed another insertion ranging in length from 2 to 21 bp. The indel regions were not included in the analysis. Individuals possessing identical sequences were combined into a single operational taxonomic unit (OTU). Sequences have been deposited in GenBank (accession nos. AF438420–AF438481). Analyses of nucleotide sequences were performed using PAUP* version 4.0b6 (Swofford 2000), using

heuristic search criteria. Seven closely related *Halictus* species (Danforth et al. 1999) were used to root the trees.

Phylogenetic analyses were conducted by using distance (neighbor joining using simple addition and uncorrected p distances; NJ), maximum-parsimony (equally weighted; MP), and maximum-likelihood (ML) criteria. We estimated the phylogeny using ML through a sequential optimization approach (Fratti et al. 1997). We first generated trees by NJ and by MP. The MP analysis used heuristic search with tree bisection-reconnection (TBR) branch-swapping and 20 replicates involving random addition sequence of taxa. The MP search yielded more than 10,000 most parsimonious trees, each 1060 steps long. We identified a 50%-majority-rule consensus tree, which proved to be identical to one of the more than 10,000 most parsimonious trees. This was the tree used in the rest of the analyses. The NJ and MP trees were used as the basis for a series of likelihood-ratio tests (Yang et al. 1995), to determine the model of evolution in ML analysis. We estimated the likelihood parameters, and calculated the likelihood score for the NJ and ML trees under four possible substitution models (Jukes-Cantor 1969, JC; Kimura [1980] two-parameter, K2P; Hasegawa-Kishino-Yano 1985, HKY; general time reversible [Yang 1994], GTR) and four possible methods of accounting for among-site rate variation (no rate variation, estimating that a proportion of sites would remain invariable, incorporating a Γ-distribution correction, which allows for among-site rate variation and combining among-site rate variation and invariable sites). The GTR + Γ + I model had a significantly higher log likelihood than any of the other models. We then used this model of molecular evolution to perform a heuristic search with 20 random addition replicates and TBR branch-swapping. The first search used parameters estimated from the data on the NJ tree, and the second used parameters estimated on the data from the MP tree. Both searches resulted in the same topology. We then estimated the ML parameters on that topology and conducted a ML analysis using those parameters and the same heuristic search criteria.

Topology Robustness and Hypothesis Testing

We conducted bootstrap analyses using distance, MP, and ML methods to estimate support for the clades found on the three topologies generated via each of those methods. One hundred bootstrap replicates were conducted using each type of analysis. We tested differences in tree scores among the three trees (NJ, MP, ML) based on ML criteria (GTR + Γ+ I) by using a one-tailed Kishino-Hasegawa test (Kishino and Hasegawa 1989).

To decipher the relationship among phylogeny, sociality, environment, and geographic distribution, we used the Mantel test (Mantel 1967) in the software package NTSYS for PC version 2.02j (Applied Biosystems, Inc.) to test for correlations among different types of distance matrices. This test can be used on both discrete and continuous data (F. J. Rohlf, pers. comm.). The Mantel statistic tests the independence of the pattern of pairwise distances between localities in one matrix from the pattern of pairwise distances in a second matrix. The Mantel statistic, Z_{XY}, was transformed into a correlation coefficient whose magnitude is not scale dependent, to permit interpretation of the magnitude of the association between the distance matrices. When the Z statistic is normalized to a product-moment correlation coefficient, it yields 1.0 if there is perfect linear dependence of the two sets of distances, and approaches 0.0 if there is no relationship between them. We applied a Dunn-Šidák correction (a variation of a Bonferroni correction; Sokal and Rohlf 1995) to limit the overall experimentwise error rate.

Data for the matrix correlation analysis included environmental, genetic, and behavioral variables, as well as information about the collection locality, for 27 ingroup OTUs representing 24 localities in North America (Vancouver, British Columbia; Mission, OR; Fernley, NV; Pyramid Lake, NV; Daniel's Reservoir, ID; Treasureton Reservoir, ID; Idaho Falls, ID; middle fork of the Clear Creek, WY; Rawlins, WY; near Fish Lake, UT; Logan, UT [two specimens]; Wellsville Mountains, UT; Scofield, UT [two specimens]; Missoula, MT [two specimens]; Big Sky, MT; near Steamboat Springs, CO; Dolores, CO; Glenwood Springs, CO; Gothic, CO; Lawrence, KS; Kananaskis, Alberta [two specimens]; Southern Pines, NC; Stony Brook, NY; Ithaca, NY). A description of each distance measure follows.

Environmental variables.—These variables include the difference in various weather-related data for a pair of sites. For each locality we collected weather information from the weather station nearest to the collection site, averaged over 5 years (usually 1996–2000, but for weather stations that are no longer operational, the data are from the most recent 5 years for which data were reported). We chose the data based on our perception of the most relevant measures of the growing season and those variables that had the most complete information for each locality available from the National Weather Service. The weather information included mean monthly temperature for each of the months of January through June, mean monthly precipitation for each of the months of January through June, and number of days in the year with snow depths greater than or equal to 1 inch.

Genetic distance based on the maximum-likelihood model.—This variable is the number of nucleotide changes per site between two OTUs based on a GTR + Γ + I model of evolution. Multiple haplotypes from a single location (Logan, UT; Scofield, UT; Missoula, MT; Kananaskis, Alberta) and multiple locations that shared a haplotype (Rawlins, WY; Idaho Falls, ID; Gothic, CO; Steamboat Springs, CO) were treated as separate OTUs in the analysis.

Geographic location.—This variable is the great circle distances (km) between a pair of collection sites based on the geographic coordinates (longitude and latitude) of the sites.

Altitude.—This variable is the difference in altitude (m) between a pair of localities.

Sociality.—This variable is the difference in social level of populations from two sites. Populations were considered to be either social or solitary (semisocial nests of this species are not known). Pairs of populations exhibiting different social organizations were coded as 1, and pairs that were similar were coded as 0. Sociality of specimens from particular locations was determined either by excavation of nests, long-term observation of nesting aggregations, seasonal phenology (solitary bees do not fly earlier than late May), or bee dissections (females with undeveloped ovaries foraging in June

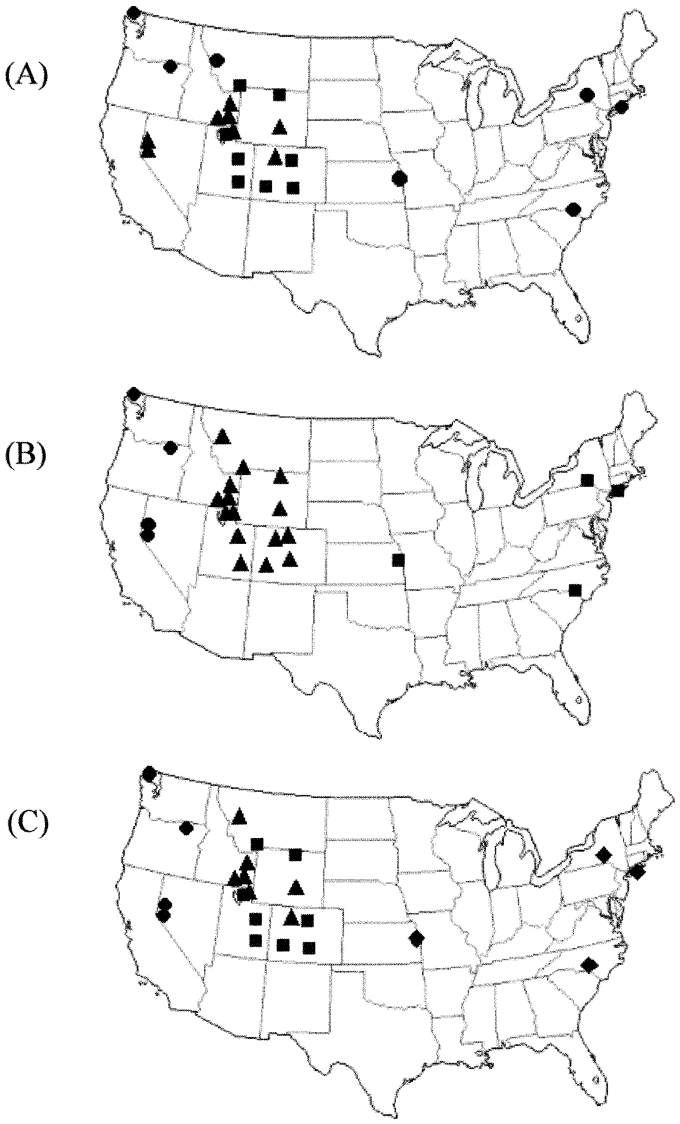

FIG. 1. Coding scheme used in the three design matrices, as hypothetical explanations for the distribution of *Halictus rubicundus* in North America. (A) Design matrix 1 is based on altitude of the locality; circles, less than 1000 m; triangles, 1000–1999 m; squares, 2000 m or above. (B) Design matrix 2 is based on the location of the collection site in relation to the Rocky Mountains; circles, west of the Rocky Mountains; triangles, in the Rocky Mountains; squares, east of the Rocky Mountains. (C) Design matrix 3 includes components of matrices 1 and 2; circles, west of the Rocky Mountains; triangles, in the Rocky Mountains less than 2000 m; squares, in the Rocky Mountains at 2000 m or above; diamonds, east of the Rocky Mountains.

and July indicate a nonreproductive worker caste). Populations that contained any workers were considered social.

Matrix Correlations

We employed design matrices to test the three alternative hypotheses regarding the biogeographic history of *H. rubicundus*. A design matrix describes the relative distances among populations expected under a particular hypothesis (e.g., Sokal et al. 1997; Waddle et al. 1998). Figure 1 shows three maps depicting the relative locations of a subset of the 24 populations used in the matrix correlation analysis. Differences among populations are described by the biogeographic hypotheses outlined in the introduction. The hypothetical relationships among pairs of populations were then converted to distance matrices as a binary variable, so that populations in the same category received a distance score of 0, and populations in different categories received a score of 1: Design 1: differences among populations are explained by three different altitudinal zones (less than 1000 m, 1000–1999 m, 2000 m or greater); design 2: differences are explained by the general geographic region in which the population is found (west of the Rocky Mountains, in the Rocky Mountains, east of the Rocky Mountains); and design 3: differences are explained by a combination of altitude and east–west differentiation (west of the Rocky Mountains, east of the Rocky Mountains, in the Rocky Mountains less than 2000 m, in the Rocky Mountains at 2000 m or higher).

RESULTS

For 61 specimens we produced an alignment spanning 1492 bp (positions 2177–3668 of the *Apis mellifera* mitochondrial genome; Crozier and Crozier 1993). This includes sequence for seven outgroups (*Halictus confusus*, *H. sexcinctus*, *H. farinosus*, *H. parallelus*, *H. crenicornis*, *H. ligatus*, and *H. quadricinctus*) and 54 individuals of *H. rubicundus* from North America ($N = 41$) and Europe ($N = 13$). There are 38 unique sequence haplotypes in the ingroup. After removing the insertion/deletion regions, the sequence yielded 411 variable positions of which 285 were parsimony informative. Among the ingroup, 135 sites were parsimony informative; 13.0% of the changes are in the first-codon position, 3.4% are second-position changes, and 83.6% are third-position changes. There is only one nonsynonymous change among the ingroup taxa, at position 2424 (corresponding to a first-codon position in the COI coding region), resulting in an amino acid substitution from leucine, present in all European specimens and all outgroups, to methionine, found in all North American specimens.

Nucleotide composition in this region is AT biased, as is common for bees (e.g., Crozier and Crozier 1993; Danforth 1999). Empirical base frequencies are A = 0.337, C = 0.142, G = 0.099, and T = 0.422. The topology resulting from the heuristic search using neighbor joining criteria is presented in Figure 2A, and the topology resulting from the search using equally weighted MP is presented in Figure 2B. The model with the highest log likelihood was a general time reversible model (GTR) with a proportion of invariable sites (I = 0.655) and among-site rate variation ($\Gamma = 1.891$) and the topology in Figure 2C. Average uncorrected and ML pairwise genetic divergences are presented in Table 2.

Topology Robustness and Hypothesis Testing

The MP approach yielded more than 10,000 trees, each with a tree length of 1060 steps. Of these, no tree is identical to the NJ or the ML trees, although several nonbinary trees are compatible. There is agreement among the different phylogenetic analyses for all well-supported nodes. The monophyly of *H. rubicundus* is supported by a bootstrap value at

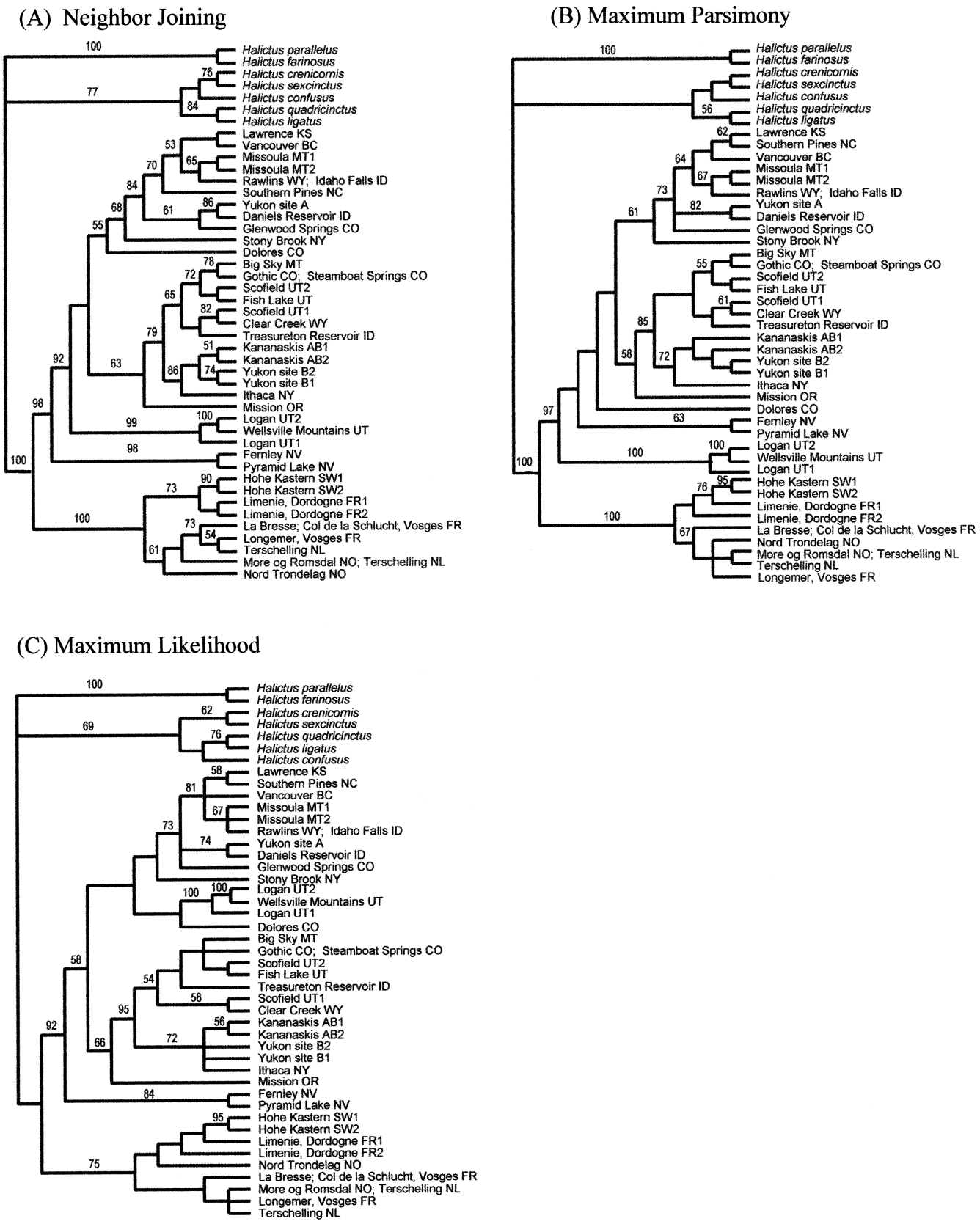

FIG. 2. Three topologies generated by different phylogenetic analyses: (A) neighbor joining; (B) maximum parsimony; (C) maximum likelihood (GTR + Γ + I). Numbers above branches indicate bootstrap support. FR, France; NO, Norway; NL, the Netherlands; SW, Switzerland; AB, Alberta, Canada.

TABLE 2. Average uncorrected and maximum-likelihood (ML; GTR + Γ + I) pairwise genetic divergences within or among selected clades (range in parentheses).

	N	Uncorrected pairwise genetic distance	ML pairwise genetic distance
Within *Halictus rubicundus*	1409	1.92% (0.0–4.76%)	2.30% (0.0–6.59%)
Within continents	1291	1.21% (0.0–3.75%)	1.39% (0.0–4.73%)
Between North American and Europe	118	2.89% (1.20–4.76%)	3.83% (2.57–6.59%)
Among species	28	10.36% (5.29–12.00%)	24.40% (6.61–32.95%)

100% in all analyses (see Fig. 2). The North American and European specimens make up two distinct clades, each with high bootstrap support in all analyses (NJ: Europe = 100%, North America = 98%; MP: Europe = 100%, North America = 97%; ML: Europe = 75%, North America = 92%). Within the North American group, there are two clades that are generally supported by all bootstrap analyses. The first includes Lawrence, Kansas; Vancouver, British Columbia; Missoula, Montana; Rawlins, Wyoming; Idaho Falls, Idaho; Southern Pines, North Carolina; Daniel's Reservoir, Idaho; Glenwood Springs, Colorado; and a low altitude site in the Yukon. Bootstrap values for this node are 84% (NJ), 73% (MP), and 73% (ML). The other clade includes Big Sky, Montana; Gothic, Colorado; Scofield, Utah; Ithaca, New York; Treasureton Reservoir, Idaho; the Middle Fork of Clear Creek in Wyoming; a location near Fish Lake, Utah; a location near Steamboat Springs, Colorado; a location near Kananaskis, Alberta; and a high-altitude site in the Yukon. Bootstrap values for this node are 79% (NJ), 85% (MP), and 95% (ML). Many of the other branches in these analyses indicate weak resolution and very short branch lengths (see Figs. 2, 3). There is little indication of any consistent structure among the European specimens.

FIG. 3. The maximum-likelihood tree indicating branch lengths. FR, France; NO, Norway; NL, the Netherlands; SW, Switzerland; AB, Alberta, Canada.

The results of the Kishino-Hasegawa test indicate that there is no significant difference in ML tree scores among the NJ, MP, and ML trees (NJ: −log likelihood = 6106.51, MP: −log likelihood = 6108.12, ML: −log likelihood = 6092.42; NJ vs. ML, $t = 1.47$, $P = 0.408$; MP vs. ML, $t = 1.26$, $P = 0.390$). No one topology is significantly more likely under the GTR + Γ + I model of evolution.

For most of the populations of *H. rubicundus*, branch lengths indicate consistent rates of evolution among lineages. The clade that includes three samples from Logan, Utah (two of which were identical), and one sample from the Wellsville Mountains in Utah (approximately 15 miles from Logan) exhibits unusually long branch lengths. Many of those sequences have an excessive number of polymorphic sites, especially the sequence labeled ''Logan, UT2.'' Because this result may be due to amplification of nuclear copies of the COI gene, the sample was removed from the analysis.

We plotted the social behaviors on the ML tree for populations of *H. rubicundus* for which the behavior is known (or inferred based on criteria described in Materials and Methods), and for the outgroups (see Fig. 4). For several populations, the social behavior is undetermined. According to a molecular phylogeny of the genus *Halictus*, the species with which *H. rubicundus* is closely allied also express social behavior, suggesting that sociality is an ancestral condition for *H. rubicundus* (Danforth et al. 1999). All of the North American populations of *H. rubicundus* that express solitary behavior are found in a single clade according to topologies derived using all three methods (NJ, MP, ML). Among the European populations, the three topologies indicate that solitary behavior has either evolved once (at the base of all European populations) with one (NJ) or two (MP and ML) transitions to social behavior (DELTRAN optimization) or it has evolved twice (MP and ML; ACCTRAN optimization)—once at the base of all European populations and once in the ancestor of the Hohe Kastern populations—with one transition to social behavior in the ancestor of the Limenie and Hohe Kastern populations.

Matrix Correlations

Although we obtained sequences for 41 North American specimens, there were only 29 OTUs used in the Mantel tests. This is because the exact location and weather information was not available for the two sites in the Yukon (four sequences), one sequence for the Logan, Utah, population was most likely a nuclear pseudogene, and there were multiple

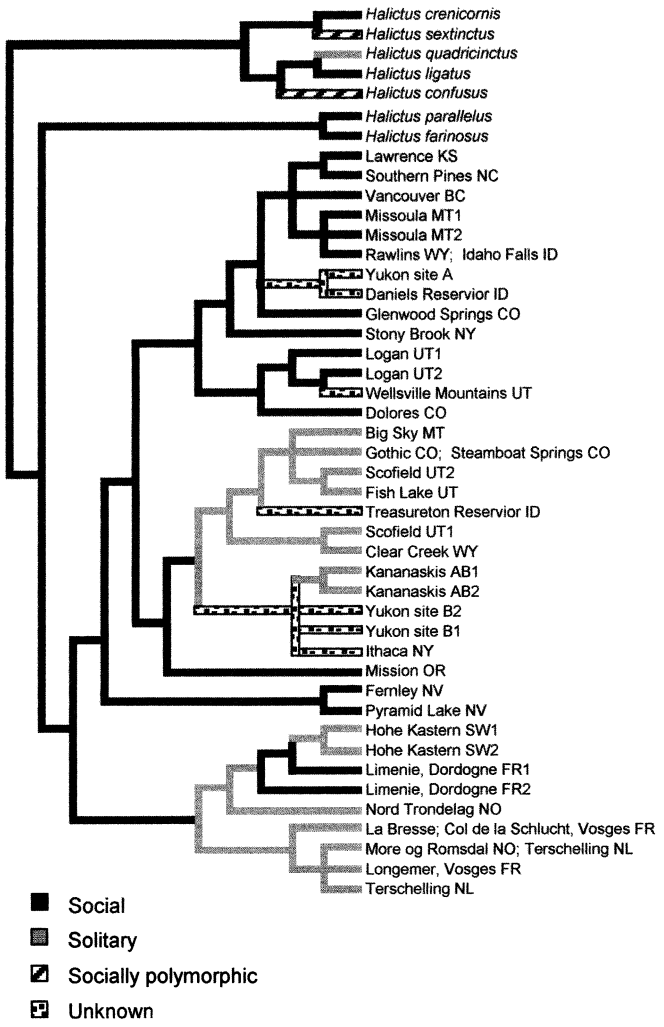

FIG. 4. The maximum-likelihood cladogram with social states mapped onto it, using ACCTRAN optimization. Behavioral categorizations were obtained by methods described in the text. FR, France; NO, Norway; NL, the Netherlands; SW, Switzerland; AB, Alberta, Canada.

samples from the same location yielding identical sequences (Stony Brook, NY; Lawrence, KS; Gothic, CO; Kananaskis, Alberta; Logan, UT; Glenwood Springs, CO; Steamboat Springs, CO; Idaho Falls, ID). Results of the Mantel tests of pairwise correlations of the genetic variable and sociality to the environmental variables, altitude and geographic location (i.e., longitude and latitude), as well as the correlations of sociality to the genetic variable, are presented in Table 3. There is no significant relationship between any of the environmental variables and the genetic variable (note that the significance of *P*-values was determined after applying Dunn-Šidák correction). Sociality is correlated only with the number of days in the year with one or more inches of snow on the ground. Sociality also is correlated with ML distance ($N = 325$, $t = 4.4253$, $P = 0.0030$). Neither altitude nor geographic location is correlated with genetic distance or sociality. Tests of pairwise correlations of ML distance and sociality to the three design matrices are presented in Table 4. Design matrix 3 is the only one that is correlated to genetic distance, and none of the design matrices are correlated to sociality.

TABLE 3. Table of Mantel statistics (*Z*) normalized as product-moment correlation coefficients. The statistics are based on Mantel tests for comparisons among genetic distance, environmental variables, altitude, location (longitude and latitude), and sociality. Variable codes: 1MNTM through 6MNTM, January through June mean temperature (°C); 1TPCP through 6TPCP, January through June total monthly precipitation (cm); DNSW, number of days in the year with snow depths greater than or equal to one inch. *P*-value is based on a one-tailed probability that a random *Z*-statistic would be greater than or equal to the observed value of *Z* in 9999 permutations of the data. Significance of *P*-value is indicated by an asterisk, based on a Dunn-Šidák correction.

	Maximum-likelihood distance		Sociality	
	Matrix correlation (*r*)	*P*	Matrix correlation (*r*)	*P*
1MNTM	−0.11815	0.1065	0.21886	0.1327
2MNTM	0.05792	0.2082	0.50976	0.0974
3MNTM	0.06792	0.1988	0.51248	0.0955
4MNTM	0.00648	0.3946	0.55265	0.0918
5MNTM	0.06247	0.2338	0.56008	0.0913
6MNTM	0.17252	0.0394	0.59032	0.0805
1TPCP	−0.03779	0.4331	0.09659	0.3608
2TPCP	−0.13170	0.0934	0.04339	0.4053
3TPCP	−0.08234	0.2650	−0.06983	0.4492
4TPCP	−0.06168	0.3250	−0.05549	0.3961
5TPCP	0.00074	0.4375	−0.00788	0.3940
6TPCP	−0.02056	0.4478	−0.08108	0.4392
DSNW	0.08548	0.1765	0.74107	0.0022*
Altitude	0.11041	0.1212	0.37807	0.3517
Location	−0.09501	0.2503	−0.06707	0.4461
Sociality	0.29499	0.0007*		

DISCUSSION

Although all of the members of the ingroup for this study are members of a single species, the analysis of DNA sequence indicated some genetic structure among populations. The North American and European lineages of this species are clearly distinct, yet the amount of pairwise sequence divergence between the two clades is in a range that is generally considered to be found within a single insect species (e.g., Vogler et al. 1993). The differentiation among populations on the two continents is clearly a result of a lack of gene

TABLE 4. Table of Mantel statistics (*Z*) normalized as product-moment correlation coefficients. The statistics are based on Mantel tests between genetic or sociality matrices. Significance of statistic is based on a one-tailed probability that a random *Z*-statistic would be greater than or equal to the observe value of *Z* in 1000 permutations of the data. A description of the three design matrices is presented in the introduction and in Figure 1. Significance of *P*-value is indicated by an asterisk, based on a Dunn-Šidák correction.

	Maximum-likelihood distance		Sociality	
	Matrix correlation (*r*)	*P*	Matrix correlation (*r*)	*P*
Design 1	0.05072	0.3775	0.34178	0.1155
Design 2	0.14560	0.1151	−0.10284	0.3659
Design 3	0.47336	0.0014*	0.27509	0.2648

flow due to geographic separation. In a study of allozyme variation in *H. rubicundus*, Packer and Taylor (1997) found five fixed differences between North American and European specimens; although the study contained only a few sample populations, the authors suggest that this is a level of differentiation incompatible with conspecificity. Divergence values between North American and European populations would suggest that these lineages are incipient species. It would be very informative to add data from Siberian populations to both of these studies to see if they are genetically intermediate between Old and New World samples.

Among the North American sites, populations expressing social and solitary behavior belong to different genetic lineages. According to the best trees using all three methods (NJ, MP, ML), all of the known solitary populations in North America are in a single well-supported clade, along with several specimens for which sociality is not known. Social populations are scattered on all trees, but many social populations form another well-supported North American clade including individuals from populations as widely dispersed as North Carolina and British Columbia. It would be quite informative to determine the social state of populations in this study for which there currently is no information. In particular, the population from Yukon site A might be solitary based on climatic conditions, and the population from Ithaca, New York, might be social. Such data may weaken the relationship between sociality and genetic distance detected in this study.

Several populations of *H. rubicundus* are genetically more similar to other populations that demonstrate the same social organization than they are to populations that are geographically closer. For instance, populations in Gothic, Colorado, and Big Sky, Montana (separated by 793 km), are more closely related to each other than the population from Gothic is to the Glenwood Springs, Colorado, population (separated by 71 km) or the population from Big Sky is to the Idaho Falls, Idaho, population (separated by 209 km). These results suggest that there are some barriers to gene flow among closely situated populations that express different modes of sociality. Clearly, the mechanisms of this barrier, whether they be differences in morphology, phenology, or some other means, require further investigation to establish whether the lack of gene flow indicates incipient speciation. Long-term behavioral observations designed to investigate differences in life histories of nearby social and solitary populations, as well as controlled mating experiments, would both be informative in this regard.

It is noteworthy that in all analyses (NJ, MP, ML) the two populations from Nevada are basal to all other North American populations. These locations may also represent the only areas in this study not affected by glaciation in the past 0.5 million years. Therefore, the genetic structuring among North American populations of *H. rubicundus* may reflect the influence of glacial advances and retreats during the Pleistocene. Indeed, using the crude estimate of 2% mitochondrial divergence per million years, cladogenesis of all North American lineages of *H. rubicundus* occurred some time during the last round of glaciation, from approximately 600,000 years ago to the present. Climatic changes in North America during the Pleistocene have been used to explain genetic structuring in many species of animals (Hafner and Sullivan 1995; Friesen et al. 1996; Byun et al. 1997; Stewart and Baker 1997; Holder et al. 1999; Nielson et al. 2001) including insects (Noonan 1990; Scudder 1993; Hewitt 1996; Tregenza et al. 2000). Therefore, it is possible that a transarctic passage through Berengia or glacial refugia in the southwestern United States also played an important part in the movement and colonization of *H. rubicundus* in North America.

We posed two hypotheses regarding control of social behavior in *H. rubicundus*. First, if sociality is genetically controlled, then pairs of populations that are genetically related will demonstrate similar social behaviors, even if they are separated by large geographic distances. With genetic control of sociality, environmental factors may be correlated with both sociality and genetic relatedness. As an alternative hypothesis, we proposed that if sociality is entirely facultative, with populations able to express a social behavior that is appropriate for the environmental conditions at the nesting site, then sociality will be correlated to environmental conditions, but not to genetic make-up. The results of the Mantel tests indicate that sociality is indeed correlated to genetic make-up but that geographic location and genetic relatedness are not correlated. These results are consistent with genetic control of sociality.

Other potential scenarios can result in the observed correlations. For example, if sociality is not under genetic control per se, but instead different genetic lineages, for some other reason, are better suited to particular environmental conditions, then one would expect genetic make-up to be correlated to environmental conditions. Given that relationship, even though all members of the species would be capable of expressing either social or solitary behavior, certain lineages would be found in regions with particular environmental conditions and those environmental conditions would lead to the expression of either social or solitary behavior. However, this scenario is not supported by the results because none of the distance matrices based on environmental variables are correlated to genetic distance. One problem with the current analysis that may account for the failure to detect relationships with environmental variables is that the weather data were obtained from weather stations near, but not always at, the collection site. One might expect that conditions may be different enough in the two locations to account for the lack of correlation.

It is intriguing that the one environmental parameter that is correlated to sociality is the number of days in the year with one or more inches of snow on the ground. This parameter has been found to correlate well with life-history variation in other diverse taxa such as ants (Evans 1996), plants, marmots, and hummingbirds (Inouye and McGuine 1991). Ultimately, snow cover may be related to the length of the growing season in many North American locales, which may be the more proximate link to the level of sociality expressed by *H. rubicundus*.

Another possible scenario accounting for the observed relationships among sociality, genetics, and environment, is that the propensity to facultatively express sociality is genetically controlled; some lineages are able to express social and solitary behavior and others express only social or solitary behavior. Those lineages that have the ability to fac-

ultatively switch between social and solitary behavior are the only ones that can persist in all climate types. Obligately social genotypes would be found only in warm regions, and obligately solitary genotypes only in regions of cool climate or at high altitudes. This could explain why sociality is linked to some measures of environmental conditions, and sociality is correlated to genetic make-up, but why genetic make-up is not necessarily related to environmental conditions.

Another possibility is that even, if sociality is genetically controlled, there may be genetic polymorphism within populations with respect to social behavior. If this were true, even if individuals themselves are not facultatively social, the population may express facultative sociality as the frequency of genetically social and solitary individuals varies over time. This polymorphism could be maintained by year-to-year variation in environmental conditions. In the present study, we coded sociality as a binary character, when in fact both social and solitary behaviors may be expressed within single populations (Yanega 1993; Eickwort et al. 1996). Short of conducting common-garden experiments with individuals from all localities to determine whether populations are social, solitary, or facultative, it may be possible to code sociality as a continuous, rather than categorical, variable. For instance, one might code sociality as the fraction of males in the first brood of each population. Yanega (1992) noticed that the fraction of males in the first brood of a nest is tightly correlated to the number of first-brood females who mate and enter diapause rather than remaining in their natal nest as workers. As the fraction of first-brood males approaches 50%, none of females remain in their natal nest as workers, and the nest is effectively solitary. When the first brood contains no males, all of the first-brood offspring are workers and the population is social. It would be helpful to measure the fraction of males in the first brood for each population included in this study, or to infer it from the fraction of mated workers in the first brood, to code sociality as a continuous variable.

We determined whether design matrices based on altitude, geography, or a combination of these parameters (Fig. 1) explained the genetic and social variation in our study populations. The results indicate that the biogeographic hypothesis described by design matrix 3 is correlated with genetic structure. Altitudinal and geographic zones alone (matrices 1 and 2, respectively) are not correlated to the genetic matrices. The fact that the genetic matrices are correlated to design matrix 3, but not to design matrices 1 and 2 or to the matrices of geographic location or altitude, implies that there is some complex geographic structuring of the population that is not correlated to a single metric. A colonization history that would generate the pattern represented by design matrix 3 is one in which a lineage that was adapted to cooler climates invaded North America, and tracked the cool conditions to areas of high altitude as the climate warmed. Another lineage, which was better suited to warmer climates, may have arisen from the cool-climate lineage and inhabited to warmer regions on the continent. The second lineage may exhibit a fair degree of panmixia within geographic zones (east, west, and within the Rockies) but slight differences in conditions and nesting phenology may limit gene flow and migration between zones. Once again, this scenario invokes a genetic differentiation among lineages and the propensity of different lineages to express either social or solitary behavior.

Several other aspects of this study require further analysis. First, insufficient sampling of European populations may have limited the resolution among clades on that continent. Only one social population from Europe, accounting for two sequence haplotypes, was included in this study. It would be informative to include more samples of both social and solitary populations from Europe, and perhaps also from Asia, to determine whether the patterns observed in North America are also found on other continents. Second, it is important to code the environmental variables using data that more accurately reflects the conditions at the nesting site to draw conclusions regarding the relationships among these and genetic distance. Third, all the DNA sequence data reflect patterns for a single maternally inherited gene. By including DNA sequence data from an independently inherited gene, such as a nuclear gene, one may determine whether the topologies presented here are likely to reflect the history of the species, the history of the females of the species, or the history of the gene alone.

As a whole, these populations reveal a genetic structure that indicates the expression of social and solitary behavior in North American populations is not independent of the evolutionary history of lineages. However, the results do not rule out the possibility that some lineages have a genetic predisposition for the environmental control of sociality. The idea that facultative sociality might be a heritable trait is also consistent with the finding that facultative sociality may have been a feature of the ancestor of *H. confusus* and *H. tumulorum* (other members of the subfamily Halictinae; Danforth et al. 1999), or the ancestor of *L.* (*E.*) *albipes* and *L.* (*E.*) *calceatum* (L. Packer, pers. comm.), and it was inherited by both daughter species. In *H. rubicundus*, the ability to demonstrate both social and solitary behavior may allow this species to inhabit regions of cool climate, whereas obligately social species may not. At the same time, an obligately social lineage of *H. rubicundus* may have higher fitness in warmer regions that are better suited for consistent social behavior than does a facultatively social lineage. The distribution of lineages across the continent and the expression of behaviors in those lineages most likely reflect a combination of geographic partitioning and environmental control of sociality.

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