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INSIGHT INTO SPECIATION FROM HISTORICAL DEMOGRAPHY IN THE PHYTOPHAGOUS BEETLE GENUS *OPHRAELLA*

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Abstract.—Speciation in phytophagous insects is commonly associated with shifts in host use. Using a phylogenetic framework to identify recently diverged taxa that have undergone a radical host shift, this study focuses on how reconstruction of the historical demography of a species, in conjunction with branching patterns between species, provides insight into mode of speciation. Analyses of mitochondrial cytochrome oxidase I sequences indicate that the leaf beetle *Ophraella communa* exhibits significant population structure, as shown by patterns of genealogical relationships among mitochondrial haplotypes and high F_{ST}-values. However, the absence of regional localization of old clades of haplotypes, negative Tajima's D, and unimodal rather than bimodal frequency distribution of the number of pairwise differences between sequences suggests an absence of long-term barriers to gene flow. Furthermore, we found no evidence of isolation by distance. This pattern of genetic variation is consistent with episodes of gene flow on a large geographic scale, perhaps owing to Pleistocene changes in climate.

Ophraella communa and its sister species *O. bilineata* diverged during the early Pleistocene. The evidence of dynamic population structure in *O. communa*, potentially including episodic but massive gene flow, suggests that reproductive isolation evolved quite rapidly on a localized geographic scale, because speciation would probably have been reversed by gene flow if the evolution of reproductive isolation had been prolonged. That is, gene flow occasioned by range shifts during the Pleistocene would likely have interrupted speciation unless it occurred very rapidly.

Sequence diversity implies a large effective population size ($> 10^6$) in both *O. communa* and *O. bilineata*. However, a model based on a drastic bottleneck did not have a lower likelihood than a model with no bottleneck, simply because the time since speciation has been great enough for coalescence to a single ancestor that existed after the speciation event. Sequence diversity in itself, without reference to the time since speciation, cannot provide evidence on the demography of speciation.

Key words.—Bottlenecks, Chrysomelidae, coalescence, historical demography, *Ophraella*, phylogeography, phytophagous insects, speciation.

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Speciation is among the most obdurately controversial topics in evolutionary biology, and few of its aspects remain more controversial than the role of population bottlenecks proposed by Mayr (1954), Carson (1982), and Templeton (1980). The "peripatric speciation," "founder-flush," and "transilience" models described respectively by these authors have in common the proposition that genetic drift in a temporarily small population (such as a colony founded by few individuals) alters allele frequencies at some loci, such that selection then moves the population to a new genetic equilibrium, or adaptive peak. According to some theoretical analyses, such peak shifts are unlikely to occur and, if they do occur, are unlikely to engender reproductive isolation (Barton and Charlesworth 1984; Barton 1989, 1996; Charlesworth 1997), whereas other models that are grounded in different assumptions lend more plausibility to the hypothesis (Wagner et al. 1994; Whitlock 1995; Gavrilets and Hastings 1996; Slatkin 1996; Gavrilets and Boake 1998). The interpretation of data on bottlenecked laboratory populations, even if considered relevant to speciation in nature, is as controversial as the hypothesis they are supposed to test (Rice and Hostert 1993; Templeton 1996). The observations that led Mayr (1942, 1954) to propose peripatric, or founder-effect, speciation (viz., the conspicuous phenotypic divergence of many peripherally isolated populations relative to their widespread presumptive ancestors) can plausibly be attributed to selection alone. Thus, it appears that the hypothesis remains difficult to evaluate.

Analyses of molecular genetic variation, however, promise to shed some light on the incidence of founder-effect speciation. The failure of some recently arisen species to meet the simple expectation that they should display little variation if they had been bottlenecked has been used as evidence against the hypothesis (e.g., Barton 1989). However, whether a bottleneck is reflected in contemporary patterns of genetic variation depends on the time and duration of the bottleneck relative to the effective population size (e.g., Eyre-Walker et al. 1998). Therefore, high genetic diversity is not necessarily in conflict with a bottleneck at speciation. Coalescent theory and the genealogical structure of DNA sequence variation afford a framework to test whether patterns of genetic variation are consistent with a particular demographic model (Slatkin and Hudson 1991; Crandall and Templeton 1993; Marjoram and Donnelly 1994; Wakeley and Hey 1997). For example, analyses of sequence variation can often indicate whether populations or species have recovered from recent severe reductions in effective population size (e.g., Rogers and Harpending 1992; Takahata 1993). Documenting that a

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species has experienced a reduction in population size cannot in itself show that the reduction played a causal role in speciation, but a consistent association between such reduction and the acquisition of reproductive isolation would strengthen the circumstantial evidence for founder-effect speciation. Conversely, it may be possible to rule out founder events as a frequent cause if most species should be found not to have experienced severe reductions early in their history.

Molecular genetic variation also provides data on the phylogeography of a species, the analysis of the geographic distribution of gene lineages (Avise 1994). Knowledge of phylogeography can contribute to the analysis of speciation by revealing historical barriers, geographic (e.g., peripheral) loci of differentiation, and patterns of gene flow that may bear on the likelihood of sympatric versus allopatric speciation. Moreover, a gene genealogy can be used to infer how rapidly the speciation process may have proceeded, that is, whether the evolution of reproductive isolation was rapid or extended over a large temporal interval (Avise and Walker 1998; Knowles 1999). For example, the structuring of population genetic variation into distinct phylads and the timing of coalescence of lineages within phylads relative to the separation of sister taxa have been used to suggest that the speciation process in many vertebrates is protracted, with a duration of at least 2 million years, on average (Avise et al. 1998).

This paper concerns the phylogeography and the demography of speciation in two closely related species of leaf beetles (Chrysomelidae). In a phylogenetic study of the genus *Ophraella*, Funk et al. (1995) obtained evidence from mitochondrial DNA sequences suggesting that the broadly distributed species *O. communa* might be paraphyletic with respect to a more narrowly, peripherally distributed species, *O. bilineata*. Similar genealogical patterns in other organisms have supported the hypothesis of speciation in peripheral isolates (e.g., Avise et al. 1983; Hey and Kliman 1993). Whereas *O. communa* appears to be geographically variable in host association, *O. bilineata* is restricted to a single host species that is only distantly related to the hosts of *O. communa*. The present study was designed to characterize the phylogeographic structure of *O. communa*, to test for peripatric speciation and evidence of a population bottleneck at the origin of *O. bilineata*, and to infer relationships among *O. bilineata* and different regional populations of *O. communa*, perhaps thereby to elucidate the history of the host shift.

Materials and Methods

The Species

Ophraella communa LeSage and *O. bilineata* (Kirby) are two of five morphologically very similar species (LeSage 1986; Futuyma 1990). Specimens referred to *O. communa* by LeSage (1986) range across all of North America from southern Canada to southern Mexico (Fig. 1). In eastern North America, *O. communa* apparently feeds only on an annual ragweed, *Ambrosia artemisiifolia* (Asteraceae, tribe Heliantheae, subtribe Ambrosiinae). It has been recorded in several western localities on two perennial *Ambrosia* species and on another ambrosiine plant, *Iva axillaris*, and it has occasionally been found on three other genera of Heliantheae (Palmer and Goeden 1991). Specimens referred to *O. bilineata* by LeSage (1986) have been collected only in the prairies of southern Canada and the northern United States, with scattered records as far south as Nebraska and Colorado (Fig. 1). The sole known host plant of *O. bilineata* is *Chrysopsis villosa* (Asteraceae, tribe Astereae).

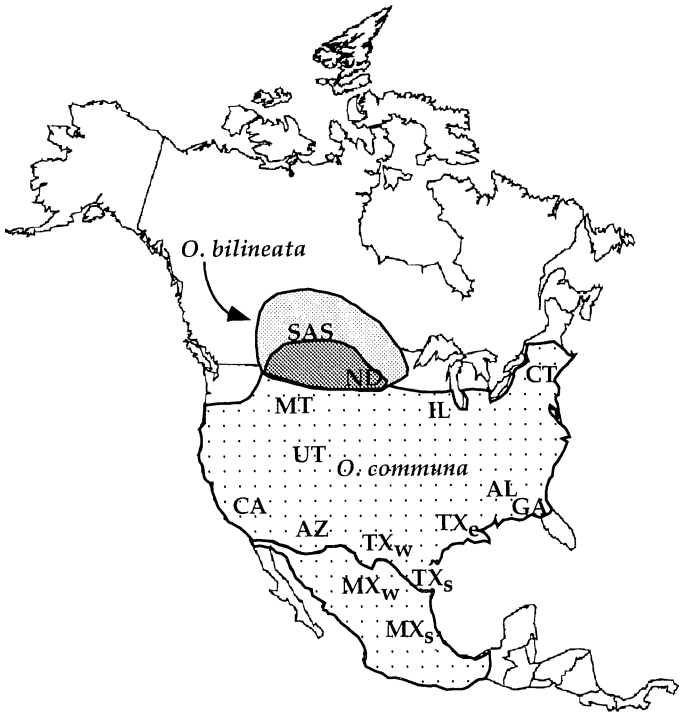

FIG. 1. Distributions of *O. communa* and *O. bilineata* identifying sampling localities (see Appendix for site information). Distributions from LeSage (1986), Futuyma (1990), and personal records.

The phylogeny of *Ophraella*, based on mtDNA sequence data (Funk et al. 1995), implies that the association of *O. communa* with ambrosiine hosts is plesiomorphic relative to the association of *O. bilineata* with *Chrysopsis*. The secondary compounds of these host plants differ substantially (Futuyma and McCafferty 1990; Jansen et al. 1991), implying a perhaps substantial adaptive change in the origin of *O. bilineata*.

Collections, DNA Amplification, and Sequencing

Specimens of *Ophraella communa* (92 individuals) were sampled from 16 localities that encompass much of the species' range and were taken from *Ambrosia artemisiifolia* (Fig. 1) (see Appendix for localities and the few collections from other host plants). Twenty-two specimens of *O. bilineata* were collected on *Chrysopsis villosa* from three localities (see Appendix). The specimens of these species listed in the study by Funk et al. (1995) are included in the present study.

Animals were collected and preserved whole in 70% ethanol. Genomic DNA was isolated using CTAB (beetle elytra were saved for species confirmation). A 473-bp fragment of cytochrome oxidase I (COI) was amplified in all individuals using primers C1-J-1718 (GGAGGATTTGGAAATTGATTAGTTCC) and C1-N-2191 (CCCGGTAAAATTAAAATA-

TAAACTTC). For a subset of individuals, primers C1-J-2183 (caacatttattttgatttttttgg) and L2-N-3014 (TCCATTGCACTA-ATCTGCCATATTA) were used to amplify an additional 831-bp fragment of COI (Simon et al. 1994). Forty amplification cycles were performed on a Idaho Technologies (Idaho Falls, ID) cycler (94°C for 15 sec, 56°C for 15 sec, and 72°C for 55 sec). Polymerase chain reaction (PCR) products were gel-purified on 1% low-melting-point agarose and stained with ethidium bromide. Target bands were excised, dissolved in ddH_20 and used in direct sequencing of double-stranded fragments (Khorana et al. 1994). Sequence was obtained from both sense and antisense strands.

Data Analysis

For each individual, we collected and analyzed a 400-bp region of sequence corresponding to positions 1718–2191 in the mitochondrial genome of *D. yakuba* (Clary and Wolstenholme 1985)(GENBANK accession nos. AF189590–AF189688). Unique sequences were determined using Maclade 3.0 (Maddison and Maddison 1992) and used in all phylogenetic analyses. Data were analyzed using maximum likelihood, generalized parsimony, and neighbor joining (PAUP*, Swofford 1998). For a subset of individuals (21 individuals), we collected and analyzed a total of about 905 bp of COI to confirm estimates of genealogical relationships between species and among clades within species.

Patterns of genetic variation and levels of genetic diversity within and among populations of *O. communa* were analyzed with HEAP BIG (Palumbi et al. 1997). Population-level differentiation was estimated using the F_{ST} approach of Hudson et al. (1992). Estimates of F_{ST} based on average sequence divergence among populations are comparable to F_{ST}-values calculated from allele frequencies (Hudson et al. 1992). Statistical significance of F_{ST}-values was determined by 1000 Monte Carlo randomizations of mtDNA sequences among populations, using HEAP BIG. Mantel tests (Sokal and Rohlf 1995) were used to test for an association between F_{ST} and geographic distance separating populations among all pairs of populations (i.e., evidence of isolation by distance). Isolation by distance was also evaluated by calculation of F_{ST}-values for groups of populations within a particular distance class to determine if there was an increase in F_{ST} as the distance separating populations increased. Six distance classes, defined by a 500-km sequentially increasing increment, were used in this structured F_{ST} analysis. Statistical significance of the Mantel tests was determined by comparing the observed *r*-value to a distribution created from 1000 random permutations. To evaluate how much these tests and estimates of F_{ST} were affected by the inclusion of populations with low diversity, both the F_{ST} estimates for the species as a whole and the tests of association between F_{ST} and geographic distance were also made omitting populations with low diversity (i.e., the California and Alabama populations). Kimura's two-parameter model (Kimura 1980) was used to calculate pairwise genetic distances.

Genetic diversities within *O. communa* and *O. bilineata* were examined, using the program SITES (Wakeley and Hey 1997), by calculating both π, the average pairwise difference between sequences (Tajima 1983), and $\hat{\theta}$, Watterson's estimator of θ, based on the number of segregating sites (Watterson 1975). Because this study is based on mitochondrial DNA, both π and $\hat{\theta}$ have expected values of $2N_f\mu$, where N_f is the effective population size of females and μ is the mutation rate per base pair per sequence per generation (μ was assumed to be 8.5×10^{-9}; Brower 1994). Tajima's *D* was used to test for deviation from the neutral equilibrium model of evolution (Tajima 1989a). Although this measure of variation can be used to examine the history of selection, it can also be used to make inferences about population demography. π and θ have the same expected value, but $\hat{\theta}$ is more greatly influenced by low-frequency polymorphisms. Thus, Tajima's *D* is expected to be negative under a model of population expansion and positive under a model of population subdivision (Tajima 1989b). The program SITES was used to calculate Tajima's *D*, as well as to test Wakeley and Hey's (1997) model of population expansion.

A likelihood approach was used to evaluate the genetic evidence for a population bottleneck associated with the speciation event that gave rise to *O. bilineata*. A coalescent process (Kingman 1982) was used to model the population genealogies expected under two different demographic scenarios: (1) a constant population size with no speciation bottleneck; and (2) a dramatic bottleneck at speciation. In the second model, it is assumed that a single individual gave rise to the species, followed by an immediate expansion to the effective population size; however, moderately larger numbers of founders and lower rates of growth produce similar results (B. Rannala, unpubl. obs.). The population size, *N*, immediately following the speciation event is assumed to be the same for both models. Thus, if *i* lineages existed at time *t* in the past that are ancestral to the *n* sampled sequences, for $t < T$, where *T* represents the time of species splitting (measured in generations), then lineages will coalesce with instantaneous rate $i\ (i - 1)/2N$ (Hudson 1990). Under the second model at time $t > T$, the remaining lineages coalesce to a single ancestral lineage with a probability of one, whereas under the first model the lineages continue to coalesce at the usual rate as given above.

The probability of observing *S* segregating sites in a sample of *n* sequences (the likelihood, when treating *S* as the observed data) was calculated under the two models for a range of population sizes, assuming an infinite sites model of sequence mutation. The probability of *S* is:

$$\Pr(S \mid \mu, T, N) = \int_{\bar{t}} \Pr(S \mid \mu, \bar{t}) \Pr(\bar{t} \mid N, T)\, d\bar{t}, \qquad (1)$$

where μ is the per sequence mutation rate (i.e., the per site mutation rate multiplied by the number of sites in the sequence) and $t = \{t_n, t_{n-1}, \ldots, t_2\}$ is a vector of the coalescence times, where t_i is the waiting time for *n* sequences to coalesce to $i-1$ ancestral sequences. Monte Carlo integration and simulation from the coalescent process $\Pr(\bar{t} \mid N, T)$ was used to evaluate the above integral. The per site mutation rate, μ, was assumed to be 8.5×10^{-9} (Brower 1994) and the length of the branch (in units of expected substitutions) separating *O. bilineata* from *O. communa* obtained from a maximum-likelihood analysis was used to estimate *T*, assuming one generation per year. A likelihood-ratio test of the molecular

TABLE 1. Number of singletons and frequency of haplotypes from the sampling localities where more than one individual was sampled (MX_s and TX_w not included because only one individual was sampled). Haplotypes shared among localities are identified by letter designation (a, b, c).

Localities	Number of individuals sampled	Number of singleton haplotypes	Number of haplotypes with incidence > 1*	Total number of haplotypes
CT	12	3	2 (8.33_a, 12.5)	5
IL	10	4	3 (4.17, 4.17, 14.58_b)	7
GA	17	4	3 (8.33, 8.33_a, 20.83_c)	7
AL	4	1	1 (6.25)	2
TX_e	7	4	2 (14.58_b 20.83_c)	6
TX_s	9	6	1 (14.58_b)	7
MX_w	11	7	1 (8.33)	8
AZ	10	4	1 (12.50)	5
CA	3	0	1 (6.25)	1
UT	7	2	2 (4.17, 6.25)	4
Total	92	35	13**	48**

* The frequency of each haplotype is given in parentheses (frequencies calculated from the total of 92 haplotypes).

** The total number of haplotypes is less than the column totals because some haplotypes are shared across localities.

clock assumption using PAUP* failed to reject the clock (logL = −2355.98 and −2342.85 with and without the clock, respectively; $P > 0.05$, df = 17).

RESULTS

Phylogenetic Relationships among Sequences

Among the 92 individual COI sequences examined from *O. communa*, 48 displayed different nucleotides at one or more sites (Table 1). In *O. bilineata*, 19 of 22 individuals sequenced had unique haplotypes. Maximum-likelihood, parsimony, and neighbor-joining analyses (using Jukes-Cantor and Kimura two-parameter models) gave similar tree topologies. In the 400-bp fragment used in these analyses there were 68 variable sites (46 informative ones). The maximum-likelihood trees (Fig. 2) are presented as networks showing the stepwise evolutionary relationships among the sequences and which sequences in the dataset are hypothesized to reside at nodes of the phylogenetic tree. The relative positions of haplotypes in the trees (e.g., a basal position vs. a tip), in conjunction with their frequency and geographic position, convey information about a species' demographic history. Thus, it is more informative to represent the phylogenetic relationships among the haplotypes as a network than as a tree with many zero branch lengths. Estimates of genealogical relationships among clades within species, as well species relationships, were confirmed by bootstrap analysis of the larger amplified fragment of COI (total of about 905 bp; 129 variable sites and 73 informative ones) for a subset of individuals (21 individuals)(Fig. 3).

Other than the single haplotype found in California (CA), the gene tree of *O. communa* consists of two major clades (A and B in Fig. 2). Sequences of *O. bilineata* form a monophyletic clade that is attached to the *O. communa* network by four mutational steps. The relationships among the *O. communa* clades and *O. bilineata* are not resolved (Fig. 3; i.e., there is a polytomy consisting of *O. bilineata* and the clades of *O. communa* marked A, B, and C in Fig. 2).

The range of sequence divergence between populations of *O. communa* is 1.04–3.60% (Table 2); the average sequence divergence weighted by the number of individuals with each haplotype is 2.29%. This between-population divergence in *O. communa* is comparable to the average divergence between *O. communa* and *O. bilineata*, 1.97%.

Population Structure and Intraspecific Phylogenetic Patterns of Ophraella communa

Variation among populations tends to be higher than variation within populations (Table 2). This heterogeneity results in high F_{ST}-values among most *O. communa* populations and a highly significant F_{ST} for the species ($F_{ST} = 0.56$, $P < 0.001$, as estimated from 1000 Monte Carlo simulations). Even when two highly divergent populations (CA and AL) with low diversity were excluded, F_{ST} remained highly significant ($F_{ST} = 0.30$, $P < 0.001$).

There is neither an overall relationship between F_{ST}-values and geographic distances separating pairs of populations ($r = 0.17$, $P > 0.20$) nor a tendency for F_{ST}-values to be greater for groups of populations separated by larger geographic distances relative to closer populations (Table 3).

The phylogenetic reconstruction likewise indicates a lack of strong phylogeographic structuring (Fig. 2). However, there is some evidence of a weak correspondence between the geographic position of some haplotypes and their genealogical relationships. For example, haplotypes from western populations (e.g., AZ and UT) tend to cluster together in derived clades (with one exception, AZ 80). In contrast, some populations contain distantly related haplotypes (e.g., CT, GA, TX_e, and IL). However, considering the relationships of the haplotypes within each of the two separate clades A and B, haplotypes from CT, GA, and TX_e populations tend to be very closely related, whereas IL haplotypes are more phylogenetically dispersed, being separated by haplotypes from other geographic areas.

MX, TX_s, and TX_e haplotypes are located at more basal or interior positions in the genealogy, with many connections to other haplotypes, suggesting their greater antiquity (Castelloe and Templeton 1994). Although not forming a monophyletic clade, MX and TX_s haplotypes are found only in one of the two deeper clades (clade B), with the exception of one haplotype (MX_w 10 in Fig. 2).

Effective Population Sizes and Species Demographies

The mean calibration rate of sequence divergence of mtDNA reported in several studies of insects and other arthropods is 2.3% per million years, and the rate for the COI gene in *Tetraopes* beetles (in the sister family of the Chrysomelidae) has been estimated as 1.7% (Brower 1994). Using these rates of sequence divergence, the divergence time between these species may be estimated as 0.9 to 1.2 million years, that is, the early to mid-Pleistocene (based on the average pairwise difference corrected for multiple hits; the maximum-likelihood estimate is 1.0 to 1.3 million years).

Although the geographic range of *O. communa* is far larger than that of *O. bilineata,* the species are similar in genetic diversity (Table 4). Based on π and $\hat{\theta}$, effective population sizes, $N_e = 2N_f$, are large: 2.3×10^6 for *O. communa* and

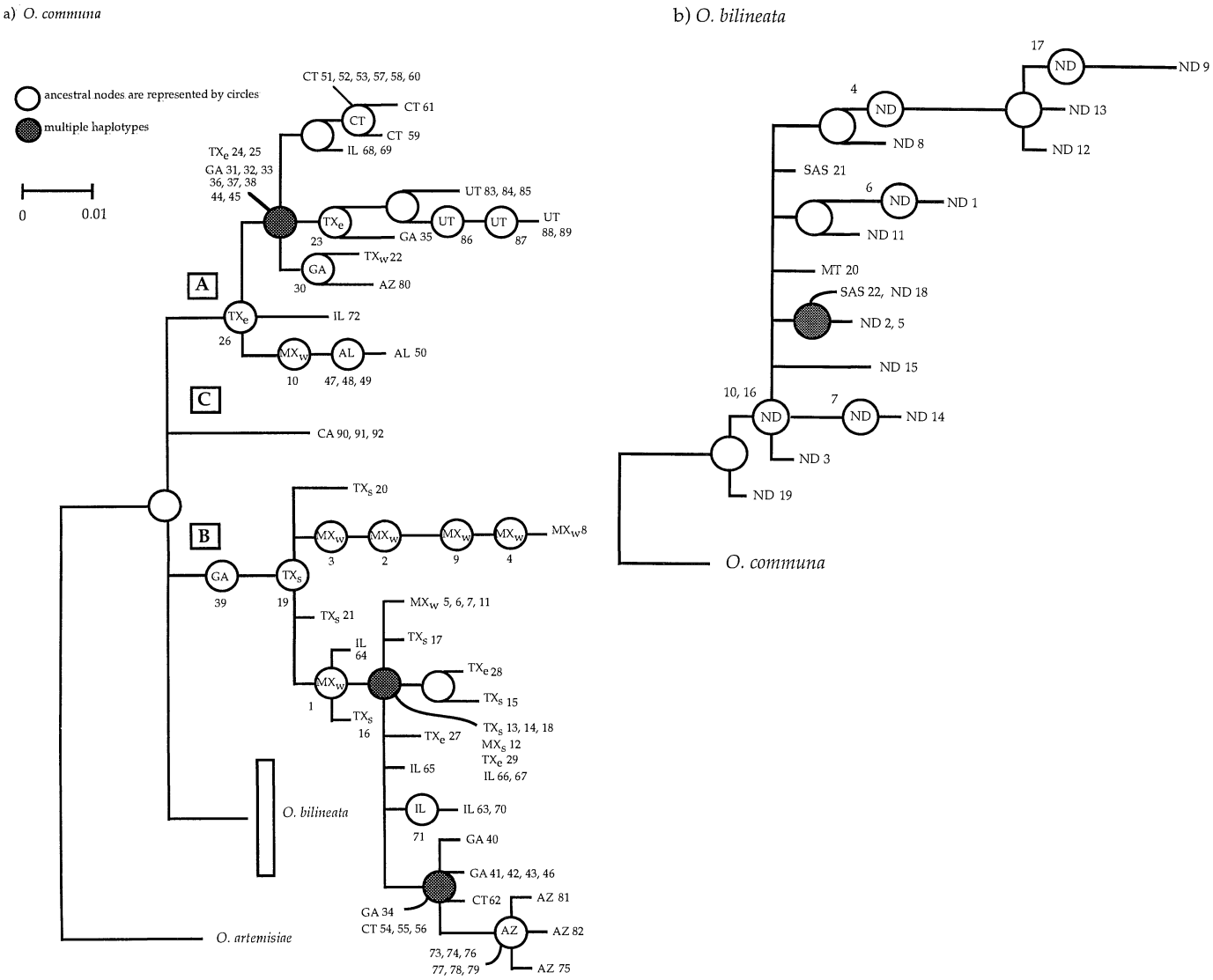

FIG. 2. Genealogical relationships (a) among *Ophraella communa* haplotypes and between *O. communa* and *O. bilineata* and (b) among *O. bilineata* haplotypes from a maximum-likelihood analysis with a gamma-distributed model of rate variation among sites (using a maximum-likelihood estimate of the shape parameter α with four rate categories). Branch lengths are proportional to the expected number of substitutions. Empty circles are hypothesized nodes not seen in our collections. Numerals next to locality designations refer to individual sequences.

2.18×10^6 for *O. bilineata*. Local samples of *O. communa*, which are comparable to the *O. bilineata* sample, also yield high estimates of N_e (Table 4). The large effective population size of *O. communa* is also reflected in the genealogical patterns of haplotypes: few haplotypes hypothesized as ancestral nodes are missing from the genealogy (Fig. 2) and many local populations include haplotypes from both major clades (A and B in Fig. 2).

Regarding the historical demography of each species, Tajima's D is negative but not significant (-0.613 and -0.317 for *O. communa* and *O. bilineata*, respectively), thus confirming the absence of any long-term geographic subdivision as reflected by the genealogical relationships among haplotypes (Fig. 2). Although a negative Tajima's D is consistent with a model of population expansion, it is not significant, nor does either species fit Wakeley and Hey's (1997) model of expansion (Fig. 4), although *O. communa* comes close.

A total of 28 segregating sites were observed in our analysis of 22 sequences of *O. bilineata*. Based on our analysis of these sequences and assuming a neutral coalescent process, we were unable to determine whether a bottleneck was associated with the origin of *O. bilineata*. There is no difference in the log- likelihood surface between models with and without a speciation bottleneck (Fig. 5). That is, given the estimate of N_e and of the time since speciation (about 1.3 million years), there has been sufficient time for the observed sequence diversity to coalesce to a single ancestor. We examined the behavior of the models over a range of biologically reasonable parameter values (e.g., over a range of $N_e = 10^5$ to 10^7 and $\mu = 1.7 \times 10^{-8}$ to 8.5×10^{-9}). In particular, we

TABLE 2. COI sequence heterogeneity within and between populations of *Ophraella communa*. Along the diagonal are within-population, average pairwise sequence diversities (shown in italics). Above the diagonal are between-population sequence divergences. Below the diagonal are estimates of F_{ST} from pairwise population comparisons (significant F_{ST}-values, $P < 0.05$ after adjusting the multiple comparisons using a Bonferroni correction, are in boldface). Percent sequence divergences among COI haplotypes corrected for multiple hits using Kimura's two-parameter model (1980). Number of specimens sequenced is given in parentheses.

	UT	CA	GA	MX_w	TX_s	TX_e	AL	CT	AZ	IL
UT (7)	*1.04*	3.38	2.11	2.96	2.66	2.06	2.74	2.34	3.05	2.65
CA (3)	**0.84**	*0.00*	3.03	3.22	2.87	2.77	3.25	3.00	3.60	3.06
GA (17)	**0.42**	**0.77**	*1.41*	1.99	1.73	1.38	1.78	1.69	2.09	1.77
MX_w (11)	**0.63**	**0.82**	**0.35**	*1.16*	1.04	1.86	2.51	2.52	2.10	1.60
TX_s (9)	**0.68**	**0.88**	**0.40**	0.12	*0.67*	1.57	2.51	2.24	1.80	1.26
TX_e (7)	**0.40**	**0.74**	0.00	**0.30**	**0.33**	*1.44*	1.64	1.71	2.21	1.63
AL (4)	**0.79**	**0.98**	**0.57**	**0.74**	**0.84**	**0.52**	*0.13*	2.02	2.58	2.25
CT (12)	**0.43**	**0.72**	0.10	**0.44**	**0.48**	0.10	**0.56**	*1.64*	2.54	2.08
AZ (10)	**0.68**	**0.88**	**0.45**	**0.52**	**0.57**	**0.48**	**0.80**	**0.50**	*0.88*	2.10
IL (10)	**0.49**	**0.73**	0.13	0.11	0.07	0.05	**0.60**	0.20	**0.39**	*1.67*

examined a range of speciation times spanning the 95% confidence interval of the maximum- likelihood estimate of the time of species splitting, $T = 1.3 \times 10^6$, obtained by phylogenetic analysis (i.e., the values of T above and below the maximum-likelihood estimate that had log-likelihood values that were less than two log-likelihoods below the log-likelihood of the maximum-likelihood estimate). No difference in the log-likelihood surface between the models was observed over the range of parameters tested.

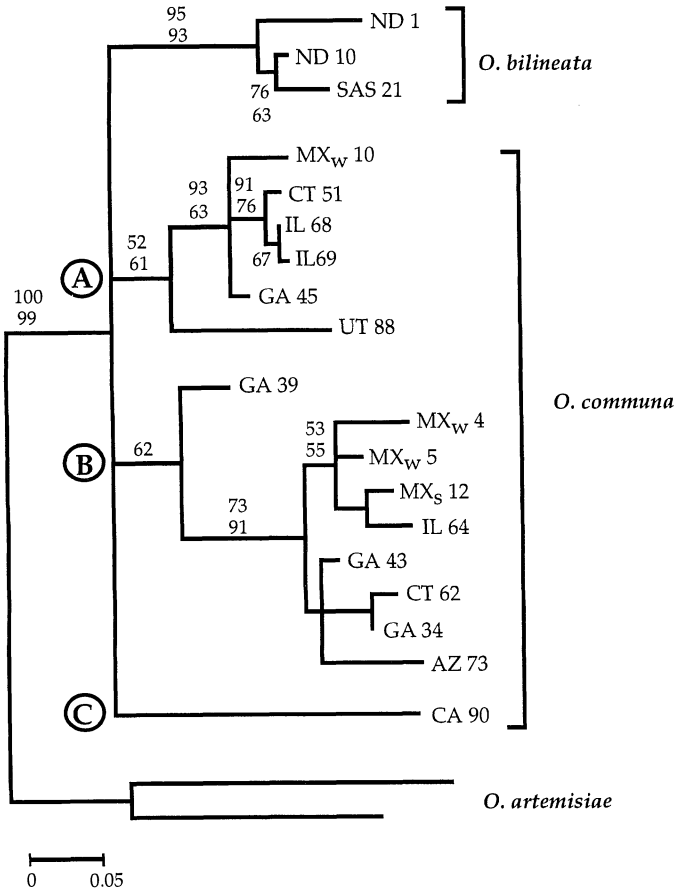

FIG. 3. Genealogical relationships among haplotypes from a maximum likelihood analysis of the larger fragment of COI (905-bp) in 21 individuals. Branch lengths are in units of expected substitutions. Two sets of bootstrap proportions are provided. The lower are from a parsimony analysis; the upper are from a neighbor-joining analysis (in those cases where the topologies of the trees obtained using the two methods agree).

DISCUSSION

This study was designed primarily to test the hypothesis that *O. bilineata* originated by peripatric (founder) speciation, and, if such a speciation event were confirmed, to infer the relationships among geographical populations of *O. communa* and *O. bilineata*. The data obtained for these purposes bear on the historical population structure and levels of gene flow in *O. communa* and illustrate how phylogeographic patterns may be used to distinguish current gene flow from population movements in the past.

Phylogeographic Patterns and Demographic History

Host-specific chrysomeloid beetles and other herbivorous insects often display significant F_{ST}-values over short distances (McCauley and Eanes 1987; McCauley et al. 1988; Mopper and Strauss 1998; Rank 1992). The value of F_{ST} we have calculated among populations of *O. communa* (0.3, excluding CA and AL) is among the highest recorded for herbivorous beetles, or indeed for insects. However, this population structure appears to reflect both historical factors and current restrictions on gene flow. If patterns of contemporary gene flow were the primary factor governing the distribution of haplotypes, we should expect F_{ST} to increase with increasing distance among populations (Slatkin and Maddison 1989), but no such relationship can be discerned (Table 3).

TABLE 3. Association of F_{ST}-values with the geographic distance between populations. F_{ST} calculated from all pairs of populations separated by a given geographic distance.

Geographic distance	Number of comparisons	F_{ST}
500–999 km	9	0.56
1000–1499 km	11	0.45
1500–1999 km	6	0.56
2000–2499 km	9	0.55
2500–2999 km	5	0.65
3000–3499 km	5	0.59

TABLE 4. Summary of diversity estimates and effective population size for both *Ophraella communa* and *O. bilineata* and each population of *O. communa*. Standard errors of estimates, per base pair, are in parentheses. Estimate of N_f based on π, where $\pi = 2N_f\mu$, $\mu = 8.5 \times 10^{-9}$.

Species/ population	π/bp	θ/bp	$N_f \times 10^{-5}$
O. communa	0.0196 (0.0018)	0.0238 (0.0064)	11.5
CT	0.0160 (0.0032)	0.0126 (0.0059)	9.4
TX_e	0.0141 (0.0026)	0.0125 (0.0056)	8.3
GA	0.0138 (0.0014)	0.0092 (0.0035)	8.1
MX_w	0.0114 (0.0023)	0.0140 (0.0058)	6.7
UT	0.0101 (0.0018)	0.0076 (0.0044)	5.9
AZ	0.0084 (0.0053)	0.0128 (0.0061)	4.9
TX_s	0.0066 (0.0015)	0.0094 (0.0045)	3.9
IL	0.0163 (0.0036)	0.0157 (0.0072)	2.1
AL	0.0013 (0.0007)	0.0014 (0.0014)	0.8
CA	0	0	0
O. bilineata	0.0186 (0.0022)	0.0203 (0.0067)	10.9

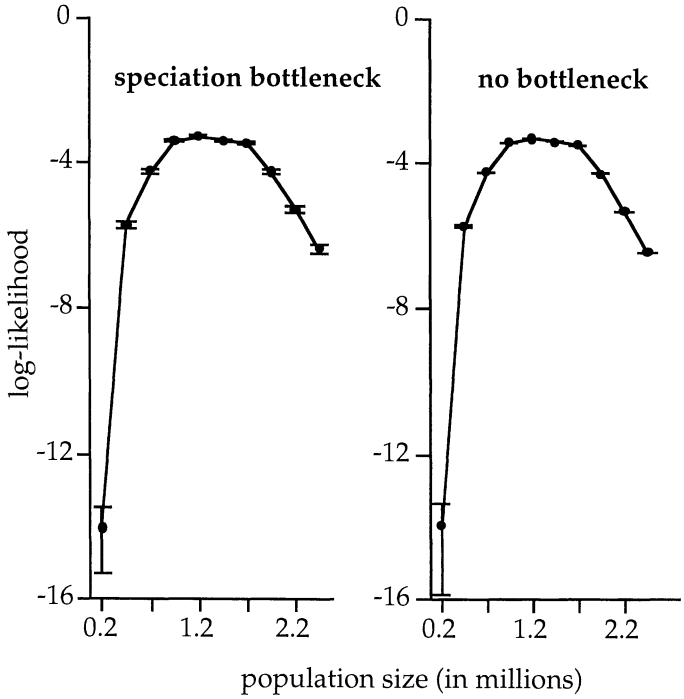

FIG. 5. Log-likelihood surface (with standard errors) of the population genealogies under the two coalescent models: (1) a constant population size with no speciation bottleneck; and (2) a dramatic bottleneck at speciation.

Moreover, no derived haplotypes (i.e., those at the tips of the genealogy) are geographically widespread. The only haplotypes that are shared among populations are located at interior nodes of the tree (Fig. 2), suggesting that haplotypes in multiple geographically separate populations reflect historical processes rather than recent gene flow (Crandall and Templeton 1993). If populations were currently exchanging genes, haplotypes that are shared among populations would be just as likely to be located at tip positions as at interior nodes. The geographic restriction of recently arisen haplotypes does indicate that gene flow in the recent past has been quite low. Furthermore, some regional samples (UT, AL, and CA) include only closely related haplotypes, implying that these populations have received few immigrants.

In contrast to many studies that have found strong phylogeographic patterns reflecting barriers to gene flow (see Avise 1994), there does not appear to be any evidence of long-term barriers to gene flow in *O. communa*. Although some regional differences among populations exist—for example, populations from southwestern and western populations include mostly haplotypes from one or the other of the major clades (CA, MX, TX_s, UT, and AZ, Fig. 2)—these regional patterns are not the product of traditional vicariance.

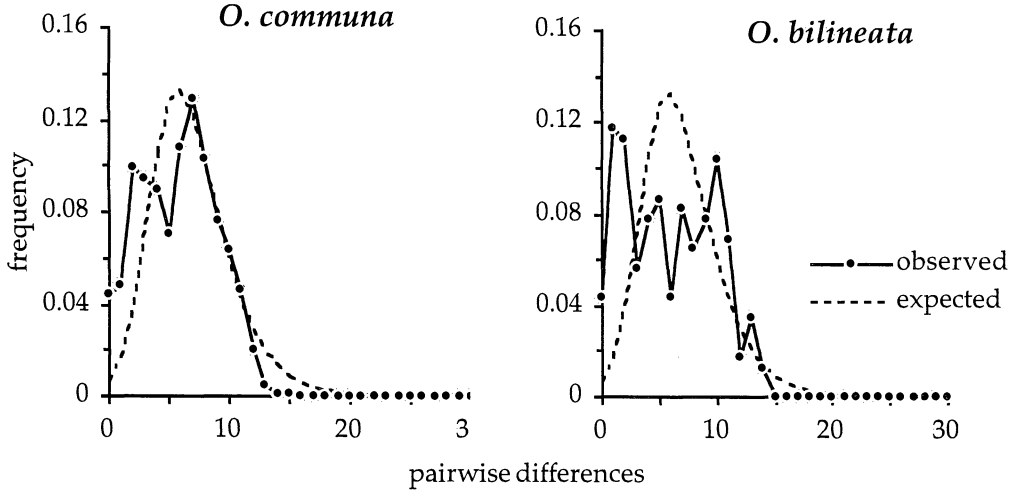

FIG. 4. Observed and expected frequency distribution of pairwise differences under Wakeley and Hey's (1997) model of expansion.

There is an absence of any regional phylogeographic pattern, in that neither clade A nor clade B is geographically coherent. For example, western populations such as UT and AZ represent genealogically discrete clades, but the two populations are derived from very divergent lineages.

If there had been any long-term geographic subdivision, mutational differences would have accumulated among clades, producing a positive Tajima's D and a bimodal frequency distribution of pairwise differences between sequences. However, the frequency distribution of pairwise differences of *O. communa* sequences is nearly unimodal and Tajima's D is negative, which is consistent with a demographic history of expansion rather than historical subdivision (Slatkin and Hudson 1991; Rogers and Harpending 1992; Marjoram and Donnelly 1994; Wakeley and Hey 1997). However, *O. communa* seems not to have experienced a recent bottleneck. Its sequence diversity implies N_e of $1-2 \times 10^6$ or more, and its haplotype tree includes two (or three, counting the CA population) distinct clades that appear as ancient as the speciation event separating *O. communa* from *O. bilineata* (Fig. 2). Of the two species, *O. communa* apparently retains the ancestral host association (Funk et al. 1995), and it may genetically approximate the common ancestor more closely than *O. bilineata* does. Furthermore, the lack of an association of F_{ST} with spatial scale and the large number of rare alleles (35 of the 48 *O. communa* haplotypes are singletons; Table 1) are consistent with a history of population expansion associated with geographic spread (Patton et. al 1996).

This process of colonization has occurred since the divergence of *O. communa* from *O. bilineata* (about 1.3 million years ago), given that the major clades A and B (Fig. 2) are evidently as old as the speciation event. Some regions, such as Utah and Arizona, have only a single clade of haplotypes, suggesting that they were founded from a localized source (or that they have had historically small population sizes, but this is not supported by the estimates of N_e in Table 4). The substantial difference between the Utah and Arizona populations, even though they are fairly close to each other, would be expected if the founders represented a small subsample of the genetic diversity in the ancestral gene pool (Nürnberger and Harrison 1995; Hagen and Hamrick 1996). Populations in other regions, such as Connecticut and Georgia, include both major clades of haplotypes, suggesting a more massive influx of migrants, perhaps from several localities (Slatkin 1987). It is plausible to suppose that some of the history of colonization was associated with Pleistocene alternation of glacial and interglacial episodes; indeed, some of the populations occupy previously glaciated areas.

Speciation

The sequence divergence between *O. communa* and *O. bilineata* suggests that speciation occurred during the Pleistocene, about 1.3 million years ago. During this time, recurrent climate changes presumably caused shifts in the distribution of *Ophraella* and its host plants, resulting in gene flow (Coope 1979; Hewitt 1996). Such gene flow, even if episodic, may be massive enough to reverse adaptive differentiation among populations, unless the integrity of populations is maintained by reproductive isolation (Futuyma 1987). As we argue above, the distribution of haplotypes in *O. communa* is indeed consistent with the hypothesis that recurrent episodes of gene flow have occurred on a broad geographic scale. Conversely, *O. communa* presents no evidence of long-term vicariant divergence between broad regional populations, of the kind described, for example, for freshwater fishes in southeastern United States (Bermingham and Avise 1986). It is likely, then, that if *O. bilineata* arose from an *O. communa*–like ancestor by allopatric speciation, it did so by rather rapid evolution of reproductive isolation in a geographically rather restricted area. If reproductive isolation had not been acquired rapidly (i.e., on a scale of 10^4 years or possibly less given recent evidence on the periodicity of climatic fluctuations; Roy et al. 1996), episodic gene flow would probably have reversed the course of divergence and prevented speciation (e.g., Hellberg 1998).

The suggestion by Funk et al. (1995) that the peripherally distributed species O. *bilineata* might be more closely related to some populations of *O. communa* than to others was not supported by the more extensive data examined in the present study. Because we have no evidence that *O. communa* is paraphyletic, we cannot determine which of the hosts used by different populations of *O. communa* may have been used by the ancestor of *O. bilineata*.

Did either species experience a bottleneck that may have facilitated speciation? Reduced sequence diversity has been taken as evidence of bottlenecks in the history of populations or species of *Drosophila* (Hey and Kliman 1993; Hilton and Hey 1996) and fishes (Ovendon and White 1990), whereas high sequence diversity and/or shared polymorphisms have supported arguments against bottleneck-induced speciation in African lake cichlids (Klein et al. 1993, 1998; Moran and Kornfield 1993; van Oppen et al. 1998), Galápagos ground finches (Vineck et al. 1997)and several species of *Drosophila*, including island endemics (Hey and Kliman 1993; Hilton and Hey 1996). If, however, high sequence diversity, rather than shared polymorphism, is the sole argument against a historical bottleneck, it is important to ask if the time since speciation has been sufficient for sequence diversity to have recovered from a bottleneck-induced nadir. If so, a highly diverse but monophyletic gene tree provides no evidence on demography at the time of speciation.

The effective population size, N_e, is estimated to be greater than two million for both *O. communa* and *O. bilineata* , and even estimates for local populations of *O. communa* are fairly large (Table 4). These estimates, which assume one generation per year, are most likely conservative because *O. bilineata* probably and *O. communa* certainly have two or more generations per year (Futuyma 1990), implying a lower mutation rate and therefore a higher effective population size. These estimates are surprisingly high (at least to us), given the presumption that insect populations in the temperate zone may fluctuate drastically in size. Indeed, some abundant species, such as *Eurosta solidaginis* (goldenrod ball gallfly) and *Ceratitis capitata* (Mediterranean fruit fly), have very low mtDNA sequence diversity and presumably have experienced substantial reductions in abundance (Brown et al. 1996; Gasparich et al. 1997). Apparently, despite the dynamic demographic history of *O. communa*, gene flow and population extinctions are not high enough to act as a homogenizing

force, or to reduce genetic diversities (Slatkin 1977, 1987). Likewise, even though the current geographic range of *O. bilineata* is much smaller than that of *O. communa*, its effective population size is nearly as great and at face value seems incompatible with the hypothesis of a bottleneck at the time of speciation.

Despite the large estimated effective population size of *O. bilineata*, however, we cannot rule out the possibility of a bottleneck at speciation. A model based on a drastic bottleneck did not have a lower likelihood than the demographic model with no bottleneck because the time since separation has been long enough for any signature of a possible bottleneck to have been erased. These results, as well as other studies (e.g., Hilton and Gaut 1998), emphasize that there is no conflict between the observation of high sequence diversity and bottlenecks. Inferences about speciation based on DNA sequence diversity are necessarily restricted by the time to coalescence of the species.

Caveats

Our conclusions about phylogeography and speciation in *Ophraella* are necessarily provisional because of the relatively short sequence used in most of the analyses. However, the major clades identified in the analyses (A, B, and C in Fig. 2) were resolved in a bootstrap analysis of the larger COI fragment (Fig. 3). Although only a subset of haplotypes was available, the gene tree based on the larger fragment of COI had much the same structure as the network based on the smaller fragment of COI. For example, the bootstrap analysis shows the same broad distribution of haplotypes for some populations (e.g., CT) as the network.

A more serious potential problem is the use of only a single gene, providing a genealogy of a locus rather than of populations. Although it is possible that the gene genealogy does not accurately reflect phylogenetic relationships among populations, our conclusions are based more on the overall shape of the genealogy and patterns of variation within populations relative to other populations than on specific relationships among populations or haplotypes. Furthermore, with regard to inferences about population bottlenecks, the smaller effective population size of mitochondrial genes makes them more sensitive to such demographic events (Chesser and Baker 1996) and a more reliable indicator of species phylogenies in some cases, particularly where there is a problem of lineage sorting (Moore 1995, 1997).

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Appendix

Sampling localities with number of individuals sampled at each site (*n*). All specimens of *Ophraella communa* were collected from *Ambrosia artemisiifolia* except as indicated (a, *Helianthus ciliaris*; b, *Ambrosia psilostachya*; c, *Iva axillaris*). All *O. bilineata* were collected from *Chrysopsis villosa.*

Species	Localities		*n*
O. communa	CT	Fairfield Co., Connecticut	7
		Suffolk Co., New York	4
	GA	Thomas Co., Georgia	10
		Leon Co., Florida	6
		Tift Co., Georgia	1
	AL	Sumter Co., Alabama	4
	TX_e	Hardin Co., Texas	7
	TX_s	Hidalgo Co., Texas	9
	TX_w	Reeves Co., Texas[a]	1
	MX_s	Tamaulipas, Mexico	1
	MX_w	Chihuahua, Mexico	11
	AZ	Pima Co., Arizona	10
	CA	Inyo Co., California[b]	2
		San Diego Co., California	1
	UT	Uintah Co., Utah[c]	7
	IL	Lake Co., Illinois	10
O. bilineata	ND	McHenry Co., North Dakota	19
	MT	Cascade Co., Montana	1
	SAS	Saskatchewan, Canada	2