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COMPARATIVE GEOGRAPHIC STRUCTURES OF TWO PARASITOID-HOST INTERACTIONS

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Abstract.—Recent studies of parasitoid-host interactions have demonstrated that parasitoids and their hosts are geographically structured for traits such as virulence and encapsulation defenses, but no studies have yet compared the geographic structure of parasitoids and hosts using neutral genetic markers. Such studies of geographic structure are needed to evaluate the underlying geographic scale at which these interactions evolve and allow assessment of the relative effects of selection and gene flow on the geographic structure observed in traits under selection. We used sequence data from the mitochondrial DNA cytochrome oxidase I and II subunits to document and compare the geographic structures of the parasitoid *Agathis thompsoni* and its moth host *Greya subalba*. We also documented the geographic structure of *G. enchrysa* and compared it to the geographic structure of its parasitoid *Agathis* n. sp. The results demonstrated that parasitoids and their hosts may have incongruent patterns of geographic structure as assessed by molecular markers. As a consequence, the geographic scale at which the interaction evolves may be different for each species involved in the interaction. Depending on the interplay of selection and gene flow, there may not be a one-to-one correspondence of traits important in the interaction between parasitoids and their hosts at the level of local populations. The geographic structures of *A. thompsoni* and *G. subalba* and *Agathis* n. sp. and *G. enchrysa* provide further evidence of the potential importance of the formation of geographic mosaics in coevolving parasitoid-host interactions and evolving interactions in general.

Key words.—Cytochrome oxidase I and II, geographic structure, parasitoid-host interactions, phylogeography, population differentiation.

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Studies examining phylogeographic patterns have demonstrated that populations within species are geographically structured (Avise 1994). This geographic structure may be viewed as sets of populations that exhibit a hierarchy of genetic relatedness that is dependent upon patterns of past and current gene flow among populations. Geographic structure influences not only the population biology of a species, but also the evolution of interspecific interactions (Thompson 1994; Burdon 1997; Hanksi and Gilpin 1997). In particular, Thompson (1994) has suggested that the geographic structure of interacting species is a major component of evolving interactions and the coevolutionary process. If conspecific populations are relatively isolated, their interspecific interactions may evolve along different trajectories in different communities (e.g., Brodie and Brodie 1991; Carroll and Boyd 1992; Wilkinson et al. 1996; Carroll et al. 1997; Benkman 1999). Conversely, a high degree of connectivity among populations homogenizes species interactions as newly evolved traits may spread rapidly among populations (e.g., Jaenike 1989; Shoemaker and Jaenike 1997). Between these extremes, moderate levels of gene flow may produce complex and continually changing geographic mosaics in interactions and may even result in local maladaptation in interactions within some local communities (Thompson 1994, 1997). Thus, differing geographic structures may fuel the ongoing geographic mosaic of coevolution between interacting species.

Numerous studies have documented the geographic structure of single species, but few studies have attempted to document the relative geographic structure of pairs of interacting species (e.g., Mulvey et al. 1991; Michalakis et al. 1993; Dybdahl and Lively 1996; Parker and Spoerke 1998). Such studies are necessary for understanding the geographic scale at which interactions evolve because incongruencies in the geographic structure of interacting species can have a profound influence on the outcome of an interaction. For example, Dybdahl and Lively (1996) showed that the snail *Potamopyrgus antipodarum* is a collection of highly structured populations, whereas its trematode parasite *Microphallus* sp. exhibits little population structure. They suggested that this difference in geographic structure favors the maintenance of sexual reproduction in the snail and prevents the development of host races in the parasite. Incongruent geographic patterns have also been detected in interactions between the weevil *Larinus cynarae* and its host plant, the thistle *Onopordum illyricum* (Michalakis et al. 1993), and in the interaction between white-tailed deer *Odocoileus virginianus* and its liver fluke *Fasciolodies magna* (Mulvey et al. 1991). Taken together, these studies suggest that incongruent patterns of geographic structure between interacting species may be common.

In this study we examine the geographic structure of the interaction between parasitoids and their hosts. As in other parasitic associations, parasitoids and their hosts exhibit geographic differences in traits important to their interaction. Parasitoid species are known to be collections of populations that differ in searching behavior and virulence (Bouletreau 1986; Kraaijeveld and van Alphen 1994; Kraaijeveld and van der Wel 1994; Kraaijeveld et al. 1994, 1995; Potting et al. 1997), and host species may also be collections of populations that exhibit different defenses to parasitoids such as encapsulation ability (Bouletreau 1986; Carton and Nappi 1991; Kraaijeveld and van Alphen 1995; Orr and Irving 1997; Kraaijeveld et al. 1998). Moreover, recent studies have uncovered local genetic variation for defenses and counterde-

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fenses within both groups, highlighting the potential for ongoing coevolution (Henter 1995; Henter and Via 1995). Unlike for other interactions, however, there have been no studies using neutral genetic markers to characterize the underlying geographic structure of parasitoid-host interactions.

We used the interaction between two parasitoids in the genus *Agathis* (Braconidae: Agathidinae) and their moth hosts in the genus *Greya* (Prodoxidae) to examine patterns of geographic structure that can arise in parasitoid-host interactions. The interaction between these parasitoids and their moth hosts is patchily distributed over the major river drainages of the Pacific Northwest. Both *Agathis* and *Greya* have been the focus of a number of studies on the potential for coevolution in interacting species (Thompson 1986, 1987, 1997; Thompson and Pellmyr 1992; Pellmyr et al. 1996; Thompson et al. 1997), and the geographic structure of *Agathis* n. sp. has been described elsewhere (Althoff and Thompson, unpubl. ms.). Here we document the geographic structure of the parasitoid *A. thompsoni* and its host *G. subalba* and also, for comparison, the geographic structure of *G. enchrysa*, which is the host of *Agathis* n. sp. We then evaluate the similarities and differences in the patterns of geographic structure for each parasitoid species and its respective host species. We also determine the geographic scale at which each parasitoid-host interaction appears to be undergoing differentiation and relate this to the evolution of these interactions.

Materials and Methods

Species

The distribution of the moth species *Greya subalba* and *G. enchrysa* extends from southwestern Canada across the Pacific Northwest to southern Oregon (Davis et al. 1992). This study compares their geographic structures in the northwestern portion of their geographic ranges where they overlap. Although there has been no formal investigation of their ranges, both parasitoids, *Agathis thompsoni* and *Agathis* n. sp., have been found at all sites where the moths occur.

Greya subalba Braun is a seed parasite of the immature seeds of five *Lomatium* species (Apiaceae). Female *G. subalba* lay one to two eggs per schizocarp and distribute their eggs across umbellets within an individual plant in a way that may minimize information on larval distributions for females of *A. thompsoni* (Thompson 1986, 1987). Larvae feed on endosperm until the end of the second instar, at which time they burrow out of the schizocarp and drop into the soil (Davis et al. 1992). Adults eclose the following spring.

Agathis thompsoni Sharkey (Braconidae: Agathidinae) attacks larvae of *G. subalba* while they feed within the schizocarps of *Lomatium*. A female wasp must probe a schizocarp with her ovipositor to determine if it contains *G. subalba* larvae. Female wasps search in a pattern that is similar to the distribution of moth larvae, and the distribution of moth larvae and the pattern of female search may have been an important target of selection in this interaction (Thompson 1986). Based on the life history of *G. subalba*, this wasp species is univoltine. No other host species have been recorded for *A. thompsoni*.

Greya enchrysa Davis and Pellmyr (Lepidoptera: Prodoxidae) is a floral parasite of the saxifrage plants *Heuchera cylindrica* Dougl., *H. grossulariifolia* Rydb., and their hybrids (Davis et al. 1992). As a female moth oviposits into the ovary of the flower, she passively transfers pollen on her abdomen to the stigma of the flower. Females are highly effective pollinators of *H. cylindrica*, accounting for up to 51% seed set during a single oviposition event (Pellmyr et al. 1996). Larvae feed on developing seeds until the second instar. At this time, they exit the seed capsule through the unfused styles or burrow through the seed capsule wall, drop into the soil, and emerge as adults the following summer. Larvae are usually located at the base of the seed capsule (Davis et al. 1992), which may afford a refuge from attack by *Agathis* n. sp. (Braconidae: Agathidinae). Female wasps insert their ovipositors into the top of the seed capsule rather than pierce the capsule wall (Althoff and Thompson, unpubl. ms.). Thus, larvae at the base of a seed capsule may avoid being detected.

Molecular Analyses

Individuals of *A. thompsoni, G. subalba*, and *G. enchrysa* were collected during the summers of 1996 and 1997 from their respective host plants (Table 1, Fig. 1). Data for *Agathis* n. sp. are from Althoff and Thompson (unpubl. ms.). Specimens were individually collected in microcentrifuge tubes, returned to the laboratory, flash frozen in liquid nitrogen, and held at −80°C. Total genomic DNA was extracted from each individual using the IsoQuick DNA isolation kit (Orca Research, Inc., Bothell, WA). Initial amplifications of a contiguous mtDNA region containing approximately half of cytochrome oxidase I (COI) and half of cytochrome oxidase II (COII) were made using the primers mtD6 (GGAGGATTTGGAAATTGATTAGTTCC) and mtD18 (CCACAAATTTCTGAACATTGACCA) from the Insect mtDNA primer kit distributed by University of British Columbia Nucleic Acid-Protein Service Unit. Amplifications were made in 50-μl reaction volumes containing 1 × PCR buffer (Gibco), 3 mM $MgCl_2$ (Gibco), 0.2 mM dNTPs, one unit *Taq* polymerase (Gibco), 5 pmoles of each primer, and 20 ng of DNA template. The PCR profile was one cycle at 95°C for 5 min, 35 cycles of 95°C for 1 min, 48°C for 45 sec, 72°C for 2 min, and one cycle at 72°C for 5 min. For *G. enchrysa* the annealing cycle was 52°C for 30 sec. PCR products were precipitated and cleaned with a 2.5 M NaCl/20% polyethylene glycol solution and resuspended in sterile water.

Sequencing reactions were in 5 μl volumes and contained 25 ng initial PCR product, 1.25 pmoles primer, and 2 μl of FS dye terminator ready reaction cycle sequencing mixture from ABI. The PCR profile was 25 cycles of 96°C for 30 sec, 45°C for 30 sec, 60°C for 4 min. Products were cleaned with Sephadex columns and sequenced on an ABI 377 Automated DNA sequencer. A total of five primers were used to sequence the COI and COII region: the two amplification primers plus three species-specific internal primers derived from sequences of each species. Two of these primers were on the same DNA strand as mtD6 and one on the same strand as mtD18. Primer sequences are available from the authors upon request.

In addition to sequencing the COI and COII region, PCR-based RFLPs of a region of the nuclear ribosomal DNA were

TABLE 1. List of sites and host plants from which collections were made for each species.

Species	Host plant	Location
Agathis thompsoni	*Lomatium grayi*	CRM: Crum (Granite Point), WA, 46°37′N, 117°22′W
		PLN: Pullman, WA, 46°44′N, 117°10′W
		SP: 1.6 km E of Nez Perce National Historic Monument, Spalding Site, ID, 46°27′N, 116°49′W
	L. ambiguum	STJ2: 4.5 km Forest Road 537, St. Joe National Forest, ID, 47°17′N, 116°7′W
	L. dissectum	SC: 6.8 km Forest Road 50, Lolo National Forest, MT, 46°42′N, 113°48′W
Greya subalba	*L. grayi*	CRM: same as above
		PLN: same as above
		KSK: junction of US routes 12 and 13, Kooskia, ID, 46°9′N, 115°58′W
		SP: same as above
	L. ambiguum	STJ2: same as above
Greya enchrysa	*Heuchera cylindrica*	ALB: Albion, WA, 46°47′N, 117°15′W
		SP: same as above
		STJ2: same as above
		STJ1: 3.7 km W of Calder, ID, St. Joe National Forest, 47°16′N, 116°15′W
		BM: Forest Road 46, Umatilla National Forest Service in the Blue Mountains of southeastern WA, 46°13′N, 117°46′W
		MC: Martin Creek Campground, Bitterroot National Forest, MT, 45°56′N, 113°44′W

examined. A region of rDNA containing the 3′ end of 18S, all of ITS-1, 5.8S, and ITS-2, and the 5′ end of 28S was amplified using PCR and cut with 10 restriction enzymes. The primers used were 18j (GCCTGCGGCTTAATTTGACTCAACACGGG) and 28z (AGACTCCTTGGTCCGTGTTTCAAGAC) from Hillis and Dixon (1991) and the PCR profiles were the same as those used for the initial amplification of COI and COII. A product of approximately 2500 bp was obtained and cut with the following 10 restriction endonucleases: *Acc*I, *Ava*I, *Ava*II, *Ban*I, *Ban*II, *BstN*I, *Hha*I, *Hinc*II, *Hpa*II, and *Nci*I. For each enzyme, PCR products were digested overnight with two units of enzyme according to the manufacturer's specifications. DNA fragments were separated on 1% agarose gels and visualized using ethidium bromide staining.

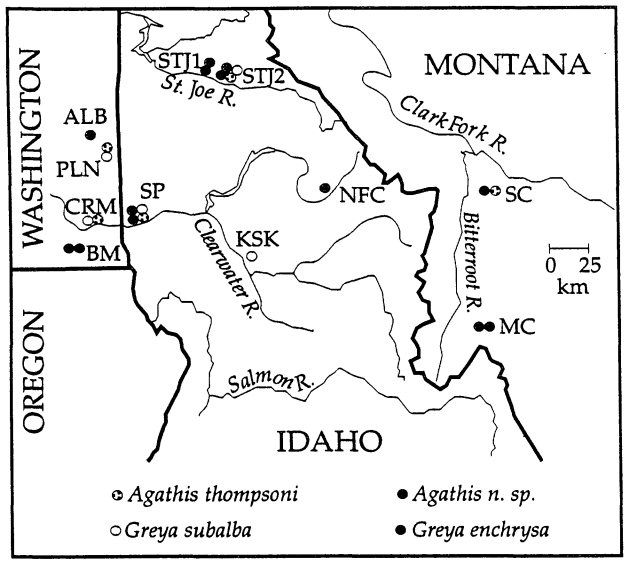

FIG. 1. Distributions of sites used to estimate geographic structure for each species. Dots next to one another represent collections from the same locality. For comparison, sites studied for *Agathis* n. sp. are included (Althoff and Thompson, unpubl. ms.). Site abbreviations are described in Table 1.

The geographic structure among sites within each species was analyzed using the parsimony algorithms in PAUP 3.1.1 (Swofford 1993) and analysis of molecular variance (AMOVA) (Excoffier et al. 1992). We used Fitch parsimony and a heuristic search with simple taxon addition and TBR branch swapping for the sequence data. One hundred bootstrap replicates were also conducted to assess support for the resulting nodes. The AMOVA program was used to estimate the variance components in haplotype diversity within sites, between sites, and among groups of sites. We used the squared number of substitutions among haplotypes as the distance matrix for each species. Significance testing of the variance component was done using a permutational approach. The AMOVA was run twice for each species. In the first analyses we did not specify an among-groups-of-sites component because we did not know a priori what the groupings should be. We used pairwise ϕ_{ST} values, an analog of F_{ST}, to infer the potential groupings among sites. We assumed, using the equation $Nm = ([1/F_{ST}]) - 1)/2$ (Hudson et al. 1992), that ϕ_{ST} values greater than 0.35 represent less than one migrant per generation between sites. This amount of gene flow is insufficient to prevent differentiation due to genetic drift (Allendorf 1983). Those sites that had pairwise ϕ_{ST} values less than 0.35 were grouped together in the second analysis to test statistically for structuring based on groups of sites.

RESULTS

As is common for insects, sequences from the three species had a high A + T content (*A. thompsoni* = 78.7%, *G. subalba* = 72.2%, and *G. enchrysa* = 72.4%). For all three species, nucleotide site variation was low (Table 2), but a large number of unique haplotypes were detected. The percentage of individuals with unique haplotypes was 65.2% for *A. thompsoni*, 95.8% for *G. subalba*, and 58.6% for *G. enchrysa*. On average, fewer than one percent of nucleotide sites were parsimony-informative. The RFLP data did not yield any par-

TABLE 2. Summary of sequence data of mtDNA COI and COII region for all species.

Species	No. localities	No. individuals	No. bp sequenced	No. sites variable	No. sites informative
Agathis thompsoni	5	23	1904	27 (1.4%)	8
Greya subalba	5	24	1877	64 (3.4%)	28
Greya enchrysa	6	29	1920	22 (1.1%)	12

simony-informative sites. Almost all individuals within each species were monomorphic for all 10 enzymes surveyed.

The resulting phylogenies for *A. thompsoni* and *G. subalba* exhibited several polytomies and some branch structuring (Figs. 2, 3). For *A. thompsoni* a majority of individuals collected from the Crum, Washington, and Pullman, Washington, sites had the same haplotype suggesting a high degree of connectedness between these two sites (Fig. 2). For *G. subalba*, a majority of haplotypes from the St. Joe River (STJ2) and Pullman (PLN) sites were grouped as clades, which suggests some structuring by site (Fig. 3). Also, a majority of individuals from the Kooskia (KSK), Spalding (SP), and Crum (CRM) sites formed a clade. For both species, however, there were no cases in which all haplotypes from one site were monophyletic. Haplotypes from geographically distant sites were sometimes more closely related to one another than haplotypes from the same sites.

The AMOVA for both species also detected some geographic structuring. The component of haplotype diversity due to the variance among groups of sites was significant (Table 3). Population groupings based on the AMOVAs produced incongruent patterns of geographic structure for both species (Fig. 4). The Spalding, Crum, and Pullman sites were grouped together for *A. thompsoni*, but were not for *G. subalba*. The St. Joe River site was also grouped differently for each species.

The parsimony analysis for *G. enchrysa* detected one population grouping (Fig. 5). All individuals from the St. Joe River 1 (STJ1) site were contained in a clade; however, individuals from other sites had the same haplotype as four of the STJ1 individuals. As with *A. thompsoni* and *G. subalba*, haplotypes from geographically distant sites were sometimes more closely related to one another than haplotypes from the same sites. The AMOVA detected a significant component of haplotype diversity due to variance among groups (Table

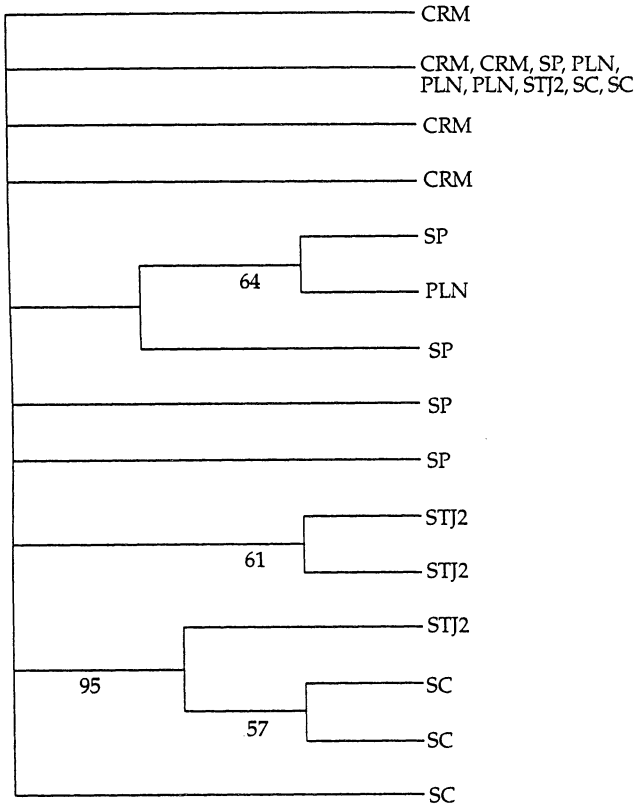

FIG. 2. One of two most parsimonious trees for *Agathis thompsoni*. Site identification as in Figure 1 and Table 1. Multiple site names per branch indicates individuals that had identical sequences. The tree has 28 steps. Bootstrap values above 50% are given below the branches. The second tree was the same except for the exclusion of the SP haplotype as the sister taxon to the PLN and SP clade with the 64% bootstrap value.

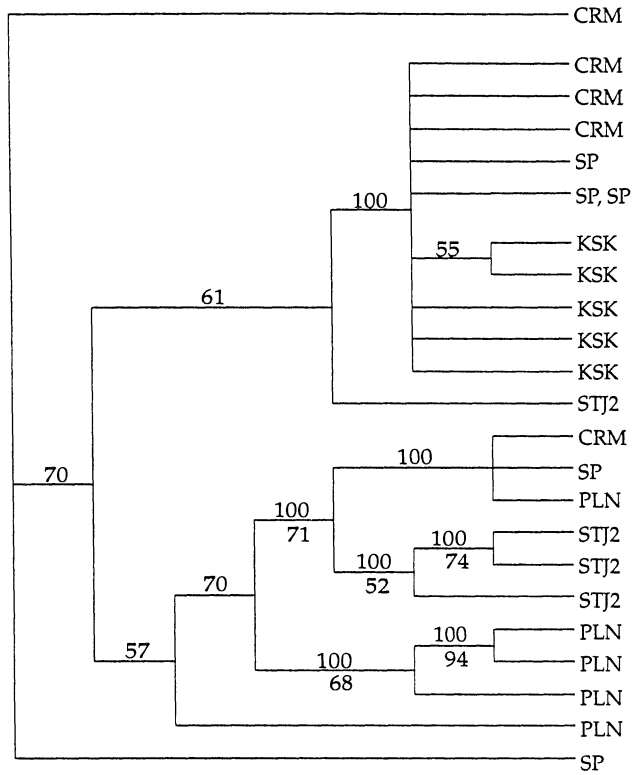

FIG. 3. Unrooted 50% majority-rule consensus tree of 4025 equally parsimonious trees for *Greya subalba*. Site identification as in Figure 1 and Table 1. The tree has 82 steps. Multiple site names per branch indicates individuals that had identical sequences. Percentage of trees containing clade are given above the branches and bootstrap values above 50% are given below branches.

TABLE 3. Analysis of molecular variance results for *Agathis thompsoni, Greya subalba,* and *Greya enchrysa.* Overall ϕ values are provided.

Source	*Agathis thompsoni*				*Greya subalba*				*Greya enchrysa*			
	Variance	% total	*P*	ϕ statistics	Variance	% total	*P*	ϕ statistics	Variance	% total	*P*	ϕ statistics
Among groups	4.37	28.43	0.001	$\phi_{CT} = 0.284$	12.81	17.10	0.001	$\phi_{CT} = 0.171$	3.82	48.52	0.001	$\phi_{CT} = 0.485$
Among populations within groups	1.46	9.55	0.101	$\phi_{SC} = 0.133$	11.73	15.66	0.041	$\phi_{SC} = 0.189$	−0.71	−9.02	0.891	$\phi_{SC} = -0.175$
Within populations	9.53	62.02	0.001	$\phi_{ST} = 0.380$	50.38	67.25	0.002	$\phi_{ST} = 0.328$	4.76	60.50	0.009	$\phi_{ST} = 0.395$

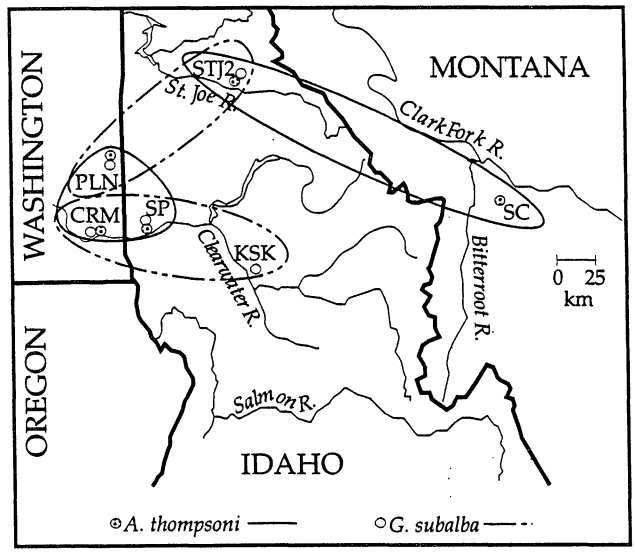

FIG. 4. Population groupings for *Agathis thompsoni* and *Greya subalba* based on analysis of molecular variance.

3). Based on this analyis there are three population groupings: individuals from Spalding, Albion, and the two St. Joe River sites formed one group; the Blue Mountain site formed another group; and the Martin Creek site formed the third (Fig. 6).

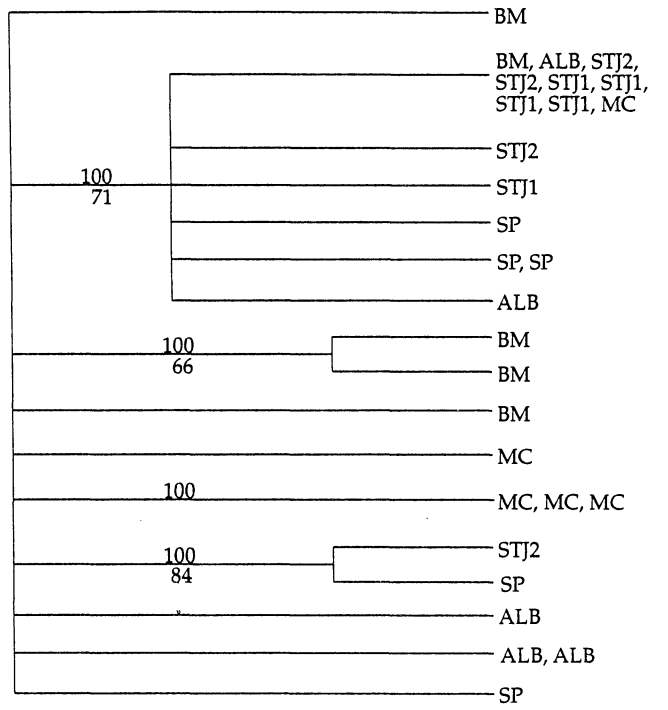

FIG. 5. Unrooted 50% majority-rule consensus tree of six equally parsimonious trees for *Greya enchrysa.* Site identification as in Figure 1 and Table 1. The tree has 23 steps. Multiple site names per branch indicates individuals that had identical sequences. Percentage of trees containing clade are given above the branches and bootstrap values above 50% are given below the branches.

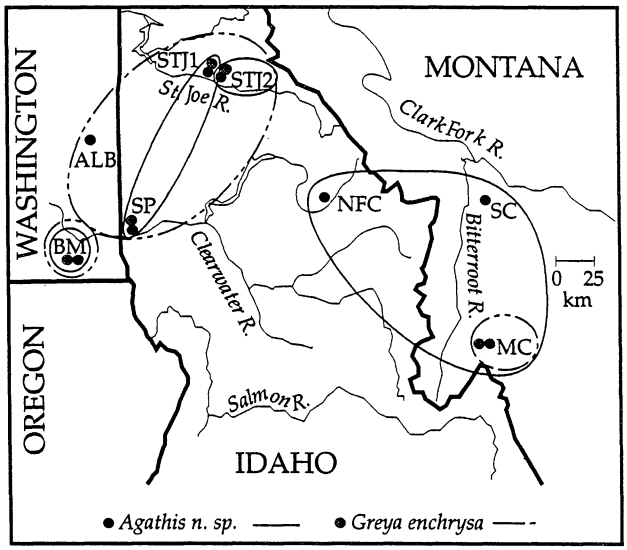

FIG. 6. Population groupings for *Greya enchrysa* based on analysis of molecular variance. For comparison, groupings determined for *Agathis* n. sp. are included (from Althoff and Thompson, unpubl. ms).

DISCUSSION

Overall Patterns of Geographic Structure

The patterns of geographic structure for *A. thompsoni, G. subalba*, and *G. enchrysa* are consistent with the structures of three other species that have been examined over the same geographic region surveyed in this study: *Agathis* n. sp. that attacks *G. enchrysa* (Althoff and Thompson, unpubl. ms.), *G. politella*, the sister species to *G. enchrysa* (Brown et al. 1997), and the saxifrage *Heuchera grossulariifolia*, which is a host plant for some populations of both *G. enchrysa* and *G. politella* (Segraves et al. 1999). For *Agathis* n. sp., only one of seven sites sampled was monophyletic even though there was significant geographic structuring in haplotype diversity and morphological and behavioral traits associated with female searching behavior (Althoff and Thompson, unpubl. ms.). Similarly, parsimony analyses of mtDNA COI and COII for populations of *G. politella* detected only geographic differences between populations in the Pacific Northwest and those from California (Brown et al. 1997). Estimates of haplotype diversity using N_{ST}, however, did show some structuring among populations in the Pacific Northwest (Brown et al. 1997). Finally, parsimony analysis of chloroplast DNA from diploid and tetraploid populations of *H. grossulariifolia* across Idaho and western Montana also detected a low degree of geographic structure. In many cases, haplotypes from geographically distant sites were more closely related to each other than to haplotypes from the same site (Segraves et al. 1999). Thus, the results obtained for *A. thompsoni, G. subalba*, and *G. enchrysa* are similar to the patterns obtained from other species in this geographic region.

The parsimony and AMOVA analyses revealed different aspects of the geographic structure for each species. For the three species, parsimony analyses suggested that there was some geographic structure, but this structure was not highly supported, and haplotypes from geographically distant sites were sometimes more closely related than haplotypes from the same site. The AMOVAs detected significant geographic structuring for each species. In some cases, these analyses also supported relationships obtained using parsimony. For example, the Crum (CRM), Spalding (SP), and Kooskia (KSK) sites for *G. subalba* formed a clade and were also grouped based on the AMOVA (Figs. 3, 4). However, the AMOVAs provided additional population groupings that were not detected using parsimony.

The differing views of geographic structure produced by the parsimony and AMOVA analyses may be the result of biases in each of the analyses. For the parsimony analyses, synapomorphies for each of the populations must be present to detect geographic structuring. Haplotype diversity within each population may be high, but all haplotypes must be united by one or more synapomorphies if the populations are geographically structured. The lack of synapomorphies uniting populations within each of the three species may be due to two factors. The first potential factor is a high level of homoplasy. Homoplasy would obscure estimation of haplotype relatedness by grouping haplotypes that had arisen in parallel. To our knowledge there have been no investigations of the levels of homoplasy in COI and COII at the phylogeographic level. A second potential factor is the amount of time populations have been isolated. Based on estimated rates of sequence divergence for mtDNA COI and COI, the evolution of synapomorphies may require a substantial amount of time (Brower 1994; Pellmyr et al. 1998). If populations have been isolated for less time than that necessary for the formation of synapomorphies, the parsimony analyses would indicate that there have been high levels of gene flow among populations within each species (e.g., Lavery et al. 1996).

Like the parsimony analyses, AMOVA would also be affected by high levels of homoplasy. Estimations of sequence divergence among haplotypes may be reduced if multiple substitutions occurred at nucleotide positions. However, the AMOVA is less sensitive to the amount of time populations have been isolated because it compares haplotype diversity within and between populations rather than using characters to unite haplotypes. Nonrandom distributions of haplotypes among populations may occur quickly if population sizes are small.

McMillan and Bermingham (1996) reported a similar discordance between parsimony and AMOVA results for populations of Dall's porpoise. They suggested that historical rates of gene flow among porpoise populations have been high, as indicated by parsimony analyses, but current distributions of haplotype diversity, as indicated by AMOVA, suggest a relatively recent decrease in gene flow among populations. This difference in historical versus recent gene flow may have also caused the discrepancy between the parsimony analyses and AMOVAs for *A. thompsoni, G. subalba*, and *G. enchrysa*.

Alternatively, a second explanation for the discrepancy between the parsimony and AMOVA approaches may be that small sample sizes were used in the latter. By chance alone, some of the haplotypes that were more common to all populations may not have been sampled. If these haplotypes were indeed missed, the estimates of variance among populations

may be inflated. Based on the current data, we cannot determine whether small sample sizes have influenced the AMOVAs.

Regardless of the discrepancy between the parsimony analyses and AMOVAs, there does appear to be some degree of geographic structuring for each of the three species. Geographic structuring detected with the sequence data is supported by the fact that the species examined are unlikely to move over the long distances between current patches of their host plants. *Agathis* n. sp. and *G. enchrysa* are dependent upon nectar from flowers of their host plants (Davis et al. 1992; Pellmyr et al. 1996), and *G. subalba* has reduced mouthparts and probably feeds very little if at all (Davis et al. 1992). None of these species have been observed to nectar on other plant species. Thus, the populations surveyed in this study would have to be connected to one another through a series of host plant patches for gene flow to occur. Second, the high degree of elevational change coupled with the differences in phenology among populations makes this connection highly unlikely. Third, the availability of flowers and hosts is approximately two weeks in duration and adults of all three species are unlikely to live much longer than this time period (Davis et al. 1992; D. M. Althoff, pers. obs.) This narrow window of time may reduce the chances of successful migration among populations. Reliance on host plants for food, coupled with a short adult life span and the patchy distribution of host plants, suggests that current gene flow among populations may be low, thus resulting in geographic structuring among populations within each species.

Comparison of Parasitoid and Host Geographic Structures

The patterns of geographic structure between *A. thompsoni* and *G. subalba* and between *Agathis* n. sp. and *G. enchrysa* demonstrate that parasitoids and their hosts may have very different geographic structures. Over the sites examined, *A. thompsoni* had a different geographic structure (as inferred from the AMOVAs) than *G. subalba*. Based on these results, the interaction between these two species may have two different foci: one in the Spalding, Crum, and Pullman area and one along the St. Joe River. Similarly, the patterns of geographic structure for *Agathis* n. sp. and *G. enchrysa* are highly incongruent. *Agathis* n. sp. has a higher degree of structuring than *G. enchrysa*. Haplotype diversity in this parasitoid was substructured in regions where *G. enchrysa* was one large group (Fig. 6). *Agathis* n. sp. has the potential to form highly divergent populations over the same area in which *G. enchrysa* is apparently one large panmictic population. For each interaction, intense selection may outweigh the effects of gene flow among sites (e.g., Chevillion et al. 1995), but our results provide a means to begin to evaluate patterns observed in traits important to the interactions and to assess the interplay of selection and population structure.

Price (1980) suggested that population subdivision is a major consequence of the parasitic lifestyle and should be typical for both parasitoids and phytophagous insects. Population subdivision has been demonstrated for many species of phytophagous insects, but fewer studies have been conducted for natural enemies such as parasitoids (Coll et al. 1994). Specialist parasitoids may be more likely to exhibit population subdivision given that they usually have lower population sizes than their hosts (Godfray 1994). Indeed, both *A. thompsoni* and *Agathis* n. sp. had higher ϕ_{ST} values than their respective host species (ϕ_{ST} = 0.59 for *Agathis* n. sp., Althoff and Thompson, unpubl. ms.), and *Agathis* n. sp. exhibited a higher degree of population structuring than its host (Fig. 6). Studies of other parasitoid-host interactions have demonstrated geographic structure in traits under selection (e.g., Kraaijeveld and van Alphen 1994, 1995), but to the best of our knowledge only one other study has used neutral genetic markers to examine geographic structure among populations of a parasitoid species (i.e., Vaughn and Antolin 1998). The use of these markers also demonstrates that parasitoids may exhibit population structure that is conducive to creating geographic mosaics in the interaction with their host species.

Conclusions

Studies of parasitoid-host interactions in the past five years have demonstrated that parasitoids and their hosts are geographically structured for selected traits such as virulence and encapsulation defenses. We have provided further evidence using neutral genetic markers that geographic structure may form in parasitoid-host interactions. Parasitoids and their hosts may exhibit incongruent patterns of geographic structure as in *A. thompsoni* and *G. subalba* and in *Agathis* n. sp. and *G. enchrysa*. These incongruences in population structure can lead to the formation of a geographic mosaic for traits under selection (such as host encapsulation ability, parasitoid virulence, and searching behavior) and highlight the need to better understand how the interplay of gene flow and selection determine the distribution of traits observed across populations of interacting species. Overall, our results suggest that the formation of geographic mosaics is an important component of the evolution of parasitoid-host interactions and perhaps interspecific interactions in general.

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Literature Cited

Allendorf, F. W. 1983. Isolation, gene flow, and genetic differentiation among populations. Pp. 51–65 *in* C. M. Shonewald-Cox, S. M. Chambers, B. MacBryde, and L. Thomas, eds. Genetics and conservation. Benjamin/Cummings, London.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

Benkman, C. W. 1999. The selection mosaic and diversifying coevolution between crossbills and lodgepole pine. Am. Nat. *In press.*

BOULETREAU, M. 1986. The genetic and coevolutionary interactions between parasitoids and their hosts. Pp. 169–200 *in* J. Waage and D. Greathead, eds. Insect parasitoids, Academic Press, London.

BRODIE, E. D., III, AND E. D. BRODIE, JR. 1991. Evolutionary response of predators to dangerous prey: reduction of toxicity of newts and resistance of garter snakes in island populations. Evolution 45:221–224.

BROWER, A. V. Z. 1994. Rapid morphological radiation and convergence in the butterfly, *Heliconius erato*, inferred from patterns of mitochondrial DNA evolution. Proc. Natl. Acad. Sci. 91: 6491–6495.

BROWN, J. M., J. H. LEEBENS-MACK, J. N. THOMPSON, O. PELLMYR, AND R. G. HARRISON. 1997. Phylogeography and host association in a pollinating seed parasite *Greya politella* (Lepidoptera: Prodoxidae). Mol. Ecol. 6:215–224.

BURDON, J. J. 1997. The evolution of gene-for-gene interactions in natural pathosystems. Pp. 245–262 *in* E. Crute, B. Holub, and J. J. Burdon, eds. The gene-for-gene relationship in plant-parasite interactions. CAB International, New York.

CARROLL, S. P., AND C. BOYD. 1992. Host race radiation in the soapberry bug: natural history with the history. Evolution 46: 1052–1069.

CARROLL, S. P., H. DINGLE, AND S. P. KLASSEN. 1997. Genetic differentiation of fitness-associated traits among rapidly evolving populations of the soapberry bug. Evolution 51:1182–1188.

CARTON, Y., AND A. NAPPI. 1991. The *Drosophila* immune reaction and the parasitoid capacity to evade it: genetic and coevolutionary aspects. Acta Oecologia 12:89–104.

CHEVILLIN, C., N. PASTEUR, M. MARQUINE, D. HEYSE, AND M. RAYMOND. 1996. Population structure and dynamics of selected genes in the mosquito culex pipiens. Evolution 49:997–1007.

COLL, M., L. GARCIA DE MENDOZA, AND G. K. RODERICK. 1994. Population genetic structure of a predatory beetle: the importance of gene flow for intertrophic level interactions. Heredity 72:228–236.

DAVIS, D. R., O. PELLMYR, AND J. N. THOMPSON. 1992. Biology and systematics of *Greya* Busck and *Tetragma*, new genus (Lepidoptera: Prodoxidae). Smiths. Contrib. Zool. no. 524.

DYBDAHL, M. F., AND C. M. LIVELY. 1996. The geography of coevolution: comparative population structure for a snail and its trematode parasite. Evolution 50:2264–2275.

EXCOFFIER, L., P. E. SMOUSE, AND J. M. QUATTRO. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

GODFRAY, H. C. J. 1994. Parasitoids, behavioral and evolutionary biology. Princeton Univ. Press, Princeton, NJ.

HANKSI, I., AND M. E. GILPIN, EDS. 1997. Metapopulation biology: ecology, genetics and evolution. Academic Press, San Diego, CA.

HENTER, H. J. 1995. The potential for coevolution in a host-parasitoid system. II. Genetic variation within a population of wasps in the ability to parasitize an aphid host. Evolution 49:439–445.

HENTER, H. J., AND S. VIA. 1995. The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. Evolution 49: 427–438.

HILLIS, D. M., AND M. T. DIXON. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol. 66:411–453.

HUDSON, R. R., M. SLATKIN, AND W. P. MADDISON. 1992. Estimation of gene flow from DNA sequence data. Genetics 132: 583–589.

JAENIKE, J. 1989. Genetic population structure of *Drosophila tripunctata*: patterns of variation and covariation of traits affecting resource use. Evolution 43:1467–1482.

KRAAIJEVELD, A. R., AND J. J. M. VAN ALPHEN. 1994. Geographical variation in resistance of the parasitoid *Asobara tabida* against encapsulation by *Drosophila melanogaster* larvae: the mechanism explored. Physiol. Entomol. 19:9–14.

———. 1995. Geographical variation in encapsulation ability of *Drosophila melanogaster* larvae and evidence for parasitoid-specific components. Evol. Ecol. 9:10–17.

KRAAIJEVELD, A. R., AND N. N. VAN DER WEL. 1994. Geographic variation in reproductive success of the parasitoid *Asobara tabida* in larvae of several *Drosophila* species. Ecol. Entomol. 19: 221–229.

KRAAIJEVELD, A. R., S. VOET, AND J. J. M. VAN ALPHEN. 1994. Geographical variation in habitat choice and host suitability in the parasitoid *Asobara rufescens*. Entomol. Exp. Appl. 72:109–114.

KRAAIJEVELD, A. R., B. NOWEE, AND R. W. NAJEM. 1995. Adaptive variation in host-selection behavior of *Asobara tabida*, a parasitoid of *Drosophila* larvae. Funct. Ecol. 9:113–118.

KRAAIJEVELD, A. R., J. J. M. VAN ALPHEN, AND H. C. J. GODFRAY. 1998. The coevolution of host resistance and parasitoid virulence. Parasitology 116:S29–S45.

LAVERY, S., C. MORITZ, AND D. R. FIELDER. 1996. Indo-Pacific population structure and evolutionary history of the coconut crab *Birgus latro*. Mol. Ecol. 5:557–570.

MCMILLAN, W. O., AND E. BERMINGHAM. 1996. The phylogeographic pattern of mitochondrial DNA variation in the Dall's porpoise *Phocoenoides dalli*. Mol. Ecol. 5:47–62.

MICHALAKIS, Y., A. W. SHEPPARD, V. NOEL, AND I. OLIVIERI. 1993. Population structure of a herbivorous insect and its host plant on a microgeographic scale. Evolution 47:1611–1615.

MULVEY, M., J. M. AHO, C. LYDEARD, P. L. LEBERG, AND M. H. SMITH. 1991. Comparative population structure of a parasite (*Fascioloides magna*) and its definitive host. Evolution 45:1628–1640.

ORR, H. A., AND S. IRVING. 1997. The genetics of adaptation: the genetic basis of resistance to wasp parasitism in *Drosophila melanogaster*. Evolution 51:1877–1885.

PARKER, M. A., AND J. M. SPOERKE. 1998. Geographic structure of lineage association in a plant-bacterial mutualism. J. Evol. Biol. 11:549–562.

PELLMYR, O., J. N. THOMPSON, J. M. BROWN, AND R. G. HARRISON. 1996. Evolution of pollination and mutualism in the yucca moth lineage. Am. Nat. 148:827–847.

PELLMYR, O., J. LEEBENS-MACK, AND J. N. THOMPSON. 1998. Herbivores and molecular clocks as tools in plant biogeography. Biol. J. Linn. Soc. 63:367–378.

POTTING, R. P. J., L. E. M. VET, AND W. A. OVERHOLT. 1997. Geographic variation in host selection behavior and reproductive success in the stemborer parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae). Bull. Entomol. Res. 87:515–524.

PRICE, P. W. 1980. Evolutionary biology of parasites. Princeton Univ. Press, Princeton, NJ.

SEGRAVES, K. A., J. N. THOMPSON, P. S. SOLTIS, AND D. E. SOLTIS. 1999. Multiple origins of polyploidy and the geographic structure of *Heuchera grossulariifolia*. Mol. Ecol. 8:253–262.

SHOEMAKER, D. D., AND J. JAENIKE. 1997. Habitat continuity and the genetic structure of *Drosophila* populations. Evolution 51: 1326–1332.

SWOFFORD, D. 1993. PAUP 3.1.1. Phylogenetic analysis using parsimony software package. Sinauer, Sunderland, MA.

THOMPSON, J. N. 1986. Oviposition and searching efficiency in a natural population of a braconid parasitoid. J. Anim. Ecol. 55: 351–360.

———. 1987. Variance in number of eggs per patch: oviposition behaviour and population dispersion in a seed parasitic moth. Ecol. Entomol. 12:311–320.

———. 1994. The coevolutionary process. Univ. of Chicago Press, Chicago.

———. 1997. Evaluating the dynamics of coevolution among geographically structured populations. Ecology 78:1619–1623.

THOMPSON, J. N., AND O. PELLMYR. 1992. Mutualism with pollinating seed parasites amid co-pollinators: constraints on specialization. Ecology 73:1780–1791.

THOMPSON, J. N., B. M. CUNNINGHAM, K. A. SEGRAVES, D. M. ALTHOFF, AND D. WAGNER. 1997. Plant polyploidy and insect/plant interactions. Am. Nat. 150:730–743.

VAUGHN, T. T., AND M. F. ANTOLIN. 1998. Population structure of an opportunistic parasitoid in an agricultural landscape. Heredity 80:152–162.

WILKINSON, H. H., J. M. SPOERKE, AND M. A. PARKER. 1996. Divergence in symbiotic compatibility in a legume-*Bradyrhizobium* mutualism. Evolution 50:1470–1477.

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