

Geographic structure in the searching behaviour of a specialist parasitoid: combining molecular and behavioural approaches

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Abstract

An increasing number of studies have shown that the traits important to species interactions may differ geographically among populations or groups of populations within a single interacting species. We examined geographic structure in the searching behaviour of a recently discovered parasitoid in the genus *Agathis* (Hymenoptera: Braconidae) by examining the pattern of population structure obtained from sequence data of mitochondrial DNA cytochrome oxidase I and the pattern of population differentiation in female searching behaviour. Analyses of population structure showed no isolation by distance and suggested long distance dispersal among populations. This pattern is consistent with recent post-glacial expansion of *Agathis* n. sp. Observations of searching behaviour demonstrated that populations of *Agathis* n. sp. differed in a subset of the behavioural traits examined and also one morphological trait. These population differences appear to be driven in part by local host plant characteristics, and based on the population structure of *Agathis* n. sp., have arisen relatively quickly in evolutionary time. This study suggests that the interaction between parasitoids and their host insects may exhibit substantial geographic variation, and studies that focus at the level of single populations or the species-level may be missing much of the evolutionary dynamics of parasitoid–host interactions.

Introduction

Studies of interspecific interactions in recent years have demonstrated substantial geographic variation in the relationships between pairs or groups of interacting species. Populations may differ in how they adapt to an interaction with another species (Kraaijeveld & van Alphen, 1994; Burdon & Thompson, 1995; Joshi & Thompson, 1996; Abrahamson & Weis, 1997), the set of species involved in an interaction may differ geographically (e.g. Arnold, 1981; Davies & de L Brooke, 1989; Carroll & Boyd, 1992; Benkman, 1993; Carroll *et al.*, 1997), and the outcome of the interaction may vary among communities (Thompson & Pellmyr, 1992; Clay, 1996; Benkman, 1999). Depending on the rate of gene

flow among populations within interacting species, traits important to an interaction may become geographically structured, and populations may only contain a subset of the traits exhibited within the entire species. The formation of geographic structure is the first step in the creation of a geographic mosaic that may drive the ongoing evolutionary dynamics of interactions, and especially coevolution (Thompson, 1994, 1997). Hence, understanding the evolution of species interactions requires not only studies of single populations, but also studies of the geographic structure of the interaction throughout a species' range.

Interactions between parasitoids and their insect hosts have the potential to exhibit complex geographic mosaics because parasitoids and their host species are often collections of genetically differentiated populations. For parasitoids, the ability of females to locate hosts and overcome host defences (virulence) is critical to their fitness (Godfray, 1994). Studies on parasitoid virulence

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have demonstrated that geographic mosaics may form across populations of a single parasitoid species. For example, the drosophilid parasitoids *Leptopilina bouhardi* (Eucolidae) and *Asobara tabida* (Braconidae) exhibit geographic differences in their ability to overcome the encapsulation responses of the one of their hosts *Drosophila melanogaster* (Bouletreau, 1986; Kraaijeveld & van Alphen, 1994). This variation is due both to the strength of the encapsulation response in *D. melanogaster* and the wasps' ability to overcome it (Bouletreau, 1986; Kraaijeveld & van Alphen, 1994, 1995; Kraaijeveld & Godfray, 1999). For *A. tabida*, populations in southern Europe have overcome this response and use *D. melanogaster* as the primary host, whereas populations in northwestern and central Europe attack the less defended species *D. subobscura* (Kraaijeveld & van der Wel, 1994). Similarly, Potting *et al.* (1997) have demonstrated that biocontrol strains of *Cotesia flavipes* differ in their ability to develop in species of stem-boring lepidoptera, primarily due to overcoming the encapsulation response of host species.

Unlike for parasitoid virulence, however, studies on female searching behaviour have not focused on differences among populations within a single species. Instead, they have focused on single populations or comparisons among closely related species. These studies have provided a solid understanding of the mechanisms responsible for eliciting searching behaviour (e.g. use of volatile chemicals and host cues), the particular mode of searching behaviour likely to be optimal for a given host distribution (e.g. Cook & Hubbard, 1977; Hubbard & Cook, 1978; Waage, 1979; Iwasa *et al.*, 1981; Weis, 1983; Green, 1984; Thompson, 1986; Casas, 1989; Janssen, 1989; Haccou *et al.*, 1991; Hemerik *et al.*, 1993; Volkl, 1994; Weisser, 1995), and the causes of behavioural differences among species (Nordlund *et al.*, 1981; Vet & van Alphen, 1985; Prevost & Lewis, 1990; Turlings *et al.*, 1990; van Alphen *et al.*, 1991; Faeth, 1994; Brodeur & Vet, 1995; Reed *et al.*, 1995). Although there are now many studies examining parasitoid searching behaviour at these two levels, relatively few studies have attempted to examine differences among populations within a single parasitoid species (Lewis *et al.*, 1990; Hopper *et al.*, 1993). Examining the evolution of parasitoid searching behaviour across groups of populations provides a bridge between the processes observed in single populations and the differences detected among closely related species.

We used the interaction between a recently discovered species of *Agathis* (Braconidae: Agathidinae) and the prodoxid moths *Greya enchrysa* and *G. piperella* to examine geographic structure in parasitoid searching behaviour. Studies of another *Agathis*–*Greya* interaction suggest that moth larval distributions and the pattern of searching behaviour by parasitoid females are an important focus of selection for coevolution between *Agathis* and *Greya* species (Thompson, 1986, 1987). We took a two-pronged approach to examine the geographic structure that may

form in parasitoid searching behaviour. First, we determined the pattern of population structure of *Agathis* n. sp. using mtDNA cytochrome oxidase I sequence and nuclear rDNA RFLP data to provide a template for examining population differences in how wasps search for hosts. Secondly, we examined the searching behaviour of wild-caught parasitoid females on natural distributions of *Greya* larvae and measured ovipositor length (a key character in host location) from four populations throughout the central range of the interaction. Specifically, we addressed the following questions: (1) What is the population structure of *Agathis* n. sp. as determined by neutral genetic markers? (2) Are there phenotypic differences in searching behaviour of females from different populations of *Agathis* n. sp., and if such differences exist, how do these compare with moth larval distributions and host plant characteristics? (3) What is the overall pattern of geographic structure in searching behaviour based on the combined surveys of neutral genetic markers and traits important to the interaction? Our overall aim is to examine geographic structure in parasitoid searching behaviour, and also to assess how two different approaches – surveys of neutral genetic markers and examination of traits important to the interaction – contribute to interpretation of the evolution of species interactions.

Natural history

The interactions between *Agathis* n. sp. and its *Greya* hosts occur on the moths' host plants, *Heuchera cylindrica* and *H. grossulariifolia*. These closely related plant species are located along the major river drainages of the Inland Pacific northwest of the United States of America, particularly in Idaho and western Montana. Both species produce multiple, spike-like inflorescences (scapes) that bear many flowers. After fertilization, the seeds mature and disperse as the seed capsules dehisce. Similarly, the flowering stems also dry and eventually are abscised from the plant. At each of the sites used in this study, only one of these *Heuchera* species was present.

Adult moths of *G. enchrysa* imbibe nectar and mate on their host plants (Davis *et al.*, 1992). When on *H. cylindrica*, female moths passively transfer pollen from their abdomens to the stigma of the flowers as they oviposit one to 25 eggs into the ovaries among the 50–200 flowers distributed across multiple scapes (Pellmyr *et al.*, 1996). The moth larvae feed on a subset of the developing seeds for approximately 2 weeks before dropping into the soil to overwinter. Ecdysis occurs the following spring (Davis *et al.*, 1992). The same life cycle occurs on *H. grossulariifolia*, although the pollination efficacy of *G. enchrysa* is undetermined for this species. Larvae are vulnerable to attack by *Agathis* n. sp. during the 2 weeks they are feeding within seed capsules. *Greya piperella* has a life history similar to *G. enchrysa*, but females oviposit into the inflorescence

stem rather than into flowers. The first and second instar larvae feed on the meristematic tissue within the stems, and then drop into the soil.

The parasitoid *Agathis* n. sp. is a braconid wasp within the subfamily Agathidinae. Species within this subfamily are all parasitoids of lepidopteran larvae. Females lay a single egg into one of the ventral nerve ganglia of the host and the larva feeds within the host body, eventually killing the host and then pupating (Balduf, 1966; Quednau, 1970; Odebiyi & Oatman, 1972, 1977; Ismail, 1981). Females of *Agathis* n. sp. search for moth larvae during the approximately 2-week period that *Greya* larvae feed in the seed capsules or flowering stems of the host plants. Like the moths, adult wasps of both sexes imbibe nectar from the host plants. Male wasps search host plants for females and attempt copulation as females probe for moth larvae (Althoff, personal observation). Females search for *G. enchrysa* larvae by first using their antennae to locate the dried stigmas of seed capsules, and then probing seed capsules with their ovipositors. They orientate their abdomens towards the stigma and slide their ovipositors into the groove between the unfused styles (Althoff, personal observation). In this way, females search the entire seed capsule without having to extract and re-insert their ovipositors. Females rarely force the ovipositor through the side of the capsule (2 of 1653 observed probes of seed capsules). Female wasps search for larvae of *G. piperella* by using their antennae to locate the oviposition scars left on the stems by female *G. piperella*, inserting their ovipositors into the scar, and probing above and below this opening. We have only observed *Agathis* n. sp. females searching on the two species of *Heuchera*.

Materials and methods

Collections

During the summers of 1996 and 1997, wasps were collected when they searched on *H. cylindrica* or *H. grossulariifolia* at the following sites (Fig. 1): Blue Mountains – Umatilla National Forest Service road FR 46 in the Blue Mountains of southeastern Washington, 46°13' N 117°46' W; Spalding – 1.6 km east of Nez Perce National Historic Monument, Spalding Site, Idaho, 46°27' N 116°49' W; St Joe R. #1 – St Joe River, 3.7 km west of Calder, Idaho, 47°16' N 116°15' W; St Joe R. #2 – St Joe River, 4.5 km on FR 537 St Joe National Forest, 47°17' N 116°7' W; North Fork Clearwater – 4.5 km on FR 711 Clearwater National Forest, 46°45' N 115°20' W; Swartz Cr. – 6.8 km on FR 502 Lolo National Forest, 46°42' N 113°48' W. Females from the Blue Mountains, Spalding, St Joe R. #1 and NF Clearwater sites were brought to the lab, used in behavioural observations (see below), and measured for ovipositor length and total body length to the nearest hundredth of a millimeter before being flash frozen in liquid nitrogen and placed in a –80 °C freezer.

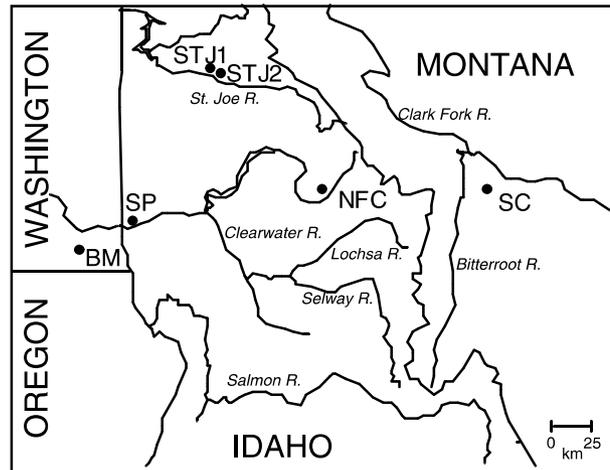


Fig. 1 Distribution of sites sampled for *Agathis* n. sp. At the NF Clearwater (NFC) site *Agathis* n. sp. searches for moth larvae of *Greya enchrysa* and *G. piperella* on *Heuchera grossulariifolia*. At all other sites *Agathis* n. sp. searches for moth larvae on *H. cylindrica*: Blue Mountains (BM), Spalding (SP), St Joe R. (STJ), and Swartz Cr. (SC).

Individuals from all other sites were flash frozen in liquid nitrogen and placed in a –80 °C freezer.

Phylogeographic analyses

Sequence data were generated for 78 individuals from six sites. All sites were represented by 15 individuals except the NF Clearwater (12 individuals) and Swartz Cr. (six individuals). Total genomic DNA from each wasp was extracted using the IsoQuick DNA Isolation kit (Orca Research, Inc., Bothell, WA, USA). Initial amplifications of a contiguous 2000 bp region containing approximately half of COI and half of COII were made using the primers mtD6 and mtD18 from the Insect mtDNA Primer Kit distributed by the University of British Columbia Nucleic Acid-Protein Service Unit. Amplifications were made in 50 µL reaction volumes containing 1X PCR buffer (Gibco, MD, USA), 3 mM MgCl₂ (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, 52 °C for 30 s, 72 °C for 2 min, and one cycle at 72 °C for 5 min. PCR products were cleaned with a 2.5-M NaCl/20% polyethylene glycol solution.

Cleaned PCR products were sequenced using an ABI Prism 377 Automated DNA Sequencer and the Dye Primer Labelling Kit and protocol provided by ABI (Foster City, CA, USA). Three primers were used to sequence the COI region: mtD6, one internal primer on the same strand as mtD6, and one internal primer on the same strand as mtD18. The internal primers were constructed from sequences obtained from *Agathis* n. sp. The primer, mtD6–1 (CTGGATTTTGGAAATTATTCA),

was located 500 bp from the 3' end of mtD6, and mtD18-1 (CATGAAATTCAATTATTAAG) was located 502 bp from the 3' end of mtD18. Sequencing reactions were in 5 μ L volumes and contained 25 ng initial PCR product, 1.25 pmole primer, and 2.5 μ L of FS dye terminator ready reaction cycle sequencing mixture from ABI. The PCR profile was 25 cycles of 96 °C for 30 s, 45 °C for 30 s, 60 °C for 4 min. Sequencing products were cleaned with Sephadex G-50 columns.

In addition to sequencing the COI region, PCR-RFLPs of an approximately 2500 bp region of the nuclear rDNA containing the 3' end of 18S, ITS-1, 5.8S, ITS-2 and the 5' end of 28S was amplified as above and digested with 10 restriction enzymes. The primers used were 18j and 28z from Hillis & Dixon (1991). The 10 enzymes used were *AccI*, *AvaI*, *AvaII*, *BanI*, *BanII*, *BstNI*, *HhaI*, *HincII*, *HpaII*, and *NciI*. PCR products were digested overnight with two units of each enzyme, and fragments were separated on 1% agarose gels and visualized using ethidium bromide staining.

We used the molecular data to analyse population structure in two ways. We performed analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992) to estimate variance in haplotype diversity within and between sites. We used the squared number of substitutions among haplotypes as the distance matrix. Significance testing of variance components was carried out using a permutational approach. The pairwise ϕ_{ST} values were then used to test for isolation by distance following Rousset (1997). We plotted pairwise $\phi_{ST}/(1-\phi_{ST})$ values against the common log of the great circle distance (distance with curvature of the earth taken into consideration) and the distance along river drainages between sites. Because the riverside habitats of *Agathis* n. sp. are separated by steep mountains, we used the distance along river drainages to mimic the dispersal pattern of this parasitoid if it were limited to movement along riparian corridors. In calculating distances between Swartz Cr. and the other sites, distance had to be estimated to the headwaters of the drainage and then over the Bitterroot Mountain range and into the next drainage. A Mantel test (Mantel, 1967) was used to statistically test if there was a positive correlation between $\phi_{ST}/(1-\phi_{ST})$ and the distance measures. We also used a nested clade analysis (Templeton *et al.*, 1995; Templeton, 1998) to separate patterns of population history from current gene flow. This method incorporates the evolutionary relationships of haplotypes and their current geographic distributions unlike traditional F_{ST} approaches that are based on current haplotype distributions. Subdivision is detected by comparing the geographic centre of all members of a clade to the geographic centres of each haplotype or subclade. An inference key (Templeton *et al.*, 1995) is then used to determine the patterns of subdivision resulting from history or gene flow. We used MINSPNET (Excoffier, 1993) to construct the haplotype network and GeoDis 2.0 (Posada *et al.*, 2000) to perform the nested clade analysis.

Nested clades were determined following the guidelines of Templeton & Sing (1993) and Templeton *et al.*, 1995. Haplotype nesting is determined by the number of mutational steps and position (tip or interior) in the haplotype network.

Phenotypic traits

We assessed the distribution of *G. enchyrsa* larvae among scapes of *H. cylindrica* and the pattern of search by *Agathis* n. sp. females at the Spalding, St Joe R. #1, and Blue Mountains sites, and also at the NF Clearwater site where the interaction occurred on *H. grossulariifolia* (Fig. 1). At each site, wasps were collected as they searched on the respective host plants. In addition, 10 plants were chosen, each at least 1 m from all other collected plants, and all the scapes with seed capsules were removed from these plants. Scapes were cut at the base and immediately placed in floral pics. Each scape was given a unique number that was coded for the plant from which it was taken. Both wasps and scapes were returned to the laboratory at Washington State University. Wasps were placed in 12 mL glass vials with foam stoppers and held at 4 °C until 20 min before the observation trials.

Searching behaviour was analysed for female wasps from St Joe R. #1 and NF Clearwater during June and July 1996, respectively, and from Blue Mountains during July 1997. Females from Spalding were observed in both June 1996, 1997 to determine if the host distributions and pattern of female search differed across years. Sites were chosen to encompass the central portion of the geographical range of the interaction as well as differences in host plant species use. Observations were conducted at the Washington State University Botany garden. All scapes collected from a single plant were randomly assigned to one of ten 3.8 l pots that contained the vegetative portion of an individual of the respective host plant that had been growing at the Washington State University Botany garden. The scapes were inserted into the soil of the pot resulting in a plant that had the vegetative base of one individual, but the field collected scapes of another. This method was chosen to realistically simulate a natural plant. When possible, the plants used as a vegetative base came from the same site as the wasps and collected scapes. The 10 pots were then randomly placed in a circle with a radius of 60 cm and a distance of 42 cm between pots. This circle was enclosed by a 2 × 2 × 4 m fine-mesh walk-in cage.

Females were observed within 3 days of being collected. In 1996, each female from Spalding, St Joe R. #1, and NF Clearwater was observed for approximately 120 consecutive minutes. Females observed in 1996 spent part of the observation period imbibing nectar rather than searching seed capsules. For 1997, females from Spalding and Blue Mountains were allowed to nectar *ad libitum* on their respective host plant species before the start of the trial to ensure that the majority of time was

spent searching rather than imbibing nectar or preening. This procedure also allowed the observation period to be reduced to 60 min for these sites in order to observe all females within 3 days of being collected.

Two females were released at the same time, and each observed by a different person. Females did not appear to interfere with each other when searching in the cages or in the field. Females have been observed to search the same capsule at the same time in the field (Althoff, personal observation). Females that did not search seed capsules within the first 15 min of being released were returned to their vials and observed at a later time. Trials took place between 10:00 and 17:00 PDT which corresponded to the time when females were most actively searching in the field (Althoff, personal observation).

We recorded the time in seconds a female spent probing each seed capsule and the scape on which the capsule was located. When a female finished probing, the capsule was marked with a felt pen with a unique colour for that female. We also recorded the frequency and time that females spent probing the inflorescence stems. After the observation period, females were returned to the lab, measured for body and ovipositor length, flash frozen in liquid nitrogen and placed in a -80°C freezer. When all females from a site had been observed, the scapes were returned to the lab and the stem and every seed capsule on a scape were dissected to determine the presence or absence of moth larvae. We were able to assess the following components of searching behaviour: the distribution of moth larvae within capsules and among scapes, whether female wasps preferentially searched capsules containing moth larvae, the time spent in searching capsules that did and did not contain moth larvae, and the pattern of search by individual females within and among scapes.

One obvious difference between host plant species was the size of seed capsules. We quantified this difference by measuring the length of seed capsules from the apex to the pedicel of the capsule for *H. grossulariifolia* from NF Clearwater and *H. cylindrica* from Spalding and St Joe R. #1. Three capsules were haphazardly chosen from the bottom, middle and top of each scape and measured to the nearest hundredth of a millimetre using a handheld dial caliper.

Data were checked for equality of variances and normality before performing parametric tests. ANOVA was used to compare the number of *G. enchrysa* larvae per capsule and the time *Agathis* n. sp. females spent searching seed capsules without larvae, an ANCOVA with body length as the covariate to compare female ovipositor length among sites, and a univariate repeated measures (ANOVAR) to compare seed capsule length. Moth larval distributions across scapes were compared with an expected Poisson distribution with a *G*-test and the coefficient of dispersion was used to characterize the larval distributions as clumped, uniform or random (Sokal & Rohlf, 1995). We used a Wilcoxon/Kruskal–Wallis test

to compare the number of seed capsules searched per stem when female wasps did and did not find moth larvae (Sokal & Rohlf, 1995).

Results

Phylogeographic analyses

Of the 1260 bp sequenced for each individual, 54 sites were variable – 14 at the first codon position, eight at the second codon position and 32 at the third codon position. Forty-two unique haplotypes were detected of the 78 individuals sequenced. Representative haplotypes were deposited in GenBank (Accession entries AF078454–AF078468). All individuals except one were monomorphic for the RFLP patterns detected with the 10 enzymes surveyed.

The AMOVA detected overall genetic structuring among sites for *Agathis* n. sp. (Table 1). Most of the haplotype variance, however, was within rather than between populations (82 and 18%, respectively), and only one of the 15 pairwise comparisons of ϕ_{ST} was significant after Bonferroni correction for multiple tests (Sokal & Rohlf, 1995). Plots of $\phi_{ST}/(1-\phi_{ST})$ vs. the common log of great circle distances or distances along river drainages between sites did not reveal any significant positive correlations indicative of isolation by distance (Fig. 2). Similarly, Mantel tests did not detect a significant association between $\phi_{ST}/(1-\phi_{ST})$ and the common log of great circle distances or distances along river drainages between sites. The nested clade analysis detected a geographical association of haplotypes for two clades (Fig. 3). For each clade, the analysis detected long distance dispersal among sites. Both the isolation by distance and nested clade analyses suggest that geographical distance has not had a major influence on the pattern of population structure for *Agathis* n. sp.

Distribution of *Greya enchrysa* larvae

Capsules with larvae averaged 2.95 larvae per capsule when all *H. cylindrica* sites were combined (means did not differ among sites or years, $F_{3,267} = 1.3$, $P = 0.28$). Larvae of *G. enchrysa* had a significantly clumped distribution across scapes of *H. cylindrica* in all three of the sites

Table 1 AMOVA pairwise ϕ_{ST} values for mtDNA cytochrome oxidase I haplotype variation in *Agathis* n. sp. Overall $\phi_{ST} = 0.18^{***}$.

Site	1	2	3	4	5	6
1. Spalding (SP)	–					
2. St Joe R. (STJ1)	0.14	–				
3. St Joe R. (STJ2)	0.35	0.10	–			
4. Blue Mountains (BM)	0.13	0.00	0.17	–		
5. NF Clearwater R. (NFC)	0.34	0.03	0.15	0.08	–	
6. Swart Cr. (SC)	0.13	0.16	0.52 ^{***}	0.08	0.44	–

*** $P < 0.001$.

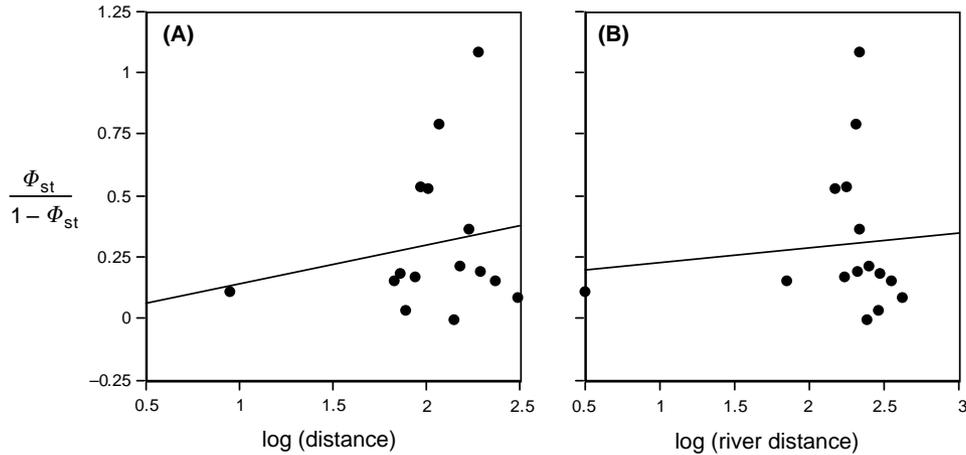


Fig. 2 Plots of pairwise $\phi_{ST}/(1-\phi_{ST})$ vs. great circle distances (A) and distances along river drainages (B) to examine isolation by distance among sites for *Agathis* n. sp. Mantel tests did not detect a significant association between $\phi_{ST}/(1-\phi_{ST})$ and either measure of distance ($r = 0.47^{ns}$; $r = -1.57^{ns}$, respectively).

examined and also between years at Spalding (Fig. 4). The observed distributions did not correspond to an expected Poisson distribution, and the coefficients of dispersion were greater than one for all sites and both years at Spalding. Dissections of seed capsules of *H. grossulariifolia* from NF Clearwater yielded too few (only eight) capsules that contained moth larvae to make estimates of larval distributions. Overall, these results

indicate that female wasps from all *H. cylindrica* sites searched for larvae that were qualitatively distributed in similar ways within capsules and among spaces.

Pattern of search by females of *Agathis* n. sp.

Agathis n. sp. females were unable to use host cues alone to find moth larvae. Females did not preferentially visit

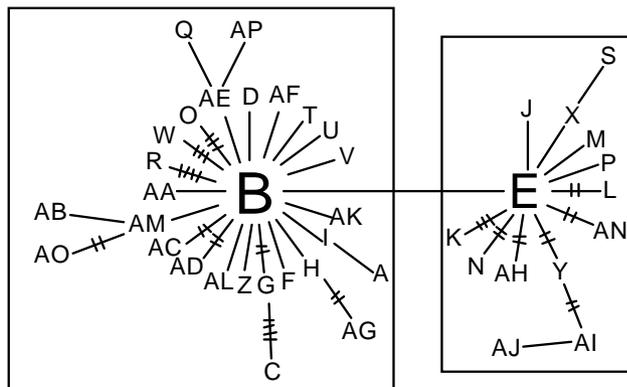


Fig. 3 Minimum spanning haplotype network for *Agathis* n. sp. used in the nested clade analysis. Lines without marks between haplotypes represent a single mutational change. Lines with marks represent the number of mutations connecting haplotypes. Intervening haplotypes were either extinct or not sampled. The two clades for which long distance dispersal was detected by the nested clade analysis are in boxes. The distribution of haplotypes among populations is given in the box below the network. Haplotypes B and E were the most common. Other haplotypes in bold-italics are shared by at least two populations. All other haplotypes were unique to their respective populations.

Blue mountains (BM)	B E AI AD AE AF AK AL AM AN AO AP
Spalding (SP)	B E J K L M N O P Q R S
St. Joe#1 (STJ1)	B E A C D S T U V W X
St. Joe#2 (STJ2)	B F G H AC T
NF clearwater (NFC)	B E I Y Z AA
Swartz Cr. (SC)	I AB AG AH AI AJ

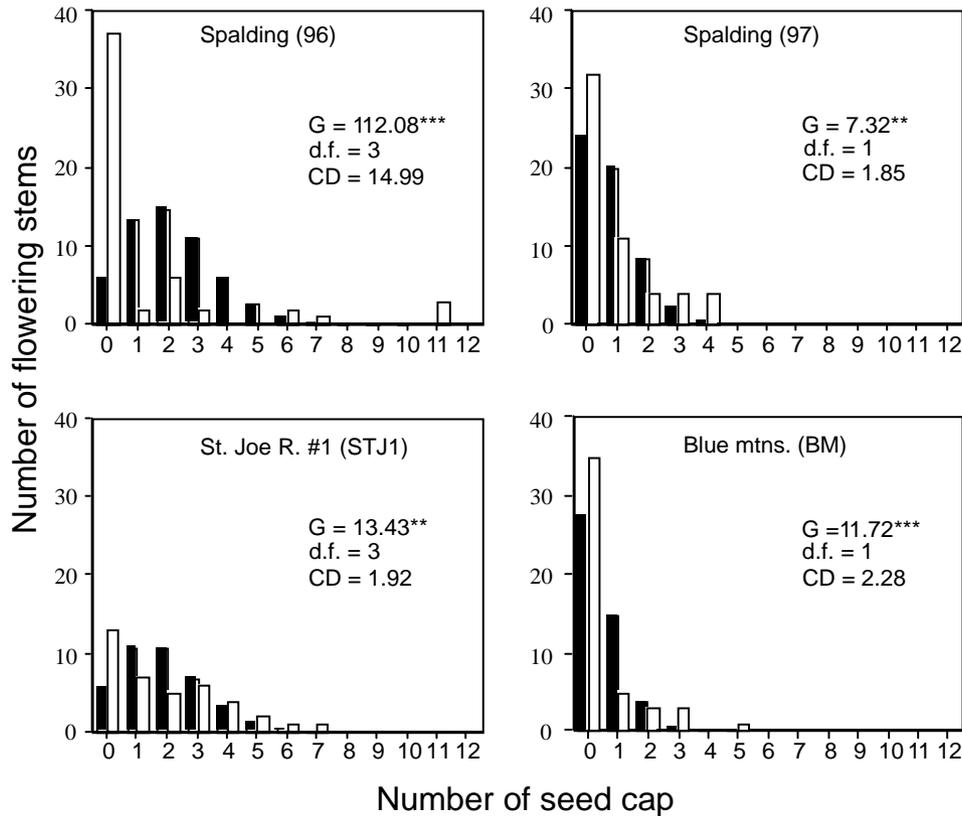


Fig. 4 Comparison of larval distributions against a Poisson distribution for populations of *Greya enchrysa*. Black bars represent expected Poisson distribution, open bars represent observed distribution. *G*-tests were used to compare observed and expected distributions. Based on coefficients of dispersion (CD), moth distributions for all populations are clumped (CDs > 1).

those scapes containing larvae (Table 2). Upon landing on a scape, females probed seed capsules to determine whether moth larvae were present. Females from NF Clearwater and Blue Mountains spent significantly less time searching individual seed capsules than females from Spalding and the St Joe R. #1 (Table 3). When females from all sites did search a capsule that contained larvae, they increased the overall number of capsules searched on that scape (Table 4).

Table 2 Results of χ^2 tests to determine whether *Agathis* n. sp. females preferentially visit scapes that contain *Greya enchrysa* larvae. H_0 : expected no. of visits = observed no. of visits. Expected no. of visits = total no. of visits by all females \times proportion of scapes with larvae.

Site	Expected no. of visits	Observed no. of visits	χ^2
SP96	28.78	27	0.110 ^{ns}
SP97	39.29	45	0.830 ^{ns}
STJ	75.37	80	0.284 ^{ns}
BM	34.68	34	0.014 ^{ns}
NFC	16.90	12	1.421 ^{ns}

In many instances, however, female wasps did not detect moth larvae that were present on a scape because they only probed capsules that did not contain larvae. In these cases, females must have decided how many capsules to search before giving up and moving to another scape. Although there could be many physiological and external factors that influence a female's decision (e.g. Mangel & Clark, 1988; Fletcher *et al.*, 1994; Mangel & Hiempel, 1998; Rosenheim, 1999), females searched on scapes in which they did not contact any host larvae for approximately 51.64 s or 2.99 capsules (grand means for *H. cylindrica* populations, differences among populations not significant, $F_{3,31} = 0.29$; $F_{3,31} = 1.73$, respectively) before leaving.

In addition to searching seed capsules of *H. cylindrica* and *H. grossulariifolia*, some *Agathis* n. sp. females also searched the inflorescence stem for larvae of *G. piperella*. Females walked down stems after searching seed capsules and searched for oviposition scars left by *G. piperella* females. Some females from Blue Mountains, St Joe R. #1, and NF Clearwater searched the stems, whereas no females from Spalding did (Table 5). *Greya piperella* larvae

Table 3 Comparisons of mean time (s) that *Agathis* n. sp. females spent searching capsules without *Greya enchrysa* larvae, female ovipositor length (mm) and host plant seed capsule length (mm). Last line shows differences among mean as determined by LSDs.

Site	Host plant	Time spent searching			Ovipositor length			Capsule length			
		<i>n</i>	Mean	SE	<i>n</i>	Mean	SE	<i>n</i>	Mean	SE	
NFC	<i>Heuchera grossulariifolia</i>	7	8.43	0.50	10	5.40	0.13	126	6.71	0.09	
BM	<i>H. cylindrica</i>	11	17.77	1.65	10	5.89	0.11				
SP96	<i>H. cylindrica</i>	11	28.71	3.62	11	7.07	0.12	80	9.70	0.13	
SP97	<i>H. cylindrica</i>	10	32.71	4.40	11	6.40	0.08				
STJ1	<i>H. cylindrica</i>	10	41.19	4.43	10	6.75	0.11	98	9.84	0.12	
			NFC = BM < SP96 = SP97 = STJ ($F_{4,48} = 30.36, P < 0.001$)			NFC < BM < SP97 < STJ1 < SP96 ($F_{4,50} = 48.36, P < 0.001$)			NFC < SP = STJ1 ($F_{3,36} = 201.81, P < 0.001$)		

Table 4 Comparison of number of seed capsules searched per scape when *Agathis* n. sp. females did and did not find *Greya enchrysa* larvae. *n* is the number of females observed. Values are mean \pm SE.

Site	<i>n</i>	No. of Capsules searched		Wilcoxon χ^2
		No larvae found	Larvae found	
SP96	11	2.699 \pm 0.260	7.200 \pm 1.471	12.712***
SP97	10	3.066 \pm 0.227	5.444 \pm 0.868	6.596**
STJ	10	2.775 \pm 0.292	6.879 \pm 0.715	32.939***
BM	11	3.414 \pm 0.317	11.125 \pm 1.950	15.868***
NFC	7	3.006 \pm 0.342	n/a	–

** $P < 0.01$, *** $P < 0.001$.

Table 5 Differences in the location of search by *Agathis* n. sp. females. All females searched for *Greya enchrysa* larvae in seed capsules; some females also searched for *G. piperella* larvae in the inflorescence stems.

Site	Stems with <i>G. piperella</i> (%)	No. of Females searching	
		Only seed capsules	Capsules and stems (%)
NFC	6/42 (14)	7	4 (57)
BM	0/47 (0)	11	2 (18)
SP96	n/a	10	0 (0)
SP97	7/39 (18)	10	0 (0)
STJ	5/55 (9)	10	5 (50)

were present in stems from all sites except Blue Mountains.

Ovipositor length

Female ovipositor length differed among sites when body size was used as a covariate (Table 3) although body size did not ($F_{4,50} = 1.93, P = 0.11$). Females searching on *H. grossulariifolia* from NF Clearwater had the shortest ovipositors. Ovipositor length also differed among females that searched on *H. cylindrica*, and also between years at Spalding. One potential explanation for the differences among NF Clearwater and the *H. cylindrica* sites is that

capsule size differs between *H. grossulariifolia* and *H. cylindrica* – *H. grossulariifolia* had smaller seed capsules than *H. cylindrica* (Table 3). Differences in seed capsule size, however, cannot explain the variation in female ovipositor length from the *H. cylindrica* sites. Seed capsule size did not differ between Spalding, St Joe R. #1 and Blue Mountains, although female ovipositor length did.

Discussion

Population structure as determined by genetic markers

The two analyses of the mtDNA COI sequence data produced similar views of population structure for *Agathis* n. sp. There was no significant isolation by distance, and *Agathis* n. sp. has undergone long distance dispersal events as detected by the nested clade analysis. These results suggest two possible alternatives for interpreting population structure: recent colonization by *Agathis* n. sp. or high levels of gene flow among existing populations (Slatkin, 1993). Both past climates and the current distribution of host plant patches suggest the former. The last North American glacial maximum occurred 18 kya during which time the mountain ranges of the Idaho panhandle supported valley glaciers (Alt & Hyndman, 1989; Graham, 1999). Shortly after this last glacial maximum, the Snake River canyon, the central panhandle of Idaho, and south-eastern Washington were scoured by floodwaters from Pleistocene Lake Missoula in Montana (Graham, 1999). Thus, suitable habitat for the host plants has only been available for less than 18 ky; both plants and insects would have had to expand their ranges since this time. As for *Agathis* n. sp., genetic surveys of *H. grossulariifolia* and another species of *Greya*, *G. politella*, have also detected very limited genetic structure over the same geographic range surveyed in this study (Brown *et al.*, 1997; Segraves *et al.*, 1999). Data from these species as well as other north western plant and animal species support a pattern of post-glacial colonization for *Agathis* n. sp. (Soltis *et al.*, 1997; Conroy & Cook, 2000; Hewitt, 2000).

If *Agathis* n. sp. has recently colonized the inland northwestern USA, host plant distribution must have been continuous enough in the past to facilitate movement along river drainages. Presently, however, both *H. cylindrica* and *H. grossulariifolia* are patchily distributed on granitic and basaltic outcroppings along river drainages (Althoff, personal observation; Thompson *et al.*, 1997). Populations of these plants differ in their flowering phenology because of altitude and riverbank aspect. Although wasps may be physically capable of flying the distances between patches, their arrival would not necessarily be synchronous with the presence of flowers or moth larvae. This coupled with the fact that populations are along rivers separated by major mountain ranges suggests that current dispersal among drainages is limited in *Agathis* n. sp. An overall significant ϕ_{ST} value of 0.18 suggests further that contemporary maternal gene flow may be limited among sites. We caution, however, that this value is for a mitochondrial gene that has a smaller effective population size and is expected to be more sensitive to subdivision than nuclear genes (Birky *et al.*, 1983). Overall, the analyses of neutral genetic markers suggest post-glacial colonization of these habitats by *Agathis* n. sp. and possibly reduced current levels of gene flow among populations. This template of population structure suggests that phenotypic differences in female searching behaviour have evolved relatively recently.

Phenotypic differences in searching behaviour

Female wasps differed in three traits which are important in searching for *Greya* host larvae: the time allocated to searching seed capsules, ovipositor length, and where they searched for moth larvae. Differences in these traits in part may be explained by variation in host plant characteristics. For example, females from NF Clearwater searched seed capsules of *H. grossulariifolia* two to five times faster than females that searched on *H. cylindrica* (Table 3). This difference is likely the result of searching on the smaller seed capsules of *H. grossulariifolia*. The same pattern was also detected for ovipositor length. NF Clearwater females had the shortest ovipositors although body length was not significantly different among sites. Interestingly, ovipositor length also differed among populations that searched on *H. cylindrica* although capsule sizes were similar. These differences may result either as a pleiotropic effect of selection on a trait unmeasured in our study, or they may result from subtle differences among populations in the distribution of *Greya* larvae within capsules.

In addition to searching time and ovipositor length, females also differed in where they chose to search for potential host larvae. Some females from all sites except for Spalding searched the inflorescence stems in addition to probing seed capsules. These females were searching for larvae of *G. piperella* that were present at all sites

except for Blue Mountains (Table 5). Females from Spalding may not be able to search stems because the stems at this site are more pubescent than stems from other *H. cylindrica* sites or *H. grossulariifolia* (Althoff, personal observation; Hitchcock & Cronquist, 1973). Stem pubescence may decrease the ability of female *Agathis* n. sp. to detect the oviposition scars left by *G. piperella* females, because wasps would be unable use their antennae to locate the necrotic plant tissue surrounding the scars.

Unlike the variation observed in host plant characteristics, moth larvae were distributed in a clumped distribution at all *H. cylindrica* sites. Consequently, females from these sites exhibited similar searching behaviours. Females probed only a small proportion of the capsules on a scape, moving soon to another scape if they found no larvae in the first few capsules they searched. If a female did contact larvae within a seed capsule, she increased her searching effort on that scape by searching more seed capsules. Hence, females intensify their searching efforts on a patch (scape) where more host individuals are present.

Geographic structure in searching behaviour

Thompson (1994) used the term geographic structure to imply that populations of a species differ in the degree of connectedness, the environments they occupy and potentially the species with which they interact. Our results for *Agathis* n. sp. suggest that the interaction with its host moths *G. enchrysa* and *G. piperella* exhibits geographic structure. The analyses of population structure showed that *Agathis* n. sp. has recently spread throughout the Inland Pacific northwest and contemporary population structure, both due to host plant patchiness and variation in flowering times is conducive to restricted gene flow among populations. Vaughn & Antolin (1998) have shown that gene flow in parasitoids can be restricted even at much smaller spatial scales (<1 km) than studied for *Agathis* n. sp. We have also documented phenotypic differences in both behaviour and morphology that are integral to the way parasitoid females search for their hosts, and that these differences have arisen fairly quickly in evolutionary time. Moreover, we have shown that females in one population of *Agathis* n. sp. do not search for and do not interact with a host insect species used by females in other populations.

Our use of wild-caught adult females and observations solely on their local host plants and natural distributions of moth larvae did not allow us to determine if the phenotypic differences detected are genetically based or learned by females over the course of their searching experiences. Some parasitoid species have been shown to change their searching behaviour with experience, and to associate microhabitat cues with host individuals (Turlings *et al.*, 1993). Vet *et al.* (1995) suggested that for specialized tritrophic interactions such as the one

studied here, the value of learning is low and parasitoids should be selected to rely on innate responses to hosts and plant cues rather than learning. Wajnberg *et al.* (1999) have demonstrated that at least one component of searching behaviour, patch-leaving tendency, is genetically based. Ultimately, the use of naïve females and observations of searching behaviour on host plants from other populations are needed to determine if these phenotypic differences are genetically based. Although we cannot rule out that learning has influenced the behavioural differences observed in *Agathis* n. sp., these phenotypic differences have direct consequences for the way *Agathis* n. sp. interacts with its host insect species, and the morphological differences remain even when scaled to body size. The results of this study in combination with studies on parasitoid virulence demonstrate that parasitoid–host interactions may exhibit geographic structure as found in other types of species interactions.

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