Molecular markers reveal strong geographic, but not host associated, genetic differentiation in *Aphidius transcaspicus*, a parasitoid of the aphid genus *Hyalopterus*

J.D. Lozier*[†], G.K. Roderick and N.J. Mills

Department of Environmental Science, Policy and Management,
Mulford Hall, University of California, Berkeley CA 94720-3114, USA

Abstract

Host plant associated genetic differentiation is a common phenomenon in phytophagous insects, but the degree to which such associations sequentially drive diversification at higher trophic levels is not as well understood. A recent study examining neutral molecular markers in *Hyalopterus* aphids revealed that genetic structure in this genus is strongly determined by primary host plant use (*Prunus* spp.). In this paper, we take a similar approach to determine whether this host plant specificity has affected genetic structure in the parasitoid *Aphidius transcaspicus*, an important natural enemy of *Hyalopterus* spp. in the Mediterranean. Mitochondrial DNA (428 bp) and seven microsatellite loci were examined in parasitoids collected from aphid populations on almond, apricot, peach and plum trees from Spain and Greece. In contrast to the previous findings for *Hyalopterus* from the same regions, here we find no evidence for host associated diversification in *A. transcaspicus* at the species level or below, though geographic structure between regional populations is exceptionally high. These findings have several implications for our understanding of the ecology and evolution of *A. transcaspicus* as well as for its use as a biological control agent for *Hyalopterus*, suggesting that a consideration of host plant specificity may be less critical than factors such as climatic suitability or geographic origins of invasive populations.

Keywords: *Aphidius transcaspicus*, *Hyalopterus*, parasitoid, population structure, host associated differentiation, mitochondrial DNA, microsatellites

(Accepted 30 March 2008)

*Author for correspondence
Fax: +01-217-244-3499
E-mail: jdlozier@life.uiuc.edu
[†]Current address: Department of Entomology, 320 Morrill Hall, University of Illinois at Urbana-Champaign, Urbana IL 61801, USA.

Introduction

Studies of resource use in insects have revealed much about the interactions between ecological and evolutionary forces, and there are now many excellent examples of the formation of cryptic species, 'biotypes' and 'host races' among populations of phytophagous insects feeding on different host plant species (Dres & Mallet, 2002; Funk *et al.*, 2002). While these plant parasites have been well studied, the process of ecological diversification in other parasitic organisms has received comparatively little attention (Huyse *et al.*, 2005). Specifically, it remains unclear to what extent host plant associated differentiation and subsequent speciation in phytophagous insects triggers co-divergence in natural enemies, such as predators and parasitoids (Cronin & Abrahamson, 2001; Stireman *et al.*, 2006; Abrahamson & Blair, 2007). Given the increasing interest in coevolutionary processes, studies examining the degree to which higher trophic levels evolve in response to genetic structure of hosts or prey (and vice versa) will be important for understanding species interactions and diversification (Singer & Stireman, 2005), as well as for applied sciences such as biological control (Roderick & Navajas, 2003; Hufbauer & Roderick, 2005).

Parasitoids are organisms that lay their eggs in or on the body of an arthropod host, which is consumed and killed by the developing parasitoid larvae (reviewed by Godfray, 1994). The Aphidiinae (Hymenoptera: Braconidae) consists of ~400 species that are solitary endoparasitoids of aphids (Homoptera: Aphididae: Starý, 1988). Host range within the subfamily varies from strict specialization to broad generalism (Starý, 1988), though it is believed that many supposedly generalist taxa comprise cryptic host specific complexes (e.g. Tremblay & Pennacchio, 1988; Atanassova *et al.*, 1998). However, while host specificity has been well-studied in these parasitoids, there is little data on how often such specialization has evolved in concert with plant-associated diversification in their aphid hosts.

Aphidius transcaspicus Telenga is an aphidiine parasitoid that is distributed across the Mediterranean basin and central Asia. In the field, the species appears highly specific to aphids in the genus *Hyalopterus* Koch (Kavallieratos *et al.*, 2004; personal communication), though it will parasitize other aphids with varying degrees of success in laboratory settings (Wang & Messing, 2006; personal observation). *Hyalopterus* species have complex life-cycles involving cyclical parthenogenesis and host alternation between primary host plants in the genus *Prunus*, on which sexual reproduction and overwintering occurs, and a secondary host plant (typically *Phragmites australis*), where populations persist clonally during the summer months. *Hyalopterus* comprises three broadly sympatric species that diverged several million years before present and exhibit strong associations with three principal primary host plants (Lozier *et al.*, 2007, 2008) – *H. amygdali* with *P. dulcis* (almond), *H. persikonus* with *P. persica* (peach), and *H. pruni* with *P. domestica* (plum). These species have maintained their genetic isolation and associations with these host plants despite sharing a secondary host plant and frequent co-occurrence on the primary host plant *P. armeniaca* (apricot) and other less commonly utilized *Prunus* spp. (Lozier *et al.*, 2007). *Aphidius transcaspicus* attacks *Hyalopterus* on each of their primary and secondary host plants throughout the Mediterranean, though at present no ecological data

are available regarding host preferences for the different *Hyalopterus* species or frequency of parasitism on different primary and secondary host plant species.

Given the host plant associated genetic differentiation documented in *Hyalopterus* and apparent specificity of *A. transcaspicus* to this aphid genus in the field (Kavallieratos *et al.*, 2004), in this study we aimed to investigate whether host plant associations have helped structure genetic diversity across trophic levels, or whether other factors, such as geographic separation, may be more important for population differentiation in this parasitoid. Beyond the implications that a better understanding of diversification at different trophic levels holds for ecology and evolution, the identification of cryptic host plant associated structure in *A. transcaspicus* also has important practical significance. *Hyalopterus pruni* is the only member of *Hyalopterus* detected in North America (Lozier *et al.*, in press) and is an important agricultural pest of dried plum in California. The discovery of parasitoid species or biotypes that are strongly associated with *Hyalopterus* on plum trees could be crucial for selecting the most specific and effective populations of *A. transcaspicus* for use in biological control (Gordh & Beardsley, 1999).

The evolution of host or host plant-specific lineages in a parasitoid, as for other insects, would require genetic polymorphism for traits associated with host use combined with barriers to gene flow and/or disruptive selection (reviewed in Via, 2001; Rundle & Nosil, 2005; Abrahamson & Blair, 2007). Broadly distributed parasitoids might exhibit host specialization because of historical vicariance and geographic heterogeneity in the distribution of host species followed by subsequent expansion into sympatry (Althoff & Thompson, 2001; Bush & Butlin, 2004; Vos & Vet, 2004). Alternatively, differentiation might occur via host shifts in the absence of physical isolation when selection associated with hosts or host plants is strong (Rundle & Nosil, 2005). The opportunity for ecologically mediated isolation may be high in aphidiine parasitoids due to a number of factors linked to their unique ecology. Importantly, host associated differentiation will likely depend both on the aphid host, which acts as a resource and a site for reproduction through oviposition, and the host plant, which provides the context in which this interaction occurs and can in some cases affect host preferences more strongly than the host itself (Storeck *et al.*, 2000; Daza-Bustamante *et al.*, 2002). It has been demonstrated in several aphidiines that chemical information obtained at adult emergence govern the host plant upon which mating and host foraging occurs (Vet & Dicke, 1992; Storeck *et al.*, 2000), promoting correlations between preferences for mating, foraging and oviposition habitats. The tendency for siblings to mate at the emergence site is also common in many parasitoids (Godfray & Cook, 1997; Mackauer & Volkl, 2002) and could enhance such prezygotic isolation by further restricting gene flow, increasing linkage disequilibrium among genes involved in habitat and mate preference or providing mating opportunities for isolated populations during the initial period following a host shift (Askew, 1968; Via, 2001; Dieckmann & Doebeli, 2004). Lastly, selection acting to increase the efficiency with which endoparasitoids respond to immune defenses or secondary symbiont communities of different host species, or to chemical differences associated with different plant species, may establish performance trade offs that can limit genetic exchange among populations (Tremblay &
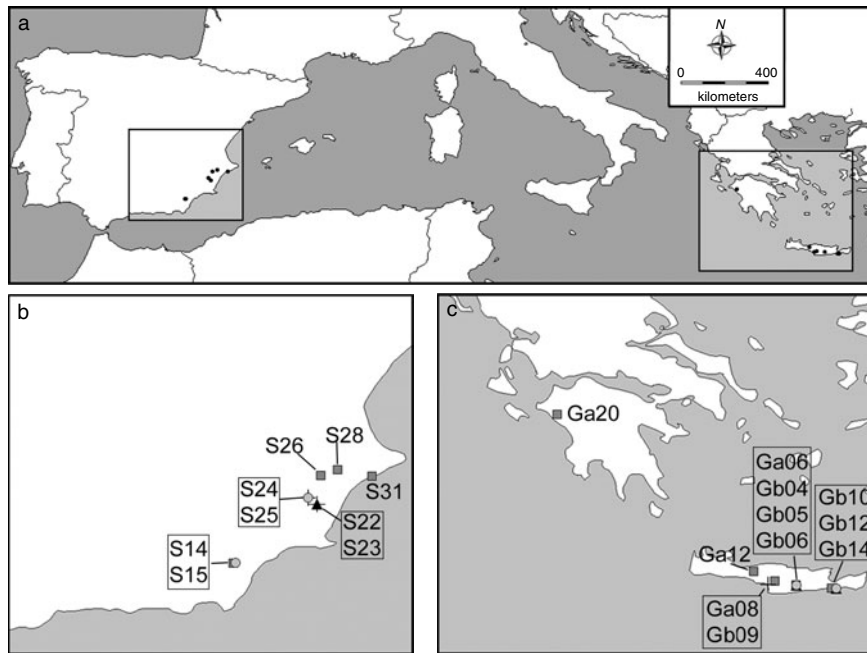
Fig. 1. (a) Regions sampled for *Aphidius transcaspicus* with detailed insets for collecting localities in (b) Spain and (c) Greece. Number of samples genotyped in Spain, June 2002 (b): S14$_{almond}$ = 10, S15$_{plum}$ = 10, S22$_{peach}$ = 14, S23$_{apricot}$ = 9, S24$_{apricot}$ = 10, S25$_{plum}$ = 12, S26$_{almond}$ = 4, S28$_{almond}$ = 10, S31$_{almond}$ = 9; for Greece, May 2003 (c): Ga20$_{almond}$ = 10; Ga06$_{almond}$ = 8, Ga08$_{almond}$ = 6, Ga12$_{almond}$ = 12; for Greece, May 2005 (c): Gb04$_{plum}$ = 13, Gb05$_{almond}$ = 12, Gb06$_{peach}$ = 12, Gb09$_{apricot}$ = 10, Gb10$_{almond}$ = 12, Gb12$_{plum}$ = 2, Gb14$_{peach}$ = 5 (■, almond; +, apricot; ▲, peach; ◯, plum).

Pennacchio, 1988; Henter, 1995; Oliver *et al*., 2005; but see Hufbauer, 2001).

Detecting cryptic lineages can be difficult in parasitoids due to a lack of good morphological characters, and identifying diversity in the genus *Aphidius* has been problematic even at the species level, with *A. transcaspicus* itself only recently validated as a distinct species (Kavallieratos & Lykouressis, 1999). However, molecular markers have proven useful at distinguishing cryptic diversity in parasitoids at the species and population level (e.g., Hoy *et al*., 2000; Smith *et al*., 2007). Thus, to assess potential cryptic differentiation in *A. transcaspicus*, we examined eight molecular markers including the mitochondrial (mtDNA) *cytochrome oxidase I* (*COI*) gene and seven microsatellites (Lozier *et al*., 2006). This combination of markers is likely to provide insights into evolutionary processes at different timescales. Genealogical relationships among *COI* sequences can be useful for detecting differences among putative insect species (e.g. Smith *et al*., 2007) or well-resolved biotypes (e.g. Boykin *et al*., 2007). Patterns of similarity among microsatellite genotypes, while of limited use for phylogenetics, can still provide support for such higher level differences at multiple independent loci and can also be used to infer relationships at finer scales between populations and individuals (Schlötterer & Pemberton, 1998). We present genetic data for parasitoids reared from *Hyalopterus* spp. collected on the four principal primary host plants (almond, apricot, peach and plum) from Spain and Greece to compare the relative importance of *Prunus* host plant species and spatial separation on patterns of genetic structure. Studies of genetic variation in host-parasite systems can reveal any of a number of potential evolutionary histories with varying

degrees of congruence between patterns found in hosts and parasites (Banks & Paterson, 2005). At one extreme is the possibility that *A. transcaspicus* will show even greater affiliation to *Prunus* species than does *Hyalopterus*, which would be supported by well-resolved parasitoid lineages or genotypic clusters associated with each of the four host plants, irrespective of sampling location. Similarly, we might observe a pattern closer to that found in *Hyalopterus* (i.e. with geographically widespread plum, almond and peach associated lineages but no lineage specific to apricot) that would be consistent with co-cladogenesis of parasitoids with their aphid hosts but would not necessarily demonstrate affiliations to particular host plants. Alternatively, we might find a generalist *A. transcaspicus* that randomly mates with respect to host plants but is strongly differentiated among geographic regions. Such a pattern would be expected for a widespread parasitoid that has failed to diverge with its host and where geographically limited dispersal (i.e. isolation by distance) or allopatric barriers lead to differentiation among regions. Finally, at the opposite extreme, if *A. transcaspicus* is highly mobile and generalist with respect to host and host plant preferences, we may see little genetic differentiation at any scale.

Materials and methods

Sampling

Aphidius transcaspicus was collected from almond, apricot, peach and plum trees from 20 locations in Spain and Greece (here, we treat each sampling event as a distinct 'population'; fig. 1). Parasitoids were collected as mummified aphids (hollowed out aphid husks representing the cocoon of the

Table 1. AMOVAs of *Aphidius transcaspicus* COI sequence data grouped by (a) geographic region and by (b) host plant/host plant without apricot samples.

AMOVA hierarchy level	df[1]	SS[2]	Covariance components	F-statistic
(a) Among regions, F_{CT}[3]	1	11.56	0.37	0.69*
Among populations within regions, F_{SC}[4]	16	5.35	0.06	0.33*
Within populations, F_{ST}[5]	57	6.37	0.11	0.79*
(b) Among host plants, F_{CT}	3/2	1.18/1.27	−0.07/−0.03	−0.23 n.s./−0.16 n.s.
Among populations within host plant groups, F_{SC}	14/12	15.73/13.73	0.27/0.25	0.71*/0.67*
Within populations, F_{ST}	57/51	6.37/6.37	0.11/0.13	0.64*/0.62*

[1] Degrees of freedom, [2] Sum of squares, [3] F_{ST} (and within population covariance component) is tested by permutation of genotypes among populations and groups, [4] F_{SC} (and among population within groups covariance component) is tested by permutation of genotypes among populations within groups and [5] F_{CT} (and among groups covariance component) is tested by permutation of populations among groups.
* $P < 0.001$; n.s., not significant.

developing parasitoid) and reared individually to emergence before being transferred to 95% ethanol. Parasitoid collections were made at the same time as the *Hyalopterus* collections previously reported (Lozier *et al.*, 2007). While identification of the *Hyalopterus* species from which individual *A. transcaspicus* emerged would have been difficult if not impossible, based on our previous results it is highly likely that parasitoids collected on almond, peach and plum would be from *H. amygdali*, *H. persikonus* and *H. pruni*, respectively; and parasitoids from apricot could come from any of these *Hyalopterus* species. We note that this approach is similar to that used in our previous study of *Hyalopterus* (Lozier *et al.*, 2007), where we used no prior taxonomic knowledge in our inference of genetic relationships and which proved effective for distinguishing the overall signal of host associated differentiation as well as more complex patterns of population admixture. However, we do consider the implications of occasional 'wrong' host plant choices by *Hyalopterus* on the genetic structure of *A. transcaspicus* (see Discussion). Lastly, we note that parasitoid mummies were encountered most frequently on almond and were unfortunately less prevalent on other *Prunus* spp., though we were able to include at least one locality for each plant species in both regions.

DNA sequencing

Genomic DNA was extracted from whole female parasitoids using a Qiagen DNEasy DNA extraction kit (Qiagen Corporation, California, USA), eluted into 100–150 μl, and stored at −20°C. We amplified COI using the primers C1-J-1718 and C1-N-2191 (Simon *et al.*, 1994). All COI polymerase chain reaction (PCR) amplifications used for sequencing were performed in 10 μl volumes as in Lozier *et al.* (2007: except with an annealing temperature of 52°C) and products were purified using *ExoSAP-IT* (USB Corporation, Ohio, USA). Both PCR strands were cycle-sequenced in 10 μl volumes using the same primers under the following cycle sequencing conditions: 0.6 μl BigDye v3.1 (ABI), 0.5 μl 5× sequencing buffer, 4.0–5.0 pmol primer, and 1.2 μl purified PCR product. Sequencing products were purified using Sephadex (Sigma-Aldrich, Missouri, USA) and electrophoresis was performed on an ABI 3730. Sequences were aligned in Sequencher 4.0 (Gene Codes Corporation, Michigan, USA) and deposited in GENBANK with the accession numbers EF541030–EF541108.

Microsatellite genotyping

We genotyped 190 *A. transcaspicus* females at nine microsatellite loci (At001, At003, At004, At005, At006, At009, At014, At016 and At017), performing PCR amplification, +A overhang removal and electrophoresis on an ABI 3730 as in Lozier *et al.* (2006, 2007). To minimize laboratory error, all reaction steps were performed simultaneously with multiple positive (known genotypes) and negative (sterile water) controls, and the qualities of allele size calls were checked manually. Any genotypes that could not be reliably scored were reamplified and, if necessary, were excluded from the study. Of the 1710 total genotypes attempted, we were unable to amplify only 15.

COI analyses

We examined the distribution of COI diversity by constructing a 95% confidence statistical parsimony network using TCS (Clement *et al.*, 2000). The network was visualized with sequences pooled either by host plant or geographic origin. We tested the significance of the association between haplotypes and host plant or geography using contingency table analysis with the Pearson X^2 statistic (the test is only performed for haplotypes A and B to avoid problems of low expected cell counts due to infrequent haplotypes; see below). We further tested population structure by examining the genetic covariance 'Among Groups' (F_{CT}), 'Among Populations within Groups' (F_{SC}), and 'Among Individuals within Populations' (F_{ST}) using analysis of molecular variance (AMOVA) implemented in ARLEQUIN 3.1 (Excoffier *et al.*, 2005). We tested the significance of two population groupings, (i) by geographic region and (ii) by host plant. Significance of each statistic was tested by 1000 permutations of the appropriate hierarchical units (table 1). To examine the possible confounding effect of apricot, which is a host plant shared by all three *Hyalopterus* species, we tested the host plant group hypothesis both with and without parasitoids from this host plant.

Basic microsatellite analyses

For the microsatellite data, we used FSTAT (Goudet, 2001) to calculate locus-specific diversity (Nei & Kumar, 2000) and F (Weir & Cockerham, 1984) statistics and to test for deviations from Hardy-Weinberg Equilibrium (HWE)

Table 2. Locus specific diversity statistics for the entire sample of *Aphidius transcaspicus* from Spain and Greece.

Locus	No. Alleles	h_O[1]	h_S[2]	h_T[3]	F_{IT} (SE)[4]	F_{ST} (SE)[4]	F_{IS} (SE)[4]
At001	5	0.081	0.092	0.547	0.83 (0.05)	0.82 (0.06)	0.10 (0.15)
At003	5	0.198	0.225	0.526	0.60 (0.11)	0.55 (0.09)	0.09 (0.12)
At004	2	0.14	0.122	0.237	0.61 (0.26)	0.62 (0.25)	−0.01 (0.10)
At005	5	0.188	0.203	0.596	0.69 (0.07)	0.66 (0.06)	0.07 (0.10)
At006	16	0.416	0.576	0.828	0.49 (0.08)	0.29 (0.04)	0.28 (0.10)***
At009	3	0.106	0.151	0.506	0.75 (0.09)	0.68 (0.09)	0.21 (0.15)
At014	17	0.53	0.643	0.863	0.37 (0.07)	0.26 (0.04)	0.15 (0.06)***
At016	10	0.279	0.309	0.65	0.58 (0.07)	0.53 (0.05)	0.10 (0.12)
At017	22	0.678	0.739	0.871	0.20 (0.06)	0.17 (0.05)	0.04 (0.07)

[1] Observed heterozygosity, [2] within population heterozygosity, [3] total heterozygosity (Nei & Kumar, 2000) and [4] correlation of alleles (F_{IT}), correlation of alleles within populations (F_{ST}), and correlation of alleles within individuals within populations (F_{IS}) (Weir & Cockerham, 1984) and their standard errors (estimated by jackknifing over populations using FSTAT).
*** Significant deviation from within-population Hardy-Weinberg Equilibrium, $P < 0.001$.

using 3600 permutations of alleles among individuals and F_{IS} as a test statistic (table 2). We used GENEPOP (Raymond & Rousset, 1995) to test for linkage disequilibrium (LD) with Fisher's method, correcting for the large number of tests with the Bonferroni method. Population structure was assessed using pairwise estimates of F_{ST}, with significance for each pair tested by randomizing multilocus genotypes among populations 1000 times and correlations with geographic distance examined using Mantel tests (5000 permutations) in FSTAT. We also used partial Mantel tests to examine the significance of host plant effects on this correlation, coding population pairs from the same plant species as 1, and from different species as 0 (a negative correlation coefficient, r, would thus indicate that population pairs from different host plants were more greatly differentiated than from the same plant species). We then used AMOVA to test the significance of partitioning genetic variance among regions and host plant groups as described above, also examining structure separately within Spain and Greece.

Clustering analyses

Standard genetic analyses like AMOVA test differences among population groups specified *a priori*. Thus, we employed an alternative model-based analysis using the program BAPS 5.0 (Corander & Marttinen, 2006), which implements a Bayesian mixture model to infer genetic clusters based solely on patterns of microsatellite variation and not on any prior grouping of populations. Patterns of clustering from such methods have proven useful for detecting cryptic host associated genetic structure in natural populations (e.g. Lozier *et al.*, 2007) and would provide evidence for host associated differentiation if assignment to clusters aligned with plant species, either across the entire sampled area (e.g. for cryptic species) or independently within either geographic region (e.g. for local differentiation). In contrast, assignment of populations from different plant species to the same cluster could be inferred as evidence against host associated differentiation. To determine optimum population partitioning, BAPS was first run using the 'Clustering Groups of Individuals' option. The number of potential clusters (K) was set from 1 to 20, with each specified K tested three times per run and results merged to obtain the optimum number of partitions based on likelihood scores. To avoid biasing results towards geographic or host associated clustering, we did not specify

locality coordinates. The resulting clusters were then used in the admixture analysis to obtain posterior estimates of the proportion of individual genotypes represented by each cluster (admixture coefficients) using the following program settings: (i) minimum number of individuals = 5; (ii) number of iterations used to estimate admixture coefficients = 100; (iii) number of reference individuals = 200; (iv) number of iterations used to estimate admixture coefficients for reference individuals = 20 (see BAPS manual; http://web.abo.fi/fak/mnf//mate/jc/software/).

Lastly, we examined relationships among individuals using a tree-based clustering analysis of the microsatellite data. We used the software MICROSAT 2 (Minch, 1997) to calculate the Cavalli-Sforza chord distance (D_C: Cavalli-Sforza & Edwards, 1967) among individuals and constructed an unrooted neighbor-joining (NJ) tree from observed distances using PHYLIP v3.67 (Felsenstein, 2004). We assessed tree support using a majority rule consensus NJ tree from 1500 bootstrap replicates (performed in MICROSAT 2) in PHYLIP, recording those clades found in more than 50% of the bootstrap replicates.

Results

COI *sequence data*

We identified only four 428 bp haplotypes in all *A. transcaspicus* individuals sequenced at *COI*. We limited our sequence analysis to a subset of 75 (out of 190 total) individuals once this low diversity became apparent in preliminary analyses, as it was deemed unlikely that increased sequencing would reveal additional structure indicative of host associated species. Haplotype A was the most frequently recovered sequence ($n = 41$), followed in order by B ($n = 28$), C ($n = 4$) and D ($n = 2$). There is no apparent pattern of host plant associations, with the two most common haplotypes present on all four *Prunus* species ($X^2 = 2.3$, $n = 69$; $P = 0.51$; fig. 2a). The association is also insignificant when apricot is excluded from the analysis ($X^2 = 2.057$, $n = 60$, $P = 0.36$). The geographic pattern is significant, however, with all four haplotypes present in Greece (+Crete), but parasitoids from Spain possessing only haplotype B ($X^2 = 44.2$, $n = 69$, $P < 0.001$; fig. 2b). The AMOVA supports these patterns, with highly significant and substantial 'Among Geographic Region' group structure ($F_{CT} = 0.69$, $P < 0.001$; table 1a) but an absence of significant 'Among Host Plant' group structure ($F_{CT} = -0.23$, $P = 0.97$; table 1b). If
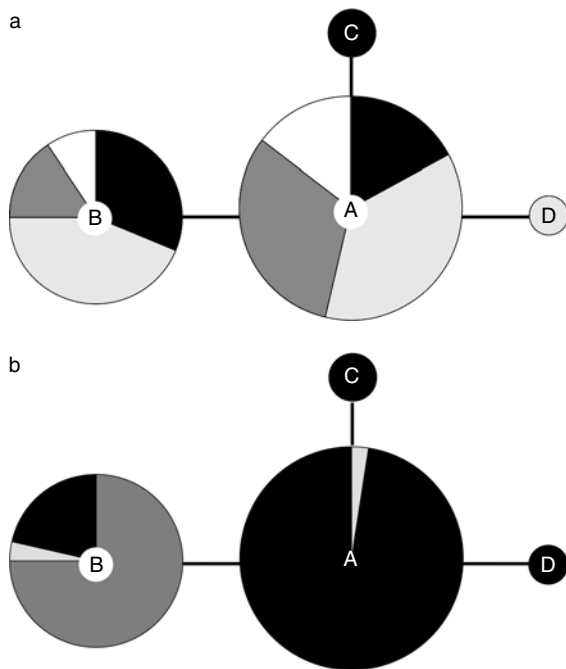
Fig. 2. *COI* haplotype network for *Aphidius transcaspicus*, with circles and edges representing unique haplotypes and single base pair mutations, respectively. Circle areas are scaled approximately by the numbers of samples that possessed a given haplotype (75 total samples), with shading representing the proportion of samples associated with (a) host plant and (b) geographical location. Sample sizes: Spain-almond: 7; Spain-apricot: 3; Spain-peach: 6; Spain-plum: 5; Greece-almond: 2; Crete-almond: 23; Crete-apricot: 6; Crete-peach: 13; Crete-plum: 11 ((a) □, almond; □, apricot; ▦, peach; ■, plum; (b) ▦, Spain; □, Greece; ■, Crete).

anything, the negative covariance and F_{CT} for 'Among Host Plant' group structure indicates greater variance among populations from the same plant species than among populations from different plant species, though it is probably more conservative to interpret these values as a lack of differentiation. However, it seems likely that the large covariance component for the 'Among Populations within Host Plant' group level (table 1b) can be attributed to the strong differentiation among populations sampled from the same plant species but in different regions. Tests performed without samples from apricot gave similar results (table 1b–d).

Microsatellite data

We found one significant case of LD among loci (At006 + At014, $P = 0.001$; corrected $\alpha = 0.0015$). These two loci also showed significant deviations from HWE (both $P < 0.001$; table 2) and 13 of the 15 un-amplifiable genotypes were also found at At006 and At014. Together, such patterns suggest the possibility of null alleles, so we removed these loci from the analyses presented here, though preliminary investigations of the complete data set suggest that their exclusion did not affect any of our conclusions.

At the remaining loci, as for the *COI* data, parasitoids from Spain and Greece show considerable geographic differentiation ('Among Geographic Regions': $F_{CT} = 0.59$,

$P < 0.001$; table 3a), but negative and insignificant 'Among Host Plant' group structure for the entire data set and separately within each region, as well as for analyses including or excluding apricot samples (table 3b–d). Pairwise F_{ST} estimates (table 4) within regions were generally relatively low, ranging from 0.000 to 0.629, which only slightly overlapped with the high range of differentiation observed in pairs from different regions (0.553–0.798). In contrast, population pairs had very similar ranges of pairwise F_{ST}, whether they were sampled from the same or from different host plant species (same: 0.000–0.798; different: 0.000–0.779). The majority of pairwise F_{ST} estimates were significant ($P < 0.05$); 30 of 31 insignificant estimates were for within-region population pairs (table 4). Poorly sampled populations did not exhibit F_{ST} levels uncharacteristic of more well-sampled populations, though the degree of significance was somewhat reduced (table 4).

The extent of geographic differentiation is made more clear when pairwise $F_{ST}/(1 - F_{ST})$ is plotted against geographic distance (fig. 3a). However, it is also clear that there is still a substantial degree of population structure within both Spain and Greece. The AMOVA showed significant differentiation 'Among Populations within Regions' for the geographically pooled samples (table 3a), and there was fairly high intra-regional differentiation when Spain ($F_{ST} = 0.11$, $P < 0.001$) and Greece ($F_{ST} = 0.22$, $P < 0.001$) were analyzed separately (table 3b, c). However, there was no significant correlation of pairwise genetic differentiation with distance within either region (fig. 3b, c), nor was there any indication from partial Mantel tests that populations sampled from different host plant species exhibited greater differentiation than populations from the same species at similar scales of spatial isolation. In fact, the positive r estimates suggest that populations from different plant species are actually more similar than those from the same plant species, significantly so in Greece (fig. 3c). While we suspect this significance is spurious (possibly due to the relatively large number of sampled almond populations), the analysis certainly provides no qualitative or quantitative support for greater genetic differentiation among populations on different host plants at any spatial scale.

Both Bayesian and D_C clustering analyses gave similar results. BAPS found that seven genetic clusters best explained the structure present in the microsatellite data (fig. 4). The clusters were cleanly split among the two geographic regions, with individuals from Greece assigned to $\{K1 + K2 + K3 + K4\}$ and those from Spain to $\{K5 + K6 + K7\}$ (fig. 4). Once again, there was no obvious relationship between *Prunus* species and the inferred genetic structure within either region. In Greece (fig. 4a), for instance, $K1$ was found on almond, peach and plum; and K4 was found on all four host plants. Likewise, in Spain (fig. 4b), parasitoids belonging to $K5$ were present on all four host plant species. The remaining four clusters were present only in single populations ($K2 = \text{Gb10almond}$, $K3 = \text{Ga20almond}$, $K6 = \text{S24apricot}$, $K7 = \text{S28almond}$).

The NJ tree for inter-individual D_C relationships also divided the samples into well-defined Spanish and Greek groups with good support (83% of bootstraps; fig. 5). In general, individuals from the different host plants were scattered throughout these two groups. There were a few possible 'monophyletic' host specific clusters apparent in the tree (marked by * in fig. 5). Closer inspection revealed that these were each comprised entirely of individuals from the

Table 3. AMOVAs of *Aphidius transcaspicus* microsatellite data grouped by (a) geographic region, (b) host plant/host plant with apricot samples removed, (c) host plant/host plant without apricot only within Spain and (d) host plant/host plant without apricot only within Greece. See Table 2 footnotes.

AMOVA hierarchy level	df	SS	Covariance components	F-statistic
(a) Among regions, F_{CT}	1	322.22	1.68	0.59*
Among populations within regions, F_{SC}	18	89.44	0.22	0.19*
Within populations, F_{ST}	360	336.28	0.93	0.67*
(b) Among host plants, F_{CT}	3/2	28.50/15.72	−0.21/−0.23	−0.11 n.s./−0.12 n.s.
Among populations within host plant groups, F_{SC}	16/14	383.16/338.34	1.25/1.27	0.57*/0.59*
Within populations, F_{ST}	360/305	336.28/271.47	0.93/0.89	0.53 */0.54*
(c) Among host plants (Spain only), F_{CT}	3/2	9.40/4.56	−0.05/−0.08	−0.04 n.s./−0.07 n.s.
Among populations within host plant groups, F_{SC}	5/4	22.49/18.19	0.19/0.20	0.15*/0.16*
Within populations, F_{ST}	167/131	183.56/138.20	1.10/1.06	0.11*/0.10*
(d) Among host plants (Greece only), F_{CT}	3/2	6.55/4.70	−0.16/−0.14	−0.16 n.s./−0.13 n.s.
Among populations within host plant groups, F_{SC}	7/7	51.00/51.00	0.38/0.39	0.32*/0.33*
Within populations, F_{ST}	193/174	152.72/133.27	0.79/0.77	0.22*/0.24*

* See table 1 footnotes

same populations (e.g. S24apricot, S28almond; also identified as distinct by BAPS, fig. 4) rather than from multiple populations as would be expected for host associated differentiation.

Discussion

Diversification of phytophagous insects in association with host plants is a remarkable and common evolutionary phenomenon (Funk *et al.*, 2002), and the ways in which these patterns of differentiation may contribute to genetic structure at higher trophic levels is beginning to be appreciated (e.g. Stireman *et al.*, 2006; Abrahamson & Blair, 2007). The unique pattern of parallel divergence between phytophagous insects and natural enemies in association with different host plants has been termed cascading (Stireman *et al.*, 2006) or sequential (Abrahamson & Blair, 2007) host associated differentiation (henceforth, sequential host associated differentiation). Aphids in the genus *Hyalopterus* have been found to exhibit strong host associated differentiation on almond, peach and plum trees but with comparatively minor effects of geographic separation on overall genetic structure (Lozier *et al.*, 2007). In this study, we were interested in determining whether the specialized parasitoid *A. transcaspicus* showed similar patterns when reared from *Hyalopterus* species on these same set of host plants. If host associated differentiation was also driving diversification in *A. transcaspicus*, we would predict reduced gene flow among populations from different host plant species compared to populations from the same host plants. In the following, we first discuss how our results for *A. transcaspicus* are incompatible with this prediction and then explore possible factors that may limit the potential for sequential host associated differentiation in this parasitoid species.

Are there distinct host associated cryptic species within A. transcaspicus?

Our primary interest in this study was to test whether host plant associated speciation in *Hyalopterus* has triggered speciation in *A. transcaspicus*. For species-level host associated differentiation, individuals sampled from the same host plant species should, on average, share a more recent

common ancestor than individuals from different host plant species, regardless of their geographic origins. In the absence of clear morphological differences or mating compatibility studies, one of the most useful ways to distinguish such a pattern is using Mallet's (1995) genotypic clusters concept. Under this definition, groups of individuals are considered as species when they are analyzed with multiple genetic markers and consistently assigned to distinct genotypic clusters with no or few intermediates, thus strongly indicating the presence of reproductive isolation. Unlike for *Hyalopterus*, none of our molecular markers revealed a level of reproductive isolation consistent with host associated species for *A. transcaspicus* using this species concept.

First, the two most common *COI* haplotypes (A and B, 92% of samples) were detected on all four *Prunus* species, and all four haplotypes were separated only by a series of single base pair substitutions. This is in stark contrast to the six haplotypes found at the same *COI* region in *Hyalopterus* individuals collected from throughout the Mediterranean, which formed three distinct clades associated with almond, peach and plum and appear to have diverged several million years before present (4–8% divergence: Lozier *et al.*, 2007). Even if we exclude apricot samples to eliminate the potentially confounding influence of a shared *Hyalopterus* host plant, *COI* variation in *A. transcaspicus* does not approach the level of host plant association or degree of differentiation observed in its hosts. Second, AMOVA, Mantel tests, and both Bayesian and distance-based clustering analyses also failed to reveal any signature of host associated differentiation at the species level in the microsatellite data. Average genetic differentiation among parasitoids grouped by host plant was less than zero in the AMOVA ($F_{CT} = -0.11$; table 3), and populations from different host plants were not more strongly differentiated than those from the same host plant at any spatial scale; if anything, the reverse was true (fig. 3). In comparison, grouping *Hyalopterus* populations by host plant explained much of the genetic variation present at microsatellite loci ($F_{CT} = 0.2$), while grouping by geographic region explained none of this variation (see Lozier *et al.*, 2007; table 3), a pattern that has been duplicated with morphological characters and has been deemed sufficient for the recognition of three distinct *Hyalopterus* species (Lozier *et al.*, 2008). Our data show that similar cryptic host plant

Table 4. Pairwise estimates of genetic differentiation among *Aphidius transcaspicus* populations, measured by F_{ST} (Weir & Cockerham, 1984).

	S14 alm	S15 plm	S22 pch	S23 apr	S24 plm	S25 alm	S26 alm	S28 alm	S31 alm	Ga20 alm	Ga12 alm	Ga6 alm	Ga8 alm	Gb10 alm	Gb12 plm	Gb14 pch	Gb4 plm	Gb5 alm	Gb6 pch
S14alm (10)	–																		
S15plm (10)	0.000	–																	
S22pch (14)	0.000	0.011	–																
S23apr (9)	0.003	0.026	0.007	–															
S24apr (10)	**0.103**	**0.143**	**0.111**	**0.103**	–														
S25plm (12)	**0.061**	**0.101**	0.046	0.000	**0.114**	–													
S26alm (4)	0.000	0.000	0.001	0.000	0.080	**0.036**	–												
S28alm (10)	*0.263*	*0.298*	*0.216*	*0.251*	*0.296*	0.236	**0.303**	–											
S31alm (9)	0.057	0.044	**0.066**	**0.066**	*0.232*	**0.121**	0.099	*0.267*	–										
Ga20alm (10)	*0.573*	*0.625*	*0.553*	*0.584*	*0.566*	*0.568*	*0.632*	*0.629*	*0.651*	–									
Ga12alm (12)	*0.690*	*0.722*	*0.669*	*0.693*	*0.651*	*0.675*	*0.719*	*0.703*	*0.737*	*0.511*	–								
Ga6alm (8)	*0.631*	*0.669*	*0.619*	*0.637*	*0.601*	*0.627*	*0.655*	*0.669*	*0.689*	*0.305*	*0.202*	–							
Ga8alm (6)	*0.659*	*0.703*	*0.640*	*0.669*	*0.625*	*0.649*	*0.709*	*0.697*	*0.728*	*0.399*	*0.228*	0.051	–						
Gb10alm (12)	*0.751*	*0.779*	*0.731*	*0.758*	*0.722*	*0.740*	*0.796*	*0.784*	*0.798*	*0.629*	*0.371*	*0.288*	*0.482*	–					
Gb12plm (2)	*0.670*	*0.724*	*0.661*	*0.682*	*0.619*	*0.665*	0.721	*0.719*	*0.758*	*0.547*	0.000	0.046	0.118	**0.228**	–				
Gb14pch (5)	*0.685*	*0.733*	*0.667*	*0.695*	*0.643*	*0.676*	*0.740*	*0.728*	*0.759*	*0.472*	*0.191*	*0.168*	*0.110*	*0.496*	0.063	–			
Gb4plm (13)	*0.623*	*0.661*	*0.609*	*0.629*	*0.598*	*0.619*	*0.658*	*0.666*	*0.680*	*0.218*	*0.295*	*0.119*	*0.132*	*0.432*	**0.222**	**0.112**	–		
Gb5alm (12)	*0.656*	*0.688*	*0.637*	*0.664*	*0.628*	*0.645*	*0.698*	*0.694*	*0.712*	*0.335*	*0.369*	*0.146*	*0.126*	*0.457*	**0.310**	**0.232**	**0.114**	–	
Gb6pch (12)	*0.628*	*0.664*	*0.617*	*0.633*	*0.602*	*0.624*	*0.653*	*0.663*	*0.681*	*0.276*	*0.170*	0.000	**0.059**	*0.267*	0.023	**0.075**	0.028	*0.125*	–
Gb9apr (10)	*0.578*	*0.621*	*0.570*	*0.588*	*0.555*	*0.577*	*0.604*	*0.620*	*0.639*	*0.195*	*0.211*	*0.076*	0.033	*0.396*	0.109	0.068	0.000	**0.094**	0.013

Sample sizes are given in parentheses next to population names, negative estimates are rounded to 0.000, estimates in bold are significant at $P < 0.05$ and estimates in bold + italics are significant following strict Bonferroni correction ($P < 0.00026$).
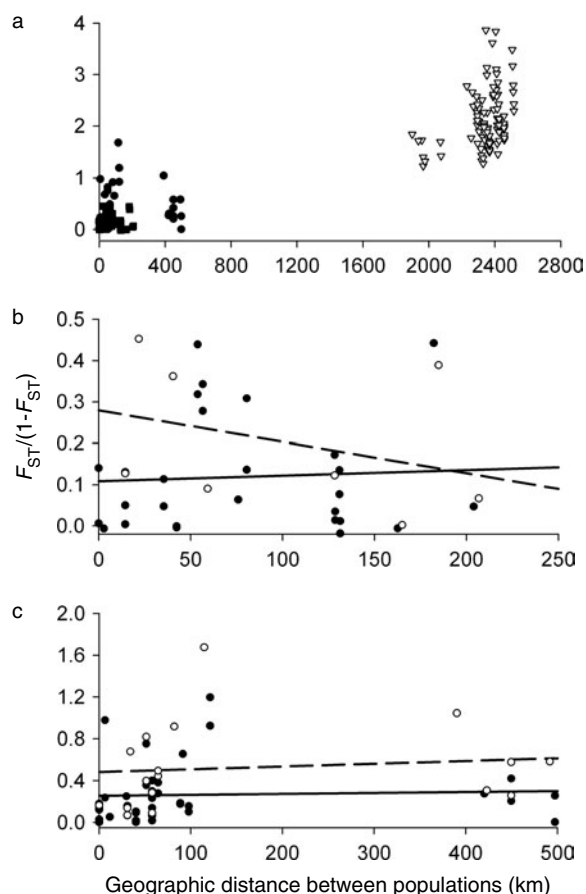
Fig. 3. Correlations between $F_{ST} \times (1 - F_{ST})^{-1}$ calculated from the microsatellite data and geographic distance for populations of *Aphidius transcaspicus*. Each point represents a single pairwise comparison of two populations visualized for (a) the entire sample (●, both populations from Greece; ▽, populations from Greece and Spain; ■, both populations from Spain) and (b) within Spain and (c) within Greece. For (b) and (c), points are differentiated based on whether the population pair was sampled from (○) the same or (●) different plant species. Regression lines are drawn through points from (- - -) the same and (—) different plant species. Mantel tests for all populations showed no significant correlation of geographic and genetic distances in either region (Spain: $r = -0.02$, $P = 0.89$; Greece: $r = 0.14$, $P = 0.31$). Partial Mantel tests using a host plant category variable ('same *Prunus* sp.'=1, 'different *Prunus* spp.' = 0) suggested that populations from the same plant species were more genetically differentiated than from different plant species, significantly so in Greece (Spain: $r_{distance} = -0.02$, $P = 0.71$, $r_{plant} = 0.24$, $P = 0.16$; Greece: $r_{distance} = 0.14$, $P = 0.60$, $r_{plant} = 0.33$, $P = 0.01$).

associated species are unlikely within *A. transcaspicus* in Spain and Greece.

Are there host races or biotypes within A. transcaspicus?

The question of whether *A. transcaspicus* exhibits intra-specific variation consistent with finer scale host associations is somewhat more difficult to address, in part because such intraspecific units are difficult to define and can encompass

a large number of intermediate steps between panmictic populations and reproductively isolated species (Dres & Mallet, 2002). While we feel that our data are more consistent with a lack of any host-associated reproductive barriers in *A. transcaspicus*, we cannot completely rule out such a scenario.

It is always difficult to rule out type II error when failing to reject a null hypothesis. In this case, the small number of aphid mummies available from some localities, for example, may have limited our power to detect certain types of fine-scale population structure. Low power will be most problematic either for very recent population divergence or in the case of very low levels of genetic differentiation where populations still undergo considerable gene flow, as would be expected for host races (Dres & Mallet, 2002; Puebla *et al.*, 2008). Otherwise, individual genotypes from a poorly sampled population should still tend to group with those from more thoroughly sampled populations on the same host plant species because they will share more recent common ancestors. Given our clear rejection of species-level differences on different host plants, we can thus conclude that if host associated differentiation has occurred in *A. transcpasicus* it must be very recent – particularly given a probable 10–12 generations per year (unpublished data) – or at a very local scale that would have been difficult to observe with our sampling. While some host associated clustering of individuals was apparent (e.g. individuals from S24 on apricot, S28 on almond, Ga20 on almond, Gb10 on almond; fig. 4), these instances are equally consistent with other hypotheses given the restriction of these clusters to single localities. For example, geographic structure (e.g. for Ga20 sampled from the Peloponessus) or the sampling of closely related individuals within localities could both elevate interpopulation genetic variance. In general, individuals or populations from the same host plants were not more similar to each other than to individuals or populations from different host plants (figs 3–5), and in no cases did we find parasitoids from multiple populations of one host plant species clustering together to the exclusion of parasitoids from different host plants (figs 2, 4 and 5). Thus, there is little evidence to conclude that host associated differentiation is occurring within either Spain or Greece, indicating that neutral genetic differentiation at the within-region scale is also likely to be maintained by some process other than affinity of parasitoid genotypes to particular host plants.

Is there evidence for geographic differentiation within A. transcaspicus?

The hypothesis that seems best supported by our data is one where gene flow is limited largely by geographic separation of *A. transcaspicus* in Spain and Greece. Significant differentiation between the two regions was consistent for both molecular data sets but was most exceptional for the microsatellites ('Among Region' $F_{CT} = 0.59$), with some pairwise comparisons reaching $F_{ST} > 0.75$. This degree of structure is striking, indicating that parasitoids in these two regions have very different sets of alleles. This geographic differentiation could be confounded by the effect of sampling in different years. However, yearly fluctuation in allele frequencies seem unlikely to generate differentiation as large as that observed between Spain (2002) and Greece (2003, 2005) (tables 1 and 2). Furthermore, we detected no differences between the 2003 and 2005 Greek samples
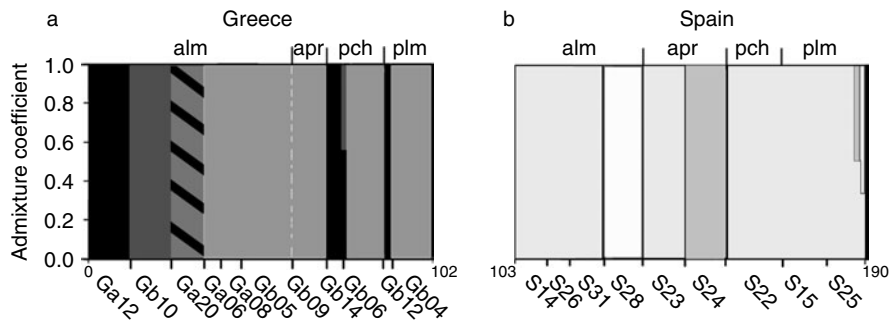
Fig. 4. Genetic structure of *Aphidius transcaspicus* inferred by the Bayesian admixture analysis implemented in BAPS, showing results for the most likely number of population partitions ($K = 7$; log marginal likelihood = $-1,878.87$). Inferred population clusters were cleanly split into two groups by geographic region, with all 102 individuals from Greece assigned to (a) $K1$–$K4$ and all 88 individuals from Spain assigned to (b) $K5$–$K7$. Population clusters (a) within Greece and (b) within Spain are both organized by plant species (top) and by collection locality (bottom, see fig. 1) and divided into *n* vertical segments, where *n* is the number of individuals. The height of each colored segment corresponds to the posterior estimate of ancestry (admixture coefficient) to one or more *K*s for that individual ((a) ■, $K1$; ■, $K2$; ▨, $K3$; ▩, $K4$; (b) □, $K5$; ▨, $K6$; □, $K7$).

consistent with such temporal effects. It, thus, seems that gene flow between Spain and Greece is rare. Significant genetic structure within each country also indicates restricted dispersal at local scales, though the absence of increases in genetic differentiation with distance within regions further suggests that *A. transcaspicus* in Spain and Greece do not necessarily represent points within a single continuous population under IBD, but may be separated by more concrete barriers to dispersal. However, until intermediate populations are analyzed, we can make no definitive statements regarding range-wide models of population structure (Templeton, 1998).

In addition to the high degree of genetic differentiation, we observed an extremely low level of genetic polymorphism, particularly for the *COI* gene. This can be explained in part by reduced effective population sizes associated with haplodiploidy (Graur, 1985), but a role for historical demographic scenarios cannot be excluded. For example, dispersal in insects associated with agriculture can be intimately tied to movements of crops by humans. Many species in the genus *Prunus* have been introduced to the Mediterranean only within the last 2000 years (Smartt & Simmonds, 1995), and it is entirely possible that both *Hyalopterus* and *A. transcaspicus* arrived concurrently. Recent non-equilibrium processes, such as founder events and subsequent range expansions, are consistent with the low level of polymorphism observed here, as well as with the failure to detect IBD within either Spain or Greece (Slatkin, 1993). We are continuing to investigate *A. transcaspicus* populations sampled over a broader and more continuous scale in an attempt to better understand these intriguing patterns of geographic structure (Lozier *et al.*, in prep.).

Sequential host associated differentiation and A. transcaspicus

Studies of phytophagous insects (*Rhopalomyia solidaginis* and *Gnorimoschema gallaesolidaginis*) and their natural enemies (*Platygaster variabilis* and *Copidosoma gelechiae*) on goldenrod (*Solidago* spp.) currently provide the best empirical evidence for sequential host associated differentiation in nature (Nason *et al.*, 2002; Stireman *et al.*, 2006). Studies of *Cotesia* parasitoids of checkerspot butterflies also lend some

support for sequential host associated differentiation, though the importance of the host plant in this radiation is not entirely clear (Kankare *et al.*, 2005). However, there are still few examples of sequential host-associated differentiation in the literature, and it appears that this mechanism will not apply to all systems (Stireman *et al.*, 2006; Abrahamson & Blair, 2007). For example, in another study on *Solidago*, Cronin & Abrahamson (2001) were unable to detect diversification of the parasitoid *Eurytoma gigantea* in response to host race formation in *Eurosta* gall flies. Similarly, a study of parasitoids of green cloverworm on alfalfa and soybean failed to detect reproductive isolation using AFLPs (Medina, 2005). We are unaware of any previous studies testing specifically for sequential host associated differentiation in the Aphidiinae. While some aphidiines certainly consist of cryptic host-specific complexes (Tremblay & Pennacchio, 1988; Atanassova *et al.*, 1998), studies of host specialization have typically examined parasitoids that attack relatively unrelated aphids rather than recently evolved cryptic species. More work is needed to test the generality of sequential host associated differentiation, and host associated differentiation overall, so that specific factors that either promote or inhibit diversification at different trophic levels and in different taxonomic groups can be identified (Abrahamson & Blair, 2007).

Given that aphidiines possess characteristics that could promote host associated differentiation (see Introduction; Abrahamson & Blair, 2007), what factors, apart from statistical concerns, might explain the apparent failure of *A. transcaspicus* to diverge with *Hyalopterus*? An important attribute of parasitic organisms is that their population structure is governed both by intrinsic properties such as dispersal and by extrinsic dynamics of host populations (Huyse *et al.*, 2005). Aphid parasitoids, for example, can disperse as adults, but also as eggs or larvae within parasitized migratory hosts (Feng *et al.*, 2007). Each summer, *Hyalopterus* populations obligately disperse from their primary *Prunus* hosts to the secondary host plant (e.g. *Phragmites*), followed in autumn by a return to primary hosts for sexual reproduction and overwintering. While the three *Hyalopterus* species are largely specific to their primary host plants (Lozier *et al.*, 2007), they co-occur on *Phragmites* (unpublished data). For *A. transcaspicus*, selection for
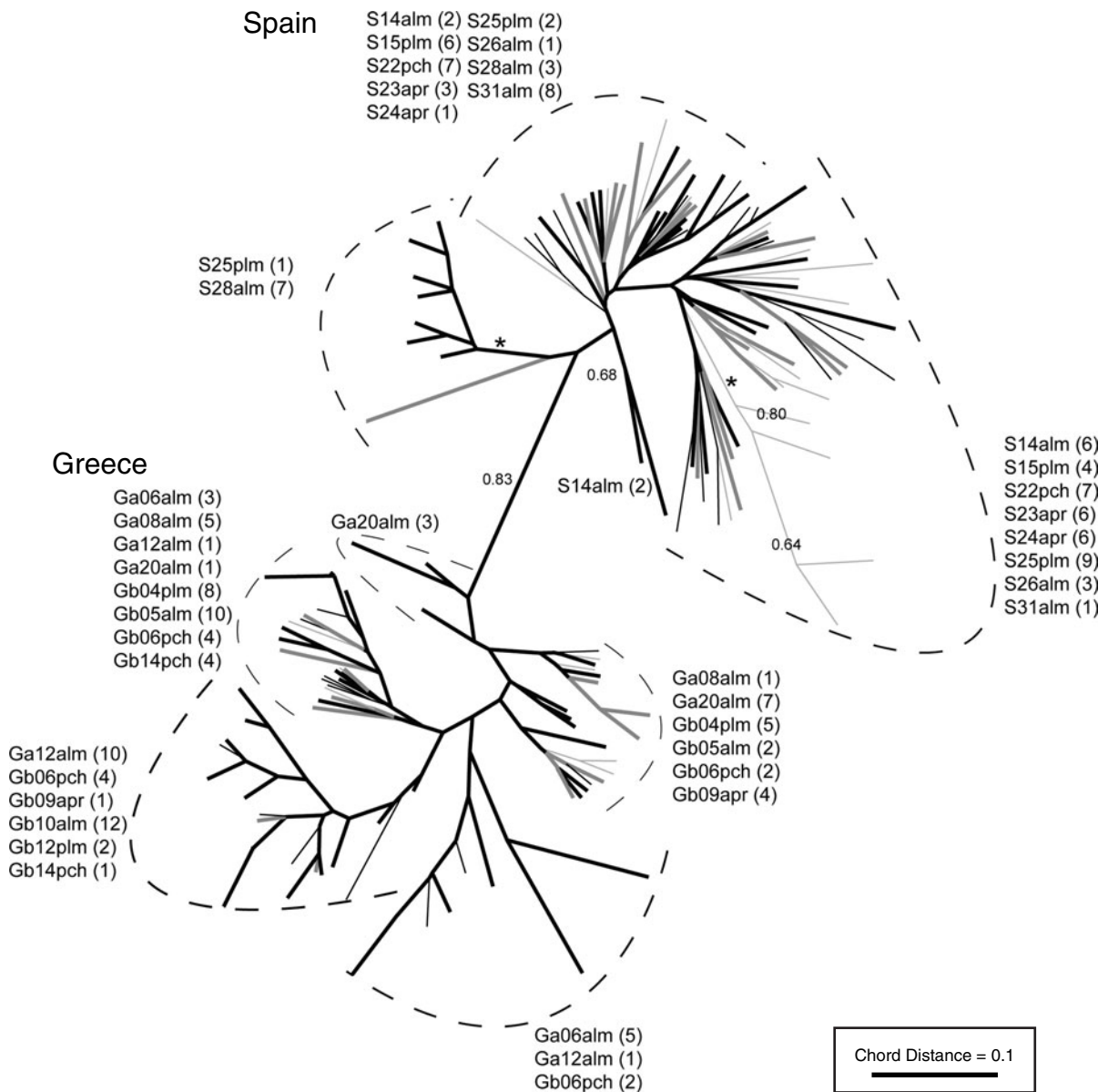
Fig. 5. Unrooted neighbor-joining tree constructed among individual *Aphidius transcaspicus* microsatellites genotypes using the Cavalli-Sforza chord distance. Terminal branches are shaded according to the host plants from which parasitoids are reared, and the population composition of various groupings is provided (with the number of individuals). Support estimates are provided for branches that were observed in greater than 50% of bootstrapped distance matrices. Branches marked by a * represent groups of individuals that were also identified as distinct clusters by BAPS and discussed in the text (S24apricot, S28almond) (━━, almond (thick black); ——, apricot (thin gray); ——, peach (thin black); ━━, plum (thick gray)).

responses to primary host plant cues (e.g. for mate or host location: Storeck *et al.*, 2000) would be ineffective at maintaining reproductive isolation when parasitoids emerge on this shared host plant. Similar patterns could arise when parasitized aphids settle on host plants shared by all three *Hyalopterus* species, such as apricot, or make the occasional 'wrong' host plant choice (Lozier *et al.*, 2007). Theoretical investigations of the conditions facilitating stable divergence of a predator following ecological divergence in the prey suggest that this secondary divergence will only occur if the new prey species are sufficiently ecologically distinct and if assortative mating is sufficiently strong (Dieckmann & Doebeli, 2004). Otherwise, the predator remains a generalist. The phenomena discussed above could promote gene flow among parasitoid populations whose ancestors had emerged on different host plants several generations earlier; and, in the face of such gene flow, ecological differences among *Hyalopterus* or *Prunus* species (e.g. quality of chemical cues, physiological response to parasitoid attack) may simply provide insufficient disruptive selection to allow the evolution of specialization and reproductive isolation below the level of host genus (Hufbauer, 2001; Bush & Butlin, 2004).

Another possibility is that the opportunity for sequential host associated differentiation in *A. transcaspicus* may be affected by resource limitation associated with fluctuations in the availability of the different *Hyalopterus* species. The evolution of specialization can benefit parasitoids by increasing efficiency and virulence or by reducing competition, but could come at the cost of reduced plasticity when the preferred host is rare or unavailable (Antolin *et al.*, 2006). Field trips to Mediterranean localities in multiple years have revealed marked differences in aphid abundance on the different *Prunus* species from year to year (N. Mills, personal observation). If encounters with the different *Hyalopterus* species are sufficiently unpredictable due to this variation, the maintenance of a partially generalist lifestyle could be favored (Berlocher & Feder, 2002; Lapchin, 2002; Antolin *et al.*, 2006).

In summary, mtDNA and microsatellite data and several analytical approaches indicate a primary role for spatial factors, rather than host plant associations, in maintaining genetic structure in *A. transcaspicus* and that, at the species level, *A. transcaspicus* should be considered a generalist with respect to its *Hyalopterus* hosts. With regard to biological control of *H. pruni* on dried plum in North America, our results suggest that targeting *A. transcaspicus* populations from aphids on *P. domestica* may be less important than considering differences among regional populations. For example, given the broad geographic distribution and high degree of genetic structure in its native range, regional *A. transcaspicus* populations are likely to possess unique adaptations to different environmental pressures. Selecting parasitoids from areas with climates most similar to California's Central Valley may, thus, be crucial for establishment, as has been the case for past biological control programs (Messenger & van den Bosch, 1971; van den Bosch *et al.*, 1979).

That said, it should be stressed that an absence of host plant or host associated population structure at neutral genetic markers does not necessarily indicate lack of differential fitness or behavioral variation in host preference in local populations. Little gene flow is needed to overcome the effects of genetic drift at neutral loci while selected loci can remain structured (Slatkin, 1987; Feder *et al.*, 1998). Indeed, host and host plant preferences have been demonstrated in several aphid parasitoids that showed no host associated differentiation at neutral genetic markers (Daza-Bustamante *et al.*, 2002; Baer *et al.*, 2004; Antolin *et al.*, 2006); and the far greater abundance of *A. transcaspicus* on almond trees suggests that aphids from this host plant may be preferred, at least in the regions sampled for this analysis. We suggest that future research effort to address the question of host preference in this parasitoid may be better spent on behavioral and ecological experiments rather than on attempts to identify the low levels of population structure that would likely be present among potential host races. If host preferences can be established experimentally, then further tests for fine-scale reproductive isolation would be warranted. Further analyses of geographic population structure throughout the Mediterranean, however, may help determine factors that can explain the extraordinarily high genetic differentiation and low genetic diversity that we have observed for Spain and Greece. Together, such studies may provide additional insights into both the evolution of host use and diversification in this parasitoid and may be of great practical importance for the selection employment of the most effective *A. transcaspicus* populations for management of *Hyalopterus* where it is a pest.

References

Abrahamson, W.G. & Blair, C.P. (2007) Sequential radiation through host race formation: Herbivore diversity leads to diversity in natural enemies. pp. 188–202 *in* Tilmon, K. (*Ed.*) *Specialization, speciation, and radiation: The evolutionary biology of herbivorous insects*. Berkeley, University of California Press.

Althoff, D.M. & Thompson, J.N. (2001) Geographic structure in the searching behavior of a specialist parasitoid: combining molecular and behavioral approaches. *Journal of Evolutionary Biology* **14**, 406–417.

Antolin, M.F., Bjorksten, T.A. & Vaughn, T.T. (2006) Host-related fitness trade-offs in a presumed generalist parasitoid, *Diaeretiella rapae* (Hymenoptera: Aphidiidae). *Ecological Entomology* **31**, 242–254.

Askew, R.R. (1968) Considerations on speciation in Chalcidoids (Hymenoptera). *Evolution* **22**, 642–645.

Atanassova, P., Brookes, C.P., Loxdale, H.D. & Powell, W. (1998) Electrophoretic study of five aphid parasitoid species of the genus *Aphidius* (Hymenoptera: Braconidae), including evidence for reproductively isolate sympatric populations and cryptic species. *Bulletin of Entomological Research* **88**, 3–13.

Baer, C.F., Tripp, D.W., Bjorksten, T.A. & Antolin, M.F. (2004) Phylogeography of a parasitoid wasp (*Diaeretiella rapae*): no evidence of host-associated lineages. *Molecular Ecology* **13**, 1859–1869.

Banks, J.C. & Paterson, A.M. (2005) Multi-host parasite species in cophylogenetic studies. *International Journal for Parasitology* **35**, 741–746.

Berlocher, S.H. & Feder, J.L. (2002) Sympatric speciation in phytophagous insects: moving beyond controversy? *Annual Review of Entomology* **47**, 773–815.

Boykin, L.M., Shatters, R.G., Rosell, R.C., McKenzie, C.L., Bagnall, R.A., De Barro, P. & Frohlich, D.R. (2007) Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences. *Molecular Phylogenetics and Evolution* **44**, 1306–1319.

Bush, G.L. & Butlin, R.K. (2004) Sympatric speciation in insects. pp. 229–248 *in* Dieckmann, U., Doebeli, M., Metz, J.A.J. & Tautz, D. (*Eds*) *Adaptive Speciation*. Cambridge, Cambridge University Press.

Cavalli-Sforza, L.L. & Edwards, A.W.F. (1967) Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics* **19**, 233–257.

Clement, M., Posada, D. & Crandall, K. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**, 1657–1660.

Corander, J. & Marttinen, P. (2006) Bayesian identification of admixture events using molecular markers. *Molecular Ecology* **15**, 2833–2843.

Cronin, J.T. & Abrahamson, W.G. (2001) Do parasitoids diversify in response to host-plant shifts by herbivorous insects? *Ecological Entomology* **26**, 347–355.

Cronin, J.T. & Reeve, J.D. (2005) Host-parasitoid spatial ecology: a plea for a landscape level synthesis. *Proceedings of the Royal Society Series B* **272**, 2225–2235.

Daza-Bustamante, P., Fuentes-Contreras, E., Rodriguez, L.C., Figueroa, C.C. & Niemeyer, H.M. (2002) Behavioural differences between *Aphidius ervi* populations from two tritrophic systems are due to phenotypic plasticity. *Entomologia Experimentalis et Applicata* **104**, 321–328.

Diekmann, U. & Doebeli, M. (2004) Adaptive dynamics of speciation: Sexual populations. pp. 76–111 *in* Dieckmann, U., Doebeli, M., Metz, J.A.J. & Tautz, D. (*Eds*) *Adaptive Speciation*. Cambridge, Cambridge University Press.

Dres, M. & Mallet, J. (2002) Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society of London Series B* **357**, 471–492.

Excoffier, L., Laval, G. & Schneider, S. (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**, 47–50.

Feder, J.L., Berlocher, S.H. & Opp, S.B. (1998) Sympatric host-race formation and speciation in *Rhagoletis* Diptera: Tephritidae: A tale of two species for Charles D. pp. 408–441 *in* Mopper, S. & Strauss, S.Y. (*Eds*) *Genetic Structure and Local Adaptation in Natural Insect Populations: Effects of Ecology, Life History, and Behavior*. New York, Chapman and Hall.

Felsenstein, J. (2004) PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Feng, M.-G., Chen, C., Shang, S.-W., Ying, S.-H., Shen, Z.-C. & Chen, X.-X. (2007) Aphid dispersal flight disseminates fungal pathogens and parasitoids as natural control agents of aphids. *Ecological Entomology* **32**, 97–104.

Funk, D.J., Filchak, K.E. & Feder, J.L. (2002) Herbivorous insects: Model systems for the comparative study of speciation ecology. *Genetica* **116**, 251–267.

Godfray, H.C.J. (1994) *Parasitoids: Behavioral and Evolutionary Ecology*. 488 pp. Princeton, Princeton University Press.

Godfray, H.C.J. & Cook, J.M. (1997) Mating systems of parasitoid wasps. pp. 212–225 *in* Choe, J.C. & Crespi, B.J. (*Eds*) *The Evolution of Mating Systems in Insects and Arachnids*. Cambridge, Cambridge University Press.

Gordh, G. & Beardsley, J.W. (1999) Taxonomy and biological control. pp. 45–55 *in* Bellows, T.S. & Fisher, T.W. (*Eds*) *Handbook of Biological Control*. San Diego, Academic Press.

Goudet, J. (2001) FSTAT, a program to estimate and test gene diversities and fixation indices. Version 2.9.3. Available from http://www.unil.ch/izea/softwares/fstat.html.

Graur, D. (1985) Gene diversity in Hymenoptera. *Evolution* **39**, 190–199.

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

Hoy, M.A., Jeyaprakash, A., Morakote, R., Lo, P.K.C. & Nguyen, R. (2000) Genomic analyses of two populations of *Ageniaspis citricola* (Hymenoptera: Encyrtidae) suggest that a cryptic species may exist. *Biological Control* **17**, 1–10.

Hufbauer, R.A. (2001) Pea aphid-parasitoid interactions: have parasitoids adapted to differential resistance? *Ecology* **82**, 717–725.

Hufbauer, R.A., Roderick, G.K. (2005) Microevolution in biological control: Mechanisms, patterns, and process. *Biological Control* **35**, 227–239.

Huyse, T., Pulin, R. & Theron, A. (2005) Speciation in parasites: a population genetics approach. *Trends in Parasitology* **21**, 469–475.

Kankare, M., van Nouhuys, S. & Hanski, I. (2005) Genetic divergence among host specific cryptic species in *Cotesia melitaerum* aggregate (Hymenoptera: Braconidae), parasitoids of checkerspot butterflies. *Annals of the Entomological Society of America* **98**, 382–394.

Kavallieratos, N.G. & Lykouressis, D.P. (1999) Redescription of *Aphidius transcaspicus* Telenga (Hymenoptera: Braconidae) and its distinction from *Aphidius colemani* Viereck (Hymenoptera: Braconidae). *Bollettino del Laboratorio di Entomologia Agraria Filippo Silvestri* **55**, 105–112.

Kavallieratos, N.G., Tomanović, Ž., Starý, P., Athanassiou, C.G., Sarlis, G.P., Petrović, O., Niketić, M. & Veroniki, M.A. (2004) A survey of aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) of Southeastern Europe and their aphid – plant associations. *Applied Entomology and Zoology* **39**, 527–563.

Lapchin, L. (2002) Host-parasitoid association and diffuse coevolution: when to be a generalist? *American Naturalist* **160**, 245–254.

Lozier, J.D., Mills, N.J. & Roderick, G.K. (2006) Di- and trinucleotide repeat microsatellites for the parasitoid wasp, *Aphidius transcaspicus*. *Molecular Ecology Notes* **6**, 27–29.

Lozier, J.D., Roderick, G.K. & Mills, N.J. (2007) Genetic evidence from mitochondrial, nuclear, and endosymbiont markers for the evolution of host pant associated species in the aphid genus *Hyalopterus* (Hemiptera: Aphididae). *Evolution* **61**, 1353–1367.

Lozier, J.D., Foottit, R.G., Miller, G.L., Mills, N.J. & Roderick, G.K. (2008) Molecular and morphological evaluation of the aphid genus *Hyalopterus* Koch (Insecta: Hemiptera: Aphididae), with a description of a new species. *Zootaxa* **1688**, 1–19.

Lozier, J.D., Roderick, G.K. & Mills, N.J. Tracing the invasion history of mealy plum aphid, *Hyalopterus pruni* (Hemiptera: Aphididae), in North America: a population genetics approach. *Biological Invasions*, in press (DOI 10.1007/s10530-008-9248-8).

Mackauer, M. & Volkl, W. (2002) Brood-size and sex-ratio variation in field populations of three species of solitary aphid parasitoids (Hymenoptera: Braconidae, Aphidiinae). *Oecologia* **131**, 296–305.

Mallet, J. (1995) A species definition for the modern synthesis. *Trends in Ecology and Evolution* **10**, 294–299.

Medina, R.F. (2005) The role of host-plant species in the differentiation of sympatric populations of hymenopteran parasitoids. PhD dissertation, University of Maryland, Maryland, USA.

Messenger, P.S. & van den Bosch, R. (1971) The adaptability of introduced biological control agents. pp. 68–92 *in* Huffaker, C.B. (*Ed.*) *Biological Control*. New York, Plenum Press.

Minch, E. (1997) MICROSAT, Version 1.5b. Stanford University Medical Center, Stanford, CA, USA.

Nason, J.D., Heard, S.B. & Williams, F.R. (2002) Host-associated genetic differentiation in the goldenrod elliptical-gall moth,

Gnorimoschema gallaesolidaginis (Lepidoptera: Gelechiidae). *Evolution* **56**, 1475–1488.

Nei, M. & Kumar, S. (2000) *Molecular Evolution and Phylogenetics*. 333 pp. New York, Oxford University Press.

Oliver, K.M., Russel, J.A., Moran, N.A. & Hunter, M.S. (2005) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences USA* **100**, 1803–1807.

Puebla, O., Bermingham, E. & Guichard, F. (2008). Population genetic analyses of *Hypoplectrus* coral reef fishes provide evidence that local processes are operating during the early stages of marine adaptive radiations. *Molecular Ecology*, **17** 1405–1415.

Raymond, M. & Rousset, F. (1995) GENEPOP Version 1.2: Population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248–249.

Roderick, G.K. & Navajas, M. (2003) Genes in novel environments: Genetics and evolution in biological control. *Nature Reviews Genetics* **4**, 889–899.

Rundle, H.D. & Nosil, P. (2005) Ecological speciation. *Ecology Letters* **8**, 336–352.

Schlötterer, C. & Pemberton, J. (1998) The use of microsatellites for genetic analysis of natural populations – a critical review. pp. 71–86 *in* DeSalle, R. & Schierwater, B. (*Eds*) *Molecular Approaches to Ecology and Evolution*. Basel, Switzerland, Birkhäuser Vertag.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87**, 651–701.

Singer, M. & Stireman, J. (2005) The tri-trophic niche concept and adaptive radiation of phytophagous insects. *Ecology Letters* **8**, 1247–1255.

Slatkin, M. (1987) Gene flow and the geographic structure of natural populations. *Science* **236**, 787–792.

Slatkin, M. (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**, 264–279.

Smartt, J. & Simmonds, N.W. (1995) *Evolution of Crop Plants*. 2nd edn. 531 pp. Essex, UK, Longman Scientific and Technical.

Smith, M.A., Wood, D.M., Janzen, D.H., Hallwachs, W. & Hebert, P.D.N. (2007) DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. *Proceedings of the National Academy of Sciences USA* **104**, 4967–4972.

Starý, P. (1988) Aphidiidae. pp. 171–184 *in* Minks, A.K. & Harrewijn, P. (*Eds*) *Aphids, Their Biology, Natural Enemies and Control. Vol. 2B of World Crop Pests*. Amsterdam, Elsevier.

Stireman, J.O., Nason, J.D., Heard, S. & Seehawer, J.M. (2006) Cascading host-associated genetic differentiation in parasitoids of phytophagous insects. *Proceedings of the Royal Society Series B* **273**, 523–530.

Storeck, A., Poppy, G.M., van Emden, H.F. & Powell, W. (2000) The role of plant chemical cues in determining host preference in the generalist aphid parasitoid *Aphidius colemani*. *Entomologia Experimentalis et Applicata* **97**, 41–46.

Templeton, A.R. (1998) Nested clade analysis of phylogeographic data: Testing hypotheses about gene flow and population history. *Molecular Ecology* **7**, 381–397.

Tremblay, E. & Pennacchio, F. (1988) Speciation in aphidiine Hymenoptera. pp. 139–146 *in* Gupta, V. (*Ed.*) *Advances in Parasitic Hymenoptera Research*. New York, Brill.

van den Bosch, R., Hom, R., Matteson, P., Frazer, B.D., Messenger, P.S. & Davis, C.S. (1979) Biological control of the walnut aphid in California, U.S.A.: Impact of the parasitoid *Trioxys pallidus*. *Hilgardia* **47**, 1–14.

Vet, L.E.M. & Dicke, M. (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology* **37**, 141–172.

Via, S. (2001) Sympatric speciation in animals: The ugly duckling grows up. *Trends in Ecology and Evolution* **16**, 381–390.

Vos, M. & Vet, L.E.M. (2004) Geographic variation in host acceptance by an insect parasitoid: genotype versus experience. *Evolutionary Ecology Research* **6**, 1021–1035.

Wang, X.-G. & Messing, R.H. (2006) Potential host range of the newly introduced aphid parasitoid *Aphidius transcaspicus* (Hymenoptera: Braconidae) in Hawaii. *Proceedings of the Hawaiian Entomological Society* **38**, 81–86.

Weir, B.S. & Cockerham, C.C. (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.