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Phylogeography of a parasitoid wasp (*Diaeretiella rapae*): no evidence of host-associated lineages

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Abstract

The exceptional diversity of insects is often attributed to the effects of specialized relationships between insects and their hosts. Parasite–host interactions are influenced by current natural selection and dispersal, in addition to historical effects that may include past selection, vicariance, and random genetic drift. Both current and historical events can lead to reduced fitness on some hosts. If trade-offs in fitness on alternate hosts are common, adaptation to one host can prevent adaptation to another, giving rise to genetic differentiation among host-associated lineages. Previous studies of *Diaeretiella rapae* (Hymenoptera: Aphidiidae), a parasitoid of aphids, have revealed additive genetic differences in performance between populations that parasitize different aphid host species. To determine whether *D. rapae* populations collected from different aphid hosts have diverged into genetically independent lineages, we constructed a haplotype network based on sequence variation in mitochondrial DNA (mtDNA). We used single strand conformation polymorphism (SSCP) analysis to examine 2041 base pairs of mtDNA and to identify nucleotide sequences of 42 unique SSCP haplotypes. We found no association between mtDNA haplotypes and host species in either the ancestral range (Europe, Mediterranean region, Middle East, Asia) or part of the introduced range (western North America). Haplotypes likely to be ancestral were geographically widespread and found on both hosts, suggesting that the ability to use both hosts evolved prior to the diversification of the mtDNA. Ongoing gene flow appears to prevent the formation of host races.

Keywords: Aphidiinae, aphids, gene flow, host use, Hymenoptera, introduced species, mtDNA, SSCP

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Introduction

The exceptional diversity of insects is well known, but its underlying causes have been the subject of considerable speculation (e.g. Hutchinson 1959). The insects' great diversity may result from their specializing on host species they depend upon for food or other resources (Strong *et al*. 1984; Mitter *et al*. 1988; Farrell 1998; Summers *et al*. 2003). If host specialization generates greater diversity, then adoption of new hosts must result in genetic divergence of lineages that shift to novel hosts, leading ultimately to reproductive isolation and the formation of new species. Further, existence of separate lineages on different hosts implies that host shifts are relatively uncommon compared to the rate of evolutionary divergence of lineages. Otherwise, ongoing gene flow would disrupt associations between particular genotypes and individual host species (e.g. Dres & Mallet 2002; Lajeunesse & Forbes 2002).

Literature on host specialization and differentiation by phytophagous insects is extensive (recently reviewed by Mopper & Strauss 1998; Via 2001; Dres & Mallet 2002), but relatively few studies provide a phylogeographical context for examining either host range or host switching. The majority of studies report at least some genetic differentiation between populations of insects inhabiting different plant hosts, although exceptions exist (Radtkey & Singer 1995;

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Brown *et al*. 1997; Kelley *et al*. 1999; Shufran *et al*. 2000; Downie *et al*. 2001). Spatial variation in coevolution can lead to divergence in host use between populations or in different parts of the species' distribution (Althoff & Thompson 2001; Thompson 1999), and several well-documented cases of cospeciation demonstrate that speciation in hosts often results in parallel diversification of their symbionts (Moran *et al*. 1993; Paterson *et al*. 2000; Itino *et al*. 2001; Ricklefs & Fallon 2002). In the same vein, introduced organisms have been found to use different hosts in their native vs. introduced ranges (Berenbaum & Zangerl 1991; Simberloff & Stiling 1996; Downie *et al*. 2001; Hufbauer 2002; reviewed in Sakai *et al*. 2001).

For insect parasitoids, which consume insect hosts during larval development and whose diversity rivals that of the other insect groups, the question of host specificity is not only pervasive but has important implications for agriculture (McEvoy 1996; Strand & Obrycki 1996) and conservation biology (Henneman & Memmot 2001; Louda *et al*. 2003). Direct evidence of parasitoid-host coevolution exists (Kraaijeveld *et al*. 2002), but remarkably little is known of host-associated phylogeography of parasitoids (Althoff & Thompson 2001; Morehead *et al*. 2001; Vink *et al*. 2003). Previously, we documented fine-scale population subdivision (Vaughn & Antolin 1998) and genetically based trade-offs in fitness-related traits of *Diaeretiella rapae* (Hymenoptera: Aphidiidae), a parasitoid of aphids, from populations in Colorado that attack different hosts (Vaughn 1997; Bjorksten *et al*. unpubl.; see Methods and Materials). We report here intraspecific phylogeography and population genetic structure of *D. rapae* based on analysis of mtDNA sequences, and relate this variation to the evolution of host-specific lineages in this wasp. Wasps were collected from the ancestral range in Europe, the Mediterranean region, the Middle East and Asia, and in part of the introduced range in western North America. Elucidating the phylogeography of *D. rapae* in both the ancestral and the introduced range allows us to address several issues related to host-parasitoid evolution: (i) Are wasp phylogeography and host use related? (ii) How common are host-shifts in the evolutionary history of *D. rapae*? and (iii) Does the relationship between host use and genotype differ between introduced populations and populations from the ancestral range?

Materials and methods

Natural history of Diaeretiella rapae

Diaeretiella rapae is a cosmopolitan endoparasitoid of aphids on a number of host plants (Mackauer & Starý 1967; Němec & Starý 1994; Pike *et al*. 1999); the complete host list includes more than 60 aphid species, but only five to six host species are commonly attacked (Pike *et al*. 1999). A ubiquitous host for *D. rapae* is the cabbage aphid, *Brevicoryne brassicae*, a pest of crucifer crops that spread throughout the world from its presumed origin in central Europe. Cereal-feeding aphids like the Russian wheat aphid, *Diuraphis noxia* ('RWA') are also commonly parasitized, especially in the wheat-growing regions of the western U.S. The distribution of RWA expanded only in the past 30 years, moving from the western Mediterranean into South Africa and South America, then into North America in 1986 (Halbert & Stoetzel 1998). Although *D. rapae* was already established in North America, a biological control effort in the late 1980s by biologists from the U.S. Department of Agriculture and several state agencies included collection of *D. rapae* from southern Europe, the Mediterranean region, the Middle East and Asia. Several hundred thousand wasps were subsequently released in the wheat-growing regions of western North America (Hopper *et al*. 1998; Prokrym *et al*. 1998), resulting in establishment and spread of new populations of *D. rapae* (Mohamed *et al*. 2000; Brewer *et al*. 2001; Burd *et al*. 2001).

The life history of *D. rapae* is typical of aphid parasitoids. Adult female *D. rapae* oviposit a single egg into the bodies of aphid hosts. Upon hatching, the wasp larvae feed on the tissues within the aphid body, killing it after two to three days. When the host dies, it becomes a 'mummy' consisting of the hardened exoskeleton of the aphid; the parasitoid larva pupates inside the mummy and emerges as an adult. Under laboratory conditions, egg-to-adult development ranges from nine to 15 days. Adult females live for 10–15 days and have lifetime fecundity as great as several hundred offspring. Males live for seven to 10 days (Hafez 1961; Simpson *et al*. 1975; Reed *et al*. 1992).

As is true for most parasitoids, the primary means of host location by *D. rapae* is olfactory. More *D. rapae* are found in crucifer patches than in noncrucifer habitats (Hafez 1961; Read *et al*. 1970), and experiments using flight tunnel assays demonstrated that *D. rapae* females are more attracted to odours from crucifer plants than from other plant types (Sheehan & Shelton 1989; Vaughn *et al*. 1996). Vaughn *et al*. (1996) found evidence of antennal receptors that respond specifically to the volatile compound allyl isothiocyanate (AITC), which is released by damaged crucifer plants. In both behavioural and physiological assays, females reared from both cabbage aphid and RWA mummies were more strongly attracted to AITC than to volatiles from cereal plants, the crucifer aphids themselves, or RWA. Although *D. rapae* is not obligate on crucifer-feeding aphids and commonly parasitizes a number of other aphid species (Pike *et al*. 1999), the consistent olfactory attraction to cruciferous plants suggests that crucifer-feeding aphids were an ancestral host species.

Effects of host species on fitness-related traits

We documented host-related tradeoffs in components of fitness in three separate experiments, which we briefly

summarize here (Vaughn 1997; Bjorksten *et al*. unpubl.). Wasps were collected from populations attacking cabbage aphid and RWA in eastern Colorado, reared on both host aphid species in the laboratory, and subsequently scored for total fecundity, larval survival, and development time. On average, wasps from cabbage aphid populations experienced a decrement in fitness of 21% compared to those raised on cabbage aphid. Similarly females from RWA populations experienced an average decrement of 35% when raised on cabbage aphid. Productivity and development time of wasps from both RWA and cabbage aphid populations also were severely reduced on a third aphid host species, the peach aphid *Myzus persicae*. Wasps taken from cabbage aphids experienced an average decrement in fitness of 40% on *M. persicae*; wasps from RWA experienced a 26% reduction in fitness. Thus, although *D. rapae* is reported from a variety of hosts, populations in eastern Colorado appear to be locally adapted to particular aphid host species. A quantitative genetic analysis of wasp performance while parasitizing cabbage aphid or RWA demonstrated that trade-offs result from additive genetic differences between populations.

Sample collection

Samples from the 'Old World', which includes Europe, the Mediterranean region, the Middle East and Asia, were obtained from localities that represent most of the historic range of *D. rapae* (Table 1, Fig. 1). The main source was from voucher samples of collections of mummies made in

Table 1 Localities where *D. rapae* were collected (see Fig. 1), listed in order from east to west. Data included are year of collection, aphid host species, sample size, and haplotype frequency (sample size, $N > 1$). For hosts, the abbreviation CAB denotes cabbage aphid (*Brevicoryne brassicae*) and RWA denotes Russian wheat aphid (*Diuraphis noxia*). See Hopper *et al*. (1998) and Prokrym *et al*. (1998) for more information on European, Mediterranean, Middle Eastern and Asian collections

Designation	Location	Latitude, Longitude	Year	Host	*N*	Haplotypes (Sample size)
CA	Altai, China	48 N, 87 E	1992	RWA	10	V(8), CC(2)
CT	Tacheng, China	47 N, 82 E	1992	RWA	9	C(9)
CW	Wuqia, China	40 N, 77 E	1991,	RWA	9	C(2), DDD, FFF, NN, S(2), T, U
			1992		9	C(4), DDD, V(3), W
KD	Dmitrievka, Kazhakhstan	43 N, 77 E	1989,	RWA	5	E(4), EEE
			1991	RWA	10	DD(2), E, GGG(3), HH(2), OO(2)
KI	Iachmen, Kyrgyzstan	42 N, 74 E	1989	RWA	7	GG(7)
PP	Parachinar, Pakistan	34 N, 73 E	1991	RWA	5	C, X(4)
UT	Tashkent, Uzbekistan	41 N, 69 E	1991	RWA	12	X(12)
IE	Ehglid, Iran	29 N, 52 E	1990	RWA	2	C, E
IF	Fars, Iran	29 N, 52 E	1991	RWA	4	A(2), AAA(2)
CB	Buddenovsk, Caucasus	45 N, 45 E	1992	RWA	10	JJ(7), M(2)
JD	Disi, Jordan	30 N, 35 E	1989	RWA	7	C(7)
SQ	Qatara, Syria	33 N, 36 E	1990	RWA	13	C, CCC, E(11)
ST	Tel. Hadya, Syria	33 N, 36 E	1992	RWA	10	A(8), C(2)
SB-102	Hrabetice, Moravia	49 N, 17 E	1996	CAB	5	BB, C(2), EE, Q
SB-104	Ceske Budejovice, Bohemia	49 N, 15 E	1996	CAB	5	A, C, LL, N, V
SB-105	Techobuz, Bohemia	49 N, 15 E	1996	CAB	5	A, C(3), EEE
HY	Hyeres, France	43 N, 7 E	1998	CAB	8	GGG, KK(7)
UK	Rothamsted, England, UK	51 N, 0.2 W	1999	CAB	19	A(2), C(3), F(6), G, J(4), KK (3)
SG	Grenada, Spain	37 N, 4 W	1992	RWA	7	C(7)
MM	Marrakech, Morocco	32 N, 8 W	1990	RWA	9	I(9)
MS	Settat, Morocco	33 N, 8 W	1992	RWA	10	C(10)
ArdW	ARDEC, Fort Collins, CO		1999	RWA	9	A(7), CCC, R
B1C	Boulder CO, 3 km S. of Longmont		1999	CAB	19	A(13), B, JJ, M, O, P, R
B1W	Boulder County, 5 km S. of Longmont		1999	RWA	6	A(6),
B2W	Boulder County, 3 km west of Erie		1999	RWA	8	A(4), B, C, EE, Z
FrWN	Fruita, CO		1999	RWA	20	A(15), C(4), FF
FrWS	Fruita, CO		1999	RWA	18	A(8), BBB, C(2), E, GG, I(4), K
FrC	Fruita, CO		1999	CAB	19	A(15), C(2), I(2)
Wa1W	Prosser, Washington		1994	RWA	9	A(4), C(5)
Wa2W	Yakima County, Washington		1996	RWA	7	A, C(5), K
Wa3C	Prosser, Washington		1995	CAB	9	A(8), L

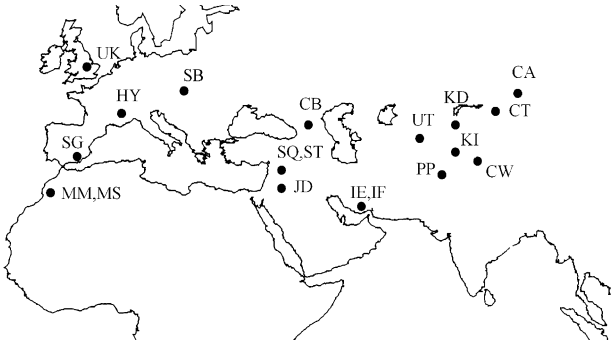

Fig. 1 Map of collection sites in the Old World in Europe, the Mediterranean region, the Middle East and Asia. Abbreviations are given in Table 1.

the Old World from RWA and housed at the USDA-APHIS Plant Protection Laboratory in Niles, Michigan (1989–92) (see Prokrym *et al*. 1998). Additional Old World samples, collected from populations of cabbage aphid, were graciously provided by several colleagues (Hyeres, France: X. Fauvergue; Rothamstead, U.K. H. Loxdale; Slovakia: P. Starý). Unfortunately, host and geography were confounded in our Old World collection. The 18 RWA samples are from the Mediterranean region, the Middle East, and Asia; five samples from cabbage aphid are from Europe.

'New World' samples from North America were collected as mummies from populations in Colorado during the summer of 1999 from agricultural fields near Ft. Collins and Boulder, CO, and from the western slope at the Colorado State University Fruita Research Center in Fruita, CO. Additional samples collected from two wheat fields and a crucifer field in eastern Washington in 1994–96 were obtained from K. Pike (Washington State University Irrigated Research and Extension Center, Prosser, WA).

Molecular methods

Evolutionary relationships between individuals were established by examining 2041 base pair (bp) nucleotide sequences of mitochondrial DNA, comprising 528 bp of the 3′ end of the 16S ribosomal RNA subunit (Domains IV and V: Misof *et al*. 2002) and a 1513 bp segment that included cytochrome oxidase I (COI — 841 bp), leucine transfer RNA (76 bp), and cytochrome oxidase II (COII — 596 bp) genes. The complete DNA sequence of each wasp was determined by single-stranded conformation polymorphism (SSCP) analysis of fragments amplified by PCR, using the primers listed in Table 2. Internal primers for the COI-COII fragment were designed after an initial amplification using the two outside primers (COI.P1, COII.M1), cloning the fragments using the Invitrogen TOPO® TA kit, and sequencing the entire 1513 bp fragment using the universal M13 sequencing primers. Internal primers for the large COI-COII region were designed to amplify fragments less than 500 bp in length because SSCP specificity is greatest for amplified fragments of that size (Hiss *et al*. 1994). Multiple representatives of each SSCP pattern from each of the smaller fragments were sequenced in both directions to establish the relationship between SSCP banding patterns and specific DNA sequences of each fragment. After excluding overlaps, sequences of the four COI — COII fragments were combined into complete 1513 bp sequences for each individual. Sequences of the 42 SSCP haplotypes we identified in this study, including both the 16S and COI — COII sequences, are in GenBank under accession numbers AY194244–AY194285.

SSCP haplotypes of the amplified fragments were determined by scoring the number and mobility of both double stranded and re-annealed single-stranded bands, which have lower mobility through the gel (Hiss *et al*. 1994).

Table 2 Primers used to amplify mtDNA for SSCP analysis and sequencing of the COI — COII region (first four sets) and the 16S rDNA (last pair), position of each primer relative to the sequence of honeybee (*Apis mellifera*), and fragment size amplified by each pair of primers. The first primer in each pair is forward, the second is reverse

Primer pair	Sequence (5′–3′)	Position*	Size (bp)
COI.P1†	TTGATTTTTTGGTCA(TC)CC(TA)GAAGT	2492	451
COI.IR	AGCTCCTATTGATAAAACATAATG	2942	
COI.IF	GGAGCTGTATTTGCTATTTTAG	2937	386
COM.R	TGGAGGATATAATTGTATTCA	3287	
COM.F	CCTCCAATAAATCATAGATATGA	3282	362
COII.IR	ATGAAGGAATTGCTATAAATACT	3851	
COII.IF	TCGTCAAATTATACATAATCAA	3787	427
COII.IF77	AGAAATTCCTTCATTGAAAATT	3837	361
COII.M1†	CC(AG)CAAATTTC(AT)GA(AG)CATTGACCA	4213	
16S.sh‡	AGATTTTAAAAGTCGAACAG	13417	528
16S.wb	CACCTGTTTATCAAAAACAT	13943	

*5′ position in the mtDNA sequence of Apis mellifera (Crozier & Crozier 1993).
† Primers COI.P1 and COII.M1 are derived from primers C1-J-2195 and C2-N-3661, respectively, from Simon *et al*. (1994).
‡ Primers are from Dowton & Austin (1994).

Sequences of all unique haplotypes were determined in both directions (Davis Sequencing, Davis CA) using the original amplification primers in the sequencing reaction. For common SSCP patterns, amplified products of at least three representatives of each pattern were sequenced to verify the mtDNA sequence that corresponded to that haplotype pattern. For the 16S fragment, five individuals were found to have a highly divergent SSCP pattern and all of these were sequenced.

After initial sequencing, it was determined that SSCP analysis of the 427 bp fragment amplified by primers COII.IF and COII.M1 failed to reveal all nucleotide sequence variation (i.e. individuals with the same SSCP pattern differed by one or two mutations). Subsequent re-amplification and SSCP analysis of a shorter fragment (361 bp) from a new primer (COII.IF77) paired with primer COII.M1 clearly differentiated fragments with different sequences.

Genomic DNA was extracted from individual wasps using salt extraction (Black & DuTeau 1996). DNA was re-suspended in 100 μL TE (10 mM Tris-HCl, 1 mM EDTA pH 8.0) and stored at −80 °C until used. Procedures for amplifying the 362–528 bp fragments of the mtDNA followed Hiss *et al*. (1994). SSCP and silver staining followed protocols in Hiss *et al*. (1994) and Black & DuTeau (1996). Samples were electrophoresed on large (35 × 50 cm), thin (0.4 mm) glycerol (5%) polyacrylamide (5%, 2% cross-linking) gels. Electrophoresis proceeded at 350 V at room temperature for approximately 15 h and gels were stained with silver nitrate to detect the mobility of the DNA conformations. Amplified products were combined with loading buffer (95% formamide, 0.1 M NaOH) at a ratio of 6 μL of product to 1 μL of buffer, melted at 95 C for five minutes, and then kept on ice until loading in gels.

Data analysis

Sequences were aligned using CLUSTAL W© software (Thompson *et al*. 1994) with default settings as implemented in BIOEDIT© (Hall 1999) and adjusted by eye as necessary. For the protein-coding regions of the COI and COII regions, putative amino acid sequences were determined by identifying open reading frames, allowing determination of synonymous vs. non-synonymous mutations. For the 16S molecule, the 16S.wb and 16S.sh primers amplify region Domains IV and V, whose sequences have been determined for a large number of insect species and whose folded secondary structure is well understood (Misof *et al*. 2002). Thus, mutations in 16S could be categorized as occurring in relatively conserved or variable sites.

A matrix of pairwise genetic distance between all haplotypes was calculated from the patristic distance (p-distance) as implemented in the MEGA© software package (Kumar *et al*. 2001). Because patristic distance does not account for transition bias or unequal nucleotide composition, we also constructed a distance matrix using Tamura's 3-parameter method (Tamura 1992) and back calculated the resulting substitution matrix; minimum spanning trees generated from the two methods did not differ. From the resulting distance matrix we determined the haplotype network (minimum spanning tree) using the 'statistical parsimony' method of Templeton *et al*. (1992) as implemented by the TCS algorithm (Clement *et al*. 2000). Out-group weights (related but not identical to root probabilities) for each haplotype were calculated from the raw sequences of the Old World data set using the method of Castelloe & Templeton (1994). Topologies of haplotype networks for the full data set (Old World and New World samples) were slightly different from those produced when only Old World haplotypes were included. We therefore present the network resulting from the full data set.

Mean pairwise nucleotide diversity, π (Nei & Li 1979), was calculated using MEGA for the Old World and New World samples and for those Old World samples collected from cabbage aphid and Russian wheat aphid. The within- and among-population components of genetic diversity, π_w and N_{ST} (Nei & Kumar 2000; p. 257), were calculated for the Old World and the New World samples separately. We calculated a pairwise genetic distance matrix using Tamura's 3-parameter distance between all individuals from Old World and New World data sets; we designate this matrix **G**. We determined the matrix of geographical (great circle) distances between all pairs of samples from coordinates describing latitude and longitude of each (Table 1), which we designate **D**. The matrix of pairwise host-use comparisons was designated **H**. An individual entry in the **H** matrix, h_{ij}, represents the relationship between the host species of individuals i and j; if i and j are found on the same host (both on cabbage aphid or both on RWA) the matrix entry $h_{ij} = 1$; if i and j are found on different hosts $h_{ij} = 0$. We used a partial Mantel test (Smouse *et al*. 1986) as implemented in ZT version 1.1 (Bonnet & Van de Peer 2002) to test for associations between host use and genetic distance while controlling for geographical distance. Formally, we calculated the partial matrix correlation $r(H, G \mid D)$, with statistical significance determined by 10 000 permutations.

Results

Haplotype diversity

Forty-two haplotypes were identified: 32 from the Old World in Europe, the Mediterranean region, the Middle East, and Asia, and an additional 10 haplotypes from the New World in Colorado and Washington. A minimum spanning tree was derived from the full data set of 42 haplotypes (Fig. 2), including all 313 individuals from both Old World and North American samples. In the Old World

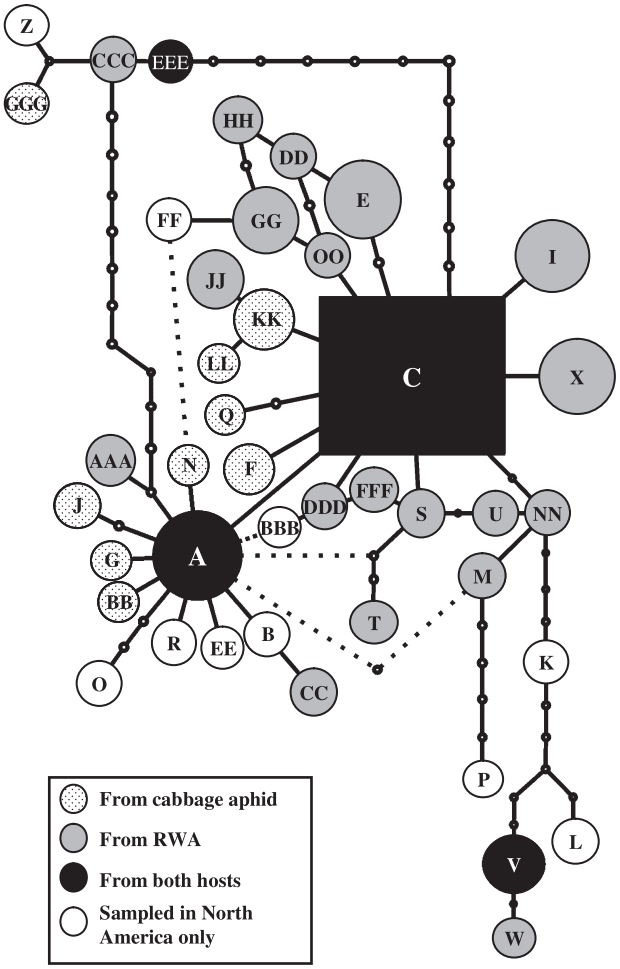

Fig. 2 Most parsimonious haplotype network that show relationships between the 42 haplotypes. Each circle represents a single haplotype, with the size of the circles proportional to the number of individuals with that haplotype. Haplotypes or 'missing haplotype nodes' (smallest circles) that differed from each other by a single nucleotide mutation are connected by lines (see text for description of broken lines). Haplotype labels are the same as in Table 1.

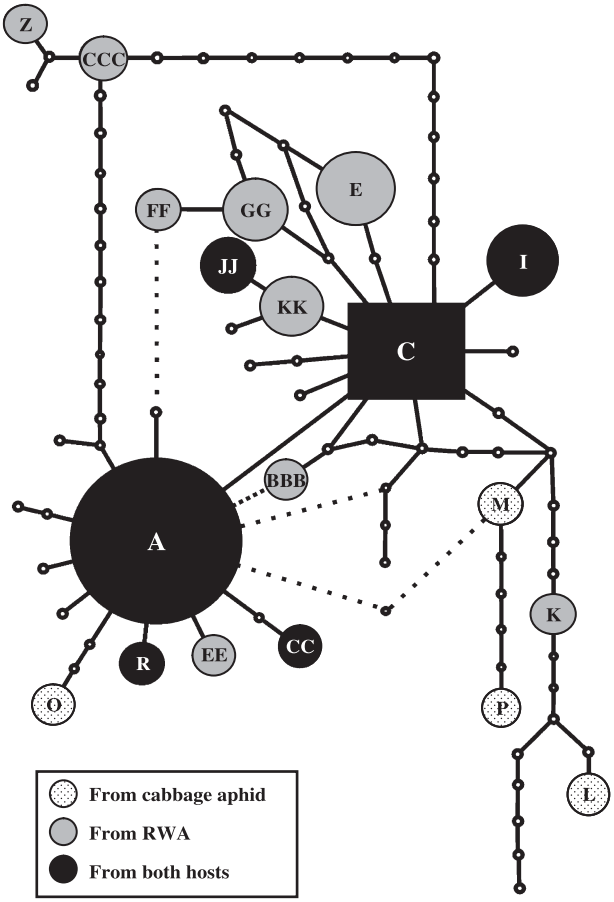

Fig. 3 Haplotype network showing the 19 haplotypes from North American samples superimposed on the same network as in Fig. 2. The same network as in Fig. 2 is depicted, with haplotypes sampled in the old world shown as 'missing haplotypes'.

samples, haplotype, C was the most common, comprising 28.7% of the sample. Most other haplotypes were represented multiple times in the samples (Table 1), only 10 haplotypes were found just once. In North American samples, only 19 haplotypes were found (Fig. 3), the A haplotype was most common (65.3% of samples), and 14 of the other haplotypes were sampled only once.

Nucleotide composition was 79.6% adenine and thymine, consistent with previous reports of AT-bias in Hymenoptera (Crozier & Crozier 1993; Dowton & Austin 1997). Fifty-two of the 2041 sites were polymorphic (2.5%), including one T-C transition in the leucine tRNA gene, 29 transitions and eight transversions in COI and COII, and 11 substitutions and three deletions in the 16S rRNA gene, In the COI and COII protein coding genes, fewer sites were variable in COI (18 of 841) than in COII (19 of 594), but the numbers of nonsynonymous and synonymous mutations were not significantly different in either COI (6 vs. 12) or COII (8 vs. 11), as determined by Fisher's exact test (Graur & Li 2000). Variable sites in the 16S gene all occurred in regions that would not influence conserved secondary structure (Misof *et al*. 2002). Nonetheless, 10 of the mutations in this fragment defined a set of divergent haplotypes (CCC, EEE, GGG, and Z) that were amplified from samples from both the Old World (France, Slovakia, Syria, Kazakhstan) and from North America (Colorado). The status of these sequences remains unresolved, as they could represent an ancient lineage within *Diaeretiella rapae*, a sibling species with no obvious morphological differences, or amplification of a nuclear translocation of the mtDNA 16S gene.

Old World phylogeography

The geographical origins of the 32 haplotypes sampled from 190 individuals in the Old World are presented in

Table 1. Haplotype C was common and widespread, four other haplotypes (A, E, EEE, and V) were collected from three or more localities, three haplotypes were found in two localities (GG, KK and X), and the remaining 24 haplotypes were sampled in one place only. Haplotype C was likely ancestral: its outgroup weight was highest, it was connected by one or two mutations to 22 other haplotypes, and it was found throughout the geographical range of *D. rapae* (Crandall & Templeton 1993; Castelloe & Templeton 1994). By the same criteria, it is likely that haplotype A also was old, although its geographical range spanned only Europe and the Middle East. The divergent clade containing haplotypes CCC, EEE, GGG, and Z connected equally to both A and C haplotypes in the network (Fig. 2).

The likely ancestral haplotype, C, differed from the other most common haplotype, A, by a single mutation, a thymine to cytosine transition at a synonymous site homologous to position 324 of the *Apis mellifera* COII gene. It is probable that the mutation distinguishing C and A occurred multiple times, and thus it is unlikely the C and A haplotypes represent exclusive lineages. The minimum spanning tree included several reticulations connecting multiple haplotypes in the network (Fig. 2). Connections (drawn as broken lines) to haplotype A from four other haplotypes (BBB, FF, M, S) were defined by similarity at position 324. However, these haplotypes were joined to the network on the basis of unique mutations they share with other adjacent haplotypes.

The haplotype network revealed some geographical structure. For example, haplotype A was collected in Europe and the Middle East, haplotype I was from Morocco, and haplotype X was from Pakistan and Uzbekistan. One group of haplotypes (O, GG, HH, DD, E) was found in Kazakhstan and Kyrgyzstan, while another group (DDD, FFF, S, U, NN, M, T, V, W) originated in western China. Despite some haplotypes being uniquely sampled from particular localities, there was no overall pattern of isolation by distance. A Mantel test of association between genetic distance and geographical distance showed no correlation; $r(G, D) = 0.026$ (one-tailed $P < 0.127$).

Old World host use

Because haplotypes C and A occurred on both cabbage aphid and RWA in multiple samples, it is parsimonious to conclude that the ability of *D. rapae* to use both hosts at some level (i.e. with fitness > 0) evolved prior to the divergence of the mtDNA lineage. Two other haplotypes (EEE, V) were collected from cabbage aphid in Slovakia but were also collected from RWA in Syria (EEE) and western China (V). Nineteen haplotypes were collected from RWA only and nine haplotypes from cabbage aphid only (Fig. 2). The great divergence of the CCC-EEE-GGG-Z clade does not change our primary conclusion that the ability to use both hosts predates the divergence of the mtDNA lineage. These haplotypes were present in low frequency over a broad geographical range and were collected from both cabbage aphid and RWA.

We did not find isolation by distance among collection sites (above), and we used the partial Mantel procedure to test the possibility of genetic isolation between populations collected from each host. After accounting for geographical distance between samples, we found no association between host-use and genetic distance; $r(H, G \mid D) = -0.035$ (one-tailed $P < 0.233$). Individuals taken from different hosts were on average no more genetically distinct than were pairs taken from the same host, even after geographical separation was accounted for.

We can also infer evolution of host use from patterns of haplotype diversity. If *D. rapae* shifted only once from an ancestral host, and that shift occurred recently, then we may expect genetic diversity of samples from the derived host to be lower than samples from the ancestral host. Further, we may expect that among-population genetic diversity would be lower in the samples from the recently colonized host (Vigilant *et al*. 1991; Slatkin 1993). Unfortunately, we cannot cleanly partition genetic variation into within and among-host components because all samples from cabbage aphid were from western and central Europe, whereas samples from RWA were from Spain, North Africa and eastward. With this caveat in mind, overall genetic diversity of collections from cabbage aphid and RWA did not differ; pairwise nucleotide diversity, π, of wasps collected from cabbage aphids was $\pi_{CA} = 0.0017$ (SE = 0.00040, $n = 42$) while diversity of wasps from RWA was $\pi_{RWA} = 0.0010$ (SE = 0.00025, $n = 148$).

Partitioning nucleotide diversity into between-sample and within-sample components showed patterns opposite to the expectation that genetic diversity would be lower on recently colonized hosts. The between-sample component of nucleotide diversity, N_{ST}, was high for RWA samples (0.64, SE = 0.033), but was effectively zero for cabbage aphid samples ($N_{ST} = -0.13$, SE = 0.129). On the other hand, the within-sample component of nucleotide diversity, π_w, was lower for RWA (0.00058, SE = 0.00013) than for cabbage aphid samples (0.0019, SE = 0.00042). This pattern is consistent with both multiple host shifts to RWA and geographical isolation of lineages from RWA samples. Geographic isolation of RWA populations might be expected, as they were sampled over a broad range spanning from Morocco to western China. European populations from the cabbage aphid spanned only from Great Britain to Slovakia.

New World samples

A list of North American samples and their associated, hosts, haplotypes, and sample sizes is presented in Table 2. Haplotype A comprised 71% of the 124 sampled individuals.

Total genetic diversity in North America (Π = 0.00066, SE = 0.00018, n = 123) was substantially lower than in Eurasia, consistent with the recent introduction of *D. rapae* into the New World (cf. Baker *et al*. 2003). Neither total genetic diversity between samples (π_{CAB} = 0.00067, SE = 0.00017, n = 47; π_{RWA} = 0.00074, SE = 0.00023, n = 76), nor within-sample genetic diversity ($\pi_{w,CAB}$ = 0.00074, SE = 0.00018; $\pi_{w,RWA}$ = 0.00067, SE = 0.00017) differed between cabbage aphid and RWA samples. To compare genetic diversity on RWA and cabbage aphid, we divided samples into three geographical regions [Washington (Wa1W, Wa2W, Wa3C), western Colorado (FrWN, FrWS, FrC), and eastern Colorado (ArdW, B1W, B2W, B1C)]. Genetic differentiation among regions (N_{ST}) for RWA samples was 0.141 (SE = 0.060) while for cabbage aphid populations was effectively zero (N_{ST} = −0.105, SE = 0.081). Although not statistically significant, the trend was the same as in the Old World samples, with cabbage aphid samples showing no genetic differentiation and RWA samples exhibiting considerable subdivision.

Tests of isolation by distance and isolation on hosts showed similar results to those from Old World samples. The partial correlation of host use and genetic distance, after accounting for geographical distance, was $r(H, G \mid D)$ = −0.003 (one-tailed $P < 0.437$); the correlation between genetic and geographical distance was $r(G, D) = 0.020$ (one-tailed $P < 0.334$). As in the Old World, individuals taken from different hosts were on average no more genetically distinct than were pairs taken from the same host, nor was there an overall pattern of isolation by distance.

As seen in Fig. 3, haplotype A was over-represented and haplotype C was under-represented relative to their frequencies in the Old World. By the criteria used to assign ancestral status, the A haplotype emerged as the apparent ancestor in the New World. Interestingly, the A haplotype in the Old World was found in western Europe in cabbage aphid samples and in the Middle East (Syria and Iran) in RWA samples. This result is consistent with most North American *D. rapae* being descended from wasps introduced from Europe and/or the Middle East, presumably via aphids inhabiting cruciferous crops.

Discussion

This study was motivated by two proximate conclusions from previous studies: (i) cabbage aphid is the probable ancestral host of *Diaeretiella rapae* (Némec & Starý 1994; Pike *et al*. 1999) and (ii) there are substantial reductions in fitness when *D. rapae* are reared on different aphid hosts than the ones in which they developed (Vaughn 1997; Bjorksten *et al*. unpubl). Our observation that mtDNA nucleotide diversity was greater within samples from cabbage aphid than from RWA reinforces the first conclusion. Our primary question of interest in this study was whether the trade-off in fitness on the different hosts has led to host race formation in *D. rapae.* Despite the cost of host switiching, our mtDNA provide no evidence of host race formation. We should also point out that we have concentrated on only two of several hosts that *D. rapae* regularly attack (Pike *et al*. 1999). Given the wide geographical distributions of many haplotypes it is unlikely that including wasp samples from other hosts would have dramatically changed the phylogeographical patterns we described. Similar to a study of *D. rapae* in Australia (Baker *et al*. 2003), genetic diversity was lower in the introduced range in North America than from samples collected in Europe and Asia.

Multiple lines of evidence argue against the existence of host races in *D. rapae*. First, and most compelling, the haplotypes that are most likely to be ancestral, C and A, were found on both hosts, in multiple samples, over vast geographical areas. If the C and A haplotypes and their descendent haplotypes represent exclusive lineages, this result strongly suggests that diversification of host use predated the diversification of the mtDNA lineage. Second, a star-like phylogeographical pattern like the one for *D. rapae* is expected in abundant species that have recently expanded their range (Avise 2000). This range expansion apparently included the ability to use several aphid host species. Third, it is conceivable we found no genetic differentiation between wasps on different hosts because incomplete lineage sorting left old polymorphisms within those populations (Avise 2000). However, the considerable differentiation among RWA populations in Europe, the Middle East, and Asia (N_{ST} = 0.64) argues against this interpretation. Fourth, the presence of a haplotype (EEE) found on both hosts in the divergent CCC-EEE-GGG-Z clade also argues against host race formation, as does the presence of a cabbage aphid haplotype from Slovakia (haplotype V) in the NN-V-W clade that was predominantly from western China. Fifth, there was no correlation between host use and genetic distance between haplotypes; we would expect such a correlation if host races were evolving independently. The lack of a correlation between host use and genetic distance implies instead that continual gene flow between hosts effectively randomizes haplotypes in populations attacking cabbage aphid and RWA in both the Old World and the New World. Finally, in several instances, wasps with haplotypes collected from only one host in the Old World were collected from the other host in the New World (haplotypes I, JJ, KK, and M). It is possible that those haplotypes occur on both hosts in the Old World and we failed to sample them, or that ecological conditions in the introduced habitat may have allowed those genotypes to use a different host than they would normally. Regardless, our results argue against the existence or formation of host races.

The conclusion that *D. rapae* has not diversified into separate lineages on the two hosts implies that gene flow

has been high enough to prevent the trade-offs in fitness between them from creating isolation. Assuming that trade-offs in fitness between hosts are characteristic of the species as a whole (they may not be: Hufbauer 2002), this also suggests that trade-offs in fitness are evolutionarily old, and that mtDNA lineages have been randomized with respect to hosts by ongoing gene flow. The possibility of ancient polymorphism for host use may not be far-fetched, as aphid parasitoid survival depends on the ability to overcome each host species' immune defenses (Henter & Via 1995; Hufbauer 2001), and ancient polymorphisms in other host-parasite contexts are well known (e.g. Hughes & Nei 1988; Hughes 1992). Theory predicts and empirical evidence confirms that strong selection can maintain genetic variation at one or a few target loci even in the presence of considerable gene flow (Berry & Kreitman 1993; Slatkin 1993; Black *et al*. 2001).

A previous study of fine-scale population structure in eastern Colorado populations of *D. rapae* revealed modest but consistent differentiation ($F_{ST} \cong 0.07$) between adjacent populations on RWA and cabbage aphid hosts (Vaughn & Antolin 1998). Even so, different populations from either RWA or cabbage aphid were also genetically distinct from each other, implying that the pattern did not result from two separate lineages isolated on the different hosts. This result, combined with evidence of fitness trade-offs between hosts and evidence of gene flow between hosts from our mtDNA data, implies that local gene flow is sufficient to prevent fixation of neutral loci but low enough to allow selection to cause populations to diverge in their ability to use both hosts (Kimura & Maruyama 1971). Conclusive resolution of the alternatives will require identification of the genes actually involved in the trade-off in performance between hosts (Hudson *et al*. 1987; Berry & Kreitman 1993; Black *et al*. 2001).

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