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Low genetic diversity among pea aphid (*Acyrthosiphon pisum*) biotypes of different plant affiliation

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Genetic diversity in the pea aphid *Acyrthosiphon pisum* was investigated by a restriction fragment length polymorphism (RFLP) analysis of three maternally inherited genomes (mitochondrial DNA and plasmids of the symbiotic bacteria *Buchnera*). Twenty-nine parthenogenetic clones of three *A. pisum* biotypes, defined by their capacity to use the legume crops pea, alfalfa and red clover, respectively, were analysed, and a total of 67 restriction sites was scored. No restriction site variation in the mitochondrial genome was obtained, but length variation at two regions (the A + T-rich region and ND3–ND5 region) was noted. One aphid clone bore a variant *Hin*dIII restriction site in the *Buchnera* leucine plasmid (pAPE*leu*), and two clones were heteroplasmic for a 0.76-kb deletion in the *Buchnera* tryptophan plasmid (pAPE*trp*). Based on arthropod nucleotide substitution rates, it is proposed that the crop-feeding biotypes of *A. pisum* may have diversified within the last 100 000 years and possibly much more recently, since the advent of agriculture.

Keywords: *Acyrthosiphon pisum*, aphid, biotype, *Buchnera*, mtDNA.

Introduction

A number of phytophagous insect species comprise individuals with different or overlapping plant ranges (Diehl & Bush, 1984). Conspecifics that differ in performance on, or preference for, particular plants are commonly described as different 'biotypes' (Diehl & Bush, 1984), a term that is used widely in relation to insect pests of agricultural crops. Elucidation of the factors contributing to intraspecific differences in plant range is central to both our understanding of the evolution of plant affiliation in phytophagous insects (Bernays & Graham, 1988) and the development of effective pest management strategies (e.g. Birch *et al.*, 1994). From various studies, it has become apparent that, although experience can influence plant range (e.g. Douglas, 1997), gene flow between populations that differ in plant affiliation is often restricted (Hales *et al.*, 1997).

DNA-based techniques are increasingly being applied to explore the genetic differences between insect biotypes, sometimes resulting in the development of biotype-specific molecular markers. These techniques are proving particularly valuable for the study of aphids (Hales *et al.*, 1997 and references therein), a group characterized by many instances of intraspecific variation in host plant affiliation (Blackman & Eastop, 1984) and low levels of intraspecific genetic variation, at least as revealed by allozymes (Hales *et al.*, 1997). For example, restriction analyses of mitochondrial DNA (mtDNA) have revealed consistent differences between greenbug *Schizaphis graminum* biotypes that use different sorghum cultivars (Powers *et al.*, 1989) and between *Therioaphis trifolii* biotypes (spotted clover or alfalfa aphids) using different legume crops (Sunnucks *et al.*, 1997a); differences in microsatellite profiles have been identified in the English grain aphid *Sitobion avenae* collected from wheat and cocksfoot (De Barro *et al.*, 1995; Sunnucks *et al.*, 1997b); and variation in ribosomal spacers has been described for the large raspberry aphid *Amphorophora idaei* infesting various raspberry cultivars (Birch *et al.*, 1994).

The purpose of this study was to explore the genetic variation in *Acyrthosiphon pisum*, the pea aphid. This species is restricted to legumes (Blackman & Eastop, 1984) and, in Europe, includes three crop-feeding biotypes: the pea biotype, alfalfa biotype and clover biotype, each of which performs poorly on the plant host of the

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other two biotypes (Sandström & Pettersson, 1994). The pea biotype [also known as ssp. *destructor* (Blackman & Eastop, 1984)] is an increasingly important pest of pea crops in the UK and western Europe (K. F. A. Walters, pers. comm.). Two lines of evidence suggest that these biotypes may have a genetic basis. First, performance of the three biotypes on pea, red clover and alfalfa is not modified by experience across multiple generations (Via, 1991a,b; T. L. Wilkinson & A. E. Douglas, unpubl. obs.). Secondly, crossing experiments reveal at least partial pre- and post-zygotic isolation between the biotypes (Müller, 1980). Gene flow between these crop biotypes and *A. pisum* populations using wild legumes is believed to be low (A. F. G. Dixon, pers. comm.), but detailed studies remain to be carried out.

There is one published molecular study of the genetic diversity of *A. pisum*: a restriction fragment length polymorphism (RFLP) analysis of mtDNA from 32 clones isolated from an alfalfa field near Lansing, New York, USA, in 1989 (Barrette *et al.*, 1994). These aphids exhibited minimal mtDNA restriction site variation, but length variation at two sites, the A + T-rich region and the ND3–ND5 region. However, the sampling design of the study gives little information on the overall genetic diversity of *A. pisum*. This is not only because just one field was sampled, but also because a very few genotypes are believed to have been introduced to North America from Europe, the native range of this species (Blackman & Eastop, 1984).

The study described here complements that of Barrette *et al.* (1994), in that it examines the mtDNA diversity of *A. pisum* of all three biotypes from Europe. Technically, it differs from that of Barrette *et al.* (1994) only in that the mtDNA was isolated by an alkali lysis technique, which extracts all covalently closed circular genomes of size < 30–40 kb [as with Barrette *et al.* (1994), this approach precludes confusion between the mitochondrial genome and mitochondrial genes translocated to the nucleus; see Sunnucks & Hales (1996)]. The alkali lysis technique used here additionally extracts two plasmids of the symbiotic bacterium *Buchnera* that are consistently recovered in the alkali extracts of *A. pisum* DNA (Birkle, 1997): pAPE*leu*, a 7.8-kb plasmid bearing the genes *leuA–D*, with high sequence similarity to the plasmid pRPE isolated from the aphid *Rhopalosiphum padi* by Bracho *et al.* (1995); and pAPE*trp*, a plasmid bearing multiple tandem repeats of the genes *trpEG*, first described in the aphid *S. graminum* by Lai *et al.* (1994). In *A. pisum*, the plasmid bears 5–10 *trpEG* repeats, varying between clones (Baumann *et al.*, 1995). Both types of plasmids from *Buchnera* are strictly maternally inherited and can be used in parallel with mtDNA to explore the genetic diversity of aphids (e.g. Martínez *et al.*, 1996).

Materials and methods

Aphid clones

This study was conducted on cultures of 29 *A pisum* clones, each isolated from a single aphid and maintained on broad bean *Vicia faba* cv. The Sutton (on which all *A. pisum* biotypes perform well) in an enclosed constant-temperature room at 20°C with an 18-h light–6 h dark cycle. Great care was taken to avoid cross-contamination of the cultures. Clone UY2 and all clones with a TLW and LMB95 prefix were isolated between 1993 and 1995 from crops of pea, broad bean, alfalfa and red clover in North Yorkshire (Birkle, 1997; Wilkinson & Douglas, 1998a). Clones OX2 and LL02 were from long-term laboratory cultures, OX2 of unknown provenance and LL02 derived from an alfalfa crop in France during 1988. The pea aphids were assigned to biotypes by the criteria of their provenance and their performance on different test plants (see Wilkinson & Douglas, 1998b). Twenty of the clones were of pea biotype (UY2, OX2, LMB95/53, LMB96/1 and all TLW clones); four were alfalfa biotype (LMB95/48, LMB95/51, LMB95/52 and LL02); and five were clover biotype (LMB95/22, LMB95/23, LMB95/28, LMB95/30 and LMB95/35). All clones were green except LL02, LMB95/23 and LMB95/28, which were red.

DNA analysis

Circular DNA molecules were isolated by alkali lysis extraction, as described by Martínez *et al.* (1992), except that the alkali solution was adjusted to pH 12.25. Restriction enzyme digestions were performed according to the manufacturers' recommendations (New England Biolabs; Promega), and the restriction fragments were electrophoresed in horizontal 1–1.3% agarose gels. To distinguish mtDNA from the bacterial plasmids (pAPE*leu* and pAPE*trp*), Southern blotting and hybridizations were carried out with mtDNA- and plasmid-specific probes, labelled with chemiluminescence by the ECL direct nucleic acid labelling and detection system (Amersham), according to the manufacturer's instructions. The probes were linearized mtDNA of *A. pisum* clone UY2 (obtained by *Pst*I digestion), a 7.5-kb *Eco*R1 fragment (p7.4E) of the leucine plasmid pRPE (Bracho *et al.*, 1995) and a 0.49-kb *trpE* fragment, obtained by polymerase chain reaction (PCR) from *A. pisum* clone UY2, using homologous 20-base primers designed to amplify bases 827–1317 of the sequence given by Lai *et al.* (1994).

The restriction analysis of mtDNA, pAPE*leu* and pAPE*trp* was conducted with 15 enzymes, chosen according to the criterion that they consistently

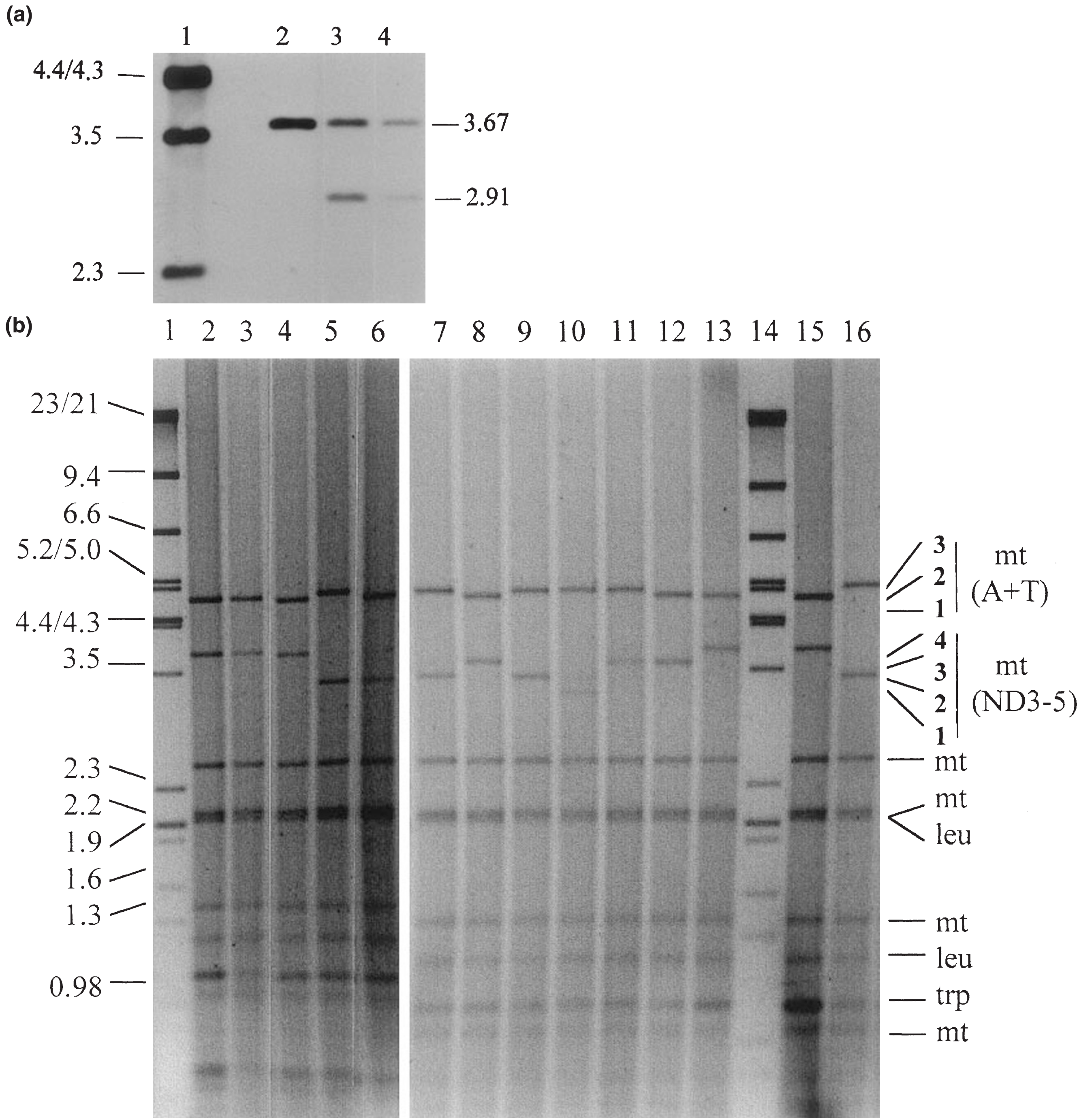

Fig. 1 Length variation in the DNA molecules extracted from *Acyrthosiphon pisum* by alkali lysis (mt, mtDNA; leu, plasmid pAPE*leu*; trp, plasmid pAPE*trp*). (a) The *Buchnera* plasmid pAPE*trp*, as revealed by digestion with *Bgl*II, on a Southern blot of the gel hybridized with *trpE* probe. Lane 1, molecular weight marker (kb); lane 2, clone UY2; lane 3, clone LMB95/53; lane 4, clone TLW94/14. (b) The aphid mtDNA, as revealed by digestion with *Taq*I. The figure shows tone-inverted agarose gels. The aphid clone and size classes of the mtDNA variable regions [A + T-rich region (1–3) and ND3–ND5 region (1–4)] are: lane 2, UY2 (1:4); lane 3, LL02 (1:4); lane 4, OX2 (1:4); lane 5 TLW94/20 (2:2); lane 6, TLW94/18 (1:2 + 4); lane 7, TLW94/17 (2:2); lane 8, TLW94/16 (1:3); lane 9, TLW94/14 (2:2) lane 10, TLW94/13 (2:1 + 2); lane 11, TLW94/12 (2:3); lane 12, TLW94/10 (1:3); lane 13, TLW94/9 (1:4); lane 15, UY2 (1:4); 16, TLW94/8 (3:2). Reference clone UY2 appears twice. Lanes 1 and 14, molecular weight markers (kb), labelled on the left.

generated DNA fragments from *A. pisum* clone UY2 of sufficient size (> 0.5 kb) for detection by ethidium bromide staining (for details see Birkle, 1997). These enzymes were: six-cutters (6-bp recognition sequences) — *Bcl*I, *Bgl*II, *Bsr*GI, *Bst*BI, *Eco*RI, *Hin*dIII, *Nde*I, *Pst*I, *Sna*BI, *Xba*I; five-cutters — *Dde*I, *Hin*fI; and four-cutters — *Dpn*II, *Msp*I and *Taq*I. The full restriction analysis was carried out on all *A. pisum* clones except clones LMB95/23, LMB95/30, LMB95/35 and LMB95/48, which were scored for mtDNA length variation with just *Taq*I.

Diversity indices of mtDNA size variation

The diversity of mtDNA length variants was considered as three hierarchical components (Rand & Harrison, 1989): the diversity within clones resulting from heteroplasmy, K_b; within biotypes, K_c; and across the total sample, K_t. Diversity values at each level were calculated as $K = 1 - \sum x_i^2$, where x_i is the frequency of the ith size class averaged across all individuals, and these estimates were apportioned to hierarchical components C_i, C_{ip} and C_{pt} corresponding to the levels of variation K_b, K_c and K_t respectively. Heteroplasmic clones were assigned to the size classes present, in proportion to their relative abundance, as estimated visually from the brightness of bands in agarose gels.

Results

The restriction analysis of DNA extracted from *A. pisum* clone UY2 revealed 43 scorable mtDNA fragments and a minimum of 45 restriction sites, allowing analysis of at least approximately 1.3% of the mtDNA sequence. There were also 24 scorable fragments from the bacterial plasmid pAPE*leu* and a minimum of 27 restriction sites scored, equivalent to at least 1.8% of its sequence. Only six restriction enzymes digested the plasmid pAPE*trp*. *Bgl*II and *Eco*RI produced a single fragment product of 3.67 kb, which hybridized with the *trpE* probe (e.g. Fig. 1a, lane 2), interpreted as the *trpEG* repeat unit (see Introduction); and *Hin*fI, *Taq*I, *Dpn*II and *Dde*I produced multiple, small fragments that could not be fully resolved.

Interclonal variation in restriction fragments was small. For the bacterial plasmid pAPE*leu*, just one variant restriction site was obtained, an additional *Hin*dIII site in clone LL02 (data not shown). Restriction fragment variation in pAPE*trp* was limited to clones TLW94/14 and LMB95/53, which produced two DNA fragments, at 3.67 kb and 2.91 kb, when digested with *Bgl*II and *Eco*RI. Both fragments hybridized strongly with the *trpE* probe (e.g. Fig. 1a, lanes 3 and 4). The smaller fragment is interpreted as evidence for a 0.76-kb deletion in a proportion of the *trpEG* copies on the plasmid in these two clones, and not as additional restriction sites, for two reasons. First, no 0.76-kb fragment was obtained, even after prolonged exposure of autoradiographs (not shown) and, secondly, it is most unlikely that *Bgl*II and *Eco*RI would generate fragments of indistinguishable size. For the mtDNA, no interclonal differences in the pattern of restriction fragments were observed for any enzyme, but length variation was apparent at two regions of the genome. The concordant patterns of differences between aphid clones in digestion profiles generated by the various restriction enzymes precluded either restriction site variation or technical artifacts such as incomplete restriction digestion. The two length-variable regions were most readily displayed by digestion with *Taq*I (see Fig. 1b), and the *Taq*I digestion profiles were identical in both the overall pattern and in the size classes of the two variable regions with those reported by Barrette *et al.* (1994), the A + T-rich region and the ND3–ND5 region [which Barrette *et al.* (1994) call regions 1 and 2 respectively]. Southern blot DNA probes specific to gene regions of the mtDNA (amplified out of pea aphid clone UY2; see Birkle, 1997) revealed corresponding positions of these genes within restriction fragments in both this study and that of Barrette *et al.* (1994) (data not shown). We have therefore interpreted the mtDNA length variation obtained in the two studies as corresponding.

The size classes for both length-variable regions of the mtDNA were determined from the *Taq*I digests for all 29 *A pisum* clones. Three different size classes were found at the A + T-rich region (from 1, the smallest, to 3, the largest, increasing in size by multiples of about 100 bp), and four size classes were scored at the ND3–ND5 region (increasing from 1 to 4, by multiples of about 200 bp). As a result of this variation, the total size of *A. pisum* mtDNA varied from 17.4 kb to 17.9 kb. The intermediate size classes were most common. Excluding the minor heteroplasmic molecules (considered below), the most frequent combination was 2:2 (A + T-rich region:ND3–ND5 region) found in 10 (34%) of the 29 aphid clones, giving a mitochondrial molecule of about 17.6 kb.

All *A. pisum* clones were homoplasmic at the A + T-rich region, i.e. each clone contained one detectable size class at this region. Two clones were heteroplasmic at the ND3–ND5 region, and each of these clones bore two size variants, one of which (the major variant) was more abundant than the minor variant (e.g. Fig. 1b, lanes 6 and 10). The size classes of the ND3–ND5 region in these heteroplasmic clones were as follows (major–minor variant, with estimated relative abundance in parentheses): 2:4 (0.85:0.15) for clone TLW94/18 and 1:2 (0.55:0.45) for clone TLW94/13.

The stability of the length-variable regions of mtDNA was analysed in two pea aphid clones, OX2 and UY2, by conducting a restriction analysis on DNA isolated from the cultures 24 months after the initial study. In the initial study, both of the clones could be assigned to class 1 for the A + T-rich region and class 4 for the ND3–ND5 region (see Fig. 1b, lanes 2 and 15 for UY2 and lane 4 for OX2). At the time of the second assay, the mtDNA of clone UY2 was unchanged and that of clone OX2 had decreased in size, as a result of a reduction at the ND3–ND5 region by four size classes, to a size class smaller than obtained for any *A. pisum* clone used in this study, precluding the possibility of cross-contamination between laboratory aphid cultures (data not shown).

The distribution of diversity in mtDNA length variants is displayed in Table 1. Population structuring with respect to biotype was not strong, accounting for 22% of the diversity in the A + T-rich region and 23% of the diversity in the ND3–ND5 region. It was also possible to compare the frequency of mtDNA size classes between the *A. pisum* clones studied here and those studied by Barrette *et al.* (1994) (Table 2). For both studies, the intermediate size classes of the ND3–ND5 region were most common but, at the A + T-rich region, the aphids used by Barrette *et al.* (1994) were dominated by size class 3, whereas size classes 1 and 2 were most prevalent in this study. The difference between the frequency distributions of size classes in the two studies was significant at the A + T-rich region ($\chi^2_2 = 37.2, P < 0.001$) but not at the ND3–ND5 region ($\chi^2_2 = 4.46, P > 0.1$).

Discussion

This study has confirmed and extended the RFLP analysis of *A. pisum* mtDNA conducted by Barrette *et al.* (1994) in two ways. First, the restriction patterns obtained in the two studies are fully consistent, indicating no substantial genetic differences in the mitochondrial genomes of *A. pisum* in Europe (this study) and North America (Barrette *et al.*, 1994). Secondly, the demonstration here of restriction site uniformity among *A. pisum* of three biotypes suggests that the lack of restriction variation obtained by Barrette *et al.* (1994) was not a consequence of their limited sampling of the *A. pisum* population in one alfalfa field, but a reflection of low genetic diversity in the mitochondrial genome of crop-feeding forms of this species.

Taken together, the data sets of Barrette *et al.* (1994) and this study indicate that the low genetic diversity found by Barrette *et al.* (1994) is unlikely to be the result of a bottleneck associated with the introduction of pea aphids to the Americas. Instead, this study shows that the maternal genomes of pea aphids in Europe and North America share low mtDNA diversity. The most plausible explanation is a population bottleneck(s) in the recent evolutionary past, although alternative interpretations, for example 'selective sweeps' of successful mtDNAs (e.g. Johnstone & Hurst, 1996), cannot be excluded. Furthermore, if the evidence for limited gene flow between the *A. pisum* biotypes is accepted (see Introduction), then these data indicate that the biotypes

Table 1 Frequencies and hierarchical diversity indices of length variants in the mtDNA of *Acyrthosiphon pisum* clones, grouped by biotype

	Size class at A + T-rich region				
Biotype (*n*)	f(1)	f(2)	f(3)	K_c	K_t
Pea (20)	0.50	0.45	0.05	0.5450	
Alfalfa (4)	0.25	0.75	0	0.3750	
Clover (5)	0.80	0.20	0	0.3200	
Mean value				0.4133	
					0.5303
Total (29)	0.517	0.448	0.034		

	Size class at ND3–ND5 region						
Biotype (*n*)	f(1)	f(2)	f(3)	f(4)	K_b	K_c	K_t
Pea (20)	0.03	0.52	0.25	0.21	0.0375	0.6285	
Alfalfa (4)	0	0.75	0	0.25	0	0.3750	
Clover (5)	0	0.80	0.20	0	0	0.3200	
Mean value					0.0259	0.4412	
							0.5694
Total (29)	0.019	0.596	0.207	0.178			

A + T-rich region: $C_{ip} = 0.7794$; $C_{pt} = 0.2206$. ND3–ND5 region: $C_i = 0.0455$; $C_{ip} = 0.7293$; $C_{pt} = 0.2251$.

Table 2 Distribution of mtDNA length variants in *Acyrthosiphon pisum* clones in this study and in Barrette *et al.* (1994) : size class (0) is included to accommodate a ND3–ND5 length variant in Barrette *et al.* (1994) that was one size class smaller than the smallest (1) in the present study

Number of aphid clones					
A + T-rich region			ND3–ND5 region*		
Size class	This study	Barrette *et al.* (1994)	Size class	This study	Barrette *et al.* (1994)
1	15	3	0	0	1
2	13	5	1	1	8
3	1	27	2	18	25
			3	6	9
			4	6	2
Total	29	35	Total	31	45

*Heteroplasmic clones are scored in both of the size classes present.

have diversified comparatively recently, possibly after (or during) population bottleneck(s). If the pairwise sequence divergence in *A. pisum* mtDNA is 2.3% per million years, as estimated for other closely related arthropods with low absolute amounts of sequence divergence (Brower, 1994), then the *A. pisum* biotypes can be predicted to have diversified within the last 100 000 years. This contrasts with an estimated divergence time of 500 000 years for the plant-affiliated biotypes of the aphids *S. graminum* (Powers *et al.*, 1989) and *T. trifolii* (Sunnucks *et al.*, 1997a) using the same divergence rate estimate. All these estimated divergence times should, however, be treated with great caution (for an example of the controversy surrounding such use of molecular clocks, see Avise *et al.*, 1994).

Plant affiliation of phytophagous insects can evolve very rapidly, especially after the introduction of novel plant species (e.g. Tabashnik, 1983). The possibility cannot be excluded that the *A. pisum* biotypes have evolved since the advent of agriculture, through specialization on monospecific stands of legume crops. An association with agricultural practices would reduce the estimated upper limit of biotype diversification time by at least an order of magnitude from 100 000 (see above) to 10 000–8500 BP, the dates when beans and peas, respectively, were first cultivated in the Mediterranean region (Vaughan & Geissler, 1997). Other agricultural crops used by the pea aphid were first cultivated more recently.

The chief source of genetic variation identified in this study was length variation, scored in both the mtDNA and bacterial plasmid pAPE*trp*. Length variation, through insertions/deletions of tandemly repeated sequences, has been reported in the mtDNA of a variety of animals including aphids, especially in the non-coding, control region (called the A + T-rich region in insects) of the molecule (Rand & Harrison, 1989; Martínez *et al.*, 1996). The variation can be attributed to slippage and mispairing of DNA strands across the tandem repeats (Moritz *et al.*, 1987). The variation at two regions of *A. pisum* mtDNA studied here was entirely consistent with the findings of Barrette *et al.* (1994), apart from the difference in frequency of the size classes at the A + T-rich region (see Table 2). The significance of this difference is uncertain, because of the small spatial scale of *A. pisum* sampled by Barrette *et al.* (1994). The similarity in the distribution of length variations at the ND3–ND5 region (Table 2) suggests that there may be saturation, a maximal level of variation, caused by a rapid transition rate between the small number of size classes. The frequency of size classes did not vary significantly between biotypes (Table 1). Further research is needed to establish whether, as in some other animal species (e.g. Rand & Harrison, 1989), length variation in mtDNA can contribute to the study of *A. pisum* population structure. The key issue limiting its usefulness may be the small number of length variants, presumably maintained, despite potentially high rates of transition between size classes, through selection against large numbers of repeats. The repeat number at the ND3–ND5 region may shift at particularly high rates; this is suggested by the several aphid clones that are heteroplasmic in this region (this study; Barrette *et al.*, 1994) and by the apparent shift in size class in one clone over 2 years (this study). (The possibility of contamination of this culture by a 'wild' aphid cannot, however, be excluded formally.)

The 0.76-kb deletion in a proportion of the *trpEG* repeats of the pAPE*trp* plasmid of two *A. pisum* clones studied here (TLW94/14 and LMB95/53) parallels recent findings in other aphid species, such as the Russian wheat aphid *Diuraphis noxia*, in which a proportion of the *trpEG* repeats are non-functional (i.e. pseudogenes), as a consequence of deletions or multiple, non-synonymous point mutations (Lai *et al.*, 1996). Curiously, pea aphid clones TLW94/14 and LMB95/53 not only share the same pAPE*trp* restriction pattern and plant affiliation (pea), but also have the same mtDNA haplotype, albeit the most common (2:2), raising the possibility that their pAPE*trp* deletions had a common origin. The amplification of *trpEG* genes on plasmids is believed to be of nutritional significance to the aphid (Lai *et al.*, 1994), which derives the essential amino acid tryptophan from its symbiotic bacteria (Douglas & Prosser, 1992). *TrpEG* codes for anthranilate

synthetase, the rate-limiting enzyme in tryptophan synthesis, and therefore the silencing of some of the *trpEG* copies could be indicative of relaxed selection pressure for high tryptophan biosynthesis by the bacteria, or even selection pressure against high rates of tryptophan synthesis. In particular, Lai *et al.* (1996) have linked the *trpEG* pseudogenes in the *Buchnera* from *D. noxia* to the high nutritional quality of this insect's diet. *Acyrthosiphon pisum* feeding from well-fertilized crop legumes, with phloem sap of very high N:C ratio (C. Awmack & A. E. Douglas, unpubl. results) may similarly have a reduced requirement for tryptophan biosynthesis. In other words, one could speculate that the persistence of the *trpEG* deletion may reflect the changing selection pressures on the aphid–bacterial symbiosis in aphids using agricultural crops.

Our understanding of the evolutionary and ecological determinants of both the *trpEG* pseudogenes and the low mtDNA diversity in crop-feeding *A. pisum* would be greatly enhanced by a parallel study of *A. pisum* clones on non-crop legumes. If agriculture is shaping the genetics of crop-feeding biotypes, then one might predict greater genetic diversity, but lower frequency of *trpEG* pseudogenes, in *A. pisum* populations using wild legumes than in the crop biotypes of this species.

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References

AVISE, J. C., NELSEN, W. S. AND SUGITA, H. 1994. A speciational history of "living fossils": molecular evolutionary patterns in horseshoe crabs. *Evolution*, **48**, 1986–2001.

BARRETTE, R. J., CREASE, T. J., HERBERT, P. D. N. AND VIA, S. 1994. Mitochondrial DNA diversity in the pea aphid *Acyrthosiphon pisum*. *Genome*, **37**, 858–865.

BAUMANN, P., LAI, C.-Y., BAUMANN, L., ROUKBAKHSH, D., MORAN, N. A. AND CLARK, M. A. 1995. Mutualistic associations of aphids and prokaryotes: biology of the genus *Buchnera*. *Appl. Environ. Microbiol.*, **61**, 1–7.

BERNAYS, E. AND GRAHAM, M. 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology*, **69**, 886–892.

BIRCH, A. N. E., FENTON, B., MALLOCH, G., JONES, A. T., PHILLIPS, M. S. AND HARROWER, B. E. *ET AL.* 1994. Ribosomal spacer length variability in the large raspberry aphid, *Amphorophora idaei*. *Insect Mol. Biol.*, **3**, 239–245.

BIRKLE, L. M. 1997. *A Molecular Characterization of the Mitochondria and Bacteria of the Pea Aphid,* Acyrthosiphon pisum. D.Phil. thesis, University of York.

BLACKMAN, R. L. AND EASTOP, V. F. 1984. *Aphids on the World's Crops: an Identification Guide*. John Wiley & Sons, New York.

BRACHO, A. M., MARTNEZ-TORRES, D., MOYA, A. AND LATORRE, A. 1995. Discovery and molecular characterization of a plasmid localized in *Buchnera* sp. bacterial endosymbiont of the aphid *Rhopalosiphum padi*. *J. Mol. Evol.*, **41**, 67–73.

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

DE BARRO, P. J., SHERRATT, T. N., BROOKES, C. P., DAVID, O. AND MACLEAN, N. 1995. Spatial and temporal genetic variation in British field populations of the grain aphid *Sitobion avenae* (F.) (Hemiptera: aphididae) studied by RAPD-PCR. *Proc. R. Soc. B*, **262**, 321–327.

DIEHL, S. R. AND BUSH, G. L. 1984. An evolutionary and applied perspective of insect biotypes. *Ann. Rev. Entomol.*, **29**, 471–504.

DOUGLAS, A. E. 1997. Provenance, experience and plant utilization by the polyphagous aphid, *Aphis fabae*. *Entomologia Exp. Appl.*, **83**, 161–170.

DOUGLAS, A. E. AND PROSSER, W. F. 1992. Synthesis of the essential amino acid tryptophan in the pea aphid (*Acyrthosiphon pisum*) symbiosis. *J. Insect Physiol.*, **38**, 565–568.

HALES, D. F., TOMIUK, J., WÖHRMANN, K. AND SUNNUCKS, P. 1997. Evolutionary and genetic aspects of aphid biology: a review. *Eur. J. Entomol.*, **94**, 1–55.

JOHNSTONE, R. A. AND HURST, G. D. D. 1996. Maternally inherited male-killing microorganisms may confound interpretation of mtDNA variation in insects. *Biol. J. Linn. Soc.*, **53**, 453–470.

LAI, C.-Y., BAUMANN, L. AND BAUMANN, P. 1994. Amplification of *trpEG*: adaptation of *Buchnera aphidicola* to an endosymbiotic association with aphids. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 3819–3823.

LAI, C.-Y., BAUMANN, P. AND MORAN, N. 1996. The endosymbiont (*Buchnera* sp.) of the aphid *Diuraphis noxia* contains plasmids consisting of *trpEG* and tandem repeats of *trpEG* pseudogenes. *Appl. Environ. Microbiol.*, **62**, 332–339.

MARTINEZ, D., MOYA, A., LATORRE, A. AND FERERES, A. 1992. Mitochondrial DNA variation in *Rhopalosiphum padi* (Homoptera: aphididae) populations from four Spanish localities. *Ann. Entomol. Soc. Am.*, **85**, 241–246.

MARTINEZ, D., SIMON, J. C., FERERES, A. AND MOYA, A. 1996. Genetic variation in natural populations of the aphid *Rhopalosiphum padi* as revealed by maternally inherited markers. *Mol. Ecol.*, **5**, 659–670.

MORITZ, C., DOWLING, T. E. AND BROWN, W. M. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Ann. Rev. Ecol. Syst.*, **18**, 269–292.

MÜLLER, F. P. 1980. Host plants, generation sequence and reproductive isolation of intraspecific forms of *Acyrthosiphon pisum*. *Entomologia Exp. Appl.*, **28**, 145–157.

POWERS, T. O., JENSEN, S. G., KINDLER, S. D., STRYKER, C. J. AND SANDALL, L. J. 1989. Mitochondrial DNA divergence among

greenbug (Homoptera: aphididae) biotypes. *Ann. Entomol. Soc. Am.*, **82**, 298–302.

RAND, D. M. AND HARRISON, R. G. 1989. Molecular population genetics of mtDNA size variation in crickets. *Genetics*, **121**, 551–569.

SANDSTRÖM, J. AND PETTERSSON, J. 1994. Amino acid composition of phloem sap and the relation to intraspecific variation in pea aphid (*Acyrthosiphon pisum*) performance. *J. Insect Physiol.*, **40**, 947–955.

SUNNUCKS, P. AND HALES, D. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the genus *Sitobion* (Hemiptera: aphididae). *Mol. Biol. Evol.*, **13**, 510–524.

SUNNUCKS, P., DRIVER, F., BROWN, W. V., CARVER, M., HALES, D. F. AND MILNE, W. M. 1997a. Biological and genetic characterization of morphologically similar *Therioaphis trifolii* (Hemiptera: aphididae) with different host utilization. *Bull. Entomol. Res.*, **87**, 425–436.

SUNNUCKS, P., DE BARRO, P. J., LUSHAI, G., MACLEAN, N. AND HALES, D. 1997b. Genetic structure of an aphid studied using microsatellites: cyclic parthenogenesis, differentiated lineages and host specialization. *Mol. Ecol.*, **6**, 1059–1073.

TABASHNIK, B. E. 1983. Host range evolution: the shift from native legume hosts to alfalfa by the butterfly, *Colias philodice eriphyle*. *Evolution*, **37**, 150–162.

VAUGHAN, J. G. AND GEISSLER, C. A. 1997. *The New Oxford Book of Food Plants*. Oxford University Press, Oxford.

VIA, S. 1991a. The genetic structure of host plant adaptation in a spatial patchwork: demographic variability among reciprocally transplanted pea aphid clones. *Evolution*, **45**, 827–852.

VIA, S. 1991b. Specialized host plant performance of pea aphid clones in not altered by experience. *Ecology*, **72**, 1420–1427.

WILKINSON, T. L. AND DOUGLAS, A. E. 1998a. Host cell allometry and regulation of the symbiosis between pea aphids, *Acyrthosiphon pisum*, and bacteria, *Buchnera*. *J. Insect Physiol.*, **44**, 629–635.

WILKINSON, T. L. AND DOUGLAS, A. E. 1998b. Plant penetration by pea aphids (*Acyrthosiphon pisum*) of different plant range. *Entomologia Exp. Appl.*, **87**, 43–50.