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Intraspecific phylogenetic congruence among multiple symbiont genomes

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Eukaryotes often form intimate endosymbioses with prokaryotic organisms. Cases in which these symbionts are transmitted cytoplasmically to host progeny create the potential for co-speciation or congruent evolution among the distinct genomes of these partners. If symbionts do not move horizontally between different eukaryotic hosts, strict phylogenetic congruence of their genomes is predicted and should extend to relationships within a single host species. Conversely, even rare 'host shifts' among closely related lineages should yield conflicting tree topologies at the intraspecific level. Here, we investigate the historical associations among four symbiotic genomes residing within an aphid host: the mitochondrial DNA of *Uroleucon ambrosiae* aphids, the bacterial chromosome of their *Buchnera* bacterial endosymbionts, and two plasmids associated with *Buchnera*. DNA sequence polymorphisms provided a significant phylogenetic signal and no homoplasy for each data set, yielding completely and significantly congruent phylogenies for these four genomes and no evidence of horizontal transmission. This study thus provides the first evidence for strictly vertical transmission and 'co-speciation' of symbiotic organisms at the intraspecific level, and represents the lowest phylogenetic level at which such coevolution has been demonstrated. These results may reflect the obligate nature of this intimate mutualism and indicate opportunities for adaptive coevolution among linked symbiont genomes.

Keywords: *Buchnera aphidicola*; co-speciation; endosymbionts; DNA sequence polymorphism; phylogenetic congruence; *Uroleucon ambrosiae* aphids

1. INTRODUCTION

Mitochondria originated as symbiotic bacteria that became metabolically co-opted by their eukaryotic hosts (Gray *et al*. 1999). Bacterial symbionts likewise provision nutrients to eukaryotic hosts in symbioses of more recent evolutionary origin (Baumann *et al*. 1997). Aphids are among the many animals obligately associated with mutualistic intracellular symbionts (Baumann *et al*. 1997; Moran & Telang 1998), harbouring the bacterium *Buchnera aphidicola*, which synthesizes amino acids for its hosts (Lai *et al*. 1994; Bracho *et al*. 1995; Douglas 1998). In many aphid species, *Buchnera* contains two types of plasmid in addition to the main chromosome (Lai *et al*. 1994; Bracho *et al*. 1995). These plasmids bear genes responsible for biosynthesis of leucine and tryptophan, essential amino acids that are limiting in the phloem-sap diet of aphids (Sandström & Moran 1999). A typical aphid host thus contains at least four potentially independent genomes of prokaryotic origin: those of the aphid mitochondrion, *Buchnera*, and two plasmids.

As in many other endosymbiotic associations, *Buchnera* are vertically transmitted from one host generation to the next via infection of progeny within mothers (Buchner 1965). If symbiont genomes are consistently co-transmitted across host generations in this manner they will become historically and genetically linked. Evidence for this historical association is provided by matching branching patterns from phylogenies of different genomes that are collected from the same set of hosts. This phylogenetic congruence may be imperfect, however, to the extent that symbiont genomes are transferred horizontally among hosts. Bacterial plasmids, for example, are well known for their potential for horizontal movement, and the plasmid-borne genes and chromosomal genes of bacteria frequently show incongruent phylogenies (Davies & Smith 1978; Valdes & Pinero 1992).

Considerable phylogenetic congruence has been observed at deeper evolutionary levels in studies of higher aphid taxa and their *Buchnera* (Moran *et al*. 1993; Rouhbakhsh *et al*. 1997; Baumann *et al*. 1999; Clark *et al*. 2000) as well as in a variety of other symbioses in plants and animals (Farrell & Mitter 1990; Reed & Hafner 1997; Mueller *et al*. 1998; Nishiguchi *et al*. 1998; Peek *et al*. 1998; Chen *et al*. 1999; Wernegreen & Riley 1999). The phylogenetic patterns observed at higher taxonomic levels may not, however, adequately expose the biological processes underlying them. For example, in studies of distantly related taxa, evolutionary congruence may be overestimated even if horizontal symbiont transfer is common, providing it only occurs among closely related hosts (Nishiguchi *et al*. 1998; Chen *et al*. 1999; Wernegreen & Riley 1999) and thus goes undetected. Thus, although previous results strongly support vertical transmission and symbiont co-speciation, the possibility of transfer among closely related lineages cannot be ruled out. Such transfer would have substantial implications for patterns of genetic polymorphism among host genomes and for the genetic structure of host and symbiont populations.

Aside from the impracticable strategy of actually witnessing vertical transfer across thousands of host generations in a natural setting, the most direct and informative evidence on the fine-scale fidelity of vertical transmission might come from phylogenetic investigations of lineages of very close relationship. The most stringent test would analyse symbiont variation within a single host

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Table 1. *Sequenced loci from symbiont genomes within* U. ambrosiae

gene(s)	genome	gene function	fragment (bp)
COI/COII	aphid mitochondrial	electron transport	1344
dnaN	bacterial chromosome	DNA replication	1107
leuB/leuC	leucine plasmid	amino-acid synthesis	1674
trpE/trpG	tryptophan plasmid	amino-acid synthesis	1200
			$\Sigma =$ 5325

species, where frequent ecological and behavioural contact and overall biological similarity should maximize opportunities for horizontal transmission.

Here, we present the first phylogenetic evidence for 'co-speciation' among genetic elements within a single host species. Our analyses treat DNA sequence polymorphisms from the mitochondrial, bacterial and plasmid genomes (table 1) within geographically widespread samples of the ragweed aphid, *Uroleucon ambrosiae*. The extreme genetic propinquity of the recovered lineages provided both a challenge and an advantage for the study of evolutionary congruence at low phylogenetic levels.

2. MATERIAL AND METHODS

U. ambrosiae aphids were collected from 20 US localities (Calhoun County, AL, October 1997; Santa Cruz County, AZ, October 1998; McIntosh County, GA, April 1999; Dade County, GA, September 1998; Wayne County, IL, October 1997; Dewitt County, IL, October 1997; Monroe County, IN, October 1997; Grant County, KY, September 1998; Madison County, LA, October 1997; Montgomery County, MD, October 1997; Wayne County, MI, September 1998; Ramsey County, MN, September 1997, September 1998; Madison County, MS, September 1998; Tompkins County, NY, October 1997; Allen County, OH, September 1998; Davidson County, TN, September 1997; Campbell County, TN, September 1998; Millard County, UT, September 1997; Albermarle County, VA, October 1997; Raleigh County, WV, October 1997). Most specimens were taken from the principal host plant, *Ambrosia trifida*, but NY, UT and McIntosh County, GA, samples were collected from other *Ambrosia* species and from the closely related *Iva frutescens*, respectively. All assayed aphids were adult, apterous, asexual females; these were identified by D.J.F. and N.A.M. and stored at $-80\,^{\circ}$C.

For a single aphid from each locality, whole genomic DNA was extracted (after Bender *et al.* 1983) and used to amplify a DNA fragment from each genome under study (table 1) using annealing temperatures of 54–60 $^{\circ}$C and primers designed from published sequences (Rouhbakhsh *et al.* 1997; Baumann *et al.* 1999; Moran *et al.* 1998). PCR, sequencing, and alignment otherwise followed previously reported protocols (Moran *et al.* 1998). These sequences can be found under GenBank accession numbers AF196354–AF196467.

The evolutionary analysis of these data included several steps. First, we separately evaluated the presence or absence of a significantly structured phylogenetic signal for each data set. This was accomplished by conducting exhaustive parsimony searches on each data set using PAUP* 4.0b2 (Swofford 1999) and comparing the resulting g1 statistics of tree-length distribution skew with critical values published by Hillis & Huelsenbeck (1992). Significantly negatively skewed distributions indicate the presence of trees shorter than would be expected by chance. As another measure of the phylogenetic robustness of the data, we conducted a bootstrap analysis of the combined data set that employed 1000 replicate branch-and-bound parsimony searches.

Second, we used PAUP* to reconstruct unrooted most parsimonious trees for each data set. Third, we investigated the possibility of conflicting phylogenetic signal among data sets. Fourth, we computed the likelihood that the four trees could have exhibited this level of phylogenetic agreement by chance (see § 3 for details).

Fifth, we inferred the minimum number of historical recombination events implied by the combined data set using the four-gamete test of Hudson & Kaplan (1985) and evaluated the degree of association between nucleotides at polymorphic sites using χ^2-tests and applying a Bonferonni adjustment for multiple comparisons (Sokal & Rohlf 1991). Both these analyses were conducted using the computer program DnaSP (Rozas & Rozas 1999).

3. RESULTS

Among the 20 multilocus haplotypes collected from individual aphid specimens in our study, 16 differed from each other at 0–2 nucleotide sites and belonged to a 'common' haplotype lineage. Each of the four remaining specimens (from McIntosh County, GA, MN, OH and UT) yielded a unique haplotype that differed from each other and the common haplotypes at 0.36–0.58% of sites overall.

Although genetic divergences were low, each data set possessed a statistically significant degree of phylogenetic signal (g_1 statistics: COI/COII, -1.50; *dnaN*, -1.26; *leuB/leuC*, -1.50; *trpE/trpG*, -1.26) ($p < 0.01$ in all cases). Altogether, ten out of the 12 total nodes across the four unrooted five-taxon trees received synapomorphic support from our data, yielding two fully resolved trees and two in which two of three nodes were resolved (figure 1). Most importantly, the phylogenetic signals of these data sets entirely agreed, yielding four gene trees that were completely topologically congruent with one another (figure 1). Bootstrap proportions of 99, 94 and 100% for the combined data set further indicated the data's robust support for the three nodes of this topology. Moreover, each and every parsimony-informative DNA polymorphism in these data sets supported a grouping of haplotypes that was consistent with this topology. That is, there was zero homoplasy in the data. Given the complete topological agreement among data sets and the complete lack of conflicting signal due to homoplasy, it is perhaps unnecessary to point out that methods for evaluating phylogenetic incongruence among data sets (Kishino & Hasegawa 1989; Huelsenbeck & Bull 1996) would not

reveal significant evidence of disagreement here. Finally, the four-gamete test provided no evidence for recombination among these four genomes.

By contrast, there is statistically significant support for the agreement shown among these tree topologies. Since there are 15 possible fully resolved unrooted five-taxon tree topologies, the probability of any fully resolved tree (e.g. that of *dnaN*) fully agreeing with another fully resolved tree selected for comparison (e.g. that of *trpE*/*trpG*) is one out of 15. Since there are three possible fully resolved COI/COII trees that are consistent with the incompletely resolved topology of COI/COII, the probability that topological agreement between COI/COII and *trpE*/*trpG* trees is due to chance is $3/15 = 1/5$; the same holds for the topologically identical *leuB*/*leuC* tree. Thus, the compound probability of these three trees showing the observed level of topological agreement with the fourth by chance is $p = 1/15 \times 1/5 \times 1/5 = 0.0027$. This phylogenetic agreement is bolstered by the detection of significant linkage disequilibrium among gene regions. Clearly, these data sets imply significantly congruent histories among symbionts.

4. DISCUSSION

This study provides intraspecific phylogenetic evidence for congruent evolution among four symbiont genomes consisting of the mitochondrial genome, the *Buchnera* chromosome, and two types of *Buchnera* plasmid. The smallest time-scale over which phylogenetic congruence has previously been demonstrated for aphids and *Buchnera* spans the radiation of species within the genus *Uroleucon*, a group originating five to 20 million years ago based on molecular clock calculations (Clark *et al.* 2000). In another investigation of closely related aphids, sexual and asexual lineages of the aphid host *Rhopalosiphum padi* were demonstrated to be genetically distinct and to exhibit complete linkage of restriction site markers from the mitochondria and *Buchnera* plasmids (Simon *et al.* 1996). The present study treats evolutionary lineages that mitochondrial sequence divergences suggest to be yet more closely related ($\leqslant 0.4\%$ versus $\geqslant 1.0\%$) and represents the first report of symbiont 'co-speciation' within a sexually reproducing host species. In addition to perfect congruence of the four trees, the data reveal a similar distribution of nucleotide changes over the phylogeny for each genome, as expected if congruence truly reflects their simultaneous divergence rather than horizontal symbiont transfer among related lineages after divergence. In combination with previous studies at deeper phylogenetic levels, our findings suggest that *Buchnera* and its associated plasmids have lost the capacity to transfer among individual hosts, except through maternally acquired infection.

The absence of similar observations in other taxa may reflect a contrast between the strong evolutionary fidelity of the aphid–*Buchnera* relationship compared with the more fluid associations of some other symbiotic systems. For example, many species of herbivorous insect use different host plant taxa across their geographical range (Thompson 1999) and legume–*Rhizobium* associations are characterized by the horizontal transfer of both bacteria and plasmids (Wernegreen & Riley 1999). Bacterial endosymbioses of invertebrates also vary in their processes

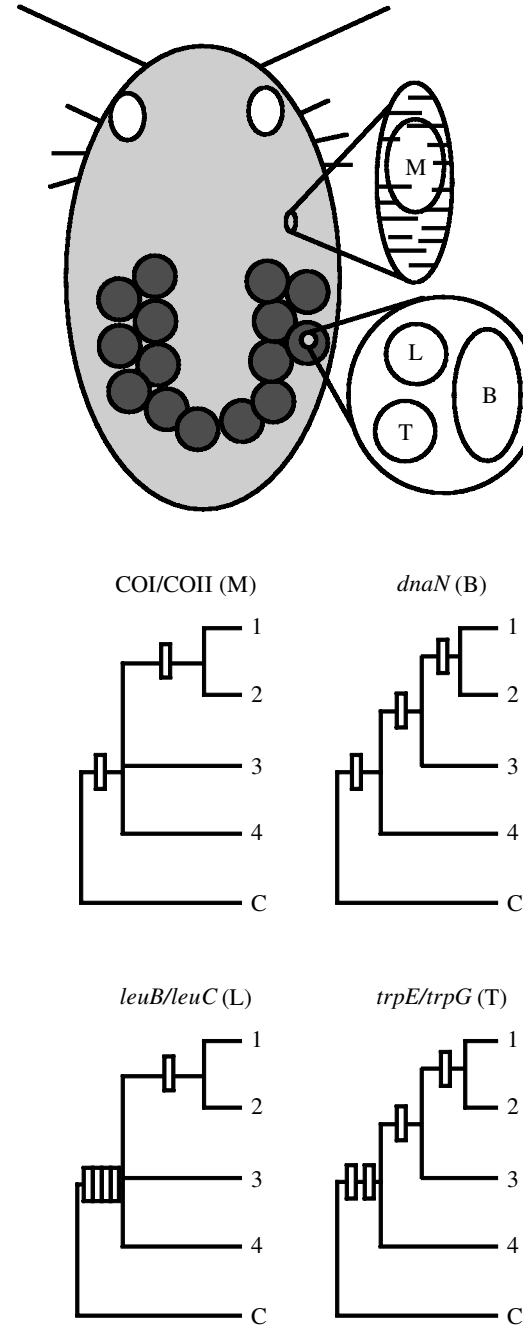

Figure 1. Perfect phylogenetic congruence among four symbiotic genomes sampled from the aphid species *U. ambrosiae* and its bacterial endosymbiont *B. aphidicola*. Four out of 20 specimens (1, GA; 2, MN; 3, UT; 4, OH) yielded unique multilocus haplotypes, while 16 specimens yielded highly similar sequences belonging to the 'common' lineage (C). Phylogenetically informative sites (tick marks) provide synapomorphic support for ten out of a possible 12 haplotype groupings across the four data sets, leaving a single node in both the mitochondrial and leucine plasmid trees unresolved. In the combined analysis, the three haplotype groupings were supported in 99, 94 and 100% (left to right) of bootstrap replicates, reflecting the absence of homoplasy within and among data sets. Most parsimonious trees from each genome are completely and significantly ($p < 0.003$) consistent with each other, supporting the hypothesis of congruent evolution and strictly vertical transmission. M, mitochondrial genome; B, bacterial chromosome; L, leucine plasmid; T, tryptophan plasmid.

of symbiont infection (Moran & Telang 1998). For example, although the bacterial parasite *Wolbachia pipientis* exhibits maternal transmission, phylogenetic studies reveal substantial levels of horizontal transfer of *Wolbachia* among distantly related invertebrate taxa (Werren 1997); similar observations apply to the 'secondary' symbionts of aphids (Chen & Purcell 1997; N. A. Moran, unpublished data). By contrast, species-level phylogenies of sepiolid squids and their bacterial vibrio symbionts are congruent even though maternal transmission is lacking (Nishiguchi *et al.* 1998). Determining how exceptional the evolutionary fidelity of the aphid–*Buchnera* symbiosis really is will require investigations of between- and within-species phylogenetics in a variety of symbiotic systems.

The current lack of within-species symbiont studies may also reflect the challenge of obtaining sufficient phylogenetically informative characters to define relationships among very recently diverged lineages. We recovered and analysed evolutionary lineages that were between one and several orders of magnitude less genetically diverse than those of typical insect and bacterial species (see references and further discussion of this issue in Funk *et al.* (2001)). Thus, this study demonstrates 'co-speciation' between entities that are intimately related even by intraspecific standards. Three strategies allowed us to obtain the data necessary to test our hypothesis: (i) assaying rapidly evolving mitochondrial and, especially, bacterial and plasmid loci; (ii) collecting sizeable (1.0–1.6 kb) sequences for each locus; and (iii) sampling sufficient individuals to obtain rare, comparatively divergent, and phylogenetically informative haplotype lineages.

These strategies yielded phylogenetically informative nucleotide polymorphisms providing significant phylogenetic signal and synapomorphic support for ten out of 12 possible nodes across four symbiont gene trees. Because of the rarity and recent origin of these mutations, multiple substitutions at single sites were not observed and the resulting data sets are completely free of homoplasy. This pattern of nucleotide variation is consistent with the infinite sites model of molecular evolution (Maynard Smith & Smith 1998), allowing us to use methods employing the infinite sites assumption (Hudson & Kaplan 1985) that indicate a lack of recombination among symbiont genomes. Even single, unambiguous mutations are often used to infer relationships in haplotype trees and are interpreted as strong evidence for lack of recombination (i.e. horizontal transfer) since other explanations are much less likely (Maynard Smith 1999).

The apparent dearth of horizontal transmission that we observed is also notable in view of the abundant time and occasions for such transfers suggested by the biology of the aphid hosts. Although the lineages we studied are very young on an evolutionary time-scale, they are ancient compared with the historical time-frames over which bacterial lineages have been observed to exchange antibiotic resistance genes via plasmids. Moreover, field observations on natural populations (including those that yielded the divergent lineages) indicate that *U. ambrosiae* exhibits sexual life-history stages throughout its range, providing regular intimate contact and plentiful opportunities for symbiont transmission. Indeed, the lack of geographical structuring and the low variation observed in the present study suggests that these aphids exhibit geographically extensive intermixing over evolutionarily short periods of time, as has been suggested for other aphid taxa (Loxdale *et al.* 1993). It thus appears that the obligate nature of this symbiosis rather than a lack of opportunity explains the observed phylogenetic congruence.

In addition to their value for understanding patterns of phylogenetic diversification in coevolving symbionts, our findings also have implications for understanding the evolutionary dynamics of coevolution in the aphid–*Buchnera* symbiosis. An absence of horizontal transfer would imply that the four genomes of our study are under complete genetic linkage. Thus, natural selection acting on a single mutation would affect allele frequencies across all symbiont genomes. Such linkage could limit the potential for adaptation at particular loci while magnifying effects of selective sweeps (Begun & Aquadro 1992) or background selection (Charlesworth *et al.* 1993). A further consequence of such linkage is an increased opportunity for co-adaptation (Bouma & Lenski 1988) among haploid genomes. Nonetheless, the linkage disequilibrium described here is itself clearly not a consequence of selection since ten out of the 14 polymorphic nucleotide sites are at synonymous positions with no effect on gene function.

Finally, our finding of complete congruence among genomes advocates using symbiont sequences as molecular markers for the study of aphid population genetics. Because synonymous sites in *Buchnera* genes evolve about twice as fast as those in the mitochondrial genes of their aphid hosts (Clark *et al.* 1999, 2000), *Buchnera* genes provide comparatively informative sources of phylogenetic data. Using symbiont sequences for this purpose may help to illuminate the little-studied population biology underlying this mutualistic association.

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REFERENCES

Baumann, L., Baumann, P., Moran, N. A., Sandström, J. & Thao, M. L. 1999 Genetic characterization of plasmids containing genes encoding enzymes of leucine biosynthesis in endosymbionts (*Buchnera*) of aphids. *J. Mol. Evol.* **48**, 77–85.

Baumann, P., Moran, N. A. & Baumann, L. 1997 The evolution and genetics of aphid endosymbionts. *Bioscience* **47**, 12–20.

Begun, D. J. & Aquadro, C. F. 1992 Levels of naturally occurring DNA polymorphism correlate with recombination rates in *D. melanogaster*. *Nature* **356**, 519–520.

Bender, W., Spierer, P. & Hogness, D. S. 1983 Chromosomal walking and jumping to isolate DNA from the ace and rosy loci and the bithorax complex in *Drosophila melanogaster*. *J. Mol. Biol.* **168**, 17–33.

Bouma, J. E. & Lenski, R. 1988 Evolution of a bacteria/plasmid association. *Nature* **335**, 351–352.

Bracho, A. M., Martineztorres, D., Moya, A. & 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.

Buchner, P. 1965 *Endosymbiosis of animals with plant microorganisms*. New York: Interscience.

Charlesworth, B., Morgan, M. T. & Charlesworth, D. 1993 The effect of deleterious mutations on neutral molecular variation. *Genetics* **134**, 1289–1303.

Chen, D. Q. & Purcell, A. H. 1997 Occurrence and transmission of facultative endosymbionts in aphids. *Curr. Microbiol.* **34**, 220–225.

Chen, X., Li, S. & Aksoy, S. 1999 Concordant evolution of a symbiont with its host insect species: molecular phylogeny of genus *Glossina* and its bacteriome-associated endosymbiont, *Wigglesworthia glossinidia*. *J. Mol. Evol.* **48**, 49–58.

Clark, M. A., Moran, N. A. & Baumann, P. 1999 Sequence evolution in bacterial endosymbionts having extreme base compositions. *Mol. Biol. Evol.* **16**, 1486–1598.

Clark, M. A., Moran, N. A., Baumann, P. & Wernegreen, J. J. 2000 Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*). *Evolution* **54**, 517–525.

Davies, J. & Smith, D. I. 1978 Plasmid-determined resistance to anti-microbial agents. *A. Rev. Microbiol.* **32**, 469–518.

Douglas, A. E. 1998 Nutritional interactions in insect–microbe symbioses: aphids and their symbiotic bacteria *Buchnera*. *A. Rev. Entomol.* **43**, 17–37.

Farrell, B. & Mitter, C. 1990 Phylogenesis of insect plant interactions—have *Phyllobrotica* leaf beetles (Chrysomelidae) and the lamiales diversified in parallel? *Evolution* **44**, 1389–1403.

Funk, D. J., Wernegreen, J. J. & Moran, N. A. 2001 Intraspecific polymorphism in symbiont genomes: bottlenecks and the aphid–*Buchnera* association. *Genetics*. (In the press.)

Gray, M. W., Burger, G. & Lang, B. F. 1999 Mitochondrial evolution. *Science* **283**, 1476–1481.

Hillis, D. M. & Huelsenbeck, J. P. 1992 Signal, noise, and reliability in molecular phylogenetic analyses. *J. Hered.* **83**, 189–195.

Hudson, R. R. & Kaplan, N. L. 1985 Statistical properties in the number of recombinational events in the history of a sample of DNA sequences. *Genetics* **111**, 147–164.

Huelsenbeck, J. P. & Bull, J. J. 1996 A likelihood ratio test to detect conflicting phylogenetic signal. *Syst. Biol.* **45**, 92–98.

Kishino, H. & Hasegawa, M. 1989 Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data and the branching order in Hominoidea. *J. Mol. Evol.* **29**, 170–179.

Lai, C. Y., Baumann, L. & Baumann, P. 1994 Amplification of *trpEG*—adaptation of *Buchnera aphidicola* to an endosymbiotic association with aphids. *Proc. Natl Acad. Sci. USA* **91**, 3819–3823.

Loxdale, H. D., Hardie, J., Halbert, S., Foottit, R., Kidd, N. A. C. & Carter, C. I. 1993 The relative importance of short-range and long-range movement of flying aphids. *Biol. Rev.* **68**, 291–311.

Maynard Smith, J. 1999 The detection and measurement of recombination from sequence data. *Genetics* **153**, 1021–1027.

Maynard Smith, J. & Smith, N. H. 1998 Detecting recombination from gene trees. *Mol. Biol. Evol.* **15**, 590–599.

Moran, N. A. & Telang, A. 1998 Bacteriocyte-associated symbionts of insects—a variety of insect groups harbor ancient prokaryotic endosymbionts. *Bioscience* **48**, 295–304.

Moran, N. A., Munson, M. A., Baumann, P. & Ishikawa, H. 1993 A molecular clock in endosymbiotic bacteria is calibrated using the insect hosts. *Proc. R. Soc. Lond.* B **253**, 167–171.

Moran, N. A., Kaplan, M. E., Gelsey, M. J., Murphy, T. G. & Scholes, E. A. 1998 Phylogenetics and evolution of the aphid genus *Uroleucon* based on mitochondrial and nuclear DNA sequences. *Syst. Entomol.* **24**, 1–9.

Mueller, U. G., Rehner, S. A. & Schulz, T. R. 1998 The evolution of agriculture in ants. *Science* **281**, 2034–2038.

Nishiguchi, M. K., Ruby, E. G. & McFall-Ngai, M. J. 1998 Competitive dominance among strains of luminous bacteria provides an unusual form of evidence for parallel evolution in sepiolid squid–vibrio symbioses. *Appl. Environ. Microbiol.* **64**, 3209–3213.

Peek, A. S., Feldman, R. A., Lutz, R. A. & Vrijenhoek, R. C. 1998 Cospeciation of chemoautotrophic bacteria and deep sea clams. *Proc. Natl Acad. Sci. USA* **95**, 9962–9966.

Reed, D. L. & Hafner, M. S. 1997 Host specificity of chewing lice on pocket gophers: a potential mechanism for cospeciation. *J. Mammal.* **78**, 655–660.

Rouhbakhsh, D., Clark, M. A., Baumann, L., Moran, N. A. & Baumann, P. 1997 Evolution of the tryptophan biosynthetic pathway in *Buchnera* (aphid endosymbionts): studies of plasmid-associated *trpEG* within the genus *Uroleucon*. *Mol. Phylogen. Evol.* **8**, 167–176.

Rozas, J. & Rozas, R. 1999 DnaSP version 3: an integrated program for molecular population genetics and molecular evolutionary analysis. *Bioinformatics* **15**, 174–175.

Sandström, J. & Moran, N. 1999 How nutritionally imbalanced is phloem sap for aphids? *Entomol. Exp. Appl.* **91**, 203–210.

Simon, J. C., Martinez Torres, D., Latorre, A., Moya, A. & Hebert, P. D. N. 1996 Molecular characterization of cyclic and obligate parthenogens in the aphid *Rhopalosiphum padi* (L). *Proc. R. Soc. Lond.* B **263**, 481–486.

Sokal, R. R. & Rohlf, F. J. 1991 *Biometry*, 3rd edn. New York: W. H. Freeman.

Swofford, D. L. 1999 *PAUP** phylogenetic analysis using parsimony (**and other methods*), v. 4. Sunderland, MA: Sinauer Associates.

Thompson, J. N. 1999 Specific hypotheses on the geographic mosaic of coevolution. *Am. Nat.* **153**, S1–S14.

Valdes, A. M. & Pinero, D. 1992 Phylogenetic estimation of plasmid exchange in bacteria. *Evolution* **46**, 641–656.

Wernegreen, J. J. & Riley, M. A. 1999 Comparison of the evolutionary dynamics of symbiotic and housekeeping loci: a case for the genetic coherence of rhizobial lineages. *Mol. Biol. Evol.* **16**, 98–113.

Werren, J. H. 1997 Biology of *Wolbachia*. *A. Rev. Entomol.* **42**, 587–609.