Evolution, 56(9), 2002, pp. 1821–1830

EVOLUTIONARY SHIFTS BETWEEN HOST OAK SECTIONS AND HOST-PLANT ORGANS IN *ANDRICUS* GALLWASPS

JAMES M. COOK,[1] ANTONIS ROKAS,[2,5] MARK PAGEL,[4] AND GRAHAM N. STONE[2,3]
[1]*Department of Biological Sciences, Imperial College, Silwood Park, Ascot, Berkshire SL5 7PY, United Kingdom*
E-mail: j.cook@ic.ac.uk
[2]*Institute of Cell, Animal and Population Biology, University of Edinburgh, Ashworth Laboratories, The King's Buildings, West Mains Road, Edinburgh EH9 3JT, United Kingdom*
[3]*E-mail: graham.stone@ed.ac.uk*
[4]*School of Animal and Microbial Sciences, University of Reading, Reading RG6 6AJ, United Kingdom*
E-mail: m.pagel@reading.ac.uk
[5]*Laboratory of Molecular Biology, R.M. Bock Laboratories, 1525 Linden Drive, University of Wisconsin, Madison, Wisconsin 53706*
E-mail: arokas@facstaff.wisc.edu

Abstract.—Gall-inducing insects have especially intimate interactions with their host plants and generally show great specificity with regard to both the host-plant species and the organ (e.g. flower, leaf) galled. However, the relative roles of shifts between host species and between host-plant organs in the diversification of gall-inducers are uncertain. We employ a novel and general maximum-likelihood approach to show that shifts between host-plant organs occur at a significantly greater rate than shifts between host oak sections in European *Andricus* gallwasps. This suggests that speciation has more often been associated with gall location shifts than with colonization of new host-plant species, and implies that it may be easier for gall-inducers to colonize new plant organs than new plant species.

Andricus gallwasps have complex life cycles, with obligate alternation of sexual and parthenogenetic generations. Our phylogenetic analyses show that a life cycle with both generations galling white oaks (section *Quercus*) is ancestral, with a single shift of the sexual generation onto black oaks (section *Cerris*) to generate a clade with a novel host-alternating life cycle. This new life cycle provided the opportunity for further speciation, but may have also increased the risk of extinction of one or both generations by the demographic requirement for co-existence of both host-plant groups. In summary, it appears that *Andricus* gallwasp radiation may be a two-level process. Speciation events often involve shifts in gall location on the same host species. However, there are only so many ways to gall an oak, and rare shifts to new oak sections may contribute greatly to long-term diversification by opening up whole new adaptive zones.

Key words.—Bayesian, Cynipidae, gall, host plant, maximum likelihood, oak, phylogeny.

Received October 9, 2001. Accepted June 21, 2002.

Insects that feed on plants contribute greatly to terrestrial biodiversity, but the role of host plants in herbivore diversification is controversial. Shifts to new host plants, or new feeding locations on existing host plants, may allow incipient insect species to escape from direct competition for resources, or to enjoy the benefits of enemy-free space (Gross and Price 1988; Lill et al. 2002). However, such shifts are likely to be constrained by factors such as plant chemistry, architecture, phenology, and genetics (Jaenike 1990; Becerra 1997). In order to establish patterns of insect radiation and the relative importance of different constraints we need to combine insect phylogenies with analysis of pertinent ecological traits.

Insect herbivory comes in many forms, of which the most intimate links gall inducers and their host plants (Cornell 1983; Price et al. 1987; Stone et al. 2002). Gall inducers are found in a wide range of insect families, and several taxa (such as the cynipid gallwasps, the cecidomyiid gallmidges, and the pemphigid aphids) contain many gall-inducing species. Gall inducers cause host-plant tissues to differentiate into novel structures, ranging from endospermlike inner nutritive tissue to woody outer protective tissues (Shorthouse and Rohfritsch 1992; Crespi et al. 1997; Nyman et al. 1998; Stone and Cook 1998; Schönrogge et al. 2000; Ronquist and Liljeblad 2001), and commonly show two types of specificity. First, most are specific to a particular taxon (species, or group of related species) of host plants (Cornell 1985, 1986; Crespi et al. 1997; Csóka 1997; Abrahamson et al. 1998; Melika et al. 2000; Nyman et al. 2000; Ronquist and Liljeblad 2001). Second, they commonly specialize in galling just one plant organ (e.g., leaves or buds) (Weis et al. 1988; Shorthouse and Rohfritsch 1992; Csóka 1997; Abrahamson et al. 1998; Melika et al. 2000).

Gall inducers can colonize new niches by switching to new host-plant species, or to new locations (organs) on the same host-plant species, but which is easier and therefore more likely to be involved in the initiation of new species? In principle, this should be predictable from an understanding of the gall induction process. However, while understanding is advancing in a few systems (Weis and Abrahamson 1986; Wood and Payne 1988; Fay and Hartnett 1991; Zantoko and Shukle 1997, 1999; Brooks and Shorthouse 1998), too little is known about the physiological basis of tissue and host specificity to make any general predictions. If a gall inducer is reliant on the results of tissue-specific gene expression (e.g., endosperm in seeds), it may be easier to switch host species than to shift between different organs on the same plant species. In contrast, if gall success is dependent on specific genetic loci in gall inducer and host (as it is in the Hessian fly; Ratcliffe et al. 1994, 2000; Formusoh et al. 1996; Zantoko and Shukle 1997, 1999), it may be easier to shift between plant organs than between plant species. In addition, it is not known whether host-plant or organ specificity gen-

erally results from a precise physiological compatibility only with certain plant tissues, from egg-laying preferences of the gall inducer, or from a combination of both (Whitham 1978; Abrahamson and Weis 1997; Stone et al. 2001).

While the gall induction process is not sufficiently understood to permit a clear prediction about the relative difficulty of shifts between host-plant species versus shifts between host-plant organs, the historical pattern can be revealed using phylogenies. We employ this approach here to study oak gallwasps in the genus *Andricus* (Hymenoptera; Cynipidae; Cynipini), and make the first statistical comparison of rates of change in gall location and host plant in any gall-inducing taxon. Although a recent study of the higher taxa (genera and tribes) of gallwasps revealed few changes in host-plant family or gall location (Ronquist and Liljeblad 2001), the oak gallwasps appeared only as a single terminal taxon associated with the plant family Fagaceae. However, with approximately 1000 species the Cynipini represents about 80% of cynipid species richness (Liljeblad and Ronquist 1998; Ronquist and Liljeblad 2001; Stone et al. 2002), and shows considerable diversity in gall induction site and host association. Within the Cynipini, *Andricus* is by far the largest and most ecologically diverse genus (approximately 100 species in Europe alone; Askew 1984). Consequently, we focus here on the importance of shifts between plant taxa and plant organs in the radiation of this genus. We use mitochondrial (cytochrome *b*) and nuclear (long-wavelength rhodopsin) DNA sequences to estimate the phylogeny of 34 European cynipids, including 32 *Andricus* species. We then map the host oak section and gall location onto the phylogeny and ask: (1) How many times have the sexual generations of *Andricus* gallwasps shifted between white and black oaks? (2) How often have *Andricus* species shifted between alternative oak organs? and (3) Is the rate of change in one trait significantly greater than that in the other trait? In order to compare rates of change, we employ a maximum-likelihood (ML) approach that should be widely applicable to evolutionary problems requiring rate comparisons (Pagel 1999a).

Finally, we also use our results to consider the evolution of complex life cycles. *Andricus* species are cyclical parthenogens with obligate alternation of sexual and parthenogenetic generations and we discuss both the opportunities offered and limitations imposed by this life cycle.

Biology of *Andricus* Gallwasps

Andricus shows considerable diversity in both gall location and host-plant use (Nieves-Aldrey 1987; Cook et al. 1998; Melika et al. 2000), as well as dramatic variation in gall form (Stone and Cook 1998). *Andricus* lifecycles primitively involve two generations each year—a sexual one in the spring, and a parthenogenetic one in the summer/autumn (Askew 1984; Cook et al. 1998; Stone et al. 2001)—and galls of a given generation are specific to a particular taxon of oaks. *Andricus* species show great host-specificity, but it is at the level of host species groups, rather than individual host species. All *Andricus* host plants belong to the subgenus *Quercus* and this is divided into four sections (Manos et al. 1999). Only two (the white oaks of section *Quercus* sensu stricto and the black oaks of section *Cerris*) are present in Europe, with 21 and ten species respectively (Camus 1936, 1938). All known parthenogenetic generations of European *Andricus* species attack white oaks (e.g., *Q. pubescens* and *Q. robur*). In contrast, their sexual generations gall either white or black oaks (e.g., *Q. cerris* and *Q. suber*), but never both. These generalizations also hold for the many *Andricus* species that are currently known from either only a sexual or only a parthenogenetic generation (Nieves-Aldrey 1987; Melika et al. 2000). Similar specificity is seen in North American *Andricus* species, which gall either white oaks or red oaks (section *Lobatae*) but not both (Cornell 1985, 1986; Abrahamson et al. 1998).

Each generation of each *Andricus* species is also specific with regard to the oak organ galled, and with very few exceptions attacks only one of buds, shoots, roots, leaves, catkins, or acorns. Sexual generations of most *Andricus* species gall buds or catkins, with a few galling stems. Parthenogenetic generations also mostly gall buds, with few on acorns, and very few species attacking catkins or roots. Few European *Andricus* species gall leaves in either generation, although some North American *Andricus* species do (Weld 1957, 1959, 1960).

Materials and Methods

Study Species

We include data for 32 European *Andricus* species and two outgroups (*Biorhiza pallida* and *Cynips quercus*) from closely related cynipid genera. The collection locations and life stages used in DNA extraction for 23 *Andricus* species and the two outgroup taxa are listed in Table 1 of Stone and Cook (1998). The data for the additional species included in this study are displayed in Figure 1. Since the study of Stone and Cook (1998), the names of two species have been revised by Melika et al. (2000): *A. malpighii* was formerly known as *A. nudus*, and *A. dentimitratus* replaces *A. viscosus*.

Host Plants and Gall Location

The database on host oak affiliation and plant organs galled was assembled from our own extensive collections of cynipid galls throughout Europe and Asia Minor during the last ten years, with additional information from the literature (Houard 1912; Buhr 1965; Sternlicht 1968; Ambrus 1974; Chodjai 1980; Nieves-Aldrey 1987; Pujade-Villar 1994; Melika et al. 2000). The host records and organs attacked by nearly all the species in this study are cataloged by Melika et al. (2000).

Our sample of *Andricus* species includes multiple representatives of each type of life cycle, host-plant affiliation and gall location, such that inferences of monophyly and trait changes are not unduly affected by unbalanced taxon sampling. We included: (1) five species known only from a sexual generation on black oaks, (2) thirteen species known only from an asexual generation on white oaks, (3) seven species with alternating sexual and parthenogenetic generations, both on white oaks, and (4) six species with alternating sexual and parthenogenetic generations on black oaks and white oaks respectively. Those known from only one generation are hereafter referred to as ''sexual generation only'' and ''parthenogenetic generation only'' species.

Molecular Methods

For each species DNA was extracted from a single adult wasp as described in Stone and Cook (1998), or by using the DNeasy Tissue kit (Qiagen, Crawley, U.K., cat. 69504) and following the manufacturer's protocol. Sequencing used Perkin-Elmer (Cambridge, U.K.) BigDye Terminator chemistry and an ABI 377 sequencer (Applied Biosystems, Freiburg, Germany). For both genes, PCR products were sequenced fully in both directions to minimize PCR artifacts, ambiguities, and base-calling errors, and the sequences were aligned by eye.

A 433-base pair fragment of the mitochondrial cytochrome *b* (cyt*b*) gene was amplified using the CB1/CB2 primer combination (Stone and Cook 1998; Rokas et al. 2001; Stone et al. 2001). Polymerase chain reactions (PCRs) were performed in 25 μl volumes, comprising 1 μl of DNA sample, 2.5 μl of 10x PARR Buffer (Hybaid, Ulm, Germany), 2 μl of $MgCl_2$ (25 mM), 0.5 μl of dNTPs (10 mM), 0.35 μl of each primer (20 mM), 0.25 μl of Taq (Promega, Southampton, U.K.) and 19.05 μl of distilled, deionized H_2O. The PCR program used was one step at 94°C for 2 minutes, followed by 35 cycles of 94°C for 30 sec, 50°C for 60 sec, 72°C for 2 min and a final extension step at 72°C for 10 min. The total volume of three PCR reactions for each individual wasp was then electrophoresed on a 1% agarose gel. The bands were cut from the gel and cleaned with the QIAQuick Gel Extraction kit (Qiagen, cat. 28704) before sequencing. All sequences were 433 bases long, with full open reading frames, and are deposited in GenBank (accession numbers AJ228448–AJ228454, AJ228456, AJ228458–AJ228461, AJ228463–AJ228472, AJ228474–AJ225475, AJ228478, AJ228481, AJ131065–AJ131068, AF481704–AF481708).

A 588–590 base pair fragment of the nuclear gene long wavelength rhodopsin (LW*Rh*) was amplified by PCR using the LW*Rh*F/LW*Rh*R primer combination (Mardulyn and Cameron 1999; Rokas et al. 2002). Polymerase chain reaction reagent volumes and cycling conditions were as for cyt*b* above, apart from a higher annealing temperature of 58°C. Polymerase chain reaction products were cloned using the TOPO TA Cloning kit (Invitrogen, Paisley, U.K., cat. 4500–01) and 2–4 clones from each specimen were sequenced. Plasmid DNA was isolated using the QIAprep Spin Miniprep kit (Qiagen, cat. 27104). All sequences are deposited in GenBank (accession numbers AF481709–AF481729), and the alignments and consensus trees from both datasets are available from TreeBASE (http://www.herbaria.harvard.edu/treebase).

Phylogenetic Analyses

We used a Bayesian Markov chain Monte Carlo (MCMC) analysis to estimate phylogenetic trees and the posterior probabilities of nodes in the trees (Larget and Simon 1999, Huelsenbeck et al. 2001, Pagel and Lutzoni 2002). The posterior probability (PP) of a node gives the probability that the node is true, conditional upon the data and the model of sequence evolution, and thus gives a direct indication of confidence in that node (Huelsenbeck et al. 2001, Lutzoni et al. 2001). The MCMC program is available from M. Pagel (School of Animal and Microbial Sciences, University of Reading, Reading, U.K.) by request.

We estimated phylogenies separately for cyt*b* and LW*Rh*, because 14 species in the cyt*b* dataset do not appear in the LW*Rh* dataset. For each gene, we used MCMC procedures to generate a population of two million trees and then sampled two independent replicates of 2000 trees to estimate the PPs of nodes. The MCMC procedure samples trees from the total ''population'' according to their probability of occurrence under the specified model of molecular evolution, while the two independent runs provide an indication of the variability between replicates. Tree sampling was delayed until after the first 100,000 trees to avoid sampling before Markov chain convergence (e.g., Lutzoni et al. 2001).

The model of sequence evolution with the best fit to the data was identified (for each gene) in two ways. First, we used likelihood-ratio (LR) tests (Huelsenbeck and Rannala 1997) in Modeltest 3.0 (Posada and Crandall 1998); and second, we compared convergence times and average likelihood values of different models (HKY, GTR) in trial runs of the MCMC program. Both methods identified the HKY model with gamma rate heterogeneity among sites and empirical base frequencies as most appropriate for both genes.

In order to compare the phylogenetic congruence of the two genes, we performed a partition homogeneity test (PTP; Swofford 1998), also known as an incongruence length difference test (ILD; Farris et al. 1994), in PAUP*4.0 (with TBR and 1000 replicates) for the 20 species that were sequenced for both genes.

Host Plant Association and Gall Location in the Sexual Generation

We mapped host-plant and gall location for the sexual generation onto a phylogeny and estimated ancestral states, using maximum likelihood (ML) incorporated in the computer programs Discrete (Pagel 1994, 1999b) and Multistate (M. Pagel). Both use a continuous time Markov model of character evolution but, whereas Discrete can only analyze binary characters, Multistate can accommodate multiple states. Gall location (three states) and host oak section (two states) were both treated as unordered characters. An advantage of ML is that it allows assessment of uncertainty in ancestral trait reconstruction. By successively fixing a given node at each of the possible states and then comparing the respective log likelihoods, one can estimate the probability that the node had each of the possible states, rather than just making the qualitative statement that a particular state is most likely, as in parsimony reconstructions (Pagel 1994, 1999b; Schluter 2000). Both Discrete and Multistate are available from M. Pagel by request.

Discrete and Multistate can use branch lengths in their calculations, but require fully bifurcating trees with no missing data. In order to include as many species as possible we used the larger cyt*b* phylogenies. Species with ''missing'' sexual generations, as well as the two outgroups (which have states not found in the ingroup), were pruned from both cyt*b* consensus cladograms, leaving 18 species for analysis. We then reconstructed the ML branch lengths in PAUP, using the HKY model with gamma rate variation and empirical

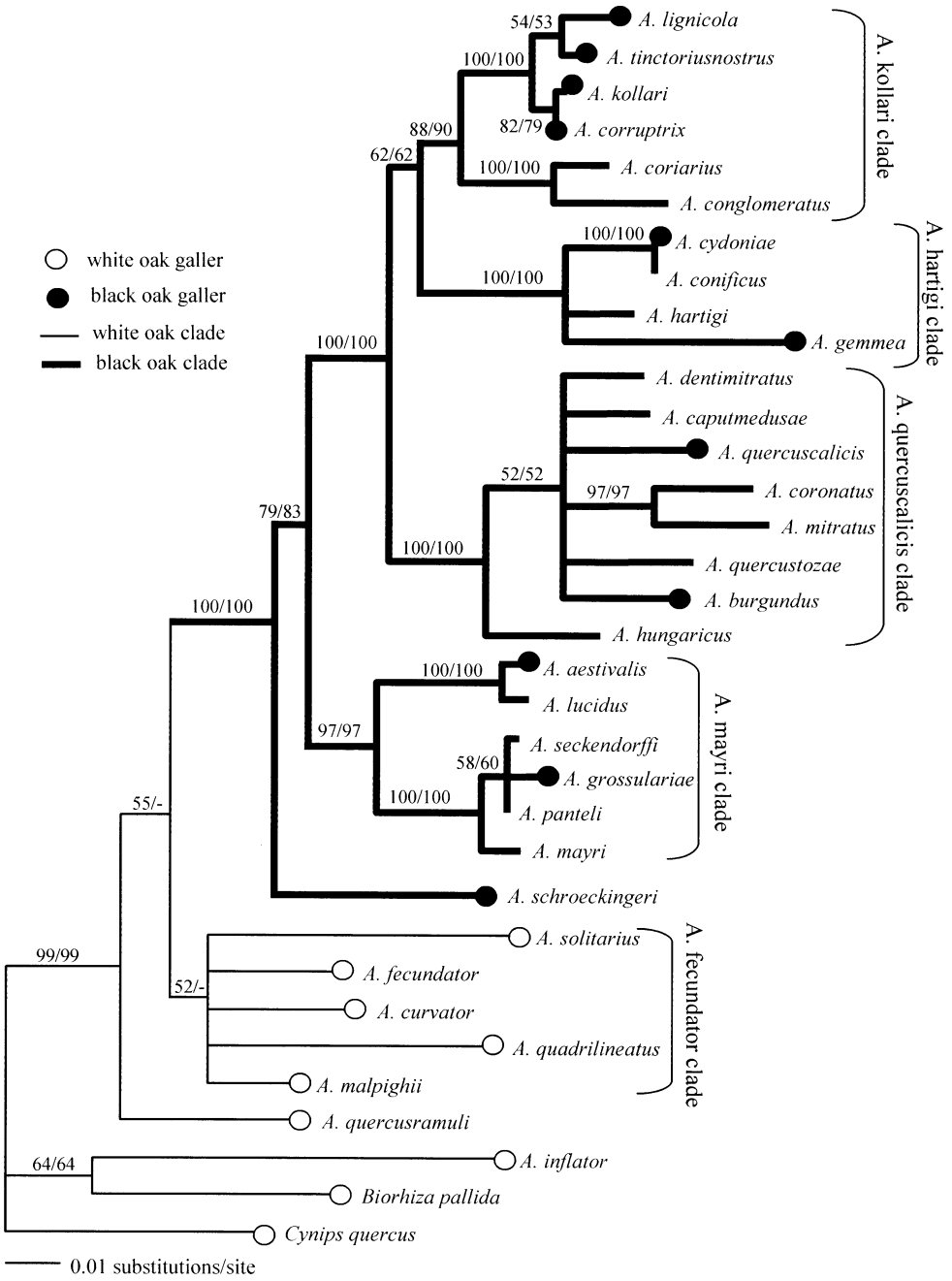

FIG. 1. The 50% majority rule consensus phylogram from Markov chain Monte Carlo (MCMC) analyses of cyt*b* sequences. Numbers show posterior probabilities (where >50%) of nodes for MCMC1/MCMC2. Host plant is shown for species with a known sexual generation. Species sequenced for the first time for this study are as follows (with collection locality): *Andricus aestivalis* (Tatabanya, Hungary), *A. cydoniae* (Mátrafüred, Hungary), *A. malpighii* (Bibulano, Italy), *A. mitratus* (Bebesli, Turkey), *A. panteli* (Poppio, Italy), *A. quadrilineatus* (Oxford, U.K.), *A. quercusramuli* (Mátrafüred, Hungary), *A. schroeckingeri* (Jászberény, Hungary), *A. tinctoriusnostrus* (Madenli, Turkey).

base frequencies. The two analyses yielded essentially the same tree (identical topology and very minor branch length differences), which is not surprising since the only difference between the two MCMC consensus trees is that one provides weak support for splitting a six-way polytomy into a pair of species and a four-way polytomy (Figure 1). Consequently, we only present results from analysis of the first tree. After ML branch length estimation, this still had a single three-way polytomy, which was resolved randomly using TreeEdit (http://evolve.zoo.ox.ac.uk/software/TreeEdit/main.html) and assigned a branch length of zero.

Comparing Rates of Change in Host Plant and Gall Location

We employed a ML approach, using Discrete and Multistate, to test whether the rate of change in gall location was significantly different from the rate of change in host oak use

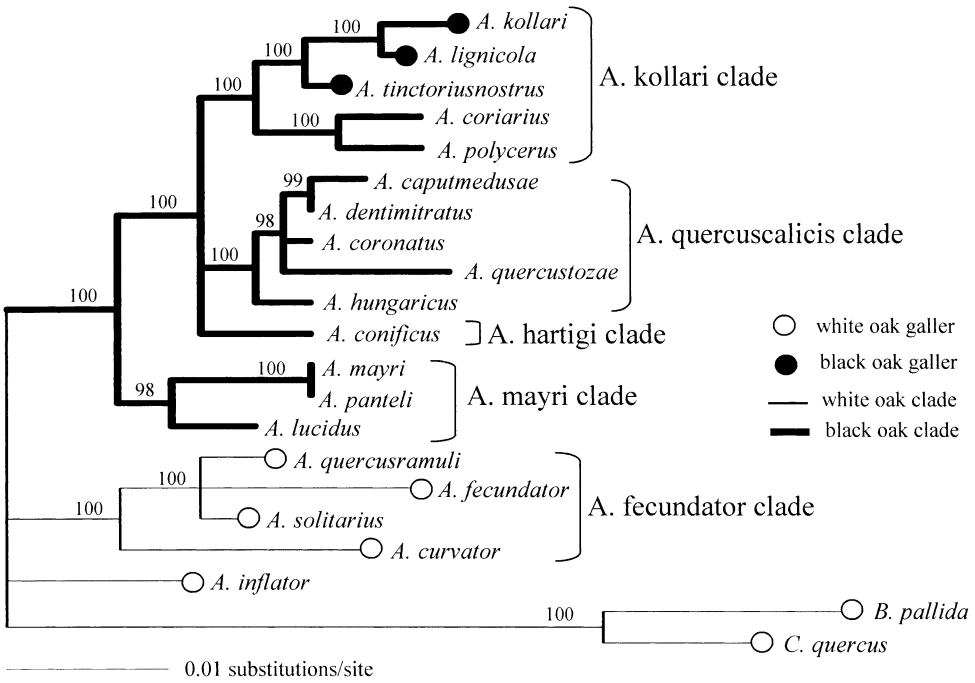

FIG. 2. The 50% majority rule consensus phylogram from Markov chain Monte Carlo (MCMC) analyses of LW*Rh* data. Numbers show posterior probabilities (where >50%) of nodes. These values were identical for MCMC1 and MCMC2. Host plant is shown for species with a known sexual generation.

in the sexual generation. Essentially, this involved estimating rates of change for each character from its own dataset and then substituting the overall location change rate parameter into the host-plant analysis and testing whether this made a significant difference in the fit of the model to the data. This approach is a general one and could be used for testing many evolutionary hypotheses requiring rate comparisons.

Host plant was treated as an unordered character with two states (white = 0, black = 1) and, therefore, two possible types of change (black to white and white to black). Initially we fitted a simple model (A) and estimated these two rate parameters. However, in this study we focus on comparing overall rates of change, so we then simplified this to model B with just one parameter (q), describing the overall rate of change in host plant use.

Gall location was treated as an unordered character with three states (bud = 0, catkin = 1 leaf = 2). We first fitted the full model (C) with six rate parameters, representing all possible changes between the three character states, and then a simplified model (D) with just one parameter (q), describing the overall rate of change. Finally, we tested whether there was a significant difference in the overall rates of change of gall location and host plant by fitting model E, which used the gall location data but the host plant rate parameter.

When simplifying model A to model B, we tested significance by comparing the change in likelihood to critical values of the χ^2 distribution with 1 df. The same test was used for simplification of model C to model D but with 5 df. However, when comparing models D (one location rate parameter) and E (one host rate parameter), the models are not nested, so we instead applied the general rule of thumb that two log likelihood units constitutes a significant difference (Edwards 1972; Pagel 1999b).

RESULTS

Phylogenies

Figures 1 and 2 show the 50% majority-rule consensus trees from the MCMC analyses of cyt*b* and LW*Rh* data, respectively. Although the PTP test suggested a significant conflict between the two data partitions ($P = 0.03$), the only substantial difference between the two phylogenies is that *A. inflator* clusters with the outgroups in cyt*b* trees but as the basal member of the *Andricus* clade in LW*Rh* trees. Even in this case, the cyt*b* placement is due to a node with relatively low support (PP = 64%) and may be attributable to long branch attraction (see Fig. 1). This reflects a general pattern that cyt*b* resolves intermediate nodes and tips quite well, but provides less resolution at deeper nodes. In contrast, the LW*Rh* trees resolve basal *Andricus* relationships (e.g., the *A. fecundator* clade) rather well (see Figs. 1 and 2). These differences are probably due to the faster rate of evolution, larger number of taxa, and smaller length of sequence in the cyt*b* dataset (Rokas et al. 2002). Nevertheless, we emphasize that no relationship supported strongly by one gene is contradicted by the other.

The two independent samples of 2000 trees from the MCMC analysis yielded identical consensus trees for LW*Rh*. The two cyt*b* consensus trees are also entirely consistent with each other, although tree two has a six-way polytomy that tree one splits into two clades with weak support (PP of 52% and 55%; see Fig. 1 for details). We label five clades of

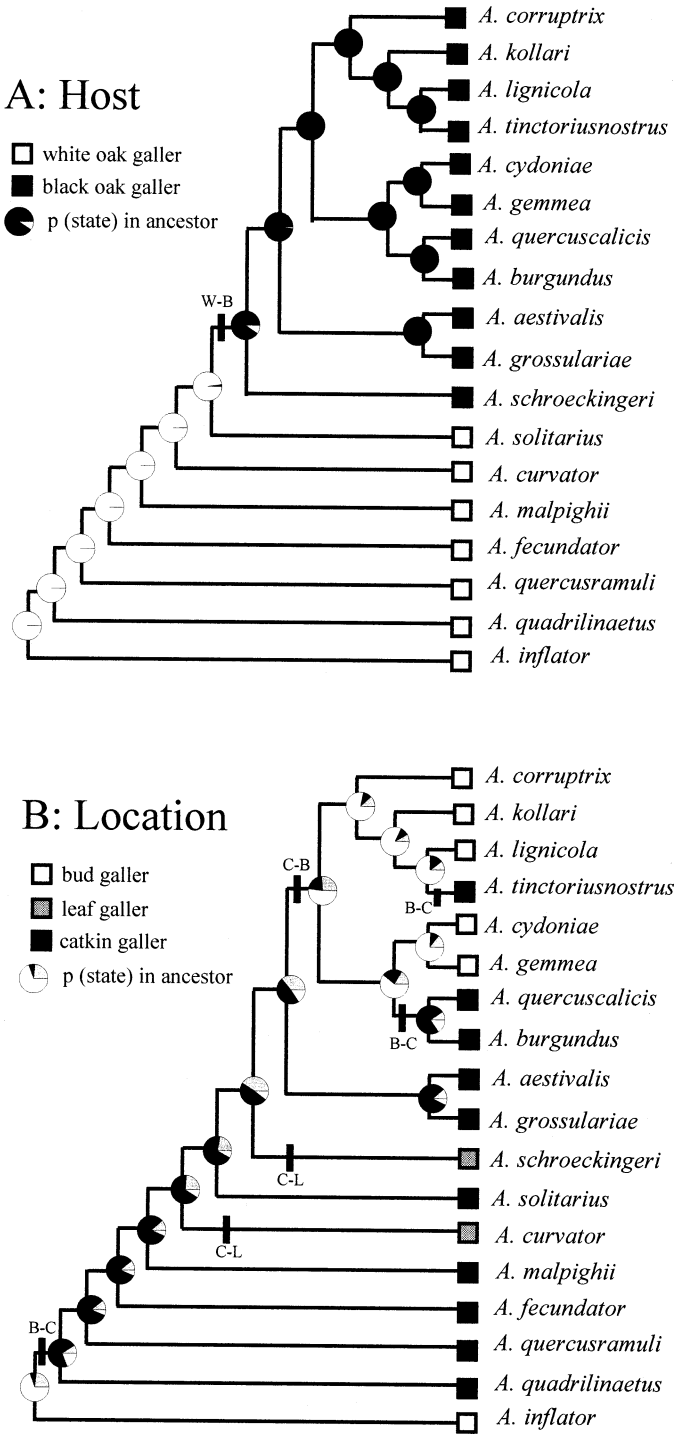

FIG. 3. (A) Host: extant and ancestral host oak associations for the sexual generation. W-B indicates the host shift from white to black oaks. (B) Location: extant and ancestral gall locations for the sexual generation. Bars indicate a possible history of changes in gall location, with B-C, for example, representing a bud to catkin shift.

Andricus species in Figures 1 and 2. These are the same clades recognized by Stone and Cook (1998), but they now contain more species.

We report in detail results of the Bayesian phylogenetic analysis. However, tree topologies were identical or very nearly so, and the same clades received weak and strong support, when standard ML phylogeny reconstruction and bootstrapping were used.

TABLE 1. Results of maximum likelihood investigation of rates of change of host plant and gall location in the sexual generation (see Figs. 1 and 3).

Model	Character	Parameters	Source	Rate (q)	Likelihood
A	Host	two	A	n/a	7.05764
B	Host	one	B	0.09633	7.92913
C	Location	six	C	n/a	14.16667
D	Location	one	D	0.38691	17.01200
E	Location	one	From B	0.09633	20.17897

Phylogenetic Patterns in Host Plant Association and Gall Location

The most striking result is that all *Andricus* species whose sexual generations induce galls on black oaks lie in a derived clade (Figs. 1, 2), whose monophyly has PP = 100% in both sequence datasets. This strongly suggests that there has been only a single shift to black oaks, and that a life cycle in which sexual generations gall white oaks is the ancestral state. This is supported further by ML reconstruction of ancestral hosts using Discrete (Fig. 3A).

Our ML reconstruction suggests six changes in gall location in the sexual generation (Fig. 3B). Most extant species gall buds or catkins and changes in both directions are implied. In addition, there were probably independent shifts from catkins to leaves in *A. curvator* and *A. schroeckingeri*. However, in contrast to host plant, there are many ancestral nodes without overwhelming support for a particular gall location. Since phylogenetic uncertainty will also have more impact on the location reconstructions, one should not yet draw any strong inferences about the exact number and sequence of changes.

Comparing Rates of Change in Host Plant and Gall Location

Initial comparison of estimates of overall rates of change (q) in models B and D suggests that gall location changes more often than host plant (Table 1). Since neither of the simplified one-parameter models was significantly worse than their respective full models (host plant LR = 0.87, alpha = 0.05, critical value for χ^2 with 1 df = 3.841; location LR = 2.84, critical χ^2 with 5 df = 11.07), we were able to compare overall rates of change in host plant and gall location.

When we substituted the host plant rate parameter (q) into the location shift analysis (model E), the likelihood was significantly worse (3.17 log units) than model D, which uses q derived from the location data. This is equivalent to stating that the 95% confidence intervals of the faster (location) rate do not include the slower (host) rate. The results were qualitatively identical, and quantitatively extremely similar, when the same analyses were performed using the MCMC2 phylogeny. In summary, changes in gall location have been sig-

nificantly more frequent than changes in host oak section during the radiation of European *Andricus* species.

A New Life-Cycle Pair?

Two insects previously regarded as separate species—the sexual only *A. cydoniae* and the parthenogenetic only *A. conificus* yielded identical DNA sequences for the cyt*b* fragment. Both ''species'' are quite rare but have similar geographic distributions and, although further studies are required, it seems likely that they are alternate generations of a single species.

Discussion

Host Shifts, Gall Location Shifts, and Gallwasp Speciation

Our results show that the rate of change in gall location has been greater than the rate of change in host oak section during the radiation of *Andricus* gallwasps in Europe. Moreover, there has been just one change in host plant from an ancestral use of white oaks to a derived use of black oaks by the sexual generation (Fig. 3A). In contrast, the organ galled by the sexual generation of *Andricus* has changed six times, with some reversions (Fig. 3B). This shows that *Andricus* speciation has been associated with shifts in gall location more often than with shifts in host oak section, suggesting that the gall induction process is less specific to particular oak tissues than to the identity of the host oak section.

Field data are consistent with this phylogenetic pattern. First, there is no substantiated case of one generation of a cynipid species galling hosts in two oak sections, but there are a few cases of gall location polymorphism. For example, sexual generation galls of *Neuroterus quercusbaccarum* are commonly found on either leaves or catkins, while *A. curvator* sexual generation galls occur on leaves or (very rarely) buds, and *A. lucidus* is able to induce parthenogenetic generation galls on buds or (very rarely) acorns. Gall success depends on two potentially constrained processes—oviposition site choice by the mother, and gall induction by the larva (Stone et al. 2002). Folliot (1964) showed that females of some *Andricus* species caged on an ''unsuitable'' oak organ of the correct host would eventually oviposit and that their larvae could, sometimes, induce galls. This suggests that location shifts are constrained primarily by oviposition behavior. However, ovipositions on the correct organ of an inappropriate oak were extremely rare, and no galls were ever induced. This suggests that host shifts are probably constrained primarily by the specificity of larval gall induction. In gall midges, host susceptibility shows a gene-for-gene correspondence with midge virulence (Ratcliffe et al. 1994; Zantoko and Shukle 1997, 1999; Ratcliffe et al. 2000), and similar specific interactions might apply in gallwasps.

The fact that gall location changes are observed within *Andricus* clades that gall a given host oak section suggests that speciation might be driven by ecological factors operating at the scale of individual host plants, such as direct competition for oviposition sites (e.g., in *A. quercuscalicis*; Hails and Crawley 1991; Atkinson et al. 2002), or indirect competition for enemy-free space (Stone et al. 2002).

If maternal choice of oviposition site is generally less constrained than larval ability to induce a gall on a given host, we would expect to see patterns similar to *Andricus* in other gall-inducing taxa. Host shifts appear rare in several taxa, including gall-inducing thrips (Crespi and Worobey 1998), aphids (Stern 1995), and higher cynipid taxa (Ronquist and Liljeblad 2001), but are far more frequent in gall-inducing sawflies (Nyman et al. 1998, 2000). However, the relevant rate comparisons have yet to be made.

Phylogenetic Conservation of Host Plant Use

In one of our study species, *A. dentimitratus*, the sexual generation insect is known, but not the gall. Based on its phylogenetic position (Fig. 1), we predict its host to be a black oak. We make the same prediction, for the same reason, for several ''parthenogenetic only'' species (*A. caputmedusae*, *A. coriarius*, *A. lucidus*, *A. panteli*, *A. quercustozae*, and *A. seckendorffi*) in which recent population genetic analyses suggest a cryptic sexual generation (Atkinson 2000; Stone et al. 2001; Atkinson et al. 2002).

The rarity of host shifts in gallwasps in general is supported by the observation that most genera (all but *Andricus* and *Callirhytis*) of oak cynipids with alternating generations use the same oak section in both generations (Weld 1957, 1959, 1960; Folliot 1964; Askew 1984; Nieves-Aldrey 1987; Melika et al. 2000). This is also true for the Pediaspidini, the sister group of the Cynipini (Folliot 1964; Melika et al. 2000). We therefore predict that host transitions between white and red oaks in the American oak cynipid fauna have also been rare.

Fossil evidence suggests that the major groups of oaks were established by 40 million years ago and molecular data support an early origin of the black oaks (Manos et al. 1999) in Eurasia. Thus, if *Andricus* originated on white oaks (the only Holarctic section) in North America (the center of gallwasp diversity; Askew 1984; Stone et al. 2002) and then spread to Europe, black oaks would already have been available to be colonized.

Gall induction on black oaks must also have evolved independently in other oak cynipids. Within the Cynipini, *Aphelonyx*, *Chilaspis*, and *Plagiotrochus* are endemic Palaearctic genera reliably known only from black oaks (Houard 1912; Buhr 1965; Sternlicht 1968; Ambrus 1974; Chodjai 1980; Nieves-Aldrey 1987; Pujade-Villar 1994; Melika et al. 2000). Meanwhile, three other genera (*Callirhytis*, *Dryocosmus*, and *Neuroterus*) with black oak galling representatives, are holarctic and gall other oak sections in North America. However, inference of the number and pattern of host shifts must await more extensive phylogenetic analyses of the Cynipini. Finally, another independent colonization of black oaks is represented by the genus *Synophrus*, which lies within the predominantly inquiline tribe Synergini, and is only distantly related to the Cynipini (Ronquist 1994, 1995; Liljeblad and Ronquist 1998).

The Evolution of Complex Life Cycles

Complex life cycles present evolutionary opportunities, but also impose constraints. No parthenogenetic *Andricus* gall is able to develop on black oaks, despite the fact that the sexual generations of some species possess the necessary traits. In-

terestingly, the opposite pattern is seen in European *Callirhytis* species, in which the parthenogenetic generation galls develop only within the acorns of black oaks, while the sexual generation galls develop under the bark of white oaks (Nieves-Aldrey 1987, 1992). Other traits of gallwasps, including gall structure and location, also show different patterns of evolutionary change in sexual and parthenogenetic generations (Stone and Cook 1998). Taken together, these patterns suggest that the evolutionary trajectories of the two generations are partly independent, a pattern that may apply generally to the evolution of components of complex lifecycles (Moran 1994). The two generations of a single *Andricus* species can be exposed to very different selective pressures, particularly in terms of host abundance and phenology, as well as attack by natural enemies that commonly inflict very high mortality (Stone et al. 2002). From an evolutionary perspective, independent generations may permit escape from limiting competition or natural enemies in one generation, without change in the other generation.

A striking feature of the host-alternating clade of *Andricus* is that it contains all of the sampled species known only from one generation (sexual or parthenogenetic). These species either possess cryptic generations, or have lost them secondarily. While facultative loss of the sexual generation has been demonstrated for *A. quadrilineatus* (Folliot 1964) and obligate loss in a Japanese *Andricus* species complex (Abe 1986), the frequency of genuine lifecycle simplification remains unknown (Stone et al. 2002). Nevertheless, the possibility of a link between use of black oaks by the sexual generation and loss of the same generation remains intriguing.

The single major clade of host-alternating *Andricus* species implies extensive speciation following the evolution of the host-alternating life cycle. This is in strong contrast with aphids, in which host-alternating lineages have arisen frequently, but often represent evolutionary dead ends (Moran 1988, 1994; von Dohlen and Moran 2000). This contrast may result from differences in the challenge represented by a host shift in the two taxa, and the relative success of populations on a new host.

Some aphids show strong fitness trade-offs (in host plant performance) associated with maintaining host alternation (Moran 1991) and this alone might favor collapse to a single host life cycle. Demographic considerations suggest a second possible reason for gallwasp life cycle simplification—populations of host-alternating *Andricus* species can persist only where suitable white and black oaks grow together. This requirement generates severe geographic range restrictions, which can be revealed dramatically when the distribution of one or both hosts is increased by human activity (Hails and Crawley 1991; Stone and Sunnucks 1993, Sunnucks and Stone 1996; Atkinson 2000; Rokas 2001; Rokas et al. 2001; Stone et al. 2001). Using the very general approximation that 2.3% sequence divergence in mitochondrial DNA indicates one million years since separation (Brower 1994), the host-alternating *Andricus* clade has been diversifying for about 10 million years (Stone and Cook 1998). Over this time scale, the distributions of oak species in Europe have varied enormously with the advance and retreat of Quaternary ice sheets (Huntley and Birks 1983; Bennett 1986; Toumi and Lumaret 1998; Hewitt 1999, 2000). Significantly for host alternators, patterns of northward range expansion also differ substantially between oak taxa, such that areas in which black oaks and white oaks exist together are both relatively restricted in area and unstable in time. This demographic constraint may have selected for repeated life cycle simplification.

Conclusions

Shifts to new feeding niches are probably pivotal to the diversification of phytophagous insects. These shifts can be new ways of feeding from the same plant species, or colonizations of novel host species. In *Andricus* gallwasps, a two-level process may have operated. Shifts to new host oak sections have been rare, but they have offered the opportunity for the evolution of a large, new, endemic European gallwasp community on black oaks. Shifts to new gall locations have been significantly more frequent, such that closely related species often (though by no means always) gall different plant organs. These location shifts have permitted radiation of *Andricus* gallwasps to exploit many feeding niches on the same host plant species.

Acknowledgments

We thank B. Crespi, J. Liljeblad, F. Ronquist, K. Schönrogge and two anonymous referees for valuable comments on earlier versions of this manuscript, and J. Nylander for sharing unpublished data and LW*Rh* PCR conditions. We are also grateful to G. Csóka, R. Folliot, G. Melika, J.-L. Nieves-Aldrey, J. Pujade-Villar and J-Y. Rasplus for their hospitality, help during fieldwork, and samples of rare species. This work was supported by grants from the Natural Environment Research Council (NERC) to JMC and GNS (GST/03/2035 and GR12847), the Royal Society to GNS (grant number 574006), and the Leverhulme Trust (F239/AG) and Biology and Biotechnology Research Council (45/G14980) to MP. JMC is supported by a NERC Advanced Fellowship. AR was partially supported by a NERC postgraduate studentship and is currently supported by a Howard Hughes Medical Institute Postdoctoral Fellowship.

Literature Cited

Abe, Y. 1986. Taxonomic status of the *Andricus mukaigawae* complex and its speciation with geographical parthenogenesis (Hymenoptera, Cynipidae). Appl. Entomol. Zool. 21:436–447.

Abrahamson, W. G., and A. E. Weis. 1997. Evolutionary ecology across three trophic levels: goldenrods, gallmakers and natural enemies. Princeton Univ. Press, Princeton, NJ.

Abrahamson, W. G., G. Melika, R. Scrafford, and G. Csóka. 1998. Gall-inducing insects provide insights into plant systematic relationships. Am. J. Bot. 85:1159–1165.

Ambrus, B. 1974. Cynipid galls. Academic Press, Budapest, Hungary.

Askew, R. R. 1984. The biology of gall wasps. Pp. 223–271 *in* T. N. Anantakrishnan, ed. Biology of gall insects. Edward Arnold, London.

Atkinson, R. J. 2000. The genetic analysis of natural history, reproductive strategy and population structure in European oak gallwasps (Hymenoptera: Cynipidae). Ph.D. diss., University of Oxford, Oxford, U.K.

Atkinson, R. J., G. A. T. McVean, and G. N. Stone. 2002. Use of population genetic data to infer oviposition behaviour: species-specific patterns in four oak gallwasps (Hymenoptera: Cynipidae). Proc. R. Soc. Lond. B 269:383–390.

Becerra, J, 1997. Insects on plants: macroevolutionary chemical trends in host use. Science 276:253–256.
Bennett, K. D. 1986. The rate of spread and population increase of forest trees during the postglacial. Philos. Trans. R. Soc. Lond. B 314:523–531.
Brooks, S. E., and J. D. Shorthouse. 1998. Developmental morphology of stem galls of *Diplolepis nodulosa* (Hymenoptera: Cynipidae) and those modified by the inquiline *Periclistus pirata* (Hymenoptera: Cynipidae) on *Rosa blanda* (Rosaceae). Can. J. Bot. 76:365–381.
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. USA 91:6491–6495.
Buhr, H. 1965. Bestimmungstabellen der gallen (zoo und phytocecidien) an pflanzen mittel- und nordeuropas. V. E. B. Gustav Fischer Verlag, Jena, Germany.
Camus, A. 1936–1938. Les chênes. Monographie du genre *Quercus*. Vol 1. Genre *Quercus*, sous-genre Cyclobalanopsis: sous-genre Euquercus (sections Cerris at Mesobalanus). Editions Lechavalier, Paris.
Camus, A. 1938–1939. Les chênes. Monographie du genre *Quercus*. Vol 2. Genre *Quercus*, sous-genre Euquercus (sections Lepidobalanus at Macrobalanus). Editions Lechavalier, Paris.
Chodjai, M. 1980. Study of the gall forming cynipid Hymenoptera of Iranian oak forests. J. Entomol. Soc. Iran. Special Suppl. no. 3.
Cook, J. M., G. N. Stone, and A. Rowe. 1998. Patterns in the evolution of gall structure and life cycles in oak gallwasps (Hymenoptera: Cynipidae). Pp. 261–279 *in* G. Csóka, W. J. Mattson, G. N. Stone, and P. W. Price, eds. The biology of gall-inducing arthropods. U.S. Dept. of Agriculture, Forest Service, North Central Research Station, St. Paul, MN.
Cornell, H. V. 1983. The secondary chemistry and complex morphology of galls formed by the Cynipinae (Hymenoptera)—Why and how. Am. Midl. Nat. 110:225–234.
———. 1985. Local and regional richness of cynipine gallwasps on California oaks. Ecology 66:1247–1260.
———. 1986. Oak species attributes and host size influence cynipine wasp species richness. Ecology 67:1582–1592.
Crespi, B., and M. Worobey. 1998. Comparative analysis of gall morphology in Australian gall thrips: the evolution of extended phenotypes. Evolution 52:1686–1696.
Crespi, B. J., D. A. Carmean, and T. W. Chapman. 1997. Ecology and evolution of galling thrips and their allies. Annu. Rev. Entomol. 42:51–71.
Csóka, G. 1997. Plant galls. Forest Research Institute, Budapest, Hungary.
Edwards, A. W. F. 1972. Likelihood. Johns Hopkins Univ. Press, London.
Farris, J. S., M. Kallersjo, A. G. Kluge, and C. Bult. 1994. Testing significance of incongruence. Cladistics 10:315–319.
Fay, P. A., and D. C. Hartnett. 1991. Constraints on growth and allocation patterns of *Silphium integrifolium* (Asteraceae) caused by a cynipid gall wasp. Oecologia 88:243–250.
Folliot, R. 1964. Contribution a l'étude de la biologie des Cynipides gallicoles (Hymenoptera: Cynipoidea). Ann. Sci. Nat. 6: 407–564.
Formusoh, E. S., J. H. Hatchett, W. C. Black, and J. J. Stuart. 1996. Sex-linked inheritance of virulence against wheat resistance gene H9 in the Hessian fly (Diptera: Cecidomyiidae). Ann. Entomol. Soc. Am. 89:428–434.
Gross, P., and P. W. Price. 1988. Plant influences on parasitism of two leaf miners: a test of enemy free space. Ecology 69: 1506–1516.
Hails, R. S., and M. J. Crawley. 1991. The population dynamics of an alien insect—*Andricus quercuscalicis* (Hymenoptera, Cynipidae). J. Anim. Ecol. 60:545–562.
Hewitt, G. M. 1999. Postglacial re-colonisation of European biota. Biol. J. Linn. Soc. 68:87–112.
———. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907–913.
Houard, C. 1912. The animal galls of southern Africa. Ann. Soc. Entomol. Fr. 81:1–43.
Huelsenbeck, J. P., and B. Rannala. 1997. Phylogenetic methods come of age: testing hypotheses in an evolutionary context. Science 276:227–232.
Huelsenbeck, J. P., F. Ronquist, R. Nielsen, and J. P. Bollpack. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294:2310–2314.
Huntley, B. H., and J. B. Birks. 1983. An atlas of past and present pollen maps for Europe: 0–13,000 years ago. Cambridge Univ. Press, Cambridge, U.K.
Jaenike, J. 1990. Host specialization in phytophagous insects. Annu. Rev. Ecol. Syst. 21:243–273.
Larget, B., and D. L. Simon. 1999. Markov chain Monte Carlo algorithms for Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. 16:750–759.
Liljeblad, J., and F. Ronquist. 1998. A phylogenetic analysis of higher-level gall wasp relationships (Hymenoptera: Cynipidae). Syst. Entomol. 23:229–252.
Lill, J. T., R. J. Marquis, and R. E. Ricklefs. 2002. Host plants influence parasitism of forest caterpillars. Nature 417:170–173.
Lutzoni, F., M. Pagel, and V. Reeb. 2001. Major fungal lineages are derived from lichen ancestors. Nature 411:937–940.
Manos, P. S., J. J. Doyle, and K. C. Nixon. 1999. Phylogeny, biogeography and processes of molecular differentiation in *Quercus* subgenus *Quercus* (Fagaceae). Mol. Phylogenet. Evol. 12: 333–349.
Mardulyn, P., and S. A. Cameron. 1999. The major opsin in bees (Insecta: Hymenoptera): a promising nuclear gene for higher level phylogenetics. Mol. Phylogenet. Evol. 12:168–176.
Melika, G., G. Csóka, and J. Pujade-Villar. 2000. Check-list of oak gall wasps of Hungary, with some taxonomic notes (Hymenoptera: Cynipidae, Cynipinae, Cynipini). Ann. Hist. Nat. Mus. Natl. Hung. 92:265–296.
Moran, N. A. 1988. The evolution of host plant alternation in aphids: evidence for specialization as a dead end. Am Nat. 132:681–706.
———. 1991. Phenotype fixation and genotypic diversity in the complex life cycle of the aphid *Pemphigus betae*. Evolution 45: 957–970.
———. 1994. Adaptation and constraint in the complex lifecycles of animals. Annu. Rev. Ecol. Syst. 25:573–600.
Nieves-Aldrey, J. L. 1987. Estado actual del conocimiento de la subfamilia Cynipinae (Hymenoptera, Parasitica, Cynipidae) en la Peninsula Iberica. Eos 63:179–195.
———. 1992. Revisíon de las especies europeas del género *Callirhytis* (Hymenoptera, Cynipidae). Graellsia 48:171–183.
Nyman, T., H. Roininen, and J. A. Vuorinen. 1998. Evolution of different gall types in willow-feeding sawflies (Hymenoptera: Tenthredinidae). Evolution 52:465–474.
Nyman, T., A. Widmer, and H. Roininen. 2000. Evolution of gall morphology and host plant relationships in willow-feeding sawflies (Hymenoptera: Tenthredinidae). Evolution 54:526–533.
Pagel, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proc. R. Soc. Lond. B 255:37–45.
———. 1999a. Inferring the historical patterns of biological evolution. Nature 401:877–884.
———. 1999b. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. Syst. Biol. 48:612–622.
Pagel, M., and Lutzoni, F. 2002. Accounting for phylogenetic uncertainty in comparative studies of evolution and adaptation. Pp. 151–164 *in* Biological evolution and statistical physics. M. Laessig and A. Valleriani, eds. Springer-Verlag, Berlin.
Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.
Price, P. W., G. W. Fernandes, and G. L. Waring. 1987. Adaptive nature of insect galls. Environ. Entomol. 16:15–24.
Pujade-Villar, J. 1994. Formes cinipo-cedidogenes detectades o que poden detectar-se, en les flors i els fruits de les fagacies a Andorra (Hymenoptera: Cynipidae: Cynipinae). Ann. Inst. Est. And. Barcelona. 1992:137–162.
Ratcliffe, R. H., G. G. Safranski, F. L. Patterson, H. W. Ohm, and

P. L. Taylor. 1994. Biotype status of Hessian fly (Diptera, Cecidomyiidae) populations from the eastern United States and their response to 14 Hessian fly resistance genes. J. Econ. Entomol. 87:1113–1121.

Ratcliffe, R. H., S. E. Cambron, K. L. Flanders, N. A. Bosque-Perez, S. L. Clement, and H. W. Ohm. 2000. Biotype composition of Hessian fly (Diptera: Cecidomyiidae) populations from the southeastern, midwestern, and northwestern United States and virulence to resistance genes in wheat. J. Econ. Entomol. 93:1319–1328.

Rokas, A. 2001. Tree-thinking, molecules and gallwasps: analysing evolutionary patterns in European gallwasps using a molecular phylogenetic approach. Ph.D. diss., University of Edinburgh, Edinburgh, U.K.

Rokas, A., R. J. Atkinson, G. S. Brown, S. A. West, and G. N. Stone. 2001. Understanding patterns of genetic diversity in the oak gallwasp *Biorhiza pallida*: demographic history or a *Wolbachia* selective sweep? Heredity 87:294–304.

Rokas, A., J. A. A. Nylander, F. Ronquist, and G. N. Stone. 2002. A maximum likelihood analysis of eight phylogenetic markers in gallwasps (Hymenoptera: Cynipidae); implications for insect phylogenetic studies. Mol. Phylogenet. Evol. 22:206–219.

Ronquist, F. 1994. Evolution of parasitism among closely related species: phylogenetic relationships and the origins of inquilism in gall wasps (Hymenoptera, Cynipidae). Evolution 48:241–266.

———. 1995. Phylogeny and early evolution of the Cynipoidea (Hymenoptera). Syst. Entomol. 20:309–335.

Ronquist, F., and J. Liljeblad. 2001. Evolution of the gall wasp-host plant association. Evolution 55:2503–2522.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford Univ. Press, Oxford, U.K.

Schönrogge, K., L. J. Harper, and C. P. Lichtenstein. 2000. The protein content of tissues in cynipid galls (Hymenoptera: Cynipidae): Similarities between cynipid galls and seeds. Plant Cell Environ. 23:215–222.

Shorthouse, J., and O. Rohfritsch. 1992. Biology of insect-induced galls. Oxford Univ. Press, Oxford, U.K.

Stern, D. L. 1995. Phylogenetic evidence that aphids, rather than plants, determine gall morphology. Proc. R. Soc. Lond. B 260: 85–89.

Sternlicht, M. 1968. The oak galls of Israel. Isr. J. Entomol. 3: 17–58.

Stone, G. N., and P. Sunnucks. 1993. Genetic consequences of an invasion through a patchy environment—the cynipid gallwasp *Andricus quercuscalicis* (Hymenoptera: Cynipidae). Mol. Ecol. 2:251–268.

Stone, G. N., and J. M. Cook. 1998. The structure of cynipid oak galls: patterns in the evolution of an extended phenotype. Proc. R. Soc. Lond. B 265:979–988.

Stone, G. N., R. Atkinson, A. Rokas, G. Csóka, and J. L. Nieves-Aldrey. 2001. Differential success in northwards range expansion between ecotypes of the marble gallwasp *Andricus kollari*: a tale of two lifecycles. Mol. Ecol. 10:761–778.

Stone, G. N., K. Schönrogge, R. J. Atkinson, D. Bellido, and J. Pujade-Villar. 2002. The population biology of oak gallwasps (Hymenoptera; Cynipidae). Annu. Rev. Entomol. 47:633–668.

Sunnucks, P. J., and G. N. Stone. 1996. Genetic structure of invading insects and the case of the knopper gallwasp. Pp. 485–496 *in* R. B. Floyd, A. W. Sheppard, and P. J. De Barro, eds. Frontiers of population ecology. CSIRO Publishing, Collingwood, Australia.

Swofford, D. L. 1998. PAUP* 4.0. Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA.

Toumi, L., and R. Lumaret. 1998. Allozyme variation in cork oak (*Quercus suber* L.): the role of phylogeography and genetic introgression by other Mediterranean oak species and human activities. Theor. Appl. Genet. 97:647–656.

von Dohlen, C. D., and N. A. Moran. 2000. Molecular data support a rapid radiation of aphids in the Cretaceous and multiple origins of host alternation. Biol. J. Linn. Soc. 71:689–717.

Weis, A. E., and W. G. Abrahamson. 1986. Evolution of host-plant manipulation by gall makers—ecological and genetic factors in the *Solidago—Eurosta* system. Am. Nat. 127:681–695.

Weis, A. E., R. Walton, and C. L. Crego. 1988. Reactive plant tissue sites and the population biology of gall makers. Annu. Rev. Ent. 33:467–486.

Weld, L. H. 1957. Cynipid galls of the Pacific slope. Privately printed, Ann Arbor, MI.

———. 1959. Cynipid galls of the Eastern United States. Privately printed, Ann Arbor, MI.

———. 1960. Cynipid galls of the Southwest. Privately printed, Ann Arbor, MI.

Whitham, T. G. 1978. Habitat selection by *Pemphigus* aphids in response to resource limitation and competition. Ecology 59: 1164–1176.

Wood, B. W., and J. A. Payne. 1988. Growth regulators in chestnut shoot galls infected with oriental chestnut gall wasp, *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae). Environ. Entomol. 17: 915–920.

Zantoko, L. and Shukle, R. H. 1997. Genetics of virulence in the Hessian fly to resistance gene H13 in wheat. J. Hered. 88: 120–123.

———. 1999. An STS linked to a Hessian fly locus controlling virulence to resistance gene H13 in wheat. J. Hered. 90:242–247.

Corresponding Editor: B. Crespi