ELSEVIER

Multiple host shifts between distantly related plants, Juglandaceae and Ericaceae, in the leaf-mining moth *Acrocercops leucophaea* complex (Lepidoptera: Gracillariidae)

Issei Ohshima *, Kazunori Yoshizawa

Systematic Entomology, Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

Received 4 April 2005; revised 8 June 2005

Abstract

Insect herbivores such as gall formers and leaf miners are often highly specialized and adapted to their respective natal host plants. Due to the specialization and adaptation, it is presumed that host shifts readily occur among closely related plant species. Leaf-mining moths, the *Acrocercops leucophaea* complex, consist of three species, *A. leucophaea*, *A. defigurata*, and *A. transecta*. Larvae of all the species of the complex feed on Juglandaceae plants, but *A. leucophaea* and *A. transecta* are also associated with an Ericaceae plant, which is quite distantly related to Juglandaceae. Such a host utilization as in this species complex is very rare among phytophagous insects. In the present study, we estimate the history of host shifts by reconstructing the phylogeny of the *A. leucophaea* complex using molecular data (partial sequence of mitochondrial COI, 12S rDNA, and ND5). Parsimony and maximum likelihood analyses indicated that the common ancestor of the *A. leucophaea* complex used Juglandaceae only, and that the association with Ericaceae has evolved in *A. leucophaea* and *A. transecta* independently. Parametric bootstrap analysis also supported multiple origins of the association with Ericaceae in this complex. These results imply that there are ecological and biochemical factors that promote host shifting between Juglandaceae and Ericaceae despite the two families being not closely related.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Phytophagous insects; Insect–plant interactions; Host shift; Host race; Speciation; Mitochondrial DNA; Phylogeny

1. Introduction

The majority of phytophagous insects are associated with one or a few closely related host plants (Schoonhoven et al., 1998; Strong et al., 1984; Symons and Beccaloni, 1999). Such mono- and oligophagous insect herbivores are highly specialized to their natal host plant species via physiological and behavioral adaptations. These adaptations to the host plants have led to differentiation in life-history traits between populations using different host plant species (Thompson, 1988; Vanbergen et al., 2003). Recent studies have suggested that there are more examples of host race formation in herbivorous insects than has previously been recognized (Abrahamson et al., 2001). Studies on host race formation in phytophagous insects have postulated that such a host specialization via host shift leads to ecological speciation (Howard and Patrik, 2005) without geographic isolation (Berlocher and Feder, 2002; Bush, 1969, 1994; Craig et al., 1997, 2001; Drès and Mallet, 2002; Feder, 1998; Wood, 1980; Wood and Guttman, 1983). Thus, host shifts may contribute as a first step to the formation of host races and subsequently to sympatric speciation.

The mechanisms and process of the host shift in phytophagous insects include numerous genetic and ecological factors (Thompson, 1991, 1999). Ehrlich and Raven (1964) particularly stressed the importance

---

* Corresponding author. Fax: +81 11 706 4939.
E-mail address: issei@res.agr.hokudai.ac.jp (I. Ohshima).

of the similarities in the plants' secondary metabolic compounds between the novel and old hosts. Closely related host plants (e.g., the same genus or family) often meet these conditions. Therefore, host shifts in phytophagous insects are generally considered to occur more readily between closely related host plants than between distantly related ones (Futuyma et al., 1995; Janz and Nylin, 1998; Lopez-Vaamonde et al., 2003).

A leaf-mining moth, the *Acrocercops leucophaea* complex, consists of three species, *A. leucophaea*, *A. defigurata*, and *A. transecta*. *A. leucophaea* and *A. defigurata* are distributed in Nepal and Northern India, while *A. transecta* is distributed in the Russian Far East, South Korea, Japan, and Taiwan. *A. leucophaea* and *A. transecta* are associated with distantly related plants, several Juglandaceae species, and *Lyonia ovalifolia* (Ericaceae), while *A. defigurata* is associated with Juglandaceae species only (Kumata et al., 1988; Ohshima, pers. obs.). Among Juglandaceae, each of *A. leucophaea* and *A. defigurata* uses only one plant species (*Engelhardtia spicata* and *Juglans regia*, respectively), but *A. transecta* is associated with *Pterocarya rhoifolia*, *Platycarya strobilacea*, and several *Juglans* plants (Kumata et al., 1988; Ohshima, pers. obs.). There are no morphological differences between the population feeding on Ericaceae and that feeding on Juglandaceae in each of *A. leucophaea* and *A. transecta* (Kumata et al., 1988). However, females exclusively prefer to oviposit on their natal host plant in *A. transecta* (Ohshima, 2005), and oviposition preference of *A. leucophaea* also differs between Juglandaceae-associated population and Ericaceae-associated population (Ohshima, unpubl. data). The Juglandaceae and Ericaceae belong to different orders, Fagales and Ericales, and the two orders are affiliated with distantly related clades, rosids and asterids, respectively (Soltis et al., 1999, 2000). These unique host associations, a single species using only two distantly related host plants, in this species complex are remarkable cases in phytophagous insects.

An increasing number of studies using molecular markers have documented genetic differentiation between host-associated populations within a single phytophagous species (Brown et al., 1996; Brunner et al., 2004; Feder et al., 1988; Lopez-Vaamonde et al., 2003; McPheron et al., 1988). These examples suggest that the two host-feeding populations in each of *A. leucophaea* and *A. transecta* could be genetically differentiated. However, they are morphologically indistinguishable, and thus molecular phylogenetic analyses are needed to evaluate genetic differentiation. Furthermore, if monophyly in each of *A. leucophaea* and *A. transecta* is supported, this extreme host association between such distantly related host plants should have evolved in the two distinct lineages independently. The alternative possibility is that *A. leucophaea* and *A. transecta* are not monophyletic. If monophyly of Juglandaceae-associated and/or Ericaceae-associated populations is supported beyond the present nominal species, this unique host association should have been formed at a single evolutionary event, indicating the paraphyly of at least one of the morphospecies.

The present study aims to test if the unique host utilization patterns shared by *A. leucophaea* and *A. transecta* have evolved independently or not by inferring the molecular phylogeny of the *A. leucophaea* complex. Determining the direction of the host shift between Juglandaceae and Ericaceae plants in this species complex is crucial for understanding the evolutionary process of the host association in this complex. This case thus provides a unique opportunity for studying the evolutionary dynamics of host–plant usage in phytophagous insects.

2. Materials and methods

2.1. Taxon sampling

This study used specimens stored in 99.5% ethanol and dried specimens (more than 20 years old). Larvae were collected with the host plant and were reared in the laboratory. Emerged adults were used for analysis except for eight larvae (voucher number IO-058, 059, 062, 064–066, 068, and 069). A total of 37 specimens were selected from among the *A. leucophaea* complex as ingroups, including 16 *A. leucophaea* (including seven samples from the Juglandaceae-associated population and nine samples from the Ericaceae-associated population), four *A. defigurata*, and 17 *A. transecta* (including 12 samples from the Juglandaceae-associated population and five samples from the Ericaceae-associated population). Three *Acrocercops* species not belonging to the *leucophaea* complex were selected as outgroups (Table 1).

2.2. Sequence determination

Total DNA was extracted using DNeasy Tissue Kit (Qiagen) following Cruickshank et al. (2001). Vouchers were slide mounted and stored in Hokkaido University. For molecular phylogenetic inference, three mitochondrial DNA regions, cytochrome oxidase subunit I (COI), NADH dehydrogenase subunit 5 (ND5), and 12S rDNA were chosen. Mitochondrial COI gene is known to be useful for inferring relationships among closely related moth species and populations (Brown et al., 1994), and NADH dehydrogenase subunit genes are more rapidly evolving than COI (Simon et al., 1994). In contrast, mitochondrial 12S rDNA gene is suitable for analyzing deeper clades

Table 1
Sampling information and GenBank accession numbers

Species name	Host plant	Collection site	Voucher number	GenBank Accession No.		
				12S	COI	ND5
Acrocercops leucophaea	*Engelhardtia spicata* (Juglandaceae)	Godawari, Nepal	IO-062	DQ080635	DQ080501	DQ080461
A. leucophaea	*E. spicata* (Juglandaceae)	Pharping, Nepal	IO-063	DQ080636	DQ080502	DQ080462
A. leucophaea	*E. spicata* (Juglandaceae)	Pharping, Nepal	IO-064	DQ080637	DQ080503	DQ080463
A. leucophaea	*E. spicata* (Juglandaceae)	Pharping, Nepal	IO-074	DQ080647	DQ080513	DQ080473
A. leucophaea	*E. spicata* (Juglandaceae)	Pharping, Nepal	IO-075	DQ080648	DQ080514	DQ080474
A. leucophaea	*E. spicata* (Juglandaceae)	Pharping, Nepal	IO-076	DQ080649	DQ080515	DQ080475
A. leucophaea	*E. spicata* (Juglandaceae)	Pharping, Nepal	IO-077	DQ080650	DQ080516	DQ080476
A. leucophaea	*Lyonia ovalifolia* (Ericaceae)	Godawari, Nepal	IO-065	DQ080638	DQ080504	DQ080464
A. leucophaea	*L. ovalifolia* (Ericaceae)	Godawari, Nepal	IO-066	DQ080639	DQ080505	DQ080465
A. leucophaea	*L. ovalifolia* (Ericaceae)	Nagarjun, Nepal	IO-067	DQ080640	DQ080506	DQ080466
A. leucophaea	*L. ovalifolia* (Ericaceae)	Pharping, Nepal	IO-070	DQ080643	DQ080509	DQ080469
A. leucophaea	*L. ovalifolia* (Ericaceae)	Pharping, Nepal	IO-072	DQ080645	DQ080511	DQ080471
A. leucophaea	*L. ovalifolia* (Ericaceae)	Pharping, Nepal	IO-073	DQ080646	DQ080512	DQ080472
A. leucophaea	*L. ovalifolia* (Ericaceae)	Pharping, Nepal	IO-068	DQ080641	DQ080507	DQ080467
A. leucophaea	*L. ovalifolia* (Ericaceae)	Pharping, Nepal	IO-069	DQ080642	DQ080508	DQ080468
A. leucophaea	*L. ovalifolia* (Ericaceae)	Pharping, Nepal	IO-071	DQ080644	DQ080510	DQ080470
A. defigurata	*Juglans regia* (Juglandaceae)	Godawari, Nepal	IO-035	DQ080631	DQ080497	DQ080457
A. defigurata	*J. regia* (Juglandaceae)	Godawari, Nepal	IO-058	DQ080632	DQ080498	DQ080458
A. defigurata	*J. regia* (Juglandaceae)	Godawari, Nepal	IO-059	DQ080633	DQ080499	DQ080459
A. defigurata	*J. regia* (Juglandaceae)	Godawari, Nepal	IO-060	DQ080634	DQ080500	DQ080460
A. transecta	*J. regia* (Juglandaceae)	Vladivostok, Russia	IO-043	DQ080616	DQ080482	DQ080442
A. transecta	*Platycarya strobilacea* (Juglandaceae)	Tsushima, Japan	IO-046	DQ080627	DQ080493	DQ080453
A. transecta	*J. ailanthifolia* (Juglandaceae)	Sapporo, Japan	IO-003	DQ080617	DQ080483	DQ080443
A. transecta	*Pterocarya rhoifolia* (Juglandaceae)	Akiu, Japan	IO-036	DQ080623	DQ080489	DQ080449
A. transecta	*J. ailanthifolia* (Juglandaceae)	Akita, Japan	IO-004	DQ080619	DQ080485	DQ080445
A. transecta	*J. ailanthifolia* (Juglandaceae)	Aomori, Japan	IO-038	DQ080618	DQ080484	DQ080444
A. transecta	*J. ailanthifolia* (Juglandaceae)	Sendai, Japan	IO-005	DQ080620	DQ080486	DQ080446
A. transecta	*J. ailanthifolia* (Juglandaceae)	Sendai, Japan	IO-006	DQ080621	DQ080487	DQ080447
A. transecta	*Pt. rhoifolia* (Juglandaceae)	Sendai, Japan	IO-037	DQ080624	DQ080490	DQ080450
A. transecta	*J. ailanthifolia* (Juglandaceae)	Maebashi, Japan	IO-040	DQ080626	DQ080492	DQ080452
A. transecta	*J. ailanthifolia* (Juglandaceae)	Sendai, Japan	IO-007	DQ080622	DQ080488	DQ080448
A. transecta	*J. ailanthifolia* (Juglandaceae)	Nagano, Japan	IO-008	DQ080625	DQ080491	DQ080451
A. transecta	*L. ovalifolia* (Ericaceae)	Sendai, Japan	IO-001	DQ080611	DQ080477	DQ080437
A. transecta	*L. ovalifolia* (Ericaceae)	Kyoto, Japan	IO-002	DQ080612	DQ080478	DQ080438
A. transecta	*L. ovalifolia* (Ericaceae)	Aichi, Japan	IO-026	DQ080613	DQ080479	DQ080439
A. transecta	*L. ovalifolia* (Ericaceae)	Shizuoka, Japan	IO-041	DQ080614	DQ080480	DQ080440
A. transecta	*L. ovalifolia* (Ericaceae)	Taiwan	IO-045	DQ080615	DQ080481	DQ080441
A. unistriata	*Quercus phillyraeoides* (Fagaceae)	Wakayama, Japan	IO-020	DQ080628	DQ080494	DQ080454
A. vallata	*Q. glauca* (Fagaceae)	Wakayama, Japan	IO-009	DQ080630	DQ080496	DQ080456
A. mantica	*Castanopsis sieboldii* (Fagaceae)	Iriomote, Japan	IO-022	DQ080629	DQ080495	DQ080455

(Moritz et al., 1987; Simon et al., 1994). Primer sets 12Sai + 12Sbi (Simon et al., 1994), L6625 + H7005 (Hafner et al., 1994), and F7081 + R7495 (Yoshizawa, 2004) were used to amplify 335 bp fragment of mitochondrial 12S rDNA, 344 bp of COI, and 413 bp of ND5, respectively. Additional primer sets L6625 + R2352 (5′-GTA TCA ATA TCT ATT CCT AC-3′) and F2314 (5′-GCT ATA ATA GCA ATT GGA TT-3′) + H7005 were used to amplify 160 and 221 bp fragments of COI, and F7081 + R7345 (5′-GCT AAT TAT GAG TTT GAT CTA AA-3′) and F7259 (5′-ATA TAG CAT GAG TTA ATA AAT G-3′) + R7495 were used to amplify 263 and 235 bp fragments of ND5 for the old specimens. Reaction cycle was 94 °C for 3 min followed by 40 cycles of 94 °C for 30 s, 45 °C (12S and COI) or 42 °C (ND5) for 30 s, and 65 °C for 45 s. For the old specimens, second PCR was conducted using first PCR products as template. Reaction cycle of second PCR was the same as the first PCR (12S), or 94 °C for 3 min followed by 5 cycles of 94 °C for 30 s, 48 °C (COI) or 47 °C (ND5) for 30 s, and 72 °C (COI) or 65 °C (ND5) for 45 s, and then followed by 35 cycles with annealing temperatures of 45 °C (COI) or 42 °C (ND5). Amplified products were purified using PCR Purification Kit (Qiagen) and then were performed a single-stranded PCR for sequencing following manufacturer's protocol. PCR products were sequenced by CEQ2000 DNA Analysis System (Beckman Coulter). Obtained sequences were aligned manually.

NEXUS files of the aligned sequences are available from the URL at <http://insect3.agr.hokudai.ac.jp/psoco-web/data/index.html> or by requested to the first author.

2.3. Phylogenetic analysis

Parsimony (MP) and maximum likelihood (ML) analyses were conducted by PAUP 4.0b10 PPC (Swofford, 2002). For MP analysis, all characters were equally weighted. MP trees were searched with 100 random addition replication using TBR branch swapping. Parameters for ML analysis were chosen based on the Akaike information Criterion (AIC) (Akaike, 1973, 1974; Sakamoto et al., 1986) as implemented in Modeltest ver 3.06 (Posada and Buckley, 2004; Posada and Crandall, 1998). As a result of Modeltest, the TVM + I + G model was selected (unequal base frequencies: A = 0.4354, C = 0.0990, G = 0.1022, T = 0.3634; six substitution categories: A–C = 3.0362, A–G = 11.2902, A–T = 1.4384, C–G = 0.6908, C–T = 11.2902, G–T = 1.0000; gamma distribution shape parameter = 1.1336; proportion of invariable sites = 0.7200; four rate categories). The ML tree was searched with TBR branch swapping using neighbor-joining tree as a starting point. To assess confidence in clades, non-parametric bootstrap tests (Felsenstein, 1985) were performed using 100 replicates with TBR branch swapping. History of host shift was parsimoniously reconstructed on the estimated MP trees using Mac Clade 4.03 (Maddison and Maddison, 2001).

2.4. Testing alternative hypotheses

We tested alternative hypotheses using parametric bootstrapping (Swofford et al., 1996), which was referred to as SOWH test by Goldman et al. (2000). We chose parametric bootstrap tests because other non-parametric methods are subject to type II statistical error (Goldman et al., 2000) and to serious biases (Shimodaira, 2002). Due to the computational complexity of the SOWH test, we subsampled 12 samples from the clades (asterisked in Fig. 1). The substitution model and the parameters were re-calculated for the 12 samples. Estimated parameters and constrained ML trees were used to generate 100 simulation data using Seq-Gen 1.5.3 (Rambaut and Grassly, 1997).

3. Results

3.1. Phylogenetic analysis

No insertion or deletion was observed in 12S rDNA, COI, and ND5 gene sequence. Of a total of 1092 bp aligned nucleotide sites, 81 were variable and 70 of the 81 bp were parsimony-informative for the ingroup (including the outgroup 185 and 101, respectively). A heuristic search detected three MP trees (length 287 steps, RI = 0.88). Of the three MP trees, two placed *A. defigurata* at the most basal position of the *A. leucophaea* complex and the two trees differed in the branching

within *A. defigurata*. The remaining MP tree placed *A. defigurata* as the sister of *A. transecta + A. leucophaea*-clade 1 subgroup. The tree obtained by ML analysis (Fig. 1) was identical with one of the former MP trees. Monophyly of *A. transecta + A. leucophaea* was recovered, although bootstrap support was low (bootstrap support <50%). Hence, we postulate that the tree shown in Fig. 1 as the best phylogenetic hypothesis because both analyses supported it.

Both analyses strongly supported monophyly of the *A. leucophaea* complex (MP 100%; ML 96%) and monophyly of *A. defigurata* (MP 100%; ML 87%). Monophyly of *A. transecta* was also fairly well supported (MP 85%; ML 62%). However, neither of the analyses supported monophyly of *A. leucophaea*, which was divided into two clearly distinguished clade. Three samples of *A. leucophaea* composed a monophyletic group with 100% bootstrap support for both analyses (clade 1), and the clade was placed as the sister group of *A. transecta* (MP 84%; ML <50%). The remaining members of *A. leucophaea* also composed a monophyletic group with high bootstrap supports (clade 2: MP 99%; ML 83%). The clade 1 consisted of the Pharping population infesting Ericaceae only, and the clade 2 contained the moths from Godawari, Nagarjun, and Pharping, which are associated with Juglandaceae or Ericaceae.

Two host-associated populations in *A. transecta* were clearly distinguished phylogenetically and showed a rather large intraspecific variation (uncorrected pairwise distance: 1.01–1.83%). Monophyly of the Juglandaceae population was strongly supported by MP analysis (79%), while ML analysis supported the monophyly less strongly (56%). Monophyly of the Ericaceae population was also well supported by both analyses (MP 74%; ML 72%). Variation in the sequences within the Juglandaceae-associated population or the Ericaceae-associated population was also rather large (0.09–1.10%, respectively). In contrast, two host-associated populations in *A. leucophaea*-clade 2 were less clearly distinguished.

We conducted the most parsimonious reconstruction (MPR) of evolutionary history of the host association on the MP tree that was selected as the best phylogenetic hypothesis (Fig. 1). Figs. 2A and B show the results of the MPR on the MP tree. Two MPRs, ACCTRAN and DELTRAN, were possible on each tree, but both showed that host shift has occurred three times within the *A. leucophaea* complex. ACCTRAN optimization indicated two host shifts from Juglandaceae to Ericaceae and one host shift in the opposite direction (Figs. 2A and C). In contrast, DELTRAN optimization suggested that host shift from Juglandaceae to Ericaceae has evolved three times (Figs. 2B and D). The results of MPR indicated that the Juglandaceae association was the most ancestral condition for
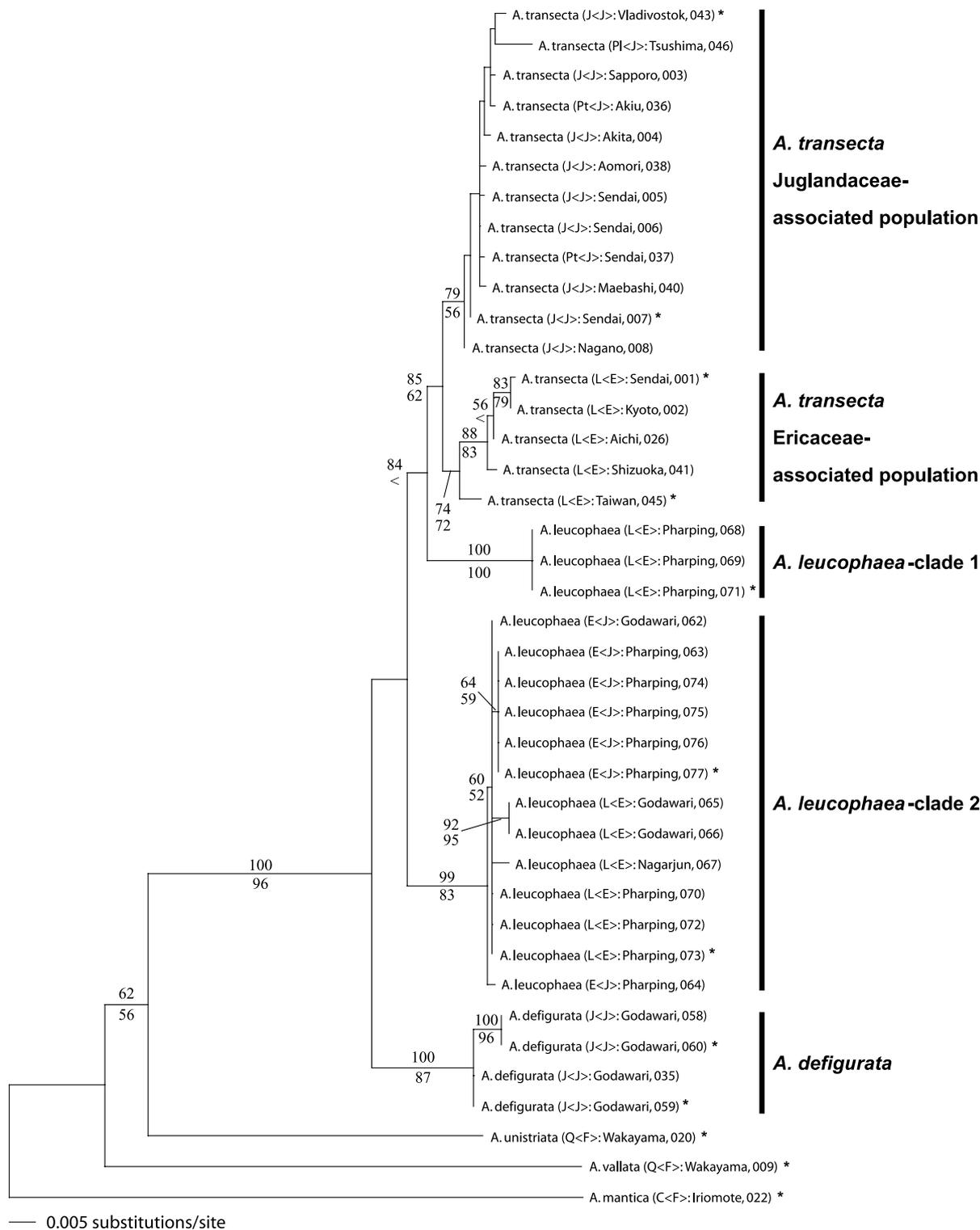
Fig. 1. A tree estimated by the ML analysis (−ln = 2901.30358). Branch length is proportional to ML estimated genetic distances. The numbers associated with branches indicate bootstrap values higher than 50% (MP: upper, ML: lower). '<' indicates bootstrap support lower than 50%. Major groups are indicated with bold bars. Within sample names, the former alphabet of < > refers to the generic name of each host plant as follows: J, *Juglans*; Pt, *Pterocarya*; Pl, *Platycarya*; E, *Engelhardtia*; L, *Lyonia*; C, *Castanopsis*; Q, *Quercus*. The alphabet included in the < > refer to the family name to which each host plant belong as follows: J, Juglandaceae; E, Ericaceae; F, Fagaceae. Asterisks indicate samples used in the SOWH test.

this species complex. The results of MP analysis also supported alternative phylogenetic hypothesis, *A. defigurata* as the sister of *A. leucophaea*-clade 1 + *A. transecta*, but the MP tree basically did not alter the interpretation on the history of host shifts in the *A. leucophaea* complex (Figs. 2C and D).
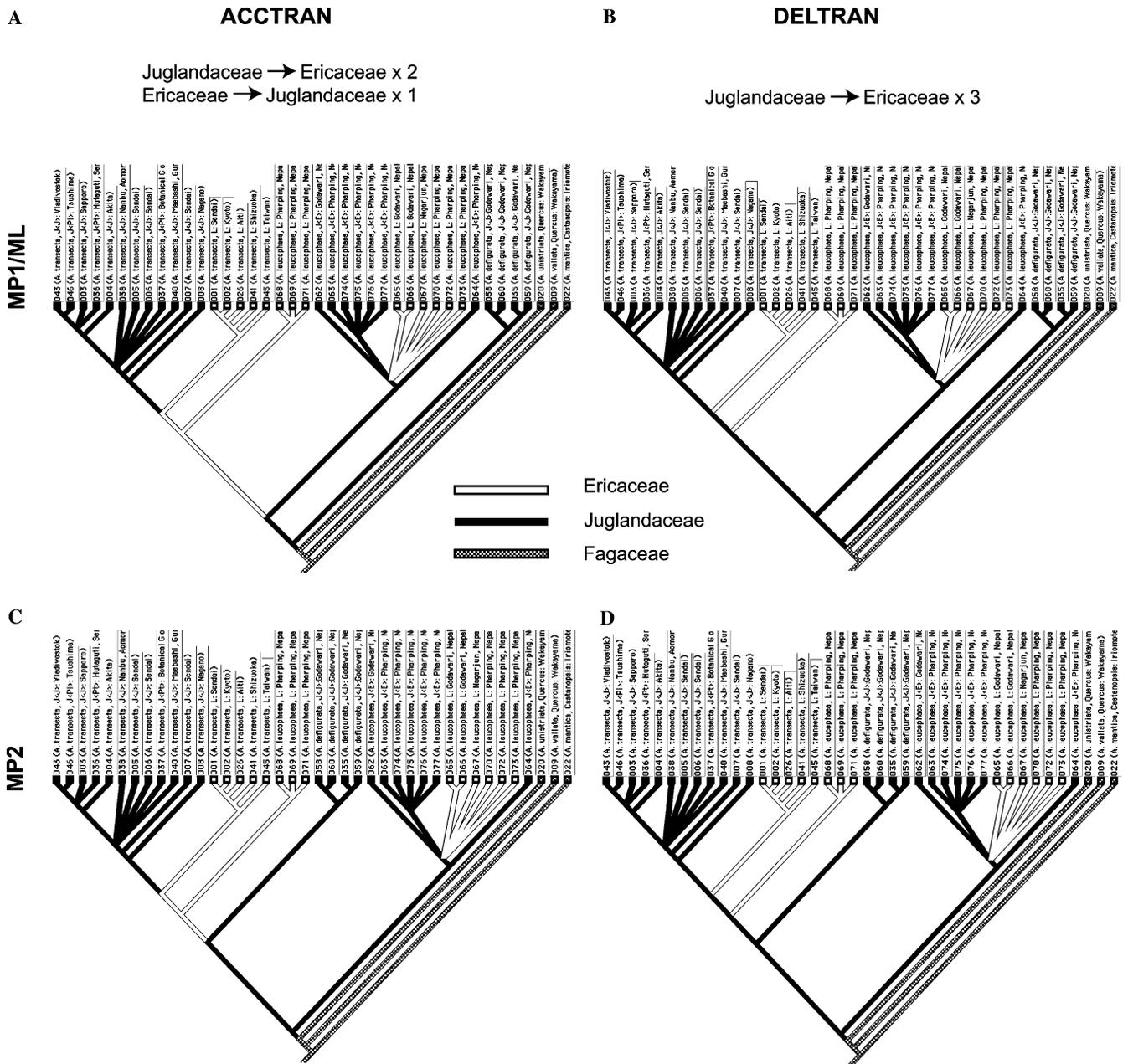
Fig. 2. Most parsimonious reconstruction (MPR) of host associations onto the two parsimony (MP) trees. These two trees differ in position of *A. defigurata*. (A) and (B) place *A. defigurata* at the most basal position of the *A. leucophaea* complex, and (C) and (D) place *A. defigurata* as the sister of *A. transecta* + *A. leucophaea*-clade 1 subgroup. (A) and (C) show ACCTRAN optimization, indicating two independent origins of Ericaceae association and one reversal from Ericaceae- to Juglandaceae-association. (B) and (D) show DELTRAN optimization, suggesting that Ericaceae association has evolved three times independently.

3.2. Tests of alternative hypotheses

To evaluate the above-mentioned results statistically, we tested the following alternative hypotheses using the SOWH test. We inferred trees using ML analysis in which either monophyly of the Juglandaceae populations or that of the Ericaceae populations was constrained. However, the single origin of Juglandaceae- and Ericaceae association was rejected ($\Delta-\ln L = 58.72319$, $p < 0.01$, Fig. 3A; $\Delta-\ln L = 57.33871$, $p < 0.01$, Fig. 3B, respectively).

Acrocercops leucophaea was clearly divided into two separated clades in the estimated trees (Figs. 1 and 2). In the tree, *A. leucophaea*-clade 1 was the sister group of *A. transecta*, indicating that *A. leucophaea* is a paraphyletic species. We inferred a ML tree with monophyly of *A. leucophaea* being constrained, but this was rejected ($\Delta-\ln L = 1.38428$, $p < 0.05$, Fig. 3C).

Both MP and ML trees indicated that *A. leucophaea*-clade 1 was the sister group of *A. transecta* (Fig. 1). This result suggests that *A. leucophaea*-clade 1 may compose
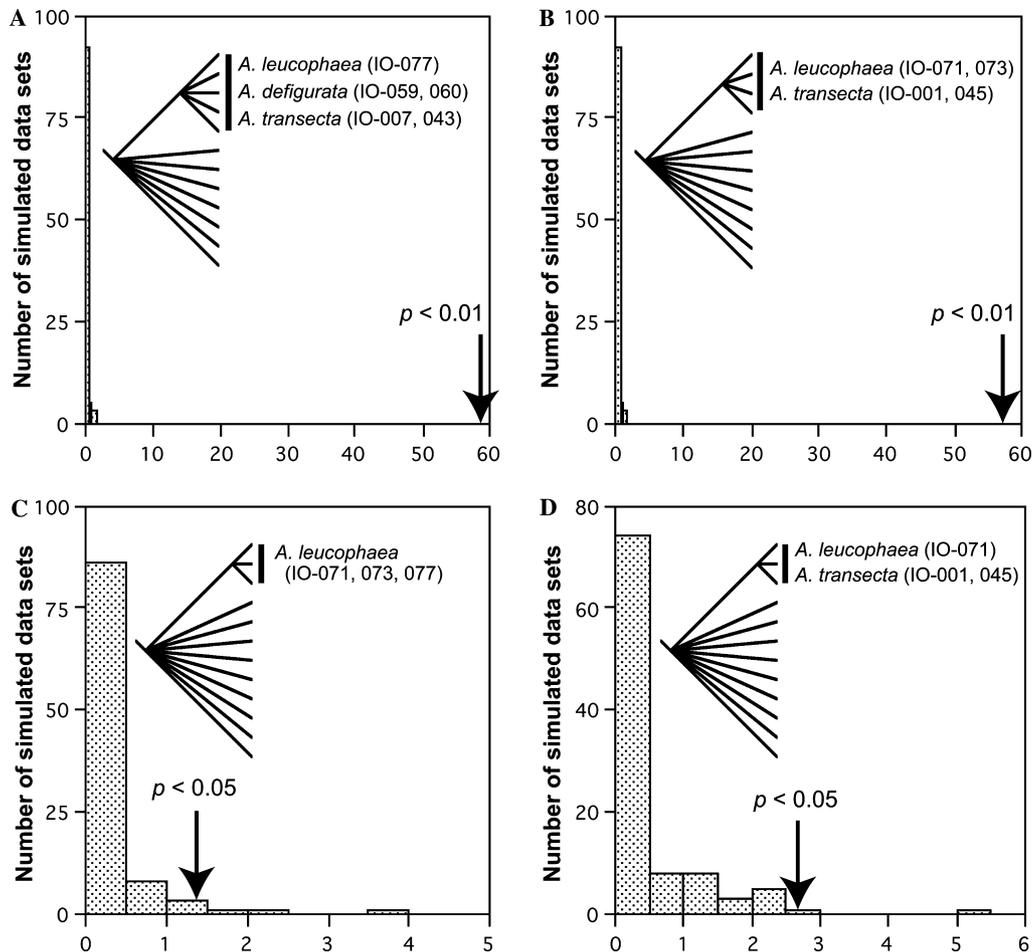
Fig. 3. Results of the parametric bootstrap analysis. The distribution of the differences in likelihood scores between the constrained (null hypothesis) and unconstrained trees for 100 replications is shown. (A) Test of monophyly of all Juglandaceae-associated populations. (B) Test of monophyly of all Ericaceae-associated populations. (C) Test of monophyly of *A. leucophaea*. (D) Test of monophyly of *A. leucophaea*-clade 1 and Ericaceae population of *A. transecta*. The former two hypotheses were rejected at $p < 0.01$, and the later two hypotheses were rejected at $p < 0.05$.

a monophyletic group with the Ericaceae population of *A. transecta*. SOWH test also rejected this possibility ($\Delta - \ln L = 2.71142$, $p < 0.05$, Fig. 3D).

4. Discussion

4.1. Phylogenetic inferences

The present analyses are based only on mitochondrial genes and thus the result represents gene tree. Phylogenetic hypotheses inferred from mitochondrial genes sometimes provide inaccurate estimates of species phylogeny due to mitochondrial introgression (Shaw, 2002). However, mitochondrial DNA is inherited maternally, so that the mitochondrial gene phylogeny directly reflects evolutionary history of female host preference and is useful for estimation of the history of host shifts (Diegisser et al., 2004).

Both MP and ML analyses based on the combined sequences provided well-resolved trees. Four major

clades, *A. leucophaea*-clade 1 and 2, *A. defigurata* and *A. transecta* were clearly detected. The present analyses also clearly distinguished two host-associated populations in *A. transecta*. However, the relative position of *A. defigurata* and *A. leucophaea*-clade 2 in *A. leucophaea* complex is rather unstable by MP analysis, although it does not influence the estimation of the history of host shifts.

Acrocercops transecta is monophyletic, and each of the Juglandaceae population and the Ericaceae population is also monophyletic. A preliminary study on oviposition preference revealed that females from different hosts exclusively oviposited on their natal host plant (Ohshima, 2005). In this regard, genetic differentiation may be suggested between the two populations. However, the two populations mated freely in the laboratory, and their hybrids are viable and fertile (Ohshima, unpubl. data). This mating compatibility indicates that these host races are maintained only by the differential host preferences of the females. Nevertheless, uncorrected pairwise distance between the two populations is rather large (1.01–1.83%). Avise (2000) pointed out that

intraspecific genetic divergences are rarely greater than 2% and most are less than 1%. In particular, uncorrected pairwise distance for COI gene between the two populations is 0.87–2.91%, which is equal to interspecies divergence (Hebert et al., 2003b). Even within each host-associated population, the maximum COI genetic distance for the Juglandaceae population is 2.62% and that for the Ericaceae population is 1.16%. These intra-population divergences are larger than the intraspecies divergence reported in several Lepidopteran families, 0.17–0.36% (Hebert et al., 2003a). An accelerated substitution rate in mitochondrial genes has been reported (Johnson et al., 2003; Page et al., 1998; Yoshizawa and Johnson, 2003). This possibility should be tested for these moths.

The two host-associated populations in *A. leucophaea*-clade 2 are not clearly distinguished in the present analyses. These results might imply that there is no host specialization in *A. leucophaea*-clade 2. However, two host-associated populations in the same locality (Goda-wari and Pharping) do not share the same haplotype, implying that host specialization has already developed within this clade. Another possibility is mitochondrial introgression from *A. leucophaea*-clade 2 to *A. leucophaea*-clade 1. Ericaceae-associated individuals in clade 2 may actually belong to clade 1. However, in *A. leucophaea*-clade 2 the Ericaceae population in Godawari or Nagarjun have a unique haplotype. Available evidence indicates that the present results were not due to mitochondrial gene introgression from *A. leucophaea*-clade 2 to clade 1. SOWH test also clearly distinguished clade 1 from clade 2 (Fig. 3C). Therefore, we conclude that the Ericaceae association has evolved in the two distinct *A. leucophaea* lineages independently.

The MPR of host plant associations suggests that the Juglandaceae association is the ancestral state for the complex. Outgroup taxa are associated with Fagaceae, which is phylogenetically closer to Juglandaceae rather than to Ericaceae (Soltis et al., 1999, 2000). Therefore, the result of MPR concerning ancestral host association in *A. leucophaea* complex is reasonable from the viewpoint of plant phylogeny. The MPR suggested two alternative hypotheses by ACCTRAN and DELTRAN optimizations (Fig. 2). Several studies on host race formation have revealed that derived races often retain adaptation for each ancestral host plant (Gross et al., 2004; Ikonen et al., 2003; Vanbergen et al., 2003). In *A. transecta*, preliminary studies on larval performance revealed that larvae from the Ericaceae population can survive on Juglandaceae plants but that larvae from the Juglandaceae population cannot survive on the Ericaceae plant (Ohshima, unpubl. data). The potential adaptation to Juglandaceae in the Ericaceae population may indicate that the population has derived from Juglandaceae population. Therefore, if above-mentioned relationships between phylogeny and host adaptation could be applicable for the case of

A. transecta, three host shifts from Juglandaceae to Ericaceae are more plausible.

The present results also provide biogeographical information for the host shift in *A. transecta*. MP and ML analyses indicated that the Juglandaceae-associated population in Vladivostok (Far East Russia) composed a monophyletic group with other Juglandaceae-associated populations in Japan, and that the Ericaceae-associated population in Taiwan also formed a monophyletic group with other Ericaceae-associated populations in Japan. These results suggest that the host shift in *A. transecta* had completed before its colonization of Japan.

4.2. Evolution of host associations

Becerra and Venable (1999) summarized three models to explain the macroevolution of insect–plant associations: (1) host shift to a new host plant that is chemically similar to the old host (Becerra, 1997; Ehrlich and Raven, 1964; Futuyma and McCafferty, 1990); (2) a parallel cladogenesis of plants and herbivorous insects (Farrell and Mitter, 1990; Mitter et al., 1991); (3) shift to new hosts that are present in the same geographic location as the range of their ancestral hosts (Bernays and Champan, 1994; Dobler et al., 1996).

The host plants of the *A. leucophaea* complex (Juglandaceae and Ericaceae) belong to different plant orders (Fagales and Ericales, respectively), and these orders are not phylogenetically closely related (Soltis et al., 1999, 2000). Therefore, the parallel cladogenesis hypothesis between these moths and host plants is rejected. In contrast, Juglandaceae plants and *L. ovalifolia* often grow side by side in the natural habitats of the *A. leucophaea* complex in Japan and Nepal (Ohshima, pers. obs.). This observation might support the same geographic location hypothesis. However, it is difficult to explain why *A. leucophaea* and *A. transecta* are exclusively associated with *L. ovalifolia* and why host shift from Juglandaceae plants to *L. ovalifolia* has occurred at least twice in different lineage. For example, several plant species, e.g., *Quercus glauca* (Fagaceae) that are closely related to Juglandaceae (Soltis et al., 1999, 2000) and some species of *Pieris* and *Vaccinium* (Ericaceae) that are closely related to *L. ovalifolia* (Kron et al., 2002), often grow sympatrically with Juglandaceae plants and *L. ovalifolia* in Japan and Nepal (Ohshima, pers. obs.). Nevertheless, the member of *A. leucophaea* species is not associated with these plants. Therefore, we hypothesize that chemical similarity (e.g., sharing of some oviposition attractant compounds) between Juglandaceae host plants and *L. ovalifolia* may have contributed to the unique host association in this species complex. Future studies should assess chemical similarity between Juglandaceae and *L. ovalifolia* to test this hypothesis.

Acknowledgments

References

Abrahamson, W.G., Eubanks, M.D., Blair, C.P., Whiipple, A.V., 2001. Gall flies, inquilines, and goldenrods: a model for host-race formation and sympatric speciation. Am. Zool. 41, 928–938.

Akaike, H., 1973. Information theory and an extension of the maximum likelihood principle. Abstracts of the Second International Symposium on Information Theory. Akademiai Kiado, Budapest, pp. 267–281.

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Control 19, 716–723.

Avise, J.C., 2000. Phylogeography. The History and Formation of Species. Harvard University Press, Cambridge, MA.

Becerra, J.X., 1997. Insects on plants: macroevolutionary chemical trends in host use. Science 276, 253–256.

Becerra, J.X., Venable, D.L., 1999. Macroevolution of insect–plant associations: the relevance of host biogeography to host affiliation. Proc. Natl. Acad. Sci. USA 96, 12626–12631.

Berlocher, S.H., Feder, J.L., 2002. Sympatric speciation in phytophagous insects: Moving beyond controversy? Annu. Rev. Entomol. 47, 773–815.

Bernays, E.A., Champan, R.F., 1994. Host–Plant Selection by Phytophagous Insects. Chapman & Hall, London.

Brown, J.M., Abrahamson, W.G., Way, P.A., 1996. Mitochondrial DNA phylogeography of host races of the goldenrod ball gall-maker, *Eurosta solidaginis* (Diptera: Tephritidae). Evolution 50, 777–786.

Brown, J.M., Pellmyr, O., Thompson, J.N., Harrison, R.G., 1994. Phylogeny of *Greya* (Lepidoptera: Prodoxidae) based on nucleotide sequence variation in mitochondrial cytochrome oxidase I and II: Congruence with morphological data. Mol. Biol. Evol. 11, 128–141.

Brunner, P.C., Chatzivassiliou, E.K., Katis, N.I., Frey, J.E., 2004. Host-associated genetic differentiation in *Thrips tabaci* (Insecta; Thysanoptera), as determined from mtDNA sequence data. Heredity 93, 364–370.

Bush, G.L., 1969. Sympatric host race formation and speciation in frugivorous flies in the genus *Rhagoletis* (Diptera: Tephritidae). Evolution 23, 237–251.

Bush, G.L., 1994. Sympatric speciation in animals: new wine in old bottles. Trends Ecol. Evol. 9, 285–288.

Craig, T.P., Horner, J.D., Itami, J.K., 1997. Hybridization studies on the host races of *Eurosta solidaginis*: implications for sympatric speciation. Evolution 51, 1552–1560.

Craig, T.P., Horner, J.D., Itami, J.K., 2001. Genetics, experience, and host–plant preference in *Eurosta solidaginis*: implications for host shifts and speciation. Evolution 55, 773–782.

Cruickshank, R.H., Johnson, K.P., Smith, V.S., Adams, R.J., Clayton, D.H., Page, R.D.M., 2001. Phylogenetic analysis of partial sequences of elongation factor 1α identifies major groups of lice (Insecta: Phthiraptera). Mol. Phylogenet. Evol. 19, 202–215.

Diegisser, T., Johannesen, J., Lehr, C., Seitz, A., 2004. Genetic and morphological differentiation in *Tephritis bardanae* (Diptera: Tephritidae): evidence for host-race formation. J. Evol. Biol. 17, 83–93.

Dobler, S., Mardulyn, P., Pasteels, J.M., Rowell-Rahier, M., 1996. Host–plant switches and the evolution of chemical defense and life history in the leaf beetle genus *Oreina*. Evolution 50, 2373–2386.

Drès, M., Mallet, J., 2002. Host-races in plant-feeding insects and their importance in sympatric speciation. Philos. Trans. R. Soc. Lond. B 357, 471–492.

Ehrlich, P.R., Raven, P.H., 1964. Butterflies and plants: a study in coevolution. Evolution 18, 586–608.

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.

Feder, J.L., 1998. The apple maggot fly, *Rhagoletis pomonella*: flies in the face of conventional wisdom about speciation. In: Howard, D.J., Berlocher, S.H. (Eds.), Endless Forms: Species and Speciation. Oxford University Press, New York, pp. 130–144.

Feder, J.L., Chilcote, C.A., Bush, G.L., 1988. Genetic differentiation between sympatric host race of *Rhagoletis pomonella*. Nature 336, 61–64.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Futuyma, D.J., McCafferty, S.S., 1990. Phylogeny and the evolution of host plant associations in the leaf beetle genus *Ophraella* (Coleoptera, Chrysomelidae). Evolution 44, 1885–1913.

Futuyma, D.J., Keese, M.C., Funk, D.J., 1995. Genetic constraints on macroevolution: the evolution of host affiliation in the leaf beetle genus *Ophraella*. Evolution 49, 797–809.

Goldman, N., Anderson, J.P., Rodrigo, A.G., 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49, 652–670.

Gross, J., Fatouros, N.E., Hilker, M., 2004. The significance of bottom-up effects for host plant specialization in *Chrysomela* leaf beetles. Oikos 105, 368–376.

Hafner, M.S., Sudman, P.D., Villablanca, F.X., Spradling, T.A., Demastes, J.W., Nadler, S.A., 1994. Disparate rates of molecular evolution in the cospeciating hosts and parasites. Science 265, 1087–1090.

Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R., 2003a. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B 270, 313–321.

Hebert, P.D.N., Ratnasingham, S., deWaard, J.R., 2003b. Barcoding animal life: cytochrome *c* oxidase subunit I divergences among closely related species. Proc. R. Soc. Lond. B 270 (Suppl.), S96–S99.

Howard, D.R., Patrik, N., 2005. Ecological speciation. Ecol. Lett., 8.

Ikonen, A., Sipura, M., Miettinen, S., Tahvanainen, J., 2003. Evidence for host race formation in the leaf beetle *Galerucella lineola*. Entomol. Exp. Appl. 108, 179–185.

Janz, N., Nylin, S., 1998. Butterflies and plants: a phylogenetic study. Evolution 52, 486–502.

Johnson, K.P., Cruickshank, R.H., Adams, R.J., Smith, V.S., Page, R.D.M., Clayton, D.H., 2003. Dramatically elevated rate of mitochondrial substitution in lice (Insecta: Phthiraptera). Mol. Phylogenet. Evol. 26, 231–242.

Kron, K.A., Judd, W.S., Stevens, P.F., Crayn, D.M., Anderberg, A.A., Gadek, P.A., Quinn, C.J., Luteyn, J.L., 2002. Phylogenetic classification of Ericaceae: molecular and morphological evidence. Bot. Rev. 68, 335–423.

Lopez-Vaamonde, C., Godfray, C., Cook, J.M., 2003. Evolution of host plant utilization in leaf mining moths: pattern and rate of host shifts. Evolution 57, 1804–1821.

Kumata, T., Kuroko, H., Ermolaev, V.P., 1988. Japanese species of the *Acrocercops*-group (Lepidoptera: Gracillariidae), part I. Ins. Matsum. n. s. 38, 1–111.

Maddison, D.R., Maddison, W.P., 2001. MacClade, Version 4.03. Sinauer, Sunderland, MA.

McPheron, B.A., Smith, D.C., Berlocher, S.H., 1988. Genetic differences between *Rhagoletis pomonella* host races. Nature 336, 64–66.

Mitter, C., Farrell, B., Futuyma, D.J., 1991. Phylogenetic studies of insect–plant interactions: insights into the genesis of diversity. Trends Ecol. Evol. 6, 290–293.

Moritz, C., Dowling, T.E., Brown, W.M., 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annu. Rev. Ecol. Syst. 18, 269–292.

Ohshima, I., 2005. Techniques for continuous rearing and assessing host preference of a multivoltine leaf-mining moth, *Acrocercops transecta* (Lepidoptera: Gracillariidae). Entomol. Sci. (in press).

Page, R.D.M., Lee, P.L.M., Becher, S.A., Griffiths, R., Clayton, D.H., 1998. A different tempo of mitochondrial DNA evolution in birds and their parasitic lice. Mol. Phylogenet. Evol. 9, 276–293.

Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of akaike information criterion and bayesian approaches over likelihood ratio tests. Syst. Biol. 53, 793–808.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Rambaut, A., Grassly, N.C., 1997. Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. Comput. Appl. Biosci. 13, 235–238.

Sakamoto, Y., Ishiguro, M., Kitagawa, G., 1986. Akaike Information Criterion Statistics. Springer, New York.

Schoonhoven, L.M., Jermy, T., Van Loon, J.J.A., 1998. Insect–Plant Biology, first ed. Chapman & Hall, London.

Shaw, K.L., 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proc. Natl. Acad. Sci. USA 99, 16122–16127.

Shimodaira, H., 2002. An approximately unbiased test of phylogenetic tree selection. Syst. Biol. 51, 492–508.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87, 651–701.

Soltis, P.S., Soltis, D.E., Chase, M.W., 1999. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. Nature 402, 402–404.

Soltis, D.E., Soltis, P.S., Chase, M.W., Mort, M.E., Albach, D.C., Zanis, M., Savolainen, V., Hahn, W.H., Hoot, S.B., Fay, M.F., Axtell, M., Swensen, S.M., Prince, L.M., Kress, W.J., Nixon, K.C., Farris, J.S., 2000. Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. Bot. J. Linn. Soc. 133, 381–461.

Strong, D.R., Lawton, J.H., Southwood, T.R.E., 1984. Insects on Plants. Harvard University Press, Cambridge, MA.

Swofford, D.L., 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4, Sinauer Associates, Sunderland, MA.

Swofford, D.L., Olsen, G.J., Waddell, P.J., Hillis, D.M., 1996. Phylogenetic inferences. In: Hillis, D.M., Mable, B.K., Moritz, C. (Eds.), Molecular Systematics, second ed. Sinauer, Sunderland, MA, pp. 407–514.

Symons, F.B., Beccaloni, G.W., 1999. Phylogenetic indices for measuring the diet breadths of phytophagous insects. Oecologia 119, 427–437.

Thompson, J.N., 1988. The evolution of diet breadth: monophagy and polyphagy in swallowtail butterflies. J. Evol. Biol. 11, 563–578.

Thompson, J.N., 1991. Evolution of oviposition behavior and host preference in Lepidoptera. Annu. Rev. Entomol. 36, 65–89.

Thompson, J.N., 1999. What we know and do not know about coevolution: insect herbivores and plants in a test case. In: Olff, H., Brown, V.K., Drent, R.H. (Eds.), Herbivores: Between Plants and Predators. Blackwell Science, Oxford, pp. 7–30.

Vanbergen, A.J., Raymond, B., Pearce, I.S.K., Watt, A., Hails, R.S., Hartley, S.E., 2003. Host shifting by *Operophtera brumata* into novel environments leads to population differentiation in life-history traits. Ecol. Entomol. 28, 604–612.

Wood, T.K., 1980. Divergence in the *Enchenopa binotata* Say complex (Homoptera: Membracidae) effected by host plant adaptation. Evolution 34, 147–160.

Wood, T.K., Guttman, S.I., 1983. *Enchenopa binotata* complex: sympatric speciation? Science 220, 310–312.

Yoshizawa, K., 2004. Molecular phylogeny of major lineages of *Trichadenotecnum* and a review of diagnostic morphological characters (Psocoptera: Psocidae). Syst. Entomol. 29, 383–394.

Yoshizawa, K., Johnson, K.P., 2003. Phylogenetic position of Phthiraptera (Insecta: Paraneoptera) and elevated rate of evolution in mitochondrial 12S and 16S rDNA. Mol. Phylogenet. Evol. 29, 102–114.