Available online at www.sciencedirect.com
SCIENCE DIRECT®

Molecular Phylogenetics and Evolution 38 (2006) 231–240

MOLECULAR PHYLOGENETICS AND EVOLUTION

www.elsevier.com/locate/ympev

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
Available online 20 July 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.

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).

1055-7903/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2005.06.010

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