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Molecular Phylogenetics and Evolution 35 (2005) 701–711

MOLECULAR
PHYLOGENETICS
AND
EVOLUTION

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Testing the trend towards specialization in herbivore–host plant associations using a molecular phylogeny of *Tomoplagia* (Diptera: Tephritidae)

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Received 11 August 2004; revised 17 February 2005
Available online 11 April 2005

Abstract

Herbivorous insects are abundant and diverse and insect–host plant associations tend to be specialized and evolutionarily conserved. Some authors suggested that generalist insect lineages tend to become specialists, with host specialization leading to an evolutionary dead-end for the parasite species. In this paper, we have examined this tendency using a phylogenetic tree of *Tomoplagia* (Diptera: Tephritidae), a parasite of asteracean plants. We have tested the trend towards specialization in different hierarchical degrees of host specialization. The topology of the tree, the inference of ancestral hosts, and the lack of directional evolution indicated that specialization does not correspond to a phylogenetic dead-end. Although most *Tomoplagia* species are restricted to a single host genus, specialization does not seem to limit further host range evolution. This work emphasizes the advantages of the use of different levels of specialization and the inclusion of occasional hosts to establish a more detailed scenario for the evolution of this kind of ecological association.

Keywords: Insect–plant interactions; Host specialization; Tephritidae; Asteraceae; Molecular phylogeny; Host shifts; Cytochrome oxidase subunit 2; Ribosomal 16S

1. Introduction

Herbivorous insects are extraordinarily diverse in tropical and temperate biomes, with roughly a quarter of all eukaryotes being insects that feed on plants (Bernays, 1998). In addition to their diversity, insect–plant associations are specialized (Bernays and Graham, 1988; Fry, 1996; Funk et al., 2002; Jaenike, 1990; Lopez-Vaamonde et al., 2003) and show a high degree of phylogenetic conservation, i.e., related insect lineages tend to feed on related plants (Benson et al., 1975; Janz and Nylin, 1998; Wahlberg, 2001).

Several theories have been developed to explain these patterns. The classic coevolutionary theory suggests that insect–plant interactions arose through successive evolutionary innovations in plant defenses and in their circumvention by insects, thus producing alternating episodes of plant and insect radiation (Ehrlich and Raven, 1964). Such an evolutionary pattern would be expected to result in close similarity in the sequence of speciation events in the plants and insects (Thompson, 1994). These similarities, however, have been rarely observed and, consequently, have been assumed to play

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doi:10.1016/j.ympev.2005.02.014

a minor role in the evolutionary associations between herbivores and their host-plants (Benson et al., 1975; Farrell and Mitter, 1993; Funk et al., 1995a,b; Janz et al., 2001; but see Farrell and Mitter, 1998; Percy et al., 2004).

On the other hand, herbivore lineages may switch their affinities to other plant groups after diversification of the host-plants, with no induction of any adaptive response in these plants (Jermy, 1984, 1993). Indeed, based on observations of host-shifts over short periods of time, many authors have proposed that, on an evolutionary scale, host affiliations of herbivorous insects may not be a consistent feature (Bernays and Graham, 1988; Rausher, 2001; Wasserman and Futuyma, 1981; but see also Feder et al., 2003). Nevertheless, if host-shifts are common, then one must explain the predominance of specialists over generalists, as well as the phylogenetic conservatism on an evolutionary scale.

Another classic hypothesis developed to explain these observations had suggested that specialization is a derived character (Brues, 1920). More specifically, any long-term association with a particular host may eventually result in the loss of the genetic variation associated with the ability to use alternate hosts, due to genetic drift or to the lack of selective pressure to maintain these alleles. This situation should culminate in an evolutionary dead-end for the parasite species. In this case, we should expect that the phylogenetic reconstruction of the parasites would show a trend towards specialization (Cope, 1896—*apud* Mayr, 1997); Futuyma and Moreno, 1988; Kelley and Farrell, 1998; Mayr, 1997; but see also Amadon, 1943; Janz et al., 2001; Termonia et al., 2001).

To properly test the tendency towards specialization in herbivorous insects using phylogenetic analysis, it is convenient to select a model in which insects and their hosts have the following characteristics: (1) the insect species should be closely related, (2) insect and plant groups should be speciose and of no economic value, (3) host ranges should be known accurately for all insect species, and (4) the insect species should show different degrees of host specialization. Unfortunately, most studies on this subject have neglected some or all of these characteristics (Dobler et al., 1996; Funk et al., 1995a; Nosil, 2002).

Therefore, in this paper, we studied the *Tomoplagia*–Asteraceae association, which shows all of the above characteristics, making it an exceptional model for studying insect–plant systems. For instance, *Tomoplagia* Coquillett is one of the most speciose and best studied genus of Neotropical tephritids (Prado and Lewinsohn, 1994; Prado et al., 2002), with 59 known species (Aczél, 1955; Prado et al., 2004). Their hosts belong to Asteraceae, the largest family of plants, with 23,000 species distributed in 1535 genera which, in turn, are subdivided into 17 tribes worldwide (Bremer, 1994). The host-records of *Tomoplagia* in Brazil reflect 17 years of an extensive inventory of endophagous insects among Asteracea (Lewinsohn, 1987, 1991; Prado et al., 2002) and are exceptionally complete and unequivocal. Finally, *Tomoplagia* can parasitize asteracean plants with different degrees of specialization (Headrick and Goeden, 1998), which means that some species are genus-, subtribe- or tribe-specialists, while others (generalists) parasitize various tribes among Asteraceae (Table 1).

We thus reconstructed the phylogeny of *Tomoplagia* based on two mitochondrial genes, *Cox*II and 16*S*, and tested the phylogenetic consistency of detailed host affiliations of *Tomoplagia*. To account for alternative explanations of the different patterns, we carefully analyzed different degrees and hierarchies of host specialization.

2. Materials and methods

2.1. The host-parasite system

The genus *Tomoplagia* belongs to the subfamily Tephritinae (Korneyev, 1999), the one with the most specialized insect–plant interactions among Tephritidae. *Tomoplagia* females, as well as the females of other endophagous insects, lay their eggs on the flower heads of the host, where the larvae develop. This characteristic is crucial to the unambiguity of the host-records of these flies, since the flower heads are sampled, transported, and maintained in laboratory until the emergence of the adults, providing accurate information about main and occasional hosts of each species. Furthermore, most of asteracean plants have no economical value, another important factor to determine their ecological habits and geographic distribution in more detail.

Since the meaning of the term “specialist” is not well defined in the literature (Kelley and Farrell, 1998), the different levels of specialization found in *Tomoplagia* are very important for testing evolutionary processes related to patterns of specialization. For instance, some authors consider as specialist a species which parasitizes a single host species, while others regard as specialist a species which is able to parasitize plants of an entire family (Nosil, 2002, and references therein).

We thus sampled 19 species of *Tomoplagia* from the Cerrado (Brazilian savannas) and Campos Rupestres (Highland grasslands) environments in the Brazilian states of Goiás (GO), Minas Gerais (MG), São Paulo (SP), and Santa Catarina (SC). The sampling sites for the individuals analyzed are shown in Fig. 1.

2.2. DNA preparation, amplification, and sequencing

Insects were identified soon after their emergence and were immediately stored in liquid N_2 until DNA extraction. Wings and terminalia of each individual were preserved for a taxonomic reevaluation, if necessary. Total