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Both host-plant phylogeny and chemistry have shaped the African seed-beetle radiation

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Abstract

For the last 40 years, many authors have attempted to characterize the main patterns of plant–insect evolutionary interactions and understand their causes. In the present work on African seed-beetles (Coleoptera: Bruchidae), we have performed a 10-year field work to sample seeds of more than 300 species of potential host-plants (from the family Fabaceae), to obtain bruchids by rearing. This seed sampling in the field was followed by the monitoring of adult emergences which gave us the opportunity to identify host-plant use accurately. Then, by using molecular phylogenetics (on a combined data set of four genes), we have investigated the relationships between host-plant preferences and insect phylogeny. Our objectives were to investigate the level of taxonomic conservatism in host-plant fidelity and host-plant chemistry. Our results indicate that phylogenetically related insects are associated with phylogenetically related host-plants but the phylogeny of the latter cannot alone explain the observed patterns. Major host shifts from Papilionoideae to Mimosoideae subfamilies have happened twice independently suggesting that feeding specialization on a given host-plant group is not always a dead end in seed-beetles. If host-plant taxonomy and chemistry in legumes generally provide consistent data, it appears that the nature of the seed secondary compounds may be the major factor driving the diversification of a large clade specializing on the subfamily Mimosoideae in which host-plant taxonomy is not consistent with chemical similarity.

Keywords: Evolution; Host shift; Host specificity; Phytophagous insects; Plant secondary compounds; Seed-beetles

1. Introduction

Endopterygote insects and especially Lepidoptera and Coleoptera have experienced a great evolutionary success that several authors have linked to the phytophagous behavior of near all (Lepidoptera) or the majority (Coleoptera) of the species of these insect orders (Farrell, 1998; Mitter et al., 1988). Likely the use of Angiosperms as feeding resources has facilitated the radiation of phytophagous Lepidoptera and Coleoptera (Farrell, 1998; Grimaldi, 1999). Thanks to PCR tune up, cheap sequencing facilities availability and improvement of tree building methods, an increasing number of comparisons between phytophagous insect and host-plant phylogenies have been performed during the last 15 years and have contributed to a better understanding of the evolution of plant–insect interactions. In addition to the classic Ehrlich and Raven's coevolutionary process (Berenbaum and Zangerl, 1998; Ehrlich and Raven, 1964; Farrell, 2001; Farrell and Mitter, 1998) which is debatable,

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phylogenetic studies in several groups of phytophagous Coleoptera and Lepidoptera have suggested several evolutionary patterns: (i) parallel evolution when the phylogeny of host-plants strongly constrained host affiliation and its evolution (Farrell and Mitter, 1990); (ii) conservatism of host-use when host shifts only occurred between closely related plants (Funk et al., 1995; Futuyma and McCafferty, 1990); (iii) diversification constrained by ecological and geographical factors (Dobler and Farrell, 1999; Dobler et al., 1996; Gomez-Zurita et al., 2000; Mardulyn et al., 1997; Menken et al., 1992); and (iv) chemical specialization when the major factor is the nature of the plants secondary compounds (Becerra, 1997; Garin et al., 1999; Swigonova and Kjer, 2004; Termonia et al., 2001; Wahlberg, 2001). However, a combination of these evolutionary patterns are not exclusive from one another to describe the diversification of a given group, highlighting the complexity of plant–insect interactions through time (Becerra and Venable, 1999; Bucheli et al., 2002; Jordal et al., 2004; Kelley and Farrell, 1998; Köpf et al., 1998). Many of the published studies focused on Chrysomelidae, a species rich family of phytophagous Coleoptera that belongs to the Chrysomeloidea super-family that also includes the longhorn-beetles (family Cerambycidae) and the seed-beetles. For the latter recent changes in taxonomy and thus nomenclature seem to favor the use of the subfamily name Bruchinae rather than Bruchidae (C.D. Johnson, pers. comm.), but for convenience (e.g., when using nomenclature ranks below the family level) we have used Bruchidae in this study. Bruchids comprise about 1700 species in about 60 genera (Johnson, 1994; Southgate, 1979). Five years after Ehrlich and Raven's (1964) classic paper, Janzen (1969) was the first to discuss the concept of coevolution in seed-beetles. The study of the evolutionary patterns driving the evolution of this highly specialized phytophagous group of Coleoptera was further discussed (Bleiler et al., 1988; Center and Johnson, 1974; Janzen et al., 1977) and Johnson (1990) presented a review of the literature related to this subject. The latter author suggested for future research on bruchid-plant associations the use of studies of systematics in conjonction with rigorous ecological and biogeographical studies. The integration of strong phylogenetic hypotheses with reliable ecological data were retained by Silvain and Delobel (1998) in their study of West African *Caryedon* and more recently by Kergoat et al. (2004) for European bruchids. The latter suggested a clear relationship between cladogenesis and host-plant association but to a certain extent only. Indeed, host shifts between non-related host-plants (from different botanical families) have occurred several times in the evolutionary history of these bruchids.

We have used molecular phylogenetics and host chemistry to investigate the radiation of African seed-beetles. These insects are known as seed-beetles because their larvae develop strictly in seeds. According to Johnson (1970), about 84% of their known host-plants (the use of host-plant refer to larvae feeding in seeds of the plants) belong to the family Fabaceae. Many species are pests of plants of economic importance and have become cosmopolitan (Johnson, 1981) whereas others are potentially important as natural enemies of invasive legumes such as *Acacia* spp. (Rohner and Ward, 1999; Van Tonder, 1985) or Scotch broom (Downey and Smith, 2000). We have focused on the large and probably paraphyletic (Johnson, 1981; Kergoat and Silvain, 2004) genus *Bruchidius* SCHILSKY. This genus is restricted to the Old World (Borowiec, 1987) and more than 250 species are known (Udayagiri and Wadhi, 1989). Since the systematic of this genus is still debated, we have chosen to include closely related genera (*Callosobruchus*, *Conicobruchus*, *Decellebruchus*, and *Tuberculobruchus*) in what is regarded as the group *Bruchidius* sensu lato, to have a better overall view. Interestingly, almost all known host-plants in the group *Bruchidius* sensu lato belong to the family Fabaceae but we have accurate records of host-plant use for at least two other botanical families (Delobel and Delobel, 2003). The evaluation of insect host-plant associations is a critical issue, as available literature usually includes many errors and unverified records (Ehrlich and Raven, 1964). According to Delobel and Delobel (2003), Jermy and Szentesi (2003), and Johnson et al. (2004), most published host records for bruchids are unreliable and literature must be used cautiously. Indeed, in earlier studies, host-plant data were frequently based on adult beetles collected on plants in nature. This emphasizes the necessity of performing extensive seed sampling in the field, and eventually monitoring adult emergences to identify host-plant associations accurately.

Regarding host-plant secondary compounds, the phytochemistry of the family Fabaceae is well documented (Bisby et al., 1994). Nitrogen-based defensive compounds (alkaloids, amino acids, cyanogenic glycosides, lectins, and proteinase inhibitors) are frequently encountered in legume seeds, and their role as an efficient defense against bruchids has been demonstrated (Birch et al., 1986; Gatehouse et al., 1990; Janzen et al., 1977; Janzen, 1981; Rosenthal, 1990). In their recent study, Wink and Mohamed (2003) have suggested a complex history of the Fabaceae chemical defense traits. For example, the observed distribution of some secondary compounds (e.g., the L-canavanine in subfamily Papilionoideae) implies many loss or gain events whereas other secondary compounds are restricted to phylogenetically related groups of taxa (e.g., the quinolizidine alkaloids in genistoids sensu lato). The repeated development of such potent chemical defense traits in the evolutionary history of the family Fabaceae has certainly influenced the evolution of the highly specialized family Bruchidae, constraining some of its members to develop key innovations (Berenbaum et al., 1996; Simpson, 1953)

allowing further shift toward well protected plants. However, until now, the influence of these secondary compounds on seed-beetles evolution has not been studied within a phylogenetic framework.

We have sequenced three mitochondrial genes (12S rRNA, cytochrome *b*, and cytochrome *c* oxidase subunit I) and a nuclear gene (D2 domain of the 28S rDNA) for 50 African bruchids species and two outgroups. The phylogenetic hypotheses obtained for the insects will subsequently be analyzed in view of existing host-plant relationships (based on published molecular phylogenetic trees) and host-plant chemistry. Thus we will investigate the level of taxonomic conservatism in host-plant fidelity, and host-plant chemistry, questioning the general assessment that host-plant taxonomy is generally consistent with chemical similarity.

2. Materials and methods

2.1. Taxon sampling

Several thousand seeds of potential host-plants (from the family Fabaceae) were collected to obtain bruchids by rearing. This extensive field work was conducted in Senegal (from 1994 to 1999), Egypt (from 2000 to 2003), and Kenya (from 2001 to 2003). The sampling in Senegal (from 95 localities) was particularly extensive, resulting in seeds from 96 potential host-plants (corresponding to 33 distinct genera). In Egypt, the seeds from 95 potential host-plants (corresponding to 49 distinct genera) were collected from 29 localities. In Kenya, the seeds from 50 potential host-plants (corresponding to 19 distinct genera) were collected from 49 localities. Seed samples were afterwards transferred to local laboratories. They were kept separately for several months at room temperature in aerated plastic bags or boxes, until emergence of adults. These were fixed and stored in 100% ethanol and transferred to France for identification and DNA extraction. Specimens corresponding to this study are kept in the "Institut de Recherche pour le Développement (IRD)" collection of the "Muséum National d'Histoire Naturelle (MNHN)" (45 rue Buffon, Paris). Slide preparations of male genitalia and external morphological key-characters were used for identification. Despite the availability of many types of African bruchids (from MNHN in France and J. Decelle collections), 17 species with unique male genitalia remain unidentified. The species analyzed, their countries of origin, the host-plant records and their systematics are listed in Table 1. For the *Acacia* species, we have followed Vassal's (1972) subgeneric classification. When necessary, host-plant name records were updated using the ILDIS (International Legume Database and Information Services: www.ildis.org) database.

2.2. DNA sequencing and alignments

DNA was extracted, amplified, and sequenced with standard protocols described elsewhere (Kergoat et al., 2004). For the amplification of 28S-D2 rDNA gene the following primers were used: (i) 28S01 (5′-GAC TACCCCCTGAATTTAAGCAT-3′); (ii) 28SR01 (5′-GACTCCTTGGTCCGTGTTTCAAG-3′). Alignment of coding sequences (COI and Cyt *b*) was unambiguous as no gap event was detected. Alignment of 12S rRNA and 28S-D2 rDNA genes were performed by using ClustalX (Thompson et al., 1997) with default settings. After alignment, the combined sequence data set was 2963 bp in length, with 899 parsimony-informative characters. The resulting sequences and Voucher information were deposited in GenBank under Accession Nos. AY390636, AY390668, AY390700, and AY625282–AY625477, and the combined sequence data set was deposited to TreeBase under Accession No. SN1978-6644.

2.3. Phylogenetic analyses

Bayesian inference was used to reconstruct phylogenetic relationships among taxa with *Pachymerus cardo* and *Gibbobruchus* sp. used as outgroup species. Preliminary analyses using incongruence length difference tests (Farris et al., 1994) have indicated that our four gene data sets were not congruent. To better take the heterogeneity of our data into account, we have chosen to perform a partitioned Bayesian analysis (Nylander et al., 2004). This analysis was carried out by using MrBayes version 3.0b4 (Huelsenbeck and Ronquist, 2001), with four partitions defined (corresponding to the four genes sequenced). For each gene, the best-fit substitution model was determined by Modeltest 3.06 (Posada and Crandall, 1998) through hierarchical likelihood ratio tests. Afterwards, a 2,000,000 generations run with four incrementally heated chains and distinct parameters estimated for each partition was conducted. Trees were saved to a file every 100 generations. The results were presented in the form of a 50% majority-rule consensus tree and the support for the nodes of this tree were given by clade posterior probability estimates.

2.4. Host-plant phylogenies

Phylogenetic hypotheses for the whole family Fabaceae were provided by recent studies (Doyle et al., 1997; Käss and Wink, 1997; Miller and Bayer, 2001; Robinson and Harris, 2000; Wink and Mohamed, 2003). According to these reviews the following assumptions can be made: (i) the family Fabaceae, the subfamilies Mimosoideae and Papilionoideae constitute monophyletic groups; (ii) the subfamily Caesalpinioideae constitutes a paraphyletic group in which tribes Cercideae and Detarieae are basal; (iii) the clade constituted by the tribes

Table 1
Material examined in this study

Species	Host-plant species	Systematic[a]	Source[b]
Bruchidius			
albopubens	*Indigofera aspera*	Pap.: Ind.	1.
	Indigofera parviflorum	Pap.: Ind.	1.
	Indigofera senegalensis	Pap.: Ind.	1.
auratopubens	*Faidherbia albida*	Mim.: Ing.	1
aurivillii	*Acacia tortilis*	Mim.: Ac.[1]	1.2.
cadei	*Faidherbia albida*	Mim.: Ing.	1.
campylacanthae	*Acacia polyacantha*	Mim.: Ac.[2]	1.
chloroticus	*Sesbania grandiflora*	Pap.: Rob.	1.
	Sesbania keniensis	Pap.: Rob.	2.
	Sesbania leptocarpa	Pap.: Rob.	1.
	Sesbania pachycarpa	Pap.: Rob.	1.
	Sesbania quadrata	Pap.: Rob.	2.
	Sesbania rostrata	Pap.: Rob.	1.
	Sesbania sesban	Pap.: Rob.	1.
centromaculatus	*Acacia farnesiana*	Mim.: Ac.[1]	3.
	Acacia nilotica	Mim.: Ac.[1]	1. 3.
	Acacia sieberiana	Mim.: Ac.[1]	1.
dichrostachydis	*Dichrostachys cinerea*	Mim.: Mim.	1.
dialii	*Dialium guineense*	Cae.: Cas.	1.
elnairensis	*Acacia dolichocephala*	Mim.: Ac.[1]	2.
fulvus	*Alhagi graecorum*	Pap.: Gal.	3.
incarnatus	*Vicia faba*	Pap.: Vic.	3.
lineatopygus	*Indigofera tinctoria*	Pap.: Ind.	1.
niokolobaensis	*Tephrosia bracteolata*	Pap.: Mil.	1.
nodieri	*Indigofera astragalina*	Pap.: Ind.	1.
	Indigofera hirsuta	Pap.: Ind.	1.
pygidiopictus	*Faidherbia albida*	Mim.: Ing.	1.
quadrisignatus	*Acacia ataxacantha*	Mim.: Ac.[2]	2.
	Acacia brevispica	Mim.: Ac.[2]	2.
raddianae	*Acacia ehrenbergiana*	Mim.: Ac.[1]	3.
	Acacia seyal	Mim.: Ac.[1]	3.
	Acacia tortilis	Mim.: Ac.[1]	1. 3.
rubicundus	*Acacia laeta*	Mim.: Ac.[2]	2.
	Acacia mellifera	Mim.: Ac.[2]	2.
	Acacia polyacantha	Mim.: Ac.[2]	2.
	Acacia thomasii	Mim.: Ac.[2]	2.
saudicus	*Acacia etbaica*	Mim.: Ac.[1]	2.
	Acacia reficiens	Mim.: Ac.[1]	2.
	Acacia zanzibarica	Mim.: Ac.[1]	2.
securiger	*Dichrostachys cinerea*	Mim.: Mim.	1.
submaculatus	*Acacia ataxacantha*	Mim.: Ac.[2]	1.
	Acacia dudgeoni	Mim.: Ac.[2]	1.
	Acacia macrostachya	Mim.: Ac.[2]	1.
	Acacia polyacantha	Mim.: Ac.[2]	1.
	Acacia senegal	Mim.: Ac.[2]	1.
uberatus	*Acacia nilotica*	Mim.: Ac.[1]	1. 3.
sp. KE01	*Faidherbia albida*	Mim.: Ing.	2.
sp. KE02	*Acacia brevispica*	Mim.: Ac.[2]	2.
sp. KE03	*Acacia etbaica*	Mim.: Ac.[1]	2.
sp. KE04	*Acacia etbaica*	Mim.: Ac.[1]	2.
	Acacia reficiens	Mim.: Ac.[1]	2.
sp. KE05	*Acacia nilotica*	Mim.: Ac.[1]	2.
sp. KE06	*Acacia nilotica*	Mim.: Ac.[1]	2.
sp. KE07	*Acacia oerfota*	Mim.: Ac.[1]	2.
sp. KE08	*Acacia zanzibarica*	Mim.: Ac.[1]	2.
sp. KE09	*Albizia grandibracteata*	Mim.: Ing.	2.
	Albizia versicolor	Mim.: Ing.	2.
sp. KE10	*Delonix elata*	Cae.: Cae.	2.
sp. KE11	*Indigofera arrecta*	Pap.: Ind.	2.
sp. KE12	*Indigofera arrecta*	Pap.: Ind.	2.
sp. KE14	*Desmodium velutinum*	Pap.: Des.	2.
sp. SE01	*Aeschynomene indica*	Pap.: Aes.	1.
	Aeschynomene sensitiva	Pap.: Aes.	1.
Callosobruchus			
chinensis	*Cajanus cajan*	Pap.: Pha.	3.
maculatus	*Vigna radiata*	Pap.: Pha.	2.
	Vigna unguiculata	Pap.: Pha.	1.
phaseoli	*Lablab purpureus*	Pap.: Pha.	3.
subinnotatus	*Vigna subterranea*	Pap.: Pha.	1.
Conicobruchus			
strangulatus	*Crotalaria comosa*	Pap.: Cro.	1.
	Crotalaria glaucoides	Pap.: Cro.	1.
	Crotalaria goreensis	Pap.: Cro.	1.
	Crotalaria perrottetii	Pap.: Cro.	1.
	Crotalaria podocarpa	Pap.: Cro.	1.
Decellebruchus			
atrolineatus	*Vigna unguiculata*	Pap.: Pha.	1.
Tuberculobruchus			
albizziarum	*Albizia lebbeck*	Mim.: Ing.	1.
babaulti	*Acacia amythethophylla*	Mim.: Ac.[1]	2.
	Acacia etbaica	Mim.: Ac.[1]	2.
natalensis	*Acacia sieberiana*	Mim.: Ac.[1]	1.
silaceus	*Acacia ataxacantha*	Mim.: Ac.[2]	1.
	Acacia macrostachya	Mim.: Ac.[2]	2.
sinaitus	*Acacia tortilis*	Mim.: Ac.[1]	1. 3.
subuniformis	*Acacia ataxacantha*	Mim.: Ac.[2]	2.
Gibbobruchus			
sp.		Cae.: Cer.	4.
Pachymerus			
cardo	*Elaeis guineense*	Arecaceae	4.

[a] Host-plant systematic was abbreviated as follows: Cae. (Caesalpinioideae), Mim. (Mimosoideae), Pap. (Papilionoideae), Ac.[1] (Acacieae: Acacia subgenus Acacia), Ac.[2] (Acacieae: Acacia subgenus Aculeiferum), Aes. (Aeschynomeneae), Cae. (Caesalpinieae), Cas. (Cassieae), Cer. (Cercideae), Cro. (Crotalarieae), Ind. (Indigofereae), Des. (Desmodieae), Ing. (Ingeae), Gal. (Galegeae), Mil. (Milletieae), Mim. (Mimoseae), Pha. (Phaseoleae), Rob. (Robinieae), and Vic. (Vicieae).

[b] Source: 1., Senegal field data; 2., Kenya field data; 3., Egypt field data; and 4., French Guyana field data.

Cassieae and Caesalpinieae (belonging to the subfamily Caesalpinioideae) and the subfamily Mimosoideae is the sister-clade of the subfamily Papilionoideae; and (iv) the monogeneric tribe Acacieae (and therefore the genus *Acacia*) constitutes a paraphyletic group.

2.5. Character optimizations

The host-plant preferences and host-plant seed chemistry were mapped parsimoniously on the same phylogenetic tree by using the program MacClade 4.05 (Maddison and Maddison, 2002) with the ACCTRAN algorithm. Host-plant preference optimizations followed a two step process. First, character optimizations at the subfamily level (Caesalpinioideae, Mimosoideae, and Papilionoideae) were performed. Then, more accurate mapping of host-plant preferences were carried out below the subfamily level. These character optimizations were exclusively based upon host-plant affiliations provided by our field data, to avoid erroneous host-plant

records. Regarding the mapping of host-plant chemistry, we focused on recognized toxic seed secondary compounds (Bleiler et al., 1988; Center and Johnson, 1974; Evans et al., 1979; Gatehouse et al., 1990; Janzen et al., 1977). Then, character optimizations for five classes of secondary compounds (amines, alkaloids, non-proteic amino acids, isoflavonoids, and proteinase inhibitors) were performed. Corresponding data on seed secondary compounds were taken from the literature (Bell et al., 1978; Bisby et al., 1994; Di Martino-Ferrer and Ferrer, 1983; Evans et al., 1977; Gatehouse et al., 1980; Ignacimuthu et al., 2000; Pando et al., 2001; Seigler, 2003; Wink and Mohamed, 2003). We deliberately choose to simplify data relative to the non-proteic amino acids composition of seeds with albizzine (Bisby et al., 1994; Evans et al., 1977, 1979; Seigler, 2003). Indeed, several other non-proteic amino acids are associated with albizzine (e.g., *S*-carboxyethylcysteine, *S*-[β-carboxyisopropyl]-L-cysteine, α-amino-β-acetylaminopropionic acid, α-amino-β-oxalylaminopropionic acid, and free αβ-diaminopropionic acid) with some variations. Consequently we choose to code this character as "albizzine and others" in our analyses. Regarding proteinase inhibitors, we have distinguished the following types of inhibitors (Pando et al., 2001): (i) Bowman-Birk inhibitors which inhibit both trypsin and chymotrypsin at independent reactive sites and are specific to the subfamily Papilionoideae; (ii) Kunitz inhibitors which have varied performances, inhibiting either trypsin or chymotrypsin or the two of them the Kunitz type inhibitors of *Delonix* spp. inhibit trypsin only (Pando et al., 2001), and are more widely distributed in Fabaceae.

3. Results

The partitioned Bayesian analysis of the combined data set was carried out with the same best-fit model of evolution that is the general time reversible model with a proportion of invariable sites and a gamma distribution (Gu et al., 1995; Lanave et al., 1984; Yang, 1994). After the 2,000,000 generations run, a burn-in period of 50,000 generations was identified, by plotting graphically likelihood values every 100 generations. The 500 trees corresponding to this burn-in period were subsequently not retained in the 50% majority-rule consensus tree shown in Fig. 1. The latter topology is well supported by the different clade posterior-probability estimates, and provides a clear picture of African *Bruchidius* sensu lato relationships. Moreover, it strongly assesses the supposed paraphyly of the genera *Bruchidius* sensu stricto and *Tuberculobruchus*. The various host-plant records (listed in Table 1) for the seed-beetles sampled in this study suggest a strong trend toward oligophagy and specialization. Not only most of the bruchids sampled in this study feed on a small number of host-plants, but they also present a high level of specificity (e.g., species feeding on genus *Acacia* present a higher level of specialization as they only feed on a given subgenus). As indicated in Table 1, each of the seed-beetles studied exclusively feeds on plants belonging to a given botanical tribe. Due to this high level of host-plant specificity, the mapping of host-plant preferences was facilitated and the inclusion of polymorphous characters was not necessary. The character optimization of host chemistry was also trivial since each bruchid species was feeding on a set of host-plants with identical secondary compounds. Yet, one species, *Bruchidius quadrisignatus*, was feeding on host-plants presenting two of the secondary compounds retained in our analyses (amine and non-proteic amino acids). Both character optimizations (host preferences and host chemistry) are presented on a mirror image cladogram (Fig. 1). We have also performed parsimonious analyses of the data sets but they have resulted in poorly supported topologies (not figured), especially for basal nodes. Character optimizations of the corresponding topologies essentially yield similar conclusions to the ones obtained by using the topology resulting from the partitioned Bayesian analysis. Since the results of character optimizations are highly dependent on the robustness of the phylogenetic hypotheses available, we have not chosen to present them.

4. Discussion

4.1. Evolution of host-plant associations

Character mapping of host-plant preferences at the plant-subfamily level reveals a strong conservatism of host use and provides valuable information on the evolutionary history of host-plant associations. Thus, each of the seed-beetles studied exclusively feeds on a given subfamily, and phylogenetically related species are generally associated with plants belonging to the same subfamily. Our analyses also suggest that the subfamily Papilionoideae is the ancestral host-plant group of the African genus *Bruchidius* sensu lato. Given that non-African members of the genus *Bruchidius* sensu lato are associated with the subfamily Papilionoideae (with some exceptions for species feeding outside the family Fabaceae), our results indicate that the subfamily Papilionoideae could be the ancestral host-plant group of all *Bruchidius* sensu lato species. Two independent host shifts from Papilionoideae toward Mimosoideae have given rise to two subsequent and successful radiations on plants of that subfamily. Finally, two species (*Bruchidius dialii* and *Bruchidius* sp. KE10) have independently shifted toward the subfamily Caesalpinioideae, from distinct mimosoid feeder ancestors belonging to the same group. Despite a large sampling of seeds from 50 Caesalpinioideae species (belonging to 18 distinct genera), we have only reared bruchids from two of these

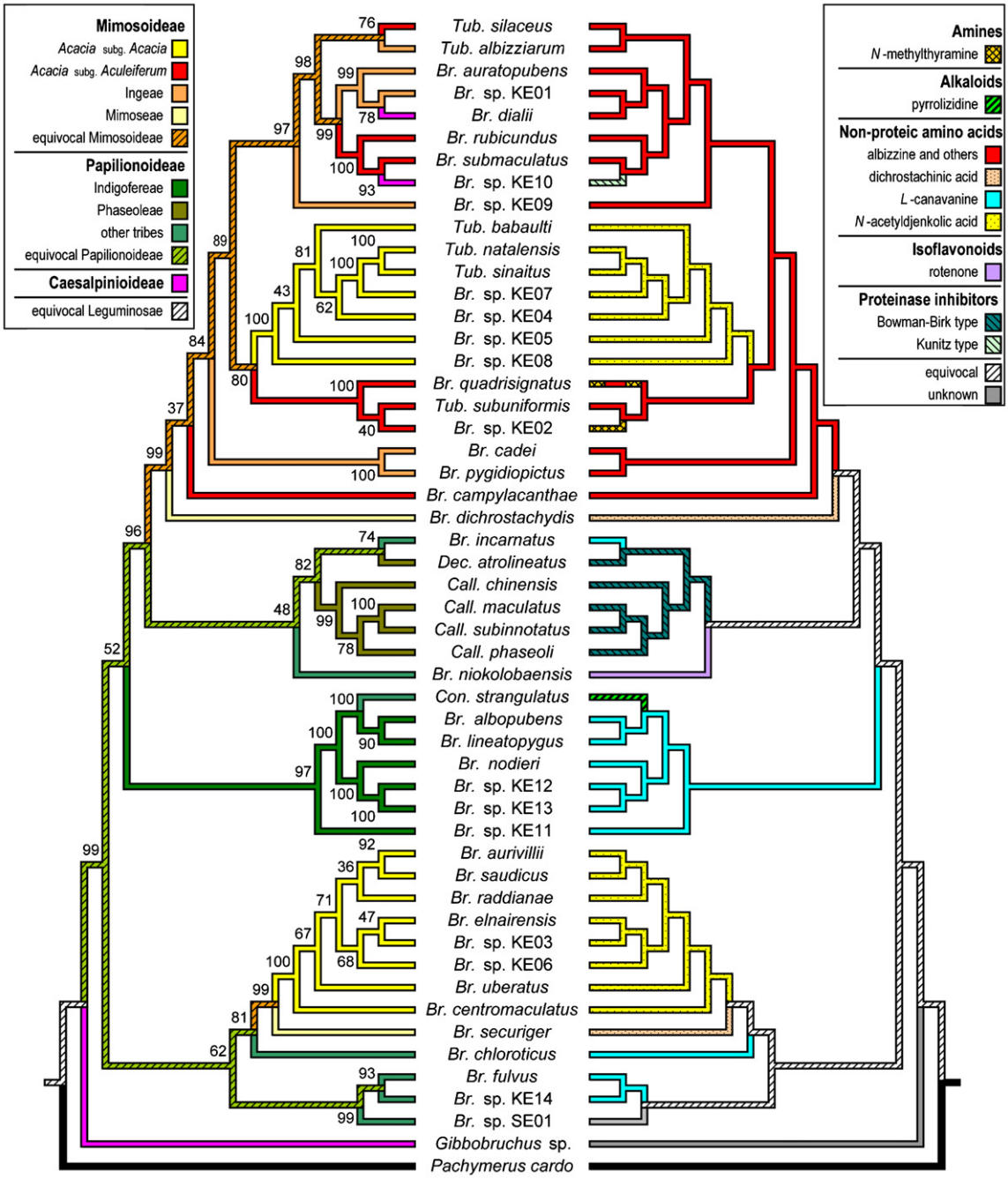

Fig. 1. Mirror image of the 50% majority-rule consensus tree from the partitioned Bayesian inference analysis of the combined data set; numbers indicate the clade posterior probability estimates for each node. On the left phylogram the parsimonious reconstruction of host-plant preferences at the subfamily level, and below the subfamily level are figured. On the right phylogram the parsimonious reconstruction of host-plant chemistry is figured. Detailed legends for both character optimizations are provided on the top of the figure.

Caesalpinioideae species (*Dialium guineense* and *Delonix elata*). In contrast with the mimosoid feeders history, host shifts toward Caesalpinioideae appear isolated as they are not followed by a successful seed-beetle radiation on other host-plants of that subfamily. This assumption is consistent with our observations in the field and host-plant data from the literature (Johnson, 1981), which seldom mention Caesalpinioideae as host-plants of *Bruchidius* seed-beetles (less than five *Bruchidius* species are known to feed on Caesalpinioideae). *Bruchidius* sensu lato offer a contrasted view of the evolutionary significance of feeding specialization in phytophagous insects (Janz et al., 2001; Kelley and Farrell, 1998; Nosil, 2002; Scheffer and Wiegmann, 2000; Termonia et al., 2001). The two independent host-shifts from Papilionoideae toward Mimosoideae show that

feeding specialization on papilionoid legumes did not hamper subsequent shifts to Mimosoideae, suggesting that feeding specialization, at least at the host-plant subfamilial level, is not always a dead end. On the contrary, shifts to Caesalpinioideae seem to have engaged the two species that experienced it in such a dead end. It is interesting to note that the hypothesis of a mimosoid origin of caesalpinioid feeders was previously suggested by Delobel et al. (2000) for seed-beetles belonging to the genus *Caryedon*. However, unlike *Bruchidius* species, the latter have undergone a successful radiation on several species of Caesalpinioideae.

The examination of host preference patterns below the subfamily level for species associated with the Papilionoideae strengthens the idea that host fidelity was a predominant factor in their evolutionary history. Thus, species associated with plants from the same tribe (Indigofereae or Phaseoleae in our study) are phylogenetically related, and this tallies with similar observations for European members of this genus (Kergoat et al., 2004). Nonetheless, for the mimosoid feeders, the observed host affiliation patterns for both tribes Ingeae and Acacieae suggest that host fidelity was lost at this level, with the exception of species associated with the subgenus *Acacia*.

Despite the strong conservatism in host-plant use revealed by the mapping of host preferences, the hypothesis of a possible co-speciation between African *Bruchidius* sensu lato and their host-plants is not supported by the comparison of their respective phylogenies. On the subfamily level, the two phylogenies are not congruent. Indeed, our analyses suggest that the subfamily Papilionoideae is the ancestral host-plant group of the African genus *Bruchidius* sensu lato and that caesalpinioid feeders came from ancestors feeding on Mimosoideae. On the contrary, phylogenetic hypotheses for the family Fabaceae indicate that the Mimosoideae and Papilionoideae both originate from the paraphyletic Caesalpinioideae group. Besides, when examining sister-clades of bruchids associated with distinct host-plant tribes, we found no evidence of a relation with plant phylogenies (e.g., species feeding on Indigofereae and Crotalarieae tribes are related but the latter tribes are phylogenetically distant).

4.2. Influence of host-plant chemistry

According to Johnson (1990), "seed toxins are one of the most robust selective agents driving bruchids to specificity to their hosts." Rotenone, for instance, is a complex isoflavonoid well-known for its strong insecticidal properties (Birch et al., 1985; Center and Johnson, 1974) and therefore very few bruchids do develop in seeds containing this compound (Gatehouse et al., 1990). Until now, only two *Bruchidius* sensu lato species (*Bruchidius nalandus* and *Bruchidius tephrosiae*) were known to feed on *Tephrosia* spp. seeds (which contain rotenone). Similarly, only two bruchid species (*Conicobruchus indicus* and *Conicobruchus strangulatus*) are known to feed on *Crotalaria* spp. seeds (which contain crotalarine, a specific pyrrolizidine alkaloid). For some of these compounds, the mechanisms of detoxification are now better understood: (i) for instance, the adaptation to L-canavanine in many bruchid species have been intensively investigated (Bleiler et al., 1988; Rosenthal, 1990) and the corresponding detoxifying biochemical pathways are known; (ii) a recent study (Oliveira et al., 2002) on the activity of various proteinase inhibitors toward bruchids suggests that some bruchid species have circumvented their deleterious effect by using serine proteinases as major digestive enzymes (instead of using cysteine proteinases).

The examination of the secondary compound distribution within a phylogenetic framework (i.e., the bruchid phylogeny) provides significant information on the evolution of the African seed-beetles. The observed pattern shows a clear correlation between host-plant chemistry and the seed-beetle phylogeny and indicates that phylogenetically related bruchids generally feed on host-plants with similar defensive traits. However, since phylogenetically related host-plants often share similar chemical defensive traits, the influence of plant phylogeny cannot be denied. For instance, the L-canavanine non-proteic amino acid is present in the seeds of almost all studied species of the genus *Indigofera* (Bell et al., 1978; Bisby et al., 1994) and consequently both character optimizations of host preferences and host chemistry yield a similar pattern. It is also the case for the various species feeding on the tribe Phaseoleae which consistently contain Bowman-Birk type proteinase inhibitors. Interestingly, the mapping of host chemistry suggest either a widespread preadaptation to many toxic compounds or multiple independent apparitions of detoxifying abilities in the evolutionary history of African seed-beetles. It is particularly the case for several lineages of seed-beetles feeding on the tribe Papilionoideae which have the ability to detoxify the widespread L-canavanine non-proteic amino acid.

As emphasized previously, the most striking pattern in our analyses is observed in the largest clade of mimosoid feeders where host fidelity has been lost. In this group, the mapping of host preferences suggests that bruchid species associated with the subgenus *Aculeiferum* are more phylogenetically related to species feeding on the tribe Ingeae or on the subfamily Caesalpinioideae than the ones associated with the subgenus *Acacia* (with the exception of a clade of three species), although *Acacia* and *Aculeiferum* are sister clades. Indeed, this pattern of host affiliation is better explained by the nature of seeds secondary compounds. Species belonging to the subgenus *Acacia* consistently contain *N*-acetyldjenkolic acid, whereas species from subgenus *Aculeiferum* have

different non-proteic amino acids (Bisby et al., 1994; Evans et al., 1977, 1979; Seigler, 2003). These non-proteic amino acids (e.g., albizzine) also occur in the seeds of the studied species from the Ingeae tribe and in the caesalpinioid *Dialium guineense*. Therefore host chemistry appears to be the major factor explaining the diversification of this clade of mimosoid feeders.

5. Conclusions

This study casts a new light on the evolution of African seed-beetles and suggests a complex evolutionary history resulting from the combination of different evolutionary patterns. Despite the strong taxonomic conservatism of host fidelity exhibited by the species studied, host shifts toward plants from different botanical subfamilies have nonetheless occurred several times in bruchid evolutionary history. This result also suggests that feeding specialization on a given host-plant group is not always a dead end in seed-beetles. The originality of the present work is to provide counter-examples to the general rule of taxonomic conservatism in host-plant use. If most bruchid lineages have consistently diversified in agreement with that rule (the phylogenetic relationships of seed-beetles reflects host-plant taxonomy which in turn is consistent to chemical similarity), one clade of bruchid has followed a different pathway. This clade originally on Papilionoideae has subsequently shifted to Mimosoideae and diversified according to host-plant chemistry which does not match the plant taxonomy. This clearly shows that the key-factor driving diversification within this clade of seed-beetles was primarily plant chemistry and not plant taxonomy. As a result, it appears that the factors prevailing in the radiation of phytophagous insects may differ even within closely related lineages.

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