

## Relationships of herbivore feeding and plant flavonoids (Coleoptera: Chrysomelidae and Anacardiaceae: *Rhus*)

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**Summary.** Eighteen leaf flavonoid compounds were isolated from several populations of *Rhus tripartita* from xeric habitats in Israel, ornamental *Schinus terebinthifolius* from Israel, and three species of *Rhus* (*vulgaris*, *natalensis*, *tenuinervis*) from mesic habitats in Kenya. Foodplant preference testing of *Rhus*-feeding Leaf Beetles (*Blepharida sacra* from Israel and *B. marginalis* and *B. conradsi* from Kenya) correlated well with the flavonoid composition of the different foodplants. *Blepharida sacra* and *B. marginalis* foodplant preferences demonstrated an herbivore sibling relationship but there is evidence, including foodplant ecology and distribution, that they are separate species. The herbivore-foodplant coevolution of the xeric *B. sacra*-*R. tripartita* is distinct from that of the mesic *B. marginalis*-*R. vulgaris*/*natalensis*, however, this study also indicates possible ancestral relationships between the herbivore species as well as between the plant species.

**Key words:** Chrysomelidae: *Blepharida* – Anacardiaceae: *Rhus* – Flavonoids – Herbivore-plant interaction

The discovery of the Sumac *Rhus tripartita* (Bernard, da Ucria) Grande as the foodplant for the Leaf Beetle *Blepharida sacra* (Weise) led to a detailed study of the biology of this herbivorous beetle species belonging to the primarily Afrotropical flea beetle genus *Blepharida* (Furth 1982). Resulting from the study of the ecological associations of *Blepharida sacra*, a comprehensive study of *Rhus tripartita* was undertaken to understand this plant as a functioning ecological system and its natural history (Furth 1985b). To this end, several relict desert and Mediterranean refuge populations of *R. tripartita* were surveyed to assess entomological and botanical differences and similarities between the two types of populations. As part of the natural history study of *R. tripartita*, a list was prepared of herbivorous arthropods recorded in the literature to feed on species of *Rhus* (*sensu lato*) and *Pistacia* (a close relative) from around the world (Furth 1985b). Research on the herbivores of *R. tripartita* proved to be the most informative aspect of its natural history because of their often close dependence on this species as a food source. The most dependent herbivores are monophagous species, e.g., *B. sacra*, *Thaumetopoea jordana* (Staudinger) (Furth and Halperin 1980), and *Peliococcus deserticola* Ben-Dov and Gerson (Furth et al.

1984), that have coevolved with the chemistry of this Sumac tree. Thus, a logical extension to the natural history and biological studies on herbivores is the examination of the foodplant chemistry and its potential effects on those herbivores.

Herbivores must overcome a variety of secondary "defense" chemicals contained in their foodplants either by de-toxifying or storing them. Specialist herbivores, i.e., monophagous species, are particularly "coevolved" in this manner with their foodplant and must either continue to overcome any slight changes in their host's chemistry or switch to another host, or go extinct. Flavonoids are probably among the most important secondary chemicals relative to herbivores (Harborne 1979), including Sumacs (*Rhus* spp. *sensu lato*). Flavonoids also have potential for demonstrating evolutionary/phylogenetic relationships among various plants groups (Harborne 1977), including *Rhus* (Young 1979). While there are other chemical groups that may be of importance to Sumac herbivores, e.g. tannins and terpenes, there has been little testing of the effects of *Rhus* flavonoids on herbivores. Therefore, a special effort was made to obtain leaf samples of *R. tripartita* from several populations at different seasons, as well as other species of *Rhus*, including some from tropical Africa.

*Rhus tripartita* is distributed in rather isolated populations across North Africa into the Middle East in relict desert or refuge Mediterranean habitats (Furth 1985b). Evidence is presented below suggesting that *R. tripartita* is related to Afro-tropical *Rhus*. *Rhus* (*sensu lato*) probably spread from Eurasia to Africa in the early Tertiary (Quezel 1979, in litt. 1985), extended southwards throughout Africa during the Pliocene and Pleistocene, and then speciated and radiated in different directions (Furth 1985b). One of these radiations may have given rise to *R. tripartita* and/or its relatives that spread from tropical African climates northward into the Sahara and whose origin became obscured by subsequent climatic fluctuations (Furth 1985b).

*Blepharida sacra* is found in the hills adjacent to the Lower Jordan River Valley and in western Samaria (present-day Israel), and in the hills near the Red Sea coast of Egypt and Sudan. It apparently exists only where there is a large population of *R. tripartita*. Life cycle studies of this beetle species in Israel (Furth 1982) revealed that adults eclose from pupae and feed from May into July, then begin a summer diapause; they break diapause in late September, feed, copulate, oviposit, and thrive into January. Larvae emerge and feed from early October through January. Stu-

dies of *B. sacra* and Afro-tropical species of *Blepharida* have suggested that *B. sacra* is closely related to the tropical African species (Furth 1982; unpublished work). Therefore, considering the monophagous relationship of *B. sacra* with *R. tripartita*, and records of two *Blepharida* on *Rhus* in Kenya (Furth 1985a), it seemed potentially revealing to test the feeding preferences of *B. sacra* and some of its African relatives using *R. tripartita* and some Afro-tropical *Rhus* species, to gain some insight into relationships of the beetles and of the plants, as well as to herbivore-plant co-evolution.

#### Methods and materials

Most leaf samples were collected at times when either *Blepharida* larvae or adults (or both) were feeding, in Israel and in Kenya. From each of the two major *R. tripartita* populations one leaf sample was taken out of *B. sacra* feeding season (Table 2, samples E and I) in order to check for significant differences in flavonoid chemistry. Also, the leaf samples from the Negev (samples J and K) were from populations without *B. sacra* and out of their feeding season. Leaf samples were collected at several times (see Table 2) during the growing season from three populations of *R. tripartita* in Israel, during 1980 and 1981: five samples were collected at Yarihiv (Western Samaria: 32°09' N, 34°58' E); four samples collected at Wadi Qilt (Judean Desert: 31°50' N, 35°22' E); and one sample each collected at two locations in the Negev Desert (Makhtesh HaQatan: 30°58' N, 35°12' E and Ma'ale Tamar: 31°00' N, 35°16' E). In addition, samples of three species of *Rhus* from Kenya were collected in June 1980: *R. natalensis* Berh. at Langata Forest (1°23' S, 35°44' E); *R. vulgaris* Meikle and *R. tenuinervis* Engler at Stony Athi (1°28' S, 37°00' E). Also, a sample from the Brazilian Pepper Tree (*Schinus terebinthifolius* Raddi) was collected at Rehovot, Israel in March, 1981. Flavonoids from the above mentioned leaf samples (air dried) were extracted with 80% aqueous methanol and concentrated under reduced pressure on a rotary evaporator. Two-dimensional paper chromatograms were prepared for each extract using standard methods (Mabry et al. 1970). Tentative identification of each compound detected on the chromatograms was based on comparisons of their  $R_f$  values and colors (under ultra-violet light, with and without ammonia vapor) with those of known compounds identified from other species of *Rhus* and related genera of Anacardiaceae, and data published in standard reference works (Harborne and Mabry 1982; Young 1976, 1979; Young and Aist 1985).

Fresh leaf material of all of the above plant species (plus *R. coriaria* Linnaeus from northern Israel) was collected to test foodplant feeding preference acceptance with three species of Leaf Beetles – *B. sacra* (Weise), *B. marginalis* Weise, and *B. conradsi* (Weise). Food preference testing was conducted in the laboratory at the Faculty of Agriculture of the Hebrew University of Jerusalem, in Rehovot, Israel during June and July, 1980. The temperature and relative humidity were approximately those of coastal Israel at that season; for June/July the average daily temperature is 23.6–25.4°C and the average daily relative humidity is 58–62%. Both adults and larvae of *B. marginalis* and *B. conradsi* and adults of *B. sacra* were tested in small plastic cages with screen tops. Fresh leaf samples of one or two plant species were placed in each cage with the beetles.

Detailed feeding observations and notes were made at least twice daily.

#### Results

The analysis of flavonoids of *R. tripartita* revealed 18 flavonoid compounds, many of which are flavonol glycosides of myricetin and quercetin (see Table 1). Although there was some variation, the eleven leaf samples of *R. tripartita* generally were quite similar in flavonoid composition. Compounds 1 and 2 were present in all samples and compounds 3, 14, 15, and 16 were in most samples of *R. tripartita* (Table 2). Only four *R. tripartita* samples were collected when no *B. sacra* adults or larvae were feeding (i.e., samples E, I, J, K) yet these samples showed no significant differences in flavonoid composition from the other inseason samples. The three species of *Rhus* from Kenya and *Schinus* (a genus native to S. America and cultivated in Israel) each had distinct flavonoid profiles, although most shared some compounds with *R. tripartita* (Table 2). *Rhus natalensis* is distinctive through the presence of flavonoid compounds 10–12. *R. vulgaris* is unique in having compounds 5 and 6, and *Schinus* is the only plant tested that contains compounds 7 and 8, and it shares 9 with *R. natalensis* (Table 2). *Rhus tenuinervis* was quite distinct in that only a single compound (no. 13) was detected in its leaves and this compound was unique to *R. tenuinervis*. Based upon  $R_f$  values and comparison with 2-D chromatograms of leaf samples of other Anacardiaceae, compound 13 most likely is a biflavonoid or a mixture of biflavonoids (Young, unpublished work). Compounds 1, 2, and 3, tentatively identified as myricetin 3-O-glucoside, quercetin 3-O-glucoside, and a quercetin diglycoside respectively, were present in most *R. tripartita* populations and in *R. natalensis*, *R. vulgaris* (the two most widespread species of Afro-tropical *Rhus*), and in *S. terebinthifolius*. These compounds also have been detected in several other species of *Rhus* and other Anacardiaceae (Young 1976, 1979, unpublished work; Young and Aist 1985).

The results of the foodplant preference testing is presented in Table 3. In most instances where both larvae and adults of a beetle species were tested with a particular foodplant species, both stages responded similarly; a notable exception was the larvae of *B. sacra* and *B. marginalis* which fed only sparsely on leaves of *Schinus*, whereas adults of both species fed voraciously on this plant. Although it was not feasible to make quantitative measurements of the feeding (e.g., limited quantities of the Kenyan foodplant species) the data in Table 3 are supported with additional information from laboratory observations. A few feeding tests were not feasible because of lack of adequate numbers of larvae.

Although not apparent from Table 3, qualitative laboratory observations indicate that both adults and larvae of *B. marginalis* showed a preference for *R. vulgaris* over *R. natalensis* and *R. tripartita*. *Blepharida sacra* (adults) also demonstrated an expected preference for its native foodplant *R. tripartita* over *R. vulgaris* and *R. natalensis*, with an apparently stronger preference for *R. vulgaris* over *R. natalensis*. Basically, the foodplant preferences of *B. sacra* and *B. marginalis* are the same, but differ significantly from the preferences of *B. conradsi* in response to *R. vulgaris* and to *Schinus*. It is noteworthy that none of the three beetle species fed at all on *R. tenuinervis* or *R. coriaria* (not tested for leaf flavonoids).



**Table 3.** Foodplant preference/acceptability tests. Leaf Beetles – *Blepharida sacra* (from Israel), *B. marginalis*, and *B. conradsi* (from Kenya) on Sumacs – *Rhus tripartita* (Rt), *R. coriaria* (Rc), and *Schinus terebinthifolius* (Sht), (from Israel), and *R. vulgaris* (RvK), *R. natalensis* (RnK), and *R. tenuinervis* (RtnK) (from Kenya)

Beetle	Rt	RvK	RnK	RtnK	Rc	Sht
<i>B. sacra</i>						
larvae	+*	0	0	0	-*	“+”*
adults	+	+	+	-	-	+
<i>B. marginalis</i>						
larvae	+	+	+	-	-	?
adults	+	+	+	-	-	+
<i>B. conradsi</i>						
larvae	+	?	-	0	0	0
adults	+	?	-	-	-	-

+ = definite feeding on leaves

- = no feeding on leaves

0 = not tested

? = some slight nibbling on leaves, but probably only random tasting

\* = tested in previous study (Furth 1982)

“+” = some true feeding, but weak

(quercetin aglycone) in samples C, D, E and K (Table 2) is probably not significant, and most likely represents the hydrolysis products of one or more of the quercetin glycosides present in the samples. The same might also apply to the variation detected in compound 14, which is myricetin aglycone. The fact that compounds 15 and 16 (rengasin and fisetin aglycones) were detected in some samples, but not others, is somewhat surprising and, perhaps, is attributable to sample size differences. In addition, these two compounds are much more commonly found in heartwood rather than leaf tissues (Young 1976, 1979). Disregarding this variation, the differences among the populations of *R. tripartita* are rather minor.

This study examined only leaf flavonoids and, of course, there are other secondary chemicals present in *Rhus* that may have a significant effect on foodplant preferences, attraction, and repellence (e.g., tannins, terpenes, etc.). However, there is reason to believe that leaf flavonoids do play an important role in Sumac herbivory and that they may indicate some relationship among plant species. Although some variation did exist, leaf samples from the three populations of *R. tripartita* examined were similar in flavonoid composition both during and after the feeding season of *B. sacra* larvae and adults. Compounds 1–3 and 14–16 were present in most of the 11 *R. tripartita* samples. Several types of flavonoids tentatively identified from leaf samples in this study are of interest. Flavonoids usually occur in living cells as glycosides and glycosylation may be important in leaf flavonoids as a protective device for keeping otherwise toxic materials in an inactive form in the plant (Harborne 1979). Reports of the toxicity of certain flavones, flavonols, and flavonol glycosides suggest that flavonoids may be feeding deterrents (Harborne 1977) or simply toxic to herbivores. Certain flavonol and other flavonoid compounds are known to be feeding attractants or stimulants for phytophagous insects (Harborne 1979). Two flavonoids present in most species in this study are quercetin and myricetin, and their glycosides. Quercetin is known to act as a feeding repellent and myricetin is usually restricted to the leaves of woody plants in association with tannins (Harborne

1979). Most of the leaf compounds found in this study are types of flavonol glycosides. Together with other secondary chemicals, a mixture of flavonol glycosides may be selectively advantageous in deterring feeding by a range of herbivorous insects. Yet a small group of herbivores could have coevolved with such a complement of flavonoids and may actually be attracted or stimulated by this same combination of secondary chemicals.

The flavonoid compound analysis (Table 2) indicates that *R. vulgaris* and *R. natalensis* are quite similar to *R. tripartita* sharing compounds 1–3 and 14, 15. Each of these two Afro-tropical species has additional flavonoid compounds (4 and 17–18, respectively) that may relate to the feeding preferences of *Blepharida* and may indicate botanical relationships among the tropical African *Rhus* and *R. tripartita*; however, this phytochemical similarity may only indicate an old (primitive) relationship. *Schinus terebinthifolius* also contains flavonoid compounds 1–3, common to *R. tripartita*, *R. vulgaris*, and *R. natalensis*, but lacks compounds 14–18 (apparently less important for the beetle's herbivory) that are present in these other species. Because *B. sacra* and *B. marginalis* will feed on the above three species of *Rhus* as well as *Schinus*, it seems possible that the flavonoid compounds 1, 2, and 3 may have feeding stimulant effects on these phytophagous insects. Flavone feeding stimulants are known for the Alligatorweed flea beetle (Zielske et al. 1972). *Rhus tenuinervis* lacks all the flavonoid compounds except 13 which may be a feeding deterrent compound (or mixture) or this plant may lack the necessary feeding stimulants. Even though *B. sacra* and *B. marginalis* demonstrated only small differences in feeding preference between *R. vulgaris* and *R. natalensis*, there is further evidence of difference in these plants indicated by their flavonoid composition (Table 2) and by the feeding tests with *B. conradsi* (Table 3). Species of *Blepharida* are able to feed on several chemically related foodplants but they still have monophagous preferences which probably play a significant role in their speciation. Of course, *Blepharida* feeding preference may also involve other secondary chemicals (i.e., feeding stimulants or deterrents, attractants or repellents). Nevertheless, there are indications that the flavonoids analyzed here play a role in the foodplant preferences of *B. sacra* and *B. marginalis*.

These results also indicate some chemical relationships between *R. tripartita* and the two Afro-tropical species, especially *R. vulgaris*. *Rhus tripartita* has other affinities with the tropical African lineage through its trifoliolate condition (possibly primitive) which is typical of Afro-tropical species of *Rhus*. Similarities of *R. tripartita* to *R. vulgaris* and *R. natalensis*, especially feeding preferences of *B. sacra* and *B. marginalis*, suggest a relationship of these species of Sumac and a possible Afro-tropical origin for *R. tripartita*. This study has provided the basis for speculations about relationships; however, it would be taxonomically and phylogenetically even more informative relative to these *Rhus* species to further analyze leaf flavonoids using additional population samples and to include a few other species, e.g., the pentafoliolate *R. pentaphylla*, the compound-leafted *R. coriaria* widespread in Europe and the Near East, as well as additional Afro-tropical species.

Scherer (in litt. and unpublished work) had indicated that *B. sacra* is possibly conspecific with *B. marginalis* or a subspecies of it. Although these two species are morphologically very similar, there are a few differences. The most

significant morphological difference is the shape of the male aedeagus (Furth, unpublished work). This morphological evidence together with the foodplant preference results described here indicate that *B. sacra* and *B. marginalis* are sibling species, very closely related and probably recently derived from a common ancestor. Possibly the most significant difference between these two beetle species is their biogeography (in a strict sense); that is, the differences of their distribution reflected by the distribution of their *Rhus* foodplants. *Blepharida sacra* is restricted to *R. tripartita* and is distributed in the Lower Jordan River Valley and western Samaria and along the Red Sea coast of Sudan (Furth 1982). Basically this distribution and that of its foodplant are in distinctly xeric (arid) habitats (Furth 1982, 1985b). *Blepharida marginalis* is known from several countries in tropical Africa, most records for it are in the vicinity of Lake Victoria: Ruanda, southern Uganda, northeastern Zaire (Scherer 1962); northern Tanzania (Weise 1906); White Nile valley emanating from Lake Victoria in southern Sudan (Scherer 1972). Also, *B. marginalis* has been recorded in south central Ethiopia in the highland lake district (Scherer 1972). In all of these localities the habitat and climate suggests a distinctively mesic (moist) environment of tropical Africa. This clearly contrasts with the xeric localities where *B. sacra* has been found in the Middle East and northern Africa. Therefore, there is a geographical separation of *B. sacra* and *B. marginalis* based on the ecological limitations of their respective foodplants, *R. tripartita* and *R. vulgaris*. The northern herbivore/plant pair (*B. sacra*/*R. tripartita*) may have coevolved more recently in isolation as xeric conditions became prevalent in North Africa and the Middle East. Thus, both plant chemistry and eco-geographic isolation may be important factors in the speciation process of these Sumacs and their flea beetle herbivores.

**Acknowledgements.** David Furth is grateful to the Department of Entomology, Faculty of Agriculture of the Hebrew University of Jerusalem, in Rehovot, Israel, for providing the laboratory facilities for the foodplant testing. Miss Christine Kabuye (botany) and Dr. Michael Clifton (entomology) of the Nairobi National Museum helped D. Furth locate collecting localities in Kenya for Sumac species and Leaf Beetles, respectively. Miss Kabuye identified the Sumac species. Prof. John Kokwaro was kind enough to show D. Furth his unpublished manuscript on east African species of *Rhus*. David Furth thanks Dr. Thomas Odhiambo, Director of the International Center for Insect Physiology and Ecology (ICIPE) for the invitation to visit ICIPE in 1980. David Furth would like to thank Dr. Gerhard Scherer (Zoologische Staatssammlung, München) for informative correspondence and discussions about *Blepharida*.

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Received October 23, 1986