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FLAVONOIDS OF HAWAIIAN ENDEMIC *LYSIMACHIA*

IN HONOUR OF PROFESSOR G. H. NEIL TOWERS 75TH BIRTHDAY

KENDRICK L. MARR, BRUCE A. BOHM,* CHRISTINA COOKE and PHILIP GUNNING

Department of Botany, University of British Columbia, Vancouver, B.C. V6T 1Z4, Canada

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Key Word Index—*Lysimachia*; subgen. *Lysimachiopsis*; Primulaceae; Hawaiian Islands; endemic species; flavonoids; interpopulational variation.

Abstract—Flavonoids of 12 of the 13 extant species of *Lysimachia* (Primulaceae) endemic to the Hawaiian Islands were isolated and identified. Whenever possible, at least two populations were sampled. The flavonoid profiles consisted of various combinations of kaempferol, quercetin and isorhamnetin 3-*O*-mono-, di- and triglycosides, vitexin and isovitexin, and eriodictyol 7-*O*-glucoside. Flavonol di- and triglycosides were the predominant compounds present in all of the profiles. Profiles were variable both within and among specimens such that taxon-specific patterns do not appear to exist in most cases. An exception is the finding that the three leaf forms of *L. remyi* subsp. *remyi*, the most variable taxon in the subgenus, exhibit the most coherent flavonoid pattern. Flavonoid variation is compared to other Hawaiian endemic species, e.g. *Bidens*. Flavonoids do not support recognition of the endemic species, currently considered to constitute subgenus *Lysimachiopsis*, at the generic level. The flavonoids of subgen. *Lysimachiopsis* have some structural features in common with each of the other subgenera for which data are available.

INTRODUCTION

Lysimachia is one of the largest genera of Primulaceae, consisting of approximately 180 species of upright or sprawling perennial or annual herbs, shrubs or subshrubs. The center of diversity is southwestern China where, according to Chen and Hu [1], there are 122 species, of which 110 are endemic to the region. Species also occur in temperate areas of the Northern Hemisphere, the southeastern Asian tropics, South America, Africa and Australia [2]. Six subgenera and 18 sections are recognized, based primarily on the work of Handel-Mazzetti [3] who emphasized floral structure, particularly the androecium. Handel-Mazzetti's [3] classification was modified somewhat in the treatment by Chen and Hu [1].

Two subgenera occur in the Hawaiian Islands, subgen. *Palladia* (Moench) Hand.-Mazz. represented by *L. mauritiana* Lam., which is an herbaceous coastline species, and the endemic subgen. *Lysimachiopsis* (Heller) Hand.-Mazz., which consists of 13 extant and three extinct species [4]. Members of *Lysimachiopsis* are perennial, scandent or upright, woody shrubs. The woody habit, 5–10 merous perianth and the tetracolporate pollen are unique in the family and strongly suggest that the subgenus is monophyletic in origin. Recent electrophoretic data support this view [5].

Adaptive radiation has resulted in species with narrow ecological ranges including wet, rocky cliffs, montane bogs, subalpine mesic shrublands, montane dry and wet forests and lowland mesic shrublands, as classified by Gagne and Cuddihy [6]. Species are found on the islands of Kauai, Oahu, Maui, Molokai and Lanai, and occur from 250 to 2300 m. Members of subgen. *Lysimachiopsis* apparently do not occur on the youngest island, Hawaii.

The objectives of the present study were to characterize the flavonoids of the endemic Hawaiian species with the aim of evaluating their potential as taxonomic markers, and to compare the findings with published information on other members of the genus. It was also of interest to document intra- and interpopulational variation in the flavonoid profiles so that comparison with other island systems could be made.

RESULTS

The major compounds in all 12 species examined were flavonol glycosides based on kaempferol, quercetin and isorhamnetin. The glycosides were predominantly 3-*O*-diglycosides and 3-*O*-triglycosides; the only flavonol monoglycoside identified was quer-

* Author to whom correspondence should be addressed.

Table 1. Occurrence of flavonoids in Hawaiian *Lysimachia*

Taxon (*N*)†	Flavonoid derivative*												
	QM1	QD1	QT1	QT2	KD1	KT1	KT2	ID1	IT1	IT2	VIT	IVT	ERI
L. daphnoides (2)	0	2	0	2	0	0	0	1	0	1	0	0	1
L. glutinosa (3)	3	3	0	3	0	0	1	0	0	0	0	0	0
L. hillebrandii (2)	0	2	0	1	0	1	0	0	0	0	0	0	0
L. filifolia (1)	0	1	0	1	0	0	0	1	0	0	0	0	0
L. iniki (1)	0	1	0	0	0	0	0	1	1	0	0	0	0
L. kalalauensis (5)	1	4	0	1	2	2	5	5	1	3	0	0	1
L. maxima (1)	0	1	0	0	0	0	0	0	0	0	0	1	1
L. ovoidea (4)	1	3	0	3	0	4	3	0	0	0	0	0	0
L. pendens (2)	0	1	0	1	1	2	0	0	0	1	1	0	0
L. remyi caliginis (5)	0	5	0	0	1	1	0	0	0	1	0	0	
L. r. kipahuluensis (5)	0	5	0	2	0	0	0	5	1	0	0	0	1
L. r. remyi narrow (3)	1	3	2	0	3	3	0	2	1	0	0	0	0
L. r. remyi interm. (4)	3	4	3	3	2	3	0	2	0	0	2	0	1
L. r. remyi broad (3)	1	3	2	2	3	3	2	2	1	0	0	0	1
L. r. subherbacea (4)	0	4	0	4	0	3	0	4	4	0	0	0	1
L. scopulensis (1)	1	1	0	1	1	0	1	0	0	0	0	0	0
L. waianaeensis (5)	0	5	0	4	2	1	3	3	1	0	1	1	1
Totals (51)‡	11	48	7	28	15	23	15	26	10	5	5	2	8

*K = Kaempferol; Q = Quercetin; I = Isorhamnetin; M = Monoglycoside; D = Diglycoside; T = Triglycoside; VIT = Vitexin; IVT = Isovitexin; ERI = Eriodictyol 7-*O*-glucoside.

†Number of individual plants examined.

‡Totals represents the total number of individuals analyzed and, under the flavonoids, the number of times each was recorded.

cetin 3-*O*-glucoside. The triglycosides gave only glucose and rhamnose upon total acid hydrolysis and consistently gave the corresponding flavonol 3-*O*-rutinosides on partial acid hydrolysis. We did not determine whether the other sugar moiety was glucose or rhamnose nor did we determine where on the disaccharide it was linked. Vitexin and isovitexin were observed in four and two species, respectively, and in only one, *L. waianaeensis*, were both present together (Table 1). Representative of the wide variation seen in the distribution of flavonoids in *Lysimachia* in this study is the observation that four specimens among five of *L. waianaeensis* had neither *C*-glycosylflavone. Eriodictyol 7-*O*-glucoside was observed in six species but not in all specimens of the species for which more than one specimen was available. Flavonoid differences were also noted between subspecies of *L. remyi* and among the "broad," "intermediate," and "narrow" leaf-shape variants of *L. remyi* subsp. *remyi*. The only taxon-specific flavonoid recorded was "QT1," which occurred only in *L. remyi* ssp. *remyi*, but not in all members of each leaf width class. Values for the total number of reports of each flavonoid for the entire data set are also given. For example, quercetin 3-*O*-rutinoside was the only compound that occurred in all of the taxa; only three specimens of 51 analyzed did not have it. The next most abundant compound can be seen to be "QT2" and so forth.

DISCUSSION

The study of island endemic species offers an excellent opportunity to address one of the most pressing questions in evolutionary biology, namely, what changes occur when an organism becomes established in an isolated location and begins to undergo adaptive radiation. The Hawaiian Islands have attracted a good deal of attention owing to the high level of species endemism in the flora. Several impressive examples of adaptive radiation are known from the islands, the most widely studied of which is probably the Silversword alliance, which involves *Argyroxiphium*, *Dubautia* and *Wilkesia* [7]. Other examples of adaptive radiation in the islands are *Bidens* [8], *Tetramolopium* [9], and members of the genera comprising the Hawaiian lobelias [10]. *Lysimachia* presents us with another situation involving a number of species that have diverged enough to be considered unique within Primulaceae. Two subgenera of *Lysimachia* occur on the Hawaiian Islands, the dozen or so species that comprise subgen. *Lysimachiopsis*, and *L. mauritiana*, a member of subgen. *Palladia* widely distributed on Pacific islands. Detailed morphometric and breeding studies [5], coupled with electrophoretic analysis of all extant endemic species of *Lysimachia* (Marr and Bohm, unpubl. data) suggest that they comprise a group of genetically closely related group of species that has resulted from a single introduction.

A study of flavonoid pigment profiles of these species was undertaken in order to see if they might assist in identifying groups of taxa within the genus. The only reasonably clear-cut situation where the flavonoid data are helpful involves *L. remyi* subsp. *remyi*. This subspecies is the most variable taxon in the subgenus and includes some densely tomentose individuals

with broad leaves that were previously classified as *L. lydgatei* Hillebr. [11], and others that are glabrous and have narrow leaves. Most populations also have individuals that are intermediate with regard to these two characters. Flavonoid profiles for "narrow," "intermediate," and "broad" leaf forms were determined. It is interesting to note that one of the few clear-cut features in the profiles is the limitation of quercetin triglycoside No. 1 (QT1) to *L. remyi* subsp. *remyi* and that the profiles of the three leaf forms are more similar to each other than they are to other species. We conclude from these observations that leaf width is a highly variable feature of this species. This was further borne out by experiments in which seeds from the broad-leafed form were germinated and grown under controlled conditions in the greenhouse. The offspring exhibited the full range of morphological variation observed in the field. It is interesting to note, however, that two of the four specimens of the intermediate-leaf form possessed vitexin, which was not seen in the other two leaf forms. Vitexin was found, however, in one of five specimens of *L. remyi* subsp. *caliginis*.

In most cases, however, the degree of variation within a species is similar to or exceeds the level of variation between different species, which renders the feature valueless for systematic purposes. This situation has been described for other Hawaiian endemic species. Very high levels of flavonoid variation were recorded in a study of all the endemic members of *Bidens* on the Hawaiian Islands including instances where flavonoid profiles of adjacent individuals within a population exhibited greater differences than was seen between pairs of species that differed significantly in morphological features [12]. In situations such as that seen in *Bidens*, and now in *Lysimachia*, ecological and morphological differentiation of species appears to have occurred without concomitant development of breeding barriers, or specialization in flavonoid chemistry. In contrast to the variable pigment patterns that characterize *Bidens* and *Lysimachia*, are the existence of nearly invariant flavonoid profiles in other Hawaiian endemic taxa: *Wilkesia gymnoxiphium* [13], *Vaccinium* [14], and *Metrosideros* (Yang and Bohm, unpubl. data).

A comparison of the flavonoid profile of the endemic Hawaiian species of *Lysimachia*, which represent subgen. *Lysimachiopsis*, with members of other subgenera shows a general agreement with regard to flavonoid class and level of glycosylation. The simplest flavonoid profile reported for any of the subgenera is that of subgen. *Naumbergia*, represented by *L. thrysifolia* [15]. The profile consisted of quercetin and isorhamnetin 3-*O*-monoglycosides. Subgenus *Palladia*, represented in the flavonoid literature by four species, is characterized by the presence of kaempferol, quercetin, and isorhamnetin 3-*O*-mono-, di- and triglycosides [16, 17, 19, 20]. Subgenus *Nummularia*, for which flavonoid information is available for three species, features kaempferol and quercetin 3-*O*-glycosides and several flavonoids characterized by 3′,4′,5′-trioxygenated flavonoids including myricetin, mearnsitrin and syringetin derivatives. Members of this subgenus do not appear to make isorhamnetin, however [21, 22, 23]. Information on three species from Subgen. *Lysimachia* shows that this group can make kaempferol, quercetin, isorhamnetin, and myricetin derivatives including all three levels of glycosylation [16, 17, 18]. These data are summarized in Table 2. Our current information on subgen. *Lysimachiopsis* shows a general resemblance to subgenera *Palladia* and *Nummularia* with regard to the capacity to accumulate kaempferol, and quercetin derivatives. *Lysimachiopsis* and *Nummularia* differ, however, in the latter's inability to make isorhamnetin, but share the capacity to make *C*-glycosylflavones. The conservative interpretation of these flavonoid data is that the Hawaiian taxa have the flavonoid profile that one would expect of the genus in general. It is entirely likely that a more thorough sampling of the other subgenera would reveal some of the "missing" compounds.

Table 2. Occurrence of flavonoid structural features in subgenera of *Lysimachia*

	Subgenus*				
Flavonoid†	Naum	Pall	Numm	Lysim	L'sis
Kaempferol	0	+	+	+	+
Quercetin	+	+	+	+	+
Isorhamnetin	+	+	0	+	+
Myricetin ders.	0	0	+	+	0
Monoglys	+	+	+	+	+
Diglys	0	+	+	+	+
Triglys	0	+	+	+	+
C-Glys	0	0	+	0	+

* Naum = *Naumbergia*; Pall = *Palladia*; Numm = *Nummularia*; Lysim = *Lysimachia*; L'sis = *Lysimachiopsis*.

†Monoglys = monoglycosides; Diglys = diglycosides; Triglys = triglycosides; *C*-Glys = *C*-glycosylflavones.

Perhaps the most challenging question concerning island endemics is where did their ancestors come from in the first place? It is considered most likely that the ancestor of Hawaiian *Lysimachia* came from the area that includes eastern Asia and the islands of the western Pacific, often referred to as "Malesia" [5, 24]. The closest relative may be *L. laxa* Baudo in the subgen. *Idiophyton*, which is thought to be the most primitive subgenus in *Lysimachia* (Hu Chi Ming, pers. comm. to K.L.M.). Unfortunately, representatives of this group have not been examined for their flavonoid chemistry. Until additional information becomes available the question of relationships is moot.

EXPERIMENTAL

Plant material. Aerial parts of plants were collected from populations of 12 species (Table 3). *Lysimachia*

Table 3. Collection sites of Hawaiian *Lysimachia* samples

Taxon	Island	Location (number of specimens)
L. daphnoides (A. Gray) Hillebr.	Kauai	Western Alakai Swamp (2)
L. glutinosa Rock	Kauai	Kaluapuhi Trail (1)
	Kauai	Below Kalalau Lookout (3)
L. iniki Marr	Kauai	Wailua River headwaters (seed)
L. kalalauensis Skottsbg.	Kauai	Maile Flats Trail (1)
	Kauai	Honopu Trail (1)
	Kauai	Awaawapuhi Trail (3)
L. ovoidea St John	Kauai	Above Limahuli Falls (1)
	Kauai	Wainiha Ridge (3)
L. pendens Marr	Kauai	Wailua River headwaters (2)
L. remyi ssp. *caliginis* (St John) Marr	East Maui	upper Koolau Gap (4)
L. remyi ssp. *kipahuluensis* (St John) Marr	East Maui	Above Paliku Cabin (1)
	East Maui	Above Lake Waianapanapa (2)
	West Maui	Iao Valley (2)
L. remyi ssp. *remyi* "narrow-leaf"	West Maui	Hale Pohaku Peak (3)
L. remyi ssp. *remyi* "intermediate-leaf"	West Maui	Hale Pohaku Peak (4)
L. remyi ssp. *remyi* "broad-leaf"	West Maui	Hale Pohaku Peak (3)
L. remyi ssp. *subherbacea* (St John) Marr	Molokai	Rim of Waikolu Valley (4)
L. scopulensis Marr	Kauai	Below Kalalau Lookout (1)
L. maxima (R. Knuth) St John	Molokai	Pelekunu Valley (seed)
L. hillebrandii Hook. F. ex A. Gray	Oahu	Palikea, Waianae Mtns. (2)
L. waianaeensis St John	Oahu	Palikea, Waianae Mtns. (1)
	Oahu	Puu Kaua, Waianae Mtns. (1)
	Oahu	Waianae Kai, Waianae Mtns. (3)
L. filifolia C.N. Forbes & Lydgate	Oahu	Waiahole Gulch, Koolau Mtns. (1)

venosa was not collected owing to difficult access, while attempts to locate *L. forbesii*, *L. kahiliensis* and *L. haupuensis*, which are probably extinct, were unsuccessful. Whenever possible, several specimens were collected in order to get some idea of interpopulation variation. In the case of *L. remyi* subsp. *remyi* sampling was more extensive in order to get representative material from the several leaf forms that occur naturally. Voucher specimens have been deposited in herbarium at the University of British Columbia (UBC) and in the herbarium of Bishop Museum (BISH) in Honolulu.

Dried plant material was extracted by soaking in 80% aqueous methanol three times for several days each at room temperature. After evaporation of the combined methanolic extract from each specimen under reduced pressure, the material remaining in the flask was extracted with boiling water, the solution filtered, and the filtrate extracted several times with water-saturated *n*-butanol. The combined *n*-butanol extract of each sample was reduced to dryness and the residue, after being taken up in a small volume of methanol, used for chromatographic analysis. Two dimensional TLC employed Polyamid 6.6 plates, a polar solvent consisting of water–*n*–butanol–acetone–dioxane (70:25:21:4), and a non-polar solvent consisting of dichloromethane–methanol–butanone–water (55:25:21:4). Compounds were located on the plates by inspection under 366 nm UV and then by spraying with diphenylboric acid ethanolamine complex and re-examination under UV. The HPLC analyses were done using a C_{18} Waters Novapak. Elution was performed using a step gradient of isopropanol–tetrahydrofuran–acetonitrile–*ortho*–phosphoric acid with a flow rate of 1.0 ml min^{-1}. Compounds were identified by comparison of retention time and TLC behavior using standard compounds and comparisons using UV spectral methods [25].

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