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Pollen Flavonoid/Phenolic Acid Composition of Four Species of Cactaceae and its Taxonomic Significance

[1]Norma Almaraz-Abarca, [2]María G. Campos, [1]Elí A. Delgado-Alvarado, [1]José A. Ávila-Reyes, [1]Jesús Herrera-Corral, [1]Laura S. González-Valdez, [1]Nestor Naranjo-Jiménez, [2]Christian Frigerio, [2]Ana F. Tomatas, [2]Ana J. Almeida, [2]Amélia Vieira and [1]José N. Uribe-Soto
[1]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional,
Instituto Politécnico Nacional, Unidad Durango,
Sigma s/n 20 de Noviembre II, Durango, Dgo. México, 34220
[2]Laboratório de Farmacognosia, Faculdade de Farmácia, Universidade de Coimbra,
3000-295 Coimbra, Portugal

Abstract: The pollen flavonoid/phenolic acid composition of 14 individual of *Stenocactus multicostatus* subsp. *zacatecasensis* (Britton et Rose) U. Guzmán et Vázquez-Benitez, comb. et stat. nov., 11 of *Echinocereus enneacanthus* Engelmann, 8 of *Echinocereus pectinatus* (Scheidweiler) Engelmann and 12 of *Mammillaria heyderi sensu lato* (all belonging to Cactaceae) was analyzed by HPLC/DAD with the aim of determining the role of pollen profiles of flavonoid/phenolic acid as valuable markers in those taxa. The flavonoid/phenolic acid composition of pollen was evaluated within and among the taxa analyzed. The results suggest that (a) the flavonoid/phenolic acid profiles of these species are among the most complex reported, (b) that quercetin, kaempferol and herbacetin glycoside derivatives are the major phenols found in the pollen of these species of cactus, (c) that some intrapopulation variability is present in all four species and (d) that these pollen flavonoid/phenolic acid profiles tend to be species-specific, so that they can represent important taxonomic markers in Cactaceae.

Key words: *Stenocactus multicostatus*, *Echinocereus enneacanthus*, *Echinocereus pectinatus*, *Mammillaria heyderi*, pollen flavonoids

INTRODUCTION

Cactaceae is a native family of American Continent; its natural distribution is from southwestern Canada to Chile. However, Mexico is where this family reaches its highest level of genera and species diversity[1]. The family Cactaceae has between 1500 and 1800 species in approximately 100 genera[2]. It is estimated that in Mexico around 707 species and 58 genera are found[3], with around 14 endemic genera and 400 endemic species[4].

The delimitation of taxa of this family and the understanding of their taxonomic relationships becomes a hard task because of many species and genera pertaining to Cactaceae, have been partially described or the description has been based solely on one individual, has not been typified or has been made without denoting the origin[5,6]. That is the case of *Stenocactus multicostatus* subsp. *zacatecasensis*[5,6], *Echinocereus enneacanthus*, *Echinocereus pectinatus* and *Mammillaria heyderi sensu lato*[5]. The great morphological variability and the high hybridization capacity are two additional factors making more difficult the taxonomy of these groups.

Stenocactus (= *Echinofossulocactus*), *Mammillaria* and *Echinocereus* belong to subfamily *Cactoideae*[7]. *Stenocactus* and *Mammillaria* share, according to morphological features, a closer taxonomic relationship, since both belong to tribe *Cacteae* and to subtribe *Cactinae*, than between either of them and *Echinocereus*, which belongs to tribe *Echinocereae*[5].

Chemical features, like flavonoid profiles, have been considered as significant taxonomic markers in the delimitation of species of different plant families[8-12]. Almost every species of plant synthesizes some kinds of flavonoids. Pollen is a very important site of synthesis and accumulation of flavonoids and phenolic acids[13,14]. Recently, reports of the taxonomic

Corresponding Author: Norma Almaraz-Abarca, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional, Unidad Durango, Sigma s/n 20 de Noviembre II, Durango, Dgo. México, 34220 Tel/Fax: (52 618) 8142091

significance of pollen flavonoid/phenolic acid profiles have appeared[14,15], this is relevant especially for chemotaxonomic studies of Cactaceae, in which many elements are fixed in some dangerous status, since the determination of those profiles represent a non plant destructive analysis.

The present study was conducted to investigate the intra- and interspecific variations in pollen flavonoid/phenolic acid compositions among one species of *Mammillaria*, one species of *Stenocactus* and two species of *Echinocereus and* establish their taxonomic significance in these taxa of Cactaceae.

MATERIALS AND METHODS

Sampling: Pollen of one species of *Stenocactus*, two of *Echinocereus* and one de *Mammillaria* were collected in different locations of Durango, Mexico (Table 1). Information about environmental conditions where each taxon develops was compiled. The flavonoid/phenolic acid composition was interpreted considering the previous identification of material based on morphological markers. A voucher of each population collected was deposited at the Herbarium CIIDIR.

Table 1: Collection sites for *Stenocactus multicostatus* subsp. *zacatecasensis*, *Mammillaria heyderi sensu lato*, *Echinocereus enneacanthus*, and *Echinocereus pectinatus*

Sample	No. Ref.	Species	Latitude N	Longitude W	Altitude (m)	Location	Date
1	559	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
2	560	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
3	561	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
4	562	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
5	563	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
6	564	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
7	565	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
8	566	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
9	568	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
10	569	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
11	571	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
12	572	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
13	573	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
14	574	*Stenocactus multicostatus* subsp. *zacatecasensis*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, Mex.	March 2005
15	567	*Mammillaria heyderi sensu lato*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, México	March 2005
16	576	*Mammillaria heyderi sensu lato*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, México	March 2005
17	577	*Mammillaria heyderi sensu lato*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, México	March 2005
18	578	*Mammillaria heyderi sensu lato*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, México	March 2005
19	579	*Mammillaria heyderi sensu lato*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, México	March 2005
20	580	*Mammillaria heyderi sensu lato*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, México	March 2005
21	581	*Mammillaria heyderi sensu lato*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, México	March 2005
22	582	*Mammillaria heyderi sensu lato*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, México	March 2005
23	583	*Mammillaria heyderi sensu lato*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, México	March 2005
24	584	*Mammillaria heyderi*	24°07'16.5''	104°45'00.1''	1969	Chupaderos,	March 2005

Table 1: continue

		sensu lato				Durango, México	
25	585	*Mammillaria heyderi sensu lato*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, México	March 2005
26	586	*Mammillaria heyderi sensu lato*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango, México	March 2005
27	609	*Echinocereus enneacanthus*	25°01'19.6''	103°35'44.5''	1550	Vallecillos, Cuencamé, Durango	March 2005
28	610	*Echinocereus enneacanthus*	25°01'19.6''	103°35'44.5''	1550	Vallecillos, Cuencamé, Durango	March 2005
29	611	*Echinocereus enneacanthus*	25°01'19.6''	103°35'44.5''	1550	Vallecillos, Cuencamé, Durango	March 2005
30	612	*Echinocereus enneacanthus*	25°01'19.6''	103°35'44.5''	1550	Cañón de San Diego,	March 2005
31	638	*Echinocereus enneacanthus*	26°41'17.7''	103°44'50.5''	1160	Mapimí, Durango	April 2005
32	639	*Echinocereus enneacanthus*	26°41'17.7''	103°44'50.5''	1160	Mapimí, Durango	April 2005
33	641	*Echinocereus enneacanthus*	26°41'17.7''	103°44'50.5''	1160	Mapimí, Durango	April 2005
34	643	*Echinocereus enneacanthus*	26°42'06.1''	103°38'04.5''	1150	Mapimí, Durango	April 2005
35	644	*Echinocereus enneacanthus*	26°41'17.7''	103°44'50.5''	1160	Mapimí, Durango	April 2005
36	645	*Echinocereus enneacanthus*	26°41'17.7''	103°44'50.5''	1160	Mapimí, Durango	April 2005
37	646	*Echinocereus enneacanthus*	26°41'17.7''	103°44'50.5''	1160	Mapimí, Durango	April 2005
38	630	*Echinocereus pectinatus*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango	April 2005
39	631	*Echinocereus pectinatus*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango	April 2005
40	632	*Echinocereus pectinatus*	24°07'16.5''	104°45'00.1''	1969	Chupaderos, Durango	April 2005
41	634	*Echinocereus pectinatus*	24°05'00''	104°40'30''	1940	5 Km N Durango-Parral	April 2005
42	635	*Echinocereus pectinatus*	24°05'00''	104°40'30''	1940	5 Km N Durango-Parral	April 2005
43	636	*Echinocereus pectinatus*	24°05'00''	104°40'30''	1949	5 Km N Durango-Parral	April 2005
44	637	*Echinocereus pectinatus*	24°05'00''	104°40'30''	1940	5 Km N Durango-Parral	April 2005
45	658	*Echinocereus pectinatus*	25°01'19.6''	103°35'44.5''	1550	Cuencamé, Durango	April 2005

HPLC/DAD analysis: Ten milligrams of dried pollen of each sample were sonicated in an ethanol-water solution (1mL, 50% v/v) for 60 min. The resultant mixtures were centrifuged at 15269 g for 10 min and the supernatants were used for HPLC/DAD analysis as previously described[14]. Extracts (20 µL) were analyzed on a Gilson 170 and Waters Spherisorb ODS2 (5µm) column (4.6×250 mm) by an acidified acetonitrile-water gradient. Standard chromatograms were plotted at λ_{max} 260 and 340 nm. Spectral data for all peaks were accumulated in the range 220-400 nm using DAD (Gilson 170). The phenolic profile of each sample was made up of all compounds resolved in its respective chromatogram. Each compound was treated as a single chemical character. The structural information was made by direct comparison of retention time and absortion spectra with standards and according to the information compiled by Campos and Markham[16].

Data analysis: The diversity of the profile of Flavonoid/phenolic acid for each taxon was derived using the Shannon index (H).

A presence-absence matrix formed by all individual samples vs. all resolved compounds (45 individuals vs. 46 compounds) was analyzed using the UPGMA method (clustering program NTSYS-pc 1.8[17].

RESULTS AND DISCUSSION

HPLC/DAD analysis: A total of 46 compounds were resolved by HPLC/DAD (Table 2). Flavonoids and phenolic acids were the only two classes of phenolics found in the pollen of all species of Cactaceae analyzed; 3-*O*-flavonolglycosides were the major structures among the flavonoids. The analysis revealed 16 kaempferol derivatives, 7 quercetin derivatives, 5 herbacetin derivatives, 4 unidentified flavonoids, 3 unidentified phenols and 11 phenolic acid derivatives.

Flavonoids are present in pollen of many species of Angiosperm and Gymnosperm and in spores of moss and ferns[18]. Particularly kaempferol and quercetin are two required flavonols to pollen tube germination and growth in tobacco[18], petunia and maize[19]. However, these two flavonols have not been shown to carry a similar function in *Arabidopsis*[20]. The abundance of kaempferol and quercetin derivatives in pollen of *Stenocactus multicostatus* subsp. *zacatecasensis*, *Echinocereus pectinatus*, *E. enneacanthus* and *Mammillaria heyderi sensu lato* could suggest that those flavonols could serve an essential function during reproduction of these species of Cactaceae.

Phenolic acids derivatives were more abundant in the pollen of *Stenocactus multicostatus* subsp. *zacatecasensis* (8 structures, Table 3) than in the pollen of *Mammillaria heyderi sensu lato* (2 structure,

Table 2: Major phenols found in pollen of *Stenocactus multicostatus* subsp. *zacatecasensis*, *Echinocereus pectinatus*, *Echinocereus enneacanthus* and *Mammillaria heyderi sensu lato*

No.of compound	Retention time (min) (X±SD)	Chemical identification
F1	25.73±0.010	Unidentified phenol
F2	29.93±0.030	Unidentified phenol
F3	30.65±0.020	Unidentified phenol
F4	32.24±0.020	Kaempferol-3-*O*-glycoside
F5	32.54±0.020	Kaempferol-3-*O*-glycoside
F6	32.56±0.033	Kaempferol-3-*O*-glycoside-7-substituted
F7	32.68±0.050	Phenolic acid
F8	33.91±0.000	Kaempfero-3-*O*-glycoside
F9	34.16±0.020	Herbacetin-3-*O*-glycoside
F10	34.18±0.010	Phenolic acid
F11	35.73±0.090	Phenolic acid
F12	35.82±0.030	Herbacetin-3-*O*-glycoside
F13	36.35±0.030	Herbacetin-3-*O*-glycoside
F14	36.40±0.000	Phenolic acid
F15	36.56±0.040	Kaempferol-3-*O*-glycoside
F16	36.80±0.000	Unidentified flavonol
F17	37.28±0.020	Quercetin-3-*O*-glycoside
F18	37.54±0.020	Herbcetin-3-*O*-glycoside
F19	37.76±0.000	Herbacetin-3-*O*-glycoside
F20	37.77±0.030	Quercetin-3-*O*-glycoside
F21	37.80±0.010	Kaempferol-3-*O*-glycoside
F22	37.82±0.010	Unidentified flavonoid
F23	37.88±0.040	Quercetin-3-*O*-glycoside
F24	38.20±0.040	Kaempferol-3-*O*-glycoside (probably susbtituted in ring A)
F25	38.24±0.020	Kaempferol-3-*O*-glycoside
F26	38.61±0.040	Kaempferol-3-*O*-glycoside
F27	38.89±0.040	Quercetin-3-*O*-glycoside
F28	38.96±0.020	Inidentified flavonol
F29	39.08±0.030	Pheolic acid
F30	39.12±0.050	Kaempferol-3-*O*-glycoside
F31	39.30±0.020	Kaempferol-3-*O*-glycoside
F32	39.42±0.000	Unidentified flavonol
F33	39.88±0.026	Kaempferol-3-*O*-glycoside
F34	40.10±0.032	Kaempferol-3-*O*-glycoside
F35	40.10±0.040	Quercetin-3-*O*-glycoside
F36	40.56±0.020	Quercetin-3-*O*-glycoside
F37	41.17±0.050	Kaempferol-3-*O*-glycoside
F38	41.55±0.020	Quercetin-3-*O*-glycoside
F39	42.56±0.020	Kaempferol-3-*O*-glycoside-7-sustituted
F40	42.57±0.050	Kaempferol-3-*O*-glycoside
F41	42.63±0.000	Phenolic acid
F42	42.90±0.010	Phenolic acid
F43	43.19±0.010	Phenolic acid
F44	43.92±0.020	Phenolic acid
F45	44.17±0.000	Phenolic acid
F46	53.30±0.020	Phenolic acid

Table 4) and *Echinocereus pectinatus* (one structure, Table 5), while in the pollen of *Echinocereus enneacanthus* no phenolic acid was found (Table 6).

The pollen phenolic profiles of four all species of Cactaceae analyzed were relatively complex (22 phenolic compounds in *Stenocactus multicostatus* subsp. *zacatecasensis*, 17 in *Mammillaria heyderi sensu lato*, 17 in *E. pectinatus* and 9 in *Echinocereus enneacanthus*). The phenols richness found in this study represents a contrast with other reports on pollen phenols obtained by the same method; for example those of *Zea mays* with six compounds, *Bidens odorata* with three[15]; *Eucalyptus globulus* with seven and *Erica australis* with two[14]. A similar complex composition has been found in the perianth parts of *Echinocereus triglochidiatus* var. *gurneyi* (Cactaceae), but in contrast to pollen composition, dihydroflavonols and dihydroflavonol 7-*O*-gycosides, besides flavonol glycosides were present[21]. Other

Table 3: Major phenols found in the pollen of *Stenocactus multicostatus* subsp. *zacatecasensis*

No. of compound	Compound	Retention time (min) (X±SD)	Samples *Stenocactus multicostatus* subsp. *zacatecasensis* 559	560	561	562	563	564	565	566	568	569	571	572	573	574
F5	Kaempferol-3-*O*-glycoside	32.54±0.020	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F13	Herbacetin-3-*O*-glycoside	36.35±0.030	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F14	Phenolic acid	36.40±0.00	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F17	Quercetin-3-*O*-glycoside	37.28±0.02	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F18	Herbacetin-3-*O*-glycoside	37.54±0.02	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F23	Quercetin-3-*O*-glycoside	37.88±0.04	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F24	Kaempferol-3-*O*-glycoside (Probably substituted in ring A)	38.20±0.04	0	0	0	1	1	0	1	0	1	1	1	1	0	0
F26	Kaempferol-3-*O*-glycoside	38.61±0.04	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F27	Quercetin-3-*O*-glycoside	38.96±0.02	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F29	Phenolic acid	39.08±0.03	1	0	0	0	0	0	0	0	0	0	0	0	0	0
F31	Kaempferol-3-*O*-glycoside	39.30±0.02	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F32	Unidentified flavonoid	39.42±0.00	1	0	0	0	0	0	0	0	0	0	0	0	0	0
F34	Kaempferol-3-*O*-glycoside	40.10±0.032	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F37	Kaempferol-3-*O*-glycoside	41.17±0.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F39	Kaempferol-3-*O*-glycoside -7-sustituted	42.56±0.02	0	0	1	1	0	1	0	1	0	0	0	0	0	0
F40	Kaempferol-3-*O*-glycoside	42.57±0.05	1	1	0	0	1	0	1	1	1	1	1	1	1	1
F41	Phenolic acid	42.63±0.00	1	0	0	0	0	0	0	0	0	0	0	0	0	0
F42	Phenolic acid	42.90±0.01	1	0	1	1	1	1	1	1	1	0	0	0	0	0
F43	Phenolic acid	43.19±0.01	1	0	0	0	0	0	0	0	1	1	1	1	1	1
F44	Phenolic acid	43.92±0.02	0	1	1	1	1	1	1	1	0	0	0	0	0	0
F45	Phenolic acid	44.17±0.00	1	0	0	0	0	0	0	0	1	1	1	1	1	1
F46	Phenolic acid	53.30±0.02	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Total	22	19	14	15	16	16	15	16	16	17	16	16	16	15	15

X: Mean; SD: Standard deviation; 1: Found; 0: Not found

Table 4: Major phenols found in the pollen of *Mammillaria heyderi sensu lato*

No. of compound	Compound	Retention time (min) (X±SD)	Samples *Mammillaria heyderi sensu lato* 567	576	577	578	579	580	581	582	583	584	585	586
F6	Kaempferol-3-*O*-glycoside -7-substituted	32.56±0.020	1	1	1	1	1	1	1	1	1	1	1	1
F7	Phenolic acid	32.68±0.050	1	1	1	1	1	1	1	1	1	1	1	1
F10	Phenolic acid	34.18±0.010	1	1	1	1	1	1	1	1	1	1	1	1
F12	Herbacetin-3-*O*-glycoside	36.32±0.030	1	1	1	1	1	1	1	1	1	1	1	1
F13	Herbacetin-3-*O*-glycoside	36.35±0.030	1	1	1	1	1	1	1	1	1	1	1	1
F16	Unidentified flavonol	36.80±0.000	0	0	1	1	1	0	0	1	0	0	0	1
F18	Herbacetin-3-*O*-glycoside	37.54±0.020	1	1	1	1	1	1	1	1	1	1	1	1
F19	Herbacetin-3-*O*-glycoside	37.76±0.000	1	0	0	1	1	0	0	0	1	0	0	0
F22	Unidentified flavonoid	37.82±0.010	1	1	1	1	1	0	1	1	1	0	0	0
F25	Kaempferol-3-*O*-glycoside	38.24±0.020	1	1	1	1	1	1	1	1	1	1	1	1
F30	Kaempferol-3-*O*-glycoside	39.12±0.050	1	1	1	1	1	1	1	1	1	1	1	1
F33	Kaempferol-3-*O*-glycoside	39.88±0.026	1	1	1	1	1	1	1	1	1	1	1	1
F35	Quercetin-3-*O*-glycoside	40.1±0.0400	1	1	1	0	1	1	1	1	1	1	0	1
F36	Quercetin-3-*O*-glycoside	40.56±0.020	1	1	1	1	1	1	1	1	1	1	1	1
F37	Kaempferol-3-*O*-glycoside	41.17±0.050	1	1	1	1	1	1	1	1	1	1	1	1
F38	Quercetin-3-*O*-glycoside	41.55±0.020	1	1	0	0	1	1	0	1	1	1	0	1
F40	Kaempferol-3-*O*-glycoside	42.57±0.050	1	1	1	1	1	1	1	1	1	1	1	1
	Total	17	16	15	15	15	17	14	14	16	16	14	12	15

Species of Cactaceae investigated for their composition of phenolics in the tepals, as a part of perianth, are *Astrophytum ornatum* Web., *Notocactus apricus* A. Berg., *Echinopsis huotii* Lab., *Aylostera pseudodeminuta* Backbg. and *Neochilenia napina* Backberg.; in them no dihydroflavonol was detected[22].

Table 5: Major phenols found in the pollen of *Echinocereus pectinatus*

No. of compound	Compound	Retention time (min) (X±SD)	Samples *Echinocereus pectinatus* 630	631	632	634	635	636	637	658
F1	Unidentified phenol	25.73±0.010	1	0	0	0	1	1	1	1
F2	Unidentified phenol	29.93±0.030	1	0	0	0	1	1	1	1
F3	Unidentified phenol	30.65±0.020	1	0	0	1	1	1	0	1
F4	Kaempferol-3-*O*-glycoside	32.24±0.020	1	0	0	1	1	1	1	1
F8	Kaempferol-3-*O*-glycoside	33.91±0.000	1	0	0	1	1	1	1	0
F9	Herbacetin-3-*O*-glycoside	34.16±0.020	1	1	1	1	1	1	1	1
F11	Phenolic acid	35.73±0.090	1	1	1	1	1	1	1	1
F13	Herbacetin-3-*O*-glycoside	36.35±0.030	1	1	1	1	1	1	1	1
F18	Herbacetin-3-*O*-glycoside	37.54±0.020	1	1	1	1	1	1	1	1
F20	Quercetin-3-*O*-glycoside	37.77±0.030	1	1	1	1	1	1	1	1
F21	Kaempferol-3-*O*-glycoside	37.80±0.010	1	1	1	1	1	1	1	1
F24	Kaempferol-3-*O*-glycoside (probably substituted in ring A)	38.20±0.040	1	1	1	1	1	1	1	1
F27	Quercetin-3-*O*-glycoside	38.89±0.040	1	1	1	1	1	1	1	1
F30	Kaempferol-3-*O*-glycoside	39.12±0.050	1	1	1	1	1	1	1	1
F33	Kaempferol-3-*O*-glycoside	39.88±0.026	1	1	1	1	1	1	1	1
F37	Kaempferol-3-*O*-glycoside	41.17±0.050	1	1	1	1	1	1	1	1
F40	Kaempferol-3-*O*-glycoside	42.57±0.050	1	1	1	1	1	1	1	1
	Total	17	17	12	12	15	17	17	16	16

X: Mean; SD: Standard deviation; 1: Found; 0: Not found

Table 6: Major phenols found in the pollen of *Echinocereus enneacanthus*

No. of Compound	Compound	Retention time (min) ($\bar{X}$ ±SD)	Samples *Echinocereus enneacanthus* 609	610	611	612	638	639	641	643	644	645	646
F9	Herbacetin-3-*O*-glycoside	34.16±0.02	1	1	1	1	1	1	1	1	1	1	1
F15	Kaempferol-3-*O*-glycoside	36.56±0.04	0	0	0	0	0	0	0	0	0	1	0
F18	Herbacetin-3-*O*-glycoside	37.54±0.02	0	0	0	0	0	0	0	1	0	1	0
F23	Quercetin-3-*O*-glycoside	37.88±0.04	1	1	1	1	1	1	1	1	1	1	1
F25	Kaempferol-3-*O*-glycoside	38.24±0.02	1	1	1	1	1	1	1	1	1	1	1
F27	Quercetin-3-*O*-glycoside	38.89±0.04	1	1	1	1	1	1	1	1	1	1	1
F33	Kaempferol-3-*O*-glycoside	39.88±0.026	1	1	1	1	1	1	1	1	1	1	1
F37	Kaempferol-3-*O*-glycoside	41.17±0.05	1	1	1	1	1	1	1	1	1	1	1
F40	Kaempnferol-3-*O*-glycoside	42.57±0.05	1	1	1	1	1	1	1	1	1	1	1
	Total	9	7	7	7	7	7	7	7	8	7	9	7

X: Mean; SD: Standard deviation; 1: Found; 0: Not found

Among the four species analyzed, *Stenocactus multicostatus* subs. *zacatecasensis* had the pollen phenolic profile most complex (Table 3), with 22 structures: 1 unidentified flavonoid, 8 phenolic acid derivatives and 13 flavonol glycosides, from which 2 are gycosilherbacetin derivatives, 2 are glycosilquercetin derivatives and 9 are glycosilkaempferol derivatives. The most simple pollen phenolic profile was that of *Echinocereus enneacanthus* (Table 6), with 9 structures, 2 glycosilherbacetin derivatives, 2 glycosilquercetin derivatives and 5 glicosilkaempferol derivatives.

Taxonomic analysis: Twelve phenolics (F5, F13, F14, F17, F18, F23, F26, F27, F31, F34, F37 and F46) among a total of 22 were always present in the pollen of every individual identified as *Stenocactus multicostatus* subsp. *zacatecasensis*; 7 (F9, F23, F25, F27, F33, F37, F40) among a total of 9 were present in every individual of *Echinocereus enneacanthus*; 12 (F9, F11, F13, F18, F20, F21, F24, F27, F30, F33, F37, F40) among a total of 17 were always present in *Echinocereus pectinatus*; while 12 phenolics (F6, F7, F10, F12, F13, F18, F25, F30, F33, F36, F37 and F40) among a total of 17 were present in the pollen of every analyzed individual of *Mammillaria heyderi sensu lato*. These figures suggest that the pollen of every these four species of Cactaceae synthesizes more than the fifty per cent of its phenolics in a very conservative way.

With regard to the richness of the various classes of the flavonoid/phenolic acid profiles as expressed by the Shannon index for each taxon, our results suggest that

Mammillaria heyderi sensu lato and *Stenocactus multicostatus* subsp. *zacatecasensis* had the relative highest variability (H = 2.254 and H = 2.107, respectively), that *Echinocereus pectinatus* has an intermediate variability (H = 1.494) and that *E. enneacanthus* was the taxa most homogeneous (H = 0.600). These figures represent the natural intrapopulation variability in the expression of pollen phenolic profiles in each taxon studied. This kind of variability has been reported for the foliar phenolic profiles of *Aspalathus linearis*[23] and for five species of *Pinus*[11,12]. In all those taxa, the phenolic profiles were significant markers for delimiting species.

Despite the observed variability, each analyzed taxon displays a unique flavonoid/phenolic acid profile (Table 7). Fifteen compounds (F5, F14, F17, F26, F29, F31, F32, F34, F39, F41, F42, F43, F44, F45 and F46) were characteristic of *Stenocactus multicostatus* subsp. *zacatecasensis*, one (F15) were found only in individuals of *Echinocereus enneacanthus*, 8 (F1, F2, F3, F4, F8, F11, F20 and F21) were exclusive of *Echinocereus pectinatus* and 10 (F6, F7, F10, F12, F16, F19, F22, F35, F36 and F38) were present solely in individuals of *Mammillaria heyderi sensu lato*. of a total of 46 compounds, three (F18, F37 and F40) were common to all analyzed taxa.

Table 7: Taxonomic distribution of pollen flavonoid/phenolic acid compounds in *Stenocactus multicostatus* subsp *zacatecasensis*, *Mammillaria heyderi sensu lato*, *Echinocereus pectinatus* and *E. enneacanthus*

No.of compound	Chemical identification	*Stenocactus multicostatus* subsp. *zacatecasensis*	*Mammillaria heyderi sensu lato*	*Echinocereus pectinatus*	*Echinocereus enneacanthus*
F1	Unidentified phenol	-	-	+/-	-
F2	Unidentified phenol	-	-	+/-	-
F3	Unidentified phenol	-	-	+/-	-
F4	Kaempferol-3-*O*-glycoside	-	-	+/-	-
F5	Kaempferol-3-*O*-glycoside	+	-	-	-
F6	Kaempferol-3-O-glycoside-7-substituted	-	+	-	-
F7	Phenolic acid	-	+/-	-	-
F8	Kaempferol-3-*O*-glycoside	-	-	+/-	-
F9	Herbacetin-3-*O*-glycoside	-	-	+/-	+/-
F10	Phenolic acid	-	+	-	-
F11	Phenolic acid	-	-	+	-
F12	Herbacetin-3-*O*-glycoside	-	+/-	-	-
F13	Herbacetin-3-*O*-glycoside	+	+	+/-	-
F14	Phenolic acid	+	-	-	-
F15	Kaempferol-3-*O*-glycoside	-	-	-	+/-
F16	Unidentified flavonol	-	+/-	-	-
F17	Quercetin-3-*O*-glycoside	+	-	-	-
F18	Herbcetin-3-*O*-glycoside	+	+	+	+/-
F19	Herbacetin-3-*O*-glycoside	-	+/-	-	-
F20	Quercetin-3-*O*-glycoside	-	-	+	-
F21	Kaempferol-3-*O*-glycoside	-	-	+	-
F22	Unidentified flavonoid	-	+/-	-	-
F23	Quercetin-3-*O*-glycoside	+	-	-	+
F24	Kaempferol-3-*O*-glycoside (probably substituted in ring A)	+/-	-	+	-
F25	Kaempferol-3-*O*-glycoside	-	+	-	+/-
F26	Kaempferol-3-*O*-glycoside	+	-	-	-
F27	Quercetin-3-*O*-glycoside	+	-	+	+
F28	Unidentified flavonol	-	-	-	-
F29	Phenolic acid	+/-	-	-	-
F30	Kaempferol-3-*O*-glycoside	-	+	+/-	-
F31	Kaempferol-3-*O*-glycoside	+	-	-	-
F32	Unidentified flavonol	+/-	-	-	-
F33	Kaempferol-3-*O*-glycoside	-	+	+	+
F34	Kaempferol-3-*O*-glycoside	+	-	-	-
F35	Quercetin-3-*O*-glycoside	-	+/-	-	-
F36	Quercetin-3-O-glycoside	-	+	-	-
F37	Kaempferol-3-*O*-glycoside	+	+	+	+
F38	Quercetin-3-*O*-glycoside	-	+/-	-	-
F39	Kaempferol-3-*O*-glycoside-7-substituted	+/-	-	-	-
F40	Kaempferol-3-*O*-glycoside	+/-	+	+	+
F41	Phenolic acid	+/-	-	-	-
F42	Phenolic acid	+/-	-	-	-
F43	Phenolic acid	+/-	-	-	-
F44	Phenolic acid	+/-	-	-	-
F45	Phenolic acid	+/-	-	-	-
F46	Phenolic acid	+	-	-	-

+: Present; -: Absent; +/-: Present or absent

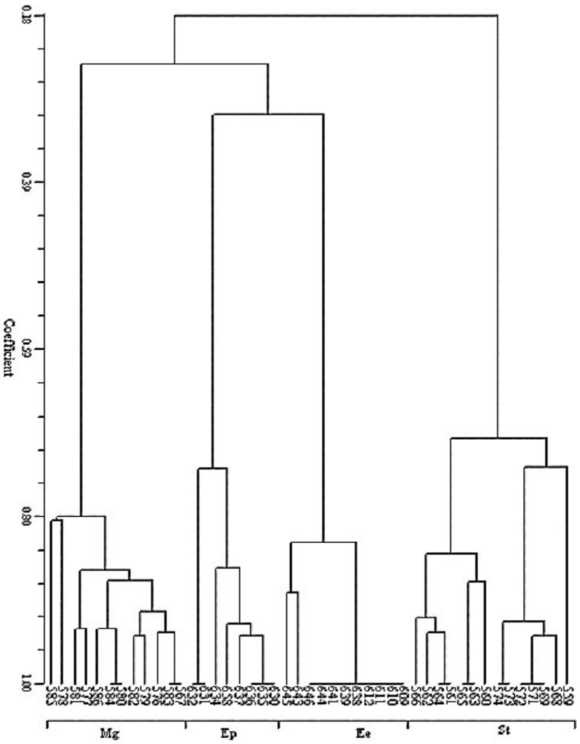

Fig. 1: Results of clustering analysis comparing pollen phenolic profiles for 45 samples of Cactaceae (Mg: *Mammillaria heyderi sensu lato*, Ep: *Echinocereus pectinatus*, Ee: *Echinocereus enneacanthus*, St: *Stenocactus multicostatus* subsp. *zacatecasensis*)

Variation among groups of individuals associated with environmental variables such as elevation, latitude or particular site was not observed. Pollen flavonoid/phenolic acid profiles of individuals of *Echinocereus enneacanthus*, which came from two different populations, separated one each from the other by around 200 Km and by orographic barriers as the Sierra El Rosario, with elevations reaching 2240 m, formed a group without subdivisions separating these two populations (Fig. 1). This supports suggestions by Kaundun *et al*.[24], Almaraz-Abarca *et al*.[12] and others that flavonoid composition is little affected by environmental fluctuations.

Mammillaria heyderi sensu lato is a complicated taxonomic group due to high polymorphism and hybridizing capacity[5]. This taxon and *Echinocereus pectinatus* both are distributed through a very broad zone, practically in a continuous way in State of Durango, Mexico. They are found either in arid zones or in template *Pinus*-*Quercus* forest. Their distributions contrast with that of *Stenocactus multicostatus* subsp. *zacatecasensis*, which is found in scarce and disjunctive populations[25]. These three species can be found as sympatric populations in zones of *Quercus* forest with few grasses and rocky substratum, like it is the growing habitats of *Stenocactus multicostatus* subsp. *zacatecasensis*. *Mammillaria heyderi sensu lato* and *Echinocereus pectinatus* both with a broad distribution, showed a relative high flavonoid/phenolic acid variability in pollen (H = 2.254 and H = 1.494, respectively). These results were expected, since according to Sosa *et al*.[26] the increment in the morphological and genetic variability is associated to the increment in the distribution area. However, the high variability in the phenolic composition of pollen of *Stenocactus multicostatus* subsp. *zacatecasensis* (H = 2.107), which is similar to that of *Mammillaria heyderi sensu lato*, can not be associated to a broad distribution, but to a high intrapopulation genetic variability. The high chemical variability found in the pollen phenol composition of *Stenocactus multicostatus* subs. *zacatecasensis* is according to the Bravo-Hollis and Sánchez-Mejorada[5]'statement about this taxon is in a present speciation process; in this taxon both chemical and morphological variability may be maintained by a heterogeneous environmental pressure of selection.

The fenetic analysis of pollen phenol profiles groups together *Mammillaria heyderi sensu lato* (Tribe *Cacteae*) and the two species of *Echinocereus* (Tribe *Echinocereae*) and place *Stenocactus multicostatus* subsp. *zacatecasensis* in a separated group, (Fig. 1). However, on a morphological basis, *Stenocactus multicostatus* subsp. *zacatecasensis* shares along to *Mammillaria heyderi sensu lato* the same tribe *Cacteae*[5]. Nevertheless, in spite of the suggestion given by these results it is necessary to investigate the distribution of pollen flavonoid/phenolic acid profiles and the correlations between chemical and morphological features, among a higher number of populations, species and genera of Cactaceae before considering a closer taxonomic relationship between *Mammillaria* and *Echinocereus* than between *Mammillaria* and *Stenocactus*.

Pollen phenolic profiles confirmed taxonomic separation of the four species made on morphological basis. The fenetic analysis (Fig. 1) clearly distinguishes four groups that correspond to the four morphologically-based taxa, *Stenocactus multicostatus* subsp. *zacatecasensis*, *Echinocereus enneacanthus*, *Echinocereus pectinatus* and *Mammillaria heyderi sensu lato*. This indicates that each of four species can be distinguished from the others by a unique flavonoid/phenolic acid profile. Pollen phenolic profiles are species-specific and each species express a variable number of unique flavonoids or phenolic acid.

In spite of the intrapopulation variability, the pollen flavonoid/phenolic acid profiles of *Stenocactus multicostatus* subsp. *zacatecasensis*, *Echinocereus enneacanthus*, *Echinocereus pectinatus* and *Mammillaria heyderi sensu lato* are so stable for each species that type profiles by species can be recognized. These profiles can be considered as valuable chemical markers at the specific level. The refereed profiles, rich in quercetin, kaempferol and herbacetin glycosil derivatives, are among the most complex reported. Although more population studies on the phenolic composition of pollen of Cactaceae are needed, over all in species with a broad distribution, these results suggest that flavonoid/phenolic acid profiles of pollen could be specific taxonomic markers in this family of plants.

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