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

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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. The family Cactaceae has between 1500 and 1800 species in approximately 100 genera. It is estimated that in Mexico around 707 species and 58 genera are found, with around 14 endemic genera and 400 endemic species.

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. That is the case of Stenocactus multicostatus subsp. zacatecasensis, Mammillaria heyderi, Echinocereus enneacanthus, Echinocereus pectinatus and Mammillaria heyderi sensu lato. 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. 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.

Chemical features, like flavonoid profiles, have been considered as significant taxonomic markers in the delimitation of species of different plant families. 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. Recently, reports of the taxonomic
significance of pollen flavonoid/phenolic acid profiles have appeared\cite{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.

| 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  | *Mammillaria heyderi* sensu lato | 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, Durango, México | March 2005 |
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-flavonoglycosides 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 quercitin 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                 | Kaempferol-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                 | Herbacetin-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 substituted 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                 | Unidentified flavonol                           |
| F29            | 39.08±0.030                 | Phenolic 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-substituted          |
| 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 2) 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<sup>15</sup>, *Eucalyptus globulus* with seven and *Erica australis* with two<sup>14</sup>. 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-glycosides, besides flavonol glycosides were present<sup>21</sup>. Other
Table 3: Major phenols found in the pollen of *Stenocactus multicostatus* subsp. *zacatecasensis*

| No. of compound | Compound | Retention time (min) (X±SD) | Samples |
|----------------|----------|-----------------------------|---------|
|                |          | 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 |
| F13            | Herbacetin-3-O-glycoside | 36.35±0.030 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| F14            | Phenolic acid | 36.40±0.000 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| F17            | Quercetin-3-O-glycoside | 37.28±0.020 | 1 | 1 | 1 | 1 | 1 | 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 | 1 | 1 | 1 | 1 | 1 |
| F23            | Quercetin-3-O-glycoside | 37.88±0.040 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| F24            | Kaempferol-3-O-glycoside | 38.20±0.040 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |

(Probably substituted in ring A)

| F26 | Kaempferol-3-O-glycoside | 38.61±0.040 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

| F27 | Quercetin-3-O-glycoside | 38.96±0.020 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| F29 | Phenolic acid | 39.08±0.030 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F31 | Kaempferol-3-O-glycoside | 39.30±0.020 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| F32 | Unidentified flavonoid | 39.42±0.000 | 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±0.050 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| F39 | Kaempferol-3-O-glycoside | 42.56±0.020 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F40 | Kaempferol-3-O-glycoside | 42.57±0.050 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| F41 | Phenolic acid | 42.63±0.000 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F42 | Phenolic acid | 42.90±0.010 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| F43 | Phenolic acid | 43.19±0.000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F44 | Phenolic acid | 43.92±0.020 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| F45 | Phenolic acid | 44.17±0.000 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| F46 | Phenolic acid | 45.30±0.020 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

Total: 22

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 huertii* Lab., *Aylostera pseudodeminita* 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) (±SD) | Samples |
|----------------|-----------------------------------|---------------------------|---------|
|                |                                   |                           | Echinocereus pectinatus |
|                |                                   |                           | 630 | 631 | 632 | 634 | 635 | 636 | 637 | 638 |
| 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   | 1   | 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             | Herbacitin-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            | Herbacitin-3-O-glycoside          | 36.35±0.030               | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   |
| F18            | Herbacitin-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          | 38.00±0.010               | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   |
| F24            | Kaempferol-3-O-glycoside          | 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  | 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) (±SD) | Samples |
|----------------|-----------------------------------|---------------------------|---------|
|                |                                   |                           | Echinocereus enneacanthus |
|                |                                   |                           | 609 | 610 | 611 | 612 | 638 | 639 | 641 | 643 | 644 | 645 | 646 |
| F9             | Herbacitin-3-O-glycoside          | 34.16±0.02                | 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   | 0   |
| F18            | Herbacitin-3-O-glycoside          | 37.54±0.02                | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 0   |
| F23            | Quercetin-3-O-glycoside           | 37.88±0.04                | 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   |
| F27            | Quercetin-3-O-glycoside           | 38.89±0.04                | 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   |
| F37            | Kaempferol-3-O-glycoside          | 41.17±0.05                | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   |
| F40            | Kaempferol-3-O-glycoside          | 42.57±0.05                | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   |
| Total          |                                   |                           | 9   | 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 flavanol glycosides, from which 2 are gycosilherbacatin derivatives, 2 are glicosilquercetin derivatives and 9 are glicosilkaempferol derivatives. The most simple pollen phenolic profile was that of *Echinocereus enneacanthus* (Table 6), with 9 structures, 2 glicosilherbacatin derivatives, 2 glicosilquercetin 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* subs. *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, 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...
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             | Herbcetin-3-O-glycoside | -                                             | -                              | +/-                    | -                        |
| F10            | Phenolic acid         | -                                             | +                              | -                      | -                        |
| F11            | Phenolic acid         | -                                             | +                              | -                      | -                        |
| F12            | Herbcetin-3-O-glycoside | -                                             | +/-                            | -                      | -                        |
| F13            | Herbcetin-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            | Herbcetin-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 | +/-                            | -                              | -                      | -                        |
| F25            | Kaempferol-3-O-glycoside | (probably substituted in ring A) | -                              | -                      | -                        |
| 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.\textsuperscript{[24]}, Almaraz-Abarca et al.\textsuperscript{[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\textsuperscript{[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\textsuperscript{[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.*\textsuperscript{[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* subsp. *zacatecasensis* is according to the Bravo-Hollis and Sánchez-Mejorada\textsuperscript{[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*\textsuperscript{[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 multicosatus* 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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REFERENCES

1. Arias, M.S., 1993. Cactáceas: conservación y diversidad en México. Rev. Soc. Mex. Hist. Nat., 44: 109-115.
2. Barthlott, W. and D.R. Hunt, 1993. Cactaceae. In: The Families and Genera of Vascular Plants, Kubitzki, K., J.G. Rohwer and V. Bittrich (Eds.). Springer Verlag, Berlin, pp: 161-197.
3. Arias, M.S., 2001. Sistemática y conservación de la familia Cactaceae en México. Memorias del XV Congreso Mexicano de Botánica, México.
4. Arias, M.S., 1997. Distribución General. In: Suculentas Mexicanas. Cactáceas, Valles, S.C. and P.L. Rodríguez (Eds.). UNAM, SEMARNAP, México, pp: 17-25.
5. Bravo-Hollis, H. and R.H. Sánchez-Mejorada, 1991. Las Cactáceas de México. Volumen No. 2. UNAM, México, pp: 404.
6. Meyrán, G.J., 1979. Discusión sobre Echinofossulocactus. Cact. Suc. Mex., 24 (3): 90-94.
7. Guzmán, C.L.U., 1997. Grupos Taxonómicos. In: Suculentas Mexicanas. Cactáceas, Valles, S.C. and P.L. Rodríguez (Eds.). UNAM-SEMARNAP, México, pp: 37-41.
8. Abdala, L.R. and P. Seeligmann, 1995. Flavonoids in Tagetes zipaquirensis and their chemosystematic significance. Biochem. Syst. Ecol., 23 (7-8): 871-872.
9. Del Pero, M.M.A., J.P. Pelotto and N. Basualdo, 1997. Distribution of flavonoid aglycones in Illex species (Aquifoliaceae). Biochem. Syst. Ecol., 25 (7): 619-622.
10. Fiasson, J.L., K. Gluchoff-Fiasson and G. Dahlgren, 1997. Flavonoid patterns in European Ranunculus L. subgenus Batrachium (Ranunculaceae). Biochem. Syst. Ecol., 25 (4): 327-333.
11. Almaraz-Abarca, N., 2000. Estudio químiotaxonómico de Pinus sección Leiophyllae (Pinaceae). Tesis Doctoral. Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México, pp: 157.
12. Almaraz-Abarca, N., M.S. González-Elizondo, J.A. Tena-Flores, J.A. Ávila-Reyes, J. Herrera-Corral and N. Naranjo-Jiménez, 2006. Foliar flavonoids distinguish Pinus leiophylla and Pinus chihuahuana (Coniferales: Pinaceae). Proc. Biol. Soc. Wash., 119 (3): 426-436.
13. Wiermann, R. and K. Vieth, 1983. Outer pollen wall, an important accumulation site for flavonoids. Protoplasma, 118 (3): 230-233.
14. Markham, K. and M.G. Campos, 1996. 7- and 8-O-methylherbacetin-3-O-sophoroside from bee pollens and some structure/activity observations. Phytochem, 43 (4): 762-767.
15. Campos, M.G. and K.R. Markham, 2007. Structure information from HPLC and on-line measured absorption spectra-flavone, flavonols and phenolic acids. Coimbra University Press, Portugal, pp: 118.
16. Rohlf, F.J., 1993. NTSyS-pc Version 1.8. Numerical taxonomy and multivariate analysis system.
17. Ylstra, B., T. Alisher, M.R.M. Benito, E. Stöger, A.J. van Tunen, O. Vicente, J.N.M. Mol and E. Heberle-Bors, 1992. Flavonols stimulate development, germination and tube growth of tobacco pollen. Plant Physiol., 100 (2): 902-907.
18. Mo, Y., C. Ángel and L.P. Taylor, 1992. Biochemical complementation of chalcone synthase mutants defines a role for flavonols in functional pollen. Proc. Nat. Acad. Sci., 89 (15): 7213-7217.
19. Burbulis, I.E., M. Iacobucci and B.W. Shirley, 1996. A null mutation in the first enzyme of flavonoid biosíntesis does not affect male fertility in Arabidopsis. Plant Cell, 8 (6): 1013-1025.
21. Miller, J.M. and B.A. Bohmt, 1982. Flavonol and dihydroflavonol glycosides of Echinocereus triglochidiatus var. gurneyi. Phytochem., 21 (4): 951-952.

22. Iwashina, T., S. Otan and K. Hayashi, 1986. Determination of minor flavonol-glycosides and sugar-free flavonols in the tepals of several species of Cereoidae (Cactaceae). J. Plant Res., 99 (1): 53-62.

23. Van Heerden, F.R., V.E. van Wyk, A.M. Viljoen and P.A. Steenkamp, 2003. Phenolic variation in wild populations of Aspalathus linearis (rooibos tea). Biochem. Syst. Ecol., 31 (8): 885-895.

24. Kaundun, Sh.Sh., Ph. Lebreton and B. Fady, 1998. Geographical variability of Pinus halepensis Mill as revealed by foliar flavonoids. Biochem. Syst. Ecol., 26 (1): 83-96.

25. Almaraz-Abarca, N., A. Delgado-Alvarado, J.A. Ávila-Reyes, N. Naranjo-Jiménez and J. Herrera-Corral, 2004b. Las cactáceas del Estado de Durango. Biotecn, 2 (4): 19-20.

26. Sosa, A.P., F.J. Batista, M.A. González-Pérez and N. Bouza, 2002. La Conservación Genética de Las Especies Vegetales Amenazadas. Técnicas de Diagnóstico Del Estado de Conservación. En: Biología de la Conservación de Especies Amenazadas, Bañares, A. (Ed.). Organismo Autónomo de Parques Nacionales, Ministerio de Medio Ambiente, España, pp: 2-27.