Introduction

The Angiosperm Phylogeny Group (APG 2016) includes the order Cucurbitales Dumort. in the Fabids, inside the Eurosid group. The order Cucurbitales Juss. ex Bercht. & J. Presl comprises herbs, climbers, shrubs and trees in 111 genera with a total of 2600 species belonging to eight families: Anisophylleaceae Ridl., Apodanthaceae Tiegh. ex Takht, Begoniaceae C. Agardh, Coriariaceae Mirb., Corynocarpacaeae Engl., Cucurbitaceae Juss. and Datisaceae L. and Tetramelaceae Airy Shaw (Airy Shaw 1964).

Abstract

Seed shape quantification in diverse species of the families belonging to the order Cucurbitales is done based on the comparison of seed images with geometric figures. Quantification of seed shape is a useful tool in plant description for phenotypic characterization and taxonomic analysis. J index gives the percent of similarity of the image of a seed with a geometric figure and it is useful in taxonomy for the study of relationships between plant groups. Geometric figures used as models in the Cucurbitales are the ovoid, two ellipses with different x/y ratios and the outline of the Fibonacci spiral. The images of seeds have been compared with these figures and values of J index obtained. The results obtained for 29 species in the family Cucurbitaceae support a relationship between seed shape and species ecology. Simple seed shape, with images resembling simple geometric figures like the ovoid, ellipse or the Fibonacci spiral, may be a feature in the basal clades of taxonomic groups.

Keywords: Cucurbitales, seed, morphology, geometry
The number of genera is unevenly distributed among families, and the most of the genera belong in the Cucurbitaceae (98 genera). Two genera belonging to the Tetramelaceae were previously included in the Datiscaceae, but recent studies have separated these two families (Thorne & Reveal 2007; APG 2009; Reveal & Chase 2011; Christenhusz & Byng 2016). The Tetramelaceae shares with Datiscaceae and Begoniaceae several features such as the presence of numerous small ovules and seeds with a large-celled surface, two-cell-layered integuments, and a collar around the funicle by an extension of the outer integument (Matthews & Endress 2004). Three families (Anisophylleaceae, Coriariaceae, Corynocarpaceae) were added lately on the basis of molecular studies (Kubitzki 2011b). Considering the number of species, the order is formed by two large families – Begoniaceae and Cucurbitaceae; one family of intermediate size – Anisophylleaceae, and five small families – Apodanthaceae, Datiscaceae, Coriariaceae, Corynocarpaceae and Tetramelaceae.

The family Apodanthaceae with 10 species of endoparasitic herbs belonging to the genera Apodanthes Poit. and Pilostyles Guill. was recently included in the order Cucurbitales in APG IV system (APG 2016).

The family Begoniaceae comprises two genera of herbs: Begonia L. with at least 1500 species and Hillebrandia Oliv. with the only species H. sandwicensis Oliv., endemic to Hawaiian islands (de Wilde 2011). The family Cucurbitaceae contains 97 genera with 940–980 species (Schaefer & Renner 2011). Most of them are annual vines, but some are woody lianas, thorny shrubs, or exceptionally, trees (e.g., Dendrosicyos Balf. f.). The family Datiscaceae contains two species of robust perennial herbs of the genus Datisca L.: D. cannabina L. and D. glomerata (C. Presl) Baill. (Swensen & Kubitzki 2011). Based on molecular studies, the Tetramelaceae was before classified within the Datiscaceae (Swensen & Kubitzki 2011), but recent studies place them separately. Two genera Octomeles Miq. and Tetrameles R. Br. have one buttressed tree species each: Octomeles sumatrana Miq. and Tetrameles nudiflora R. Br.

The family Anisophylleaceae comprise four genera: Anisophylla R. Br. ex Sabine; Combretocarpus Hook. f.; Poga Pierre, and Polygonanthus Ducke, with 71 species of shrubs and medium to fairly large trees. The family Coriariaceae includes 14 species of shrubs, subshrubs, or rarely perennial herbs in a single genus Coriaria L. (Kubitzki 2011a). The family Corynocarpaceae includes six species of evergreen trees in another unique genus Corynocarpus J.R. Forst. & G. Forst (Kubitzki 2011c).

Seed shape is an important characteristic in plants. It is related with their ecology and reproduction (especially seed dispersal), but the use of shape as a taxonomic criteria requires special methods of quantification. Quantification of seed shape may be based on automated programs that give magnitudes such as the diameter or axial distances of a figure, perimeter, circularity index or roundness (Sonka et al. 2008). But giving the similarity of seed images to a range of geometric figures in our analysis is based on the comparison of surface shared in bi-dimensional images. The comparison is by means of the J index, a magnitude that gives the percent of similarity between the seed image and a chosen geometric figure (Cervantes et al. 2012; also see Cervantes et al. 2016 for a recent review). The method was first applied to seeds of the model plant Arabidopsis thaliana (L.) Heynh., comparing their images to a modified cardioid (elongated in parallel to the symmetry axis by a factor of Phi, the Golden Ratio...
Seed shape quantification in the order Cucurbitales

= 1.618, approximately). This was used in the identification of differences in seed shape between genotypes (Cervantes et al. 2010) as well as during seed germination, showing that J index reaches a maximum value early upon imbibition (Martín et al. 2014). A cardioid was also the geometric figure used in the quantification of the model legume Lotus japonicus L. (Cervantes et al. 2012) as well as for Capparis spinosa L. (Saadaoui et al. 2013). A modified cardioid (elongated in perpendicular to the symmetry axis by a factor of Phi) was the geometric model for the quantification of seeds in other model legume Medicago truncatula Gaertn. In the family Euphorbiaceae Juss., seed shape has been quantified using as the geometric model an ellipse in Ricinus communis L. (Martín et al. 2016) and Jatropha curcas L. (Saadaoui et al. 2015), and variation in values of J index was evaluated in diverse cultivars.

A clear relation exists between seed shape and ecological adaptations. In ecology the organisms may be classified according to their structural complexities and life cycle in two types: r and K (Begon et al. 2005). The former r type contains species with small individuals having rapid life cycles and not much structural complexities. The K type, on the contrary, contains species with large individuals, slow cycles and specialised organs (structural complexities). Plants that follow the r type strategy tend to have simple seeds resembling geometric figures. For example we found seeds whose images adjust well to the cardioid in model small plants with rapid life cycles and without specialised structures (Cervantes et al. 2010, 2012). In addition, in the context of complex plant families we also found seeds adjusting well to cardioids in the species that have shorter life cycles and simplest structures, such as in the case of Rhus tripartita (Ucria) Grande in the complex of family Anacardiaceae R. Br. (Saadaoui et al. 2017). The analysis of seed shape at the level of order may reveal the validity of these assumptions.

This work is a first approach to seed shape quantification in species of the order Cucurbitales. The hypothesis is that seeds having single, well defined shapes resembling well defined geometrical figures will appear more frequently in herbs than in trees.

Material and methods

Seed image observation

Seed images belonging to six of the eight families (Anisophylleaceae, Begoniaceae, Coriariaceae, Corynocarpaceae, Cucurbitaceae and Datiscaceae) in the Cucurbitales were analysed. Seeds from Apodanthaceae and Tetramelaceae were not analysed. Seed images were taken from sources indicated in Tab. 1. Individual seeds of Ecballium elaterium (L.) A. Rich. were placed over a flat surface with their micropile oriented to the right. Photographs of orthogonal views of the seeds were taken with a digital camera Leica C Type 112.

Image analysis

Composed images containing the geometric model and each seed were elaborated with Corel Photo-Paint X7. Quantification of areas was done with Image J. Individual seed images were used for determination of J index. To obtain the J index areas in two regions were compared: the regions shared by the model and the seed image (i.e. common regions C) and the regions D not shared between both areas (Fig. 1). The index of adjustment (J) is defined by:
Table 1. Analyzed seeds and image sources.
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J = (area C) / (area C+ area D) \times 100

Note that J is a measure of seed shape, not of its area. It ranges between 0 and 100, decreasing when the size of the non-shared region grows and equals 100 when the geometric model and the seed image areas coincide, that is, when area D is zero.

Models used were the ovoid, ellipses and the outline of Fibonacci’s spiral. Two ellipses were used with different x/y relationship – x/y = 2.16 and x/y = 1.76.

Results

Seed images of many species In the Cucurbitaceae resemble an ovoid such as for example species of Cucurbita L. (Fig. 2), as well as species of other genera such as Coccinia Wight & Arn. (Fig. 3), Bryonia L., Trichosanthes L., Citrullus Schrad. ex Eckl. & Zeyh., Melothria L., Sicyos L., Acanthosicyos Welw. ex Hook. f. and Dendrosicyos (Fig. 4).

Still in the Cucurbitaceae, the internal part of the seed of the woody liana Alsomitra macrocarpa (Blume) M. Roem. adjusts well to an ovoid (J index = 93.5), nevertheless the seed contains an external part and a wing that deviate it from ovoid shape (Fig. 5).

Species in other Cucurbitaceae genera such as Cucumis L. adjust better to an ellipse with x/y ratio of 2.16 (Fig. 6). The same ellipse is useful for the adjustment of Echinocystis lobata (Michx.) Torr. & A. Gray and Lagenaria sphaerica (Sond.) Naudin from Cucurbitaceae, as well as Datisca cannabina from Datisaceae (Fig. 7).

Seeds in other Cucurbitaceae genera adjust better to an ellipse with x/y ratio of 1.76 such as, for example, Ecballium elaterium Figure 1. J index represents the percent of similarity between seed image and a geometric figure: A – ovoid; B – seed image; C – total area occupied by the ovoid and the seed image superimposed (shown in black is the area not shared); D – area shared between both figures the ovoid and the seed image. J index is the ratio between shared area and total area (× 100).
Cervantes & Martín Gómez

Figure 2. Seeds of the genus *Cucurbita* adjust well to an ovoid. Values of J index are given between parentheses. Bars represent 5 mm.

![Cucurbita seeds](image)

*Figure 2. Se...* (93.9)  
*Figure 2. Se...* (90.3)  
*Figure 2. Se...* (87.8)

![Cucurbita seeds](image)

*Figure 2. Se...* (87.1)  
*Figure 2. Se...* (86.3)

(L.) A. Rich., *Citrullus colocynthis* (L.) Schrad., *Luffa acutangula* (L.) Roxb., *Thladiantha dubia* Bunge and *Momordica charantia* L. (Fig. 8). The same model is useful for *Corynocarpus laevigatus* J.R. Forst. & G. Forst from the family Corynocarpaceae and *Anisophyliella apetala* Scort. ex King from Anisophylleaceae (Fig. 9), as well as for *Begonia* species from Begoniaceae (Fig. 10).

The model based on the outline of Fibonacci’s spiral was applied as a morphological model to the Coriaraiceae including *Coriaria arborea*, *C. japonica* and *C. myrtifolia* (Fig. 11).

Finally, there are Cucurbitaceae species that do not adjust well to any of proposed models, such as *Lagenaria siceraria* (Molina) Standl., *Cyclanthera pedata* (L.) Schrad. and *Cyclantheropsis parviflora* (Cogn.) Harms (Fig. 12). These three species are not included in Tab. 2, because the images of their seeds do not correspond well with the geometrical figures used here as the models (ovoid, ellipses or Fibonacci’s spiral).

![Cucurbita seeds](image)

*Figure 3. Se...* (90.5)  
*Figure 3. Se...* (88.5)  
*Figure 3. Se...* (88.0)

![Cucurbita seeds](image)
High values of J index were found in Cucumis species with $x/y = 2.16$ ratio ellipse as a model, as well as in Begonia, Corynocarpus and Anisophyllea, and species of other genera with the ellipse of a ratio $x/y = 1.76$ as a model. Thus Cucumis melo L., C. miryocarpus Naudin and C. metuliferus E. Mey. ex Naud. gave values for J index of 94.3, 93.4 and 92.2 respectively. Cucumis anguria L. and C. dipsaeus Ehrenb. gave J index values over 90, of 91.5 and 90.7 respectively.

The model based on the ellipse of a ratio $x/y = 1.76$ gave values over 90 for J index in the following species: Corynocarpus laevigatus (94.2), Begonia wallichiana Lehm. (94.1), B. leptotricha C. DC. (93.9), B. labordei H. Lév. (93.7), B. catharinensis Brade (93.6), B. dryadis Irmsch. (93.6), B. semperflorens Link & Otto (92.9), B. malabarica Lam. (92.8) and B. acida (92.7), Anisophyllea apetala (92.8), Echbalium elaterium (92.3), Citrullus colocynthis (92.2), Luffa acutangula (91.0) and Thladiantha dubia (90.2).

Taking as a model the ovoid, maximum J index values were obtained for Cucurbita maxima Duchesne (93.9), Bryonia alba L. (92.5), Ruthalia eglandulosa (Hook. f.) C. Jeffrey (90.5), Cucurbita digitata A. Gray (90.3) and Trichosanthes subvelutina F. Muell. ex Cogn. (90.3).

The model based on the outline of Fibonacci’s spiral gave J index values of 91.4 for Coriaria arborea, 89.6 for C. japonica and 88.0 for C. myrtifolia.

**Discussion**

Seed shape is an important factor in descriptive taxonomy and mutant phenotyping, as well as in plant ecology. Seed
shape analysis often involves computer-assisted methods based on automated vision and the use of different algorithms (Rovner & Gyulai 2007). Modern methods of seed shape analysis have been applied to seed classification in diverse species (Sahai et al. 1997; Venora et al. 2007; Mattana et al. 2008; Grillo et al. 2012), but they tend to be completely automated and thus do not regard the similarity of seeds with geometrical figures, while our method is semi-automated and is designed to obtain the percent of similarity of a seed image with model geometric figures (Cervantes et al. 2016).

Model species usually are represented by small plants with rapid life cycles and lack of specialised structures. Previously we found, that in such plants as Arabidopsis thaliana, Medicago truncatula and Lotus japonicus, seed shape can be quantified on the basis of cardioid models (Cervantes et al. 2010, 2012). Now we have made a first approach to the relationship between seed shape and ecology in the order Cucurbitales. In particular, the model based on the outline of Fibonacci’s spiral could only be applied to the family Coriariaceae, what distinguish it from other investigated Cucurbitales. Interestingly this model can also be applied to Actaea spicata L. seeds from Ranunculaceae Juss. (Martín Gómez & Cervantes, in preparation).
Figure 8. Seeds of Cucurbitaceae that adjust well to an ellipse with \(x/y\) ratio of 1.76. Values of J index are given between parentheses. Bars represent 5 mm.

Figure 9. Seeds of Corynocarpus laevigatus (Corynocarpaceae) and Anisophyllea apetala (Anisophylleaceae) adjust well to an ellipse with \(x/y\) ratio of 1.76. Values of J index are given between parentheses. Bar represents 5 mm.

Figure 10. Seeds of Begonia (Begoniaceae) species adjust well to an ellipse with \(x/y\) ratio of 1.76. Values of J index are given between parentheses. Bar represents 5 mm.
Corynocarpus laevigatus, the only species tested of the Corynocarpaceae gives a high value of J index with model based on the ellipse of a ratio x/y = 1.76. Corynocarpus is a tree. Interesting that Anisophylella apetala is another tree from Anisophilleaceae, which gave high values of J index within this model.

Our analysis has been more complete in the family Cucurbitaceae. It involved in this family the calculation of J index values for a total of 29 species of diverse life forms and ecological strategies (Tab. 2). In this family, lower values of J index were observed for species having adaptations in their seeds (mean J index for 12 species with adaptations is 89.5), while mean J index for species without adaptations was higher (90.6 for 17 species). Also, values of J index in the Cucurbitaceae are higher for seeds belonging to species of the r strategy (mean J index for 26 species is 90.3) than those of the K strategy (mean J index for 3 species is 88.4). The total mean values of the species with K strategy appear higher just because of high value for the J index of Alsomitra macrocarpa (93.5). Seed of A. macrocarpa has a wing that conceals it from classification into the ovoid model, but it fits well to an ovoid if take just the internal part without wing. In addition, A. macrocarpa is fast growing and parasitic woody liana, and cannot serve as a really good example of K type plant.

Thus, we can assume that an overall trend is maintained in the Cucurbitaceae with higher J index values in seeds without special adaptations. Interesting, that the Coriariacceae present a similar trend with lower J index values in seeds having rugose structure of the testa (Tab. 3).
Our results support a relationship between seed shape and species ecology and maintain the hypothesis that in large families the simple seed shapes with images resembling simple geometric figures like the ovoid, diverse ellipses or Fibonacci’s spiral, are associated with characteristics of the plants of r type (rapid growth, short life cycle, absence of specialised structures). This may be a feature in the basal clades of taxonomic groups, such that departing from small plants with rapid cycles, evolutionary divergence could give rise to larger plants with special adaptations. We are investigating this possibility in other Angiosperm families.
Table 3. Life forms, ecological strategies, special adaptations and J index values in the families Datiscaceae, Corynocarpaceae, Anisophyllaceae, Begoniaceae and Coriariaceae.

<table>
<thead>
<tr>
<th>Family/Species</th>
<th>Life form</th>
<th>Strategy</th>
<th>Seed adaptations</th>
<th>Geometric model</th>
<th>J index</th>
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</thead>
<tbody>
<tr>
<td>Datiscaceae</td>
<td></td>
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<td>r</td>
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<td>93.6</td>
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References


