Studying flowers in 3D using photogrammetry

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October 26, 2022

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Abstract

- Flowers are intricate and integrated three-dimensional structures predominantly studied in 2D due to the difficulty in quantitatively characterising their morphology in 3D. Given the recent development of analytical methods for high-dimensional data, the reconstruction of flower models in three dimensions represents the limiting factor to studying flowers in 3D.

- We developed a floral photogrammetry protocol to reconstruct 3D models of flowers based on images taken with a digital single-lens reflex camera, a turntable and a portable lightbox.

- We demonstrate that photogrammetry allows a rapid and accurate reconstruction of 3D models of flowers from 2D images. It can reconstruct all visible parts of flowers and has the advantage of keeping colour information. We illustrated its use by studying the shape and colour of 18 Gesneriaceae species.

- Photogrammetry is an affordable alternative to micro-computed tomography (microCT) that requires minimal investment and equipment, allowing it to be used directly in the field. It has the potential to stimulate research on the evolution and ecology of flowers by providing a simple way to access three-dimensional morphological data from a variety of flower types.

Keywords — Comparative morphology, Flower colour, Floral shape, Geometric morphometrics, Ultra close-range photogrammetry, Three-dimensional flower models
1 Introduction

Flower shape, size and colour influence the attraction of pollinators, the way pollinators access floral rewards, and contingently the exchange of pollen between anthers and stigmas (Faegri and Van Der Pijl [1979]; Fenster et al. [2004]; Willmer [2011]). Flower shape is also important in wind-pollinated species (anemophily) as it influences interactions with air flows and so determines efficient pollen release, dispersal and capture (Timerman and Barrett [2019]). Because flowers are three-dimensional structures that interact with a three-dimensional biotic and abiotic environment for conspecific exchange of pollen, characterising flower shape and colour in 3D is important to promote a comprehensive understanding of flower development and the role of flower shape in the ecology and evolution of species.

Only recently has it become feasible to study the variation of flower shape in three dimensions (3D) due to the development of methods to build 3D flower models. The first reconstruction of flowers in 3D used micro-computed tomography (microCT, or HRCT for high-resolution CT) to acquire and visually render digital three-dimensional shape data of both surfaces and internal structures (Stuppy et al. [2003]). MicroCT helps to visualise minute plant structures and to study their external 3D morphology and internal structures qualitatively and quantitatively. The characterisation and comparison of these 3D flower models using geometric morphometrics (Rohlf and Marcus [1993]) has opened a vast array of possibilities for the study of flowers in 3D, which was deemed to represent a “revolution” for the study of flowers (van der Niet et al. [2010]). Though other 3D modelling techniques are available such as laser-scanning and structured light that record surfaces, microCT scanning remains the most common 3D digitization technique applied to plant specimens (Mathys et al. [2013]; Davies et al. [2017]).

Despite the fact that several studies recently used 3D flowers models (Gamisch et al. [2013]; Wang et al. [2015]; Dellinger et al. [2019]; Hsu et al. [2020]; Reich et al. [2020]; Artuso et al. [2021, 2022]), the widespread analysis of 3D flowers has not occurred. Geometric morphometrics studies of flowers in 3D are still limited compared to the mass of literature in the fields of anthropology, zoology and paleontology. This could be due in part to the difficulties of using microCT on the soft tissues of flowers, even though solutions for optimising HRCT scanning of flowers have been proposed (e.g., Staedler et al. [2013]; Dellinger et al. [2019]). In addition, and perhaps more importantly, the high cost of microCT techniques (Mathys et al. [2013]) contributes to reducing their accessibility. Lastly, the fact that flower colour is lost when reconstructing 3D models using X-ray scanning technologies (Mathys et al. [2013]) limits the use of this technique for studies interested in colour or colour patterns.

Recently, research based on 3D imagery has evolved rapidly and has received considerable attention (e.g., Katz and Friess [2014]; Cunliffe et al. [2016]; Evin et al. [2016]; Strobel et al. [2018]; Christiansen et al. [2019]; Giacomini et al. [2019]; Florey and Moore [2019]; Iglhaut et al. [2019]; Medina et al. [2020]). A 3D technique of interest is photogrammetry (or structure from motion), which uses a collection of digital images to reconstruct a 3D model (see Linder [2009]; Luhmann et al. [2013]). Photogrammetry was originally used to reconstruct models of landscapes, buildings or large objects, but it can also be used for medium (close-range photogrammetry) or small objects (ultra-close-range photogrammetry). In short, photogrammetry begins by taking pictures of an object from all angles, ensuring that all aspects of the object are present in several overlapping photos. The sets of photos are then aligned using the relative position of homologous points in the overlapping pictures in a 3D space, and picture information is then used to reconstruct a 3D model with colour (see Floral photogrammetry protocol and Fig. 1 for more detailed information). Although used in many fields of biological sciences, photogrammetry has not yet been applied to the study of flowers.

The objective of this study is to demonstrate the potential of photogrammetry to reconstruct 3D models of flowers to facilitate studies of floral shape and colour. We describe an affordable and portable photogrammetric setup that could be used in the field, and outline a detailed protocol for reconstructing 3D photographic models of flowers of various shapes, colours and sizes. To illustrate the approach, we present an example of application in the study of the shape and colour of flowers from species of the Gesneriaceae.
2 Floral photogrammetry protocol

Here, we provide a summary of the photogrammetry protocol we developed. The full protocol is available from Github [https://github.com/plantevolution/photogrammetry-protocol] and details of the source and costs of materials, tools and software are provided as Supporting Information (Table S1). Specific terms in photography, 3D modelling and geometric morphometrics are defined in the glossary (Box 1). Our objective is not to provide a unique and final protocol, but to provide guidelines for users to employ photogrammetry to 3D model flowers and guide them on how to adapt this approach for their own system.

2.1 Image acquisition

The first step of photogrammetry involves acquiring photos encapsulating flower details for later modelling in 3D. This step is perhaps the most important as high quality images are key to produce high quality 3D models. We capture images using a digital single-lens reflex (DSLR) camera and a fixed focal-length macro lens. We save images in RAW format using an aperture of F16 (highest field depth without deteriorating the image quality), lowest ISO (e.g. 100) to avoid image noise created by the sensor, and a shutter speed adjusted to allow the appropriate amount of light to reach the camera’s sensor to result in a well-exposed image (see Supporting Information Table S2 for a summary of the settings we used).

To facilitate the photo capture of the flower from all directions, we use a turntable and automated remote camera control (Fig. 1a,b). To help later photo processing and mask the background in the pictures, we recommend using a uniform background. Good lighting conditions are also necessary for optimal picture quality. These conditions can be recreated in the field using a portable lightbox (see Supporting Information Table S1).

The flower to be photographed is fixed at the centre of the turntable using pins or clamps, or could be placed in a tube or a cut pipette tip depending on the structure and stiffness of the flower (see example Fig. 1c). A scale should be placed so that it is visible in several photographs to allow scaling of the resulting model.

To capture the entire flower surface and details, we take a 360° series of photos of flowers placed in normal and inverted positions (e.g., ventrally and dorsally). Typically 20 photos per rotation were taken at 3 different camera heights and angles of approximately 0°, 30° and 60° for each side of a flower (see Fig. 1b), for a total of 120 photos per flower. Depending on the flower complexity, the number of photos, camera angles and flower positions can be adjusted to capture all visible floral details. It is also possible to add close-ups photos to enhance the model and reveal concealed and minute parts (e.g., reproductive organs). If using a variable focal lens, it is preferable that the focal length is kept identical for all the pictures, and ideally at the minimum or maximum focal length possible to avoid optical deformations (Agisoft LLC [2021]).

2.2 Colour and exposure calibration

Photographs must be colour-calibrated to adjust the reflectance and colour of an object and allow accurate comparison between flowers [Troschianko and Stevens [2015]]. To calibrate multiple photos with the same parameters, we use DNG (Digital Negative) colour profiles created from an additional RAW photo of a standardised colour chart, taken for each series of photos of a flower under the same light conditions and camera parameters. We convert the colour chart in a DNG profile (e.g. using Adobe Digital Negative converter) and standardise the series of photos corresponding to the colour chart (using e.g. Adobe Lightroom (Adobe Inc., San Jose, California, USA)), providing an accurate reproduction of the flower colour for subsequent analyses of pigmentation patterns. We also standardise the photo exposure using a 75% grey colour chip from the colour chart. Exposure calibration can also be performed at a later stage directly using the colour file (texture) of the 3D model. From the calibrated RAW photos, we export JPG images for the model reconstruction (Fig. 1c,d,e).
2.3 3D model reconstruction

The procedure we use to obtain a 3D model from photogrammetry includes photo alignment, which results in a sparse three-dimensional point cloud, surface generation through depth maps calculation, and texture generation using the projection of photos onto the surface of the model. Our protocol uses the commercial software Agisoft Metashape Professional Edition version 1.7 (Agisoft LLC., St. Petersburg, Russia), but open-source photogrammetry software also exist (see Medina et al. (2020) for more details).

During photo alignment (also referred as camera alignment), source images are positioned by searching for common points in the photos (tie points) and by using the triangulation of the matching points. The alignment procedure can customarily be done in a single step, by attributing images from different flower positions to distinct camera groups, or by separating images from different flower positions into different chunks. Treating sets of images separately can be useful for merging models of different flower parts (e.g. for modelling a complete flower by merging the flower with and without its perianth). Chunks of images can also be treated separately during the alignment procedure when the overlap between pairs of images isn’t optimal to facilitate the alignment calculation (Fig. 1j,g,h,i, showing an example with two chunks of images). Prior to aligning images, masks can be captured manually or automatically to separate the flower from the background and restrict the searching of common points between images during the alignment procedure to the flower itself (Fig. 1g). The picture alignment (Fig. 1h) generates a three dimensional cloud of matching tie points for each set of images (Fig. 1i). When different chunks of images are used separately, they need to be aligned together and then merged either automatically or by using manually-placed markers on distinctive features on the flowers on several images (e.g. tips of petals or sepals, anthers). If manual markers are used, a minimum of 3 markers spaced on the flower is required. The merging of images or groups of images results in a single tie point cloud (Fig. 1j).

Once all the images are aligned around a single tie point cloud, the model (mesh) can be generated, using depth maps generated for each photo that represent the distance of the flower surface on the z axis for each camera positions (Fig. 1k). The mesh is composed of vertices, edges and faces, together forming polygons (Fig. 1l). During mesh reconstruction, the interpolated colour of the mesh polygons is calculated from images when using depth maps as source information (Fig. 1m). The resulting mesh may need minor touch-ups, such as removing unwanted portions of the inflorescence or the pin used to attach the flower.

We then scale the model by manually positioning landmarks on the scale bar in the original images and defining these landmarks as being spaced by the length of the scale, which resizes the model accordingly. Finally we build the texture (detailed colour) of the model by using the 2D picture’s information to generate a realistic visualisation of the flower surface in 3D (Fig. 1n,o). The flower surface mesh can subsequently be used in geometric morphometric applications (Fig. 1p,q) and the three-dimensional textured surface can be exported as a 2D layout of the 3D surface, used in quantification of flower colour using each pixel colour information (Fig. 1r,s).
Figure 1: Graphical workflow of the photogrammetric approach used to study floral morphology and colour in three dimensions (3D). Flowers are attached to a 360° turntable that automatically triggers a camera as the turntable rotates in steps of a few degrees and are photographed using three camera angles for both ventral and dorsal view (a-b). All RAW images (c) are calibrated identically using a colour chart (d) to obtain realistic colour representation of flowers (e). Masks are applied to remove the background (f-g) before aligning the images (h), which results in a tie point cloud of homologous pixels detected in multiple photos (i). The separate sets of photos are then aligned and merged to give a unique tie points cloud (j). Using depth maps (k), a 3D mesh is reconstructed (l) and the interpolated colour of the mesh polygons is calculated from images (m). A more realistic 3D model is obtained by building the texture from the original photos (n), providing a finely detailed and coloured 3D model on the outer and inner surfaces of the flower (o). Landmarks (in red) and semi-landmarks are positioned on the flower model for curves (in blue) on the petal margin, petal base, dorsal and ventral corolla curvature, and the base of the sepals, as well as on the simplified truncated cone template. Surface semi-landmarks (in green) are automatically applied on flowers according to the template (p-q). The flower texture wrapping the model can be extracted as a 2D representation of the 3D surface (r) and used to analyse and quantify colour variation of the entire flower surface (s).
3 Performance of the photogrammetry approach

The above protocol was applied to diverse flowers selected to represent a variety of floral forms, colours and complexity. These were taken from species belonging to different Angiosperm families such as the Fabaceae (*Phaseolus coccineum*), Cactaceae (*Schlumbergera sp.*), Lamiaceae (*Salvia nemorosa*), as well as 19 Gesneriaceae species from the living collections of the Montreal Botanical Garden, mainly from the genera *Rhytidophyllum* and *Gesneria* (see example below for more information on these specimens). Flowers from 5 species modelled by photogrammetry were also scanned using microCT to compare models originating from both approaches.

3.1 3D flower reconstruction

The overall 3D reconstruction process using photogrammetry took from 0.5 to 2h depending on the complexity of the project and the computer resources. The 3D models, the RAW photogrammetry image series to generate these models, and the corresponding colour charts and calibrated textures can be accessed from MorphoSource (https://www.morphosource.org/) under the project “Gesneriaceae of the Montreal Botanical Garden”.

The photogrammetric approach generated models of high quality that accurately represent the shape and colour of flowers of different structures, sizes (2cm to 8cm), and symmetry (see Fig. 2 for a sample of flower models). The flower models presented in figure 2 were also deposited in SketchFab (sketchfab.com/plantevolution), an online platform for hosting and visualising 3D models with textures, to allow a closer inspection of the models. In many cases, very minute details could be modelled, such as the delicate petal margins of *Rhytidophyllum vernicosum* (Fig.
or the styles and stamens of several species (Fig. 2c, d, e, f). Overall, the flower shape and colour of the models were very accurate and were essentially identical to the real flowers.

Some structures are more difficult to reconstruct, particularly those that are slender, translucent or reflective. Pillose organs were also challenging, in part due to the difficulty of applying masks. Structures that are very close to each other such as overlapping petals (Schlumbergera sp.; Fig. 2b) or styles that are very close to petals (Fig. 2f, h, i) were also difficult to reconstruct independently from each other. Finally, very thin surfaces or parts that were partly concealed depending on the camera angles may require more source images to be further improved such as below the basal petal surfaces on Schlumbergera sp., the petal margins inside the corolla on Phaseolus coccineum, and the dorsal midrib of the sepal on Salvia nemorosa. Despite the occurrence of slight imperfections in some of the models, the global shape and colour of flowers reconstructed should allow most downstream applications.

Figure 2: 3D textured models derived from non-Gesneriaceae (a-c) and Gesneriaceae flowers (d-i) using photogrammetry. Phaseolus coccineum, Fabaceae (a), Schlumbergera sp., Cactaceae (b), Salvia nemorosa, Lamiaceae (c), Petrocosmea minor (d), Aeschynanthus splendidus (e), the hybrid Rhytidophyllum x vernicosum (f), Rhytidophyllum vernicosum (g), Rhytidophyllum bicolor (h), and Gesneria cornuta (i), Gesneriaceae. The model of the inflorescence of S. nemorosa is illustrated under two different viewing angles (c).
3.2 Photogrammetry vs. microCT comparison

To compare the models obtained by photogrammetry with models obtained by microCT scanning, the flowers of five species (Gesneria acaulis 1328-2021, Kohleria sp. 1828-2013, Paliavana prasinata 1432-2010, Rhytidophyllum exsertum 112-1991 and Rhytidophyllum tomentosum 1327-2021) were sent to the Integrated Quantitative Biology Initiative (IQBI) platform at McGill University for microCT scanning and model reconstruction. Flowers were collected at the Montreal Botanical Garden and fixed in 4% Paraformaldehyde (PFA) in 1X Phosphate-buffered saline (PBS). The samples were gradually transferred to 70% ethanol from water and placed in 2% ethanolic phosphotungstic acid (EPTA) in 70% ethanol stain for 15 days. Flowers were scanned in 1% agarose in 15 ml tubes at resolution 22 µm at voltage 60V. The microCT models were reconstructed in Dragonfly (version 2020.2). The reconstruction of the models obtained from microCT took between 0.5 to 5h.

To allow a visual comparison of the models obtained by photogrammetry and microCT, we presented the flower models of three species side-by-side in different figures (Fig. 3 and Supporting Information Figs. S1 and S2). All microCT models were also analysed alongside the photogrammetry models in a geometric morphometric analysis (see application example below). The microCT models were deposited in Morphosource in the same project as the photogrammetry models.

The surface meshes of models reconstructed with the microCT scans included holes as well as bumps due to the detection of hairs during the scanning step, but overall represented the morphology of flowers similarly to photogrammetry (see details in the floral shape analysis application example). Due to the softening of tissues during the staining process, flower parts were occasionally distorted and flowers with naturally recurved petals and/or sepals during the anthesis were slightly unfolded on the CT-based model compared to the photogrammetry model and the real flower (see for instance Paliavana prasinata in Fig. 3b,c,d). Similar distortions of the corolla and sepals in microCT models are evident on the models of G. rupincola and R. exsertum (Supporting Information Fig. S1, and S2). Internal and non-visible organs were properly modelled using microCT scan, which could not be accurately reproduced using photogrammetry (Fig. 3e,f). However, anther and stigma position can only be captured when visible from the flower opening (see Figs. 2 and 3).
4 Application example

4.1 Material and methods

To demonstrate the potential use of flower models obtained from photogrammetry, we studied the shape and colour of 26 flowers from 18 species and one hybrid (*Rhytidophyllum auriculatum × vernicosum*) from the Gesneriaceae family that belong to three pollination syndromes: bird pollination, bat pollination, and mixed-pollination (see details in Supporting Information Notes S1 and Table S3). We provide a brief description of the methods used here, but detailed materials and methods are available as supplementary information.

4.2 Floral shape analysis

The perianth of each flower was reconstructed in 3D using the described photogrammetry protocol above. We then used geometric morphometrics to compare the 3D flower shapes. Landmarks as well as semi-landmarks for curves and surfaces were placed on the flowers (see Supporting Information Methods S1, Fig. S3, and Table S4). After shape alignment using a generalized Procrustes analysis (GPA), the coordinates of the landmarks and semi-landmarks were
projected onto the tangent space using principal component analysis (PCA) (Supporting Information Methods S1). The resulting morphospace places individuals from the same species very close to each other and allows the distinction of the pollination syndromes (Fig. 4 and Supporting Information Fig. S4). The estimated mean 3D shape of each syndrome also shows the main differences in shape between them (Supporting Information Fig. S5). The inclusion of the microCT flower models to the same morphospace shows that they fall very close to the photogrammetry models (Supporting Information Fig. S6).

Figure 4: 3D floral morphospace and corresponding shape variation along the axes for individuals belonging to the Gesneria, Rhytidophyllum, Nematanthus, Aeschynanthus, Kohleria, and Paliavana genera. Specialists for bat (blue circles) and hummingbird pollination (red triangles) as well as mixed-pollination strategies (green squares) are represented along the first and second dimensions of the principal component analysis (PCA) (a). The mean 3D flower shape, as well as the maximum and minimum configurations of the 3D flower shape are shown for the first and second dimensions of the PCA (b).

4.3 Colour analysis

To illustrate the potential of the photogrammetric approach to study flower colour, the colour profiles of flowers were compared alongside the phylogeny of the 18 species of Gesneriaceae (see Supporting Information Methods S2 for phylogeny reconstruction). The colour of each flower surface texture was quantified in eight categories (bins) in terms of red, blue, and green, which then allowed to compute a colour distance between flowers (see Supporting Information Methods S3 and Fig. S7). The resulting distance matrix was used to build a phenogram and compared to the phylogenetic relationships (Fig. 5). This example highlights that species tend to group by pollination syndromes when considering flower colour and that similar colour patterns show evolutionary convergence in the Gesneriaceae.
Figure 5: Tanglegram linking the phylogenetic relations of Gesneriaceae species (left) with the colour distance dendrogram from flower specimen colour using Ward’s clustering method (right) according to their respective pollination syndromes.

5 Discussion

5.1 Relevance of ultra-close-range photogrammetry for the study of flowers

Flower shape has attracted much interest in several sub-fields of plant sciences, but relatively few studies have used 3D flower models, despite the importance of precisely quantifying the size, shape and colour of flowers in 3D given that the vast majority of flowers have to interact in a three dimensional environment to be fertilised. The main aspects of the methods currently used that may limit the number of 3D geometric morphometric studies on floral shape are the cost and lack of portability of microCT technologies. Although flowers can be fixed in the field in highly concentrated ethanol for later microCT scanning, doing so can damage or shrink flowers depending on their structure and thickness (Staedler et al., 2013; Dellinger et al., 2019). Moreover, contrasting reagents used to infiltrate the flower tissues prior to scanning may also produce additional leaching artefacts (Staedler et al., 2013). We observed slight anatomical distortions in the flower models obtained with CT scans following fixation such as the unfolding of petals and sepals, distorting the shape and the relative positioning of the floral structures. Our microCT protocols could be improved to provide better and more accurate results; however, the use of standard protocols combined with an external service offers a fair comparison with our photogrammetry approach.

An array of flower sizes and colours for species belonging to the Gesneriaceae, Fabaceae, Lamiaceae, and Cactaceae were successfully reconstructed using photogrammetry. Various sizes of flowers as well as inflorescences can be rendered accurately and in detail and we were able to model all visible parts of the flowers, including the stigma and anthers in many species. The entire physical set-up (turntable, lightbox, tripod and colour chart) required to implement this method costs less than 600 US$, making this approach affordable for most laboratories that already own a suitable digital camera. Computer requirements are relatively reasonable for photogrammetry applications (16-32 GB...
of Random Access Memory (RAM), and 4-8 central processing units (CPUs)). The academic professional edition of
the Agisoft Metashape software we used to reconstruct the 3D models currently costs 549 US$, although cheaper or
open-source alternatives are available (see Medina et al. (2020)).

Ultra-close-range photogrammetry has several clear advantages over microCT—the gold standard in the field—
for reconstructing 3D models of flowers. It is portable, affordable, time efficient both for image acquisition and model
building, and can reconstruct the colour of the flower (see Table 1). Yet, photogrammetry is also subject to some
limitations (Table 1). Most obviously, flower models can be reconstructed for only externally visible parts of the
flower. Hidden structures, such as reproductive organs in some species, may remain hidden in particularly closed
corollas. Floral dissection might in some cases provide the means to characterise concealed structures in 3D using
photogrammetry, e.g., removal of the corolla, or simply cutting the sepals to reveal the base of the corolla, as we did
in the application example using Gesneriaceae. In addition, concealed organs can be photographed and thus be visible
on the model texture, allowing them to be analysed. But if concealed organs are of main interest for a given study,
microCT is clearly the best option.

Some flower characteristics also likely complicate the 3D model reconstruction with photogrammetry. One of
these is the often very thin surfaces of flowers. Missing surfaces or edges in the final 3D model often result from a
miscalculation of the position of sufficiently close outer and inner surfaces of an object to be modelled. Depth data
from sets of images may intersect, thus creating holes in the 3D mesh. A contrasting background and accurate masking
of flowers allow recovery of thin structures at the extremities of the models such as fringed petals or stamen filament
(e.g. see interactive flowers of Rhytidiophyllum vernicosum and Salvia nemorosa). Shiny or translucent surfaces also
represented a challenge because the reflection of light or the features that are detected behind the translucent petals
are not fixed features on the surface, but are still captured in images. This issue can be solved by using a softer
light during the photography step. The presence of dense hairs on the flower also complicates model reconstruction
because of the difficulty of adequately removing the background on photographs and because the resulting model
is often rugged, although it can generally be corrected by smoothing the 3D surface. Finally, radially symmetrical
or spherical objects with no clear visual landmarks are typically difficult to reconstruct using photogrammetry (Ijiri
et al., 2018), such as some actinomorphic flowers. A straightforward solution is placing a few visually distinct markers
on such flowers (i.e. coloured dots) to add artificial distinct features to help the software to align the photographs.

Overall, ultra-close-range photogrammetry is an useful alternative to microCT scanning and could be valuable for
studies interested in the non-occluded parts of a flower and its colour. One interesting avenue is to combine CT-based
and image-based approaches to get the advantages of both approaches, as demonstrated by Ijiri et al. (2018).
### Table 1: Summary of the capabilities of both the microCT scan and photogrammetry approaches. Details on costs associated with photogrammetry are available in the Supporting Information Table S1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Photogrammetry</th>
<th>microCT scan</th>
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<tbody>
<tr>
<td>3D reconstruction of occluded areas and internal structures</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Model colour and texture</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Portability</td>
<td>Yes</td>
<td>No, but flowers can be fixed on site</td>
</tr>
<tr>
<td>Adaptable to any type of flower</td>
<td>Yes, but very soft and hairy flowers and those with few clear visual landmarks are more difficult to reconstruct</td>
<td></td>
</tr>
<tr>
<td>Deformation of structures</td>
<td>no</td>
<td>slight</td>
</tr>
<tr>
<td>Cost</td>
<td>from $2000</td>
<td>$200,000 to $1,000,000</td>
</tr>
<tr>
<td>Time requirements per flower</td>
<td>Setup and photography: 30min</td>
<td>Staining: typically &gt; 14 days</td>
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<tr>
<td></td>
<td>Colour calibration: 10min</td>
<td>Scanning: 1h</td>
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<tr>
<td></td>
<td>Reconstruction: 30min to 3h</td>
<td>Segmentation: 30min to 5h</td>
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5.2 Perspectives for floral morphology studies

5.2.1 Studying high-dimensional floral shape evolution

One obvious application of 3D flower models obtained by photogrammetry is to study floral shape. Different methods exist for characterising biological shapes in 3D. The most popular type of methods are geometric morphometrics that are based on the positioning of landmarks (homologous points) and semi-landmarks (points that are positioned relative to others, along curves or surfaces). Landmark-free methods have also been developed and could be useful for smooth or featureless 3D surfaces for which landmark placement is not appropriate (Pomidor et al., 2016). Our worked example on Gesneriaceae showed how photogrammetry can be used to study the three-dimensional morphology of flowers. The accurate three-dimensional reconstruction of flowers combined with landmark-based geometric morphometrics allowed better discrimination and understanding of the 3D structure of the distinct pollination syndromes (Fig. 4) compared to what can be obtained using 2D shape information from flowers in profile view (Joly et al., 2018).

5.2.2 Studying flower colour in 3D

One obvious advantage of photogrammetry over other approaches is to provide a very accurate reconstruction of flower colour in 3D. This opens many study opportunities as flower pigmentation is a major display signal for animal-pollinated plants. The complexity of floral colouration, including nectar guides, help pollinators limit the time they spend locating rewards, thus improving their foraging efficiency (Leonard and Papaj, 2011). Moreover, both biotic factors, such as pollinator abundance and the colour of co-occurring plants in the community, and abiotic factors such as solar radiation and low precipitation, can influence the colour perception and patterns of flowers (Dalrymple et al., 2020).

We showed that photogrammetry is a valuable tool to recover calibrated colour information from the entire 3D surface of flowers. 3D textures generated directly from calibrated high-quality photos in colour analyses can account for the totality of variation in flower pigmentation, avoiding biases caused by overlooked concealed surfaces or the...
distorted importance given certain regions of the flower (surfaces perpendicular to the camera compared to those that are more parallel) when using only a few 2D images in such analyses. Moreover, the presence of colour on 3D models could facilitate the distinction of structures that vary in colour but not so much in shape. For example, some species of Merianieae have stamen appendages that are distinguished from anthers primarily by their colour. Retaining colour on 3D models could thus help the positioning of these structures to test alternative hypotheses of floral modularity (Dellinger et al., 2019). The use of 3D models of flowers that retain colour information would greatly assist in distinguishing organs that differ in function and colour, but are difficult to distinguish based on shape.

Photography is a convenient way to collect morphological and reflectance data (colour) from specimens, and to facilitate research in ecology and evolution. However, the lack of tools to make objective colour measurements and the fact that cameras generally used in scientific studies produce uncalibrated photographs unreliable for quantitative colour measurements (Troscianko and Stevens, 2015). For this reason, a particular attention needs to be given to photo calibration and linearisation.

In addition, photography is not restricted to the visible spectrum (wavelength from 400 to 700 nm). Light can be detected by camera sensors in the UV range (320 to 400 nm) and IR (also called "heat radiation") or near-infrared (NIR) range (over 700 nm to about 1 mm), making it possible to incorporate these components of the light spectrum in 3D models. This could be important to understand patterns of reflectance evolution as most insect pollinators possess UV receptors (Chittka et al., 2001; Schiestl and Johnson, 2013), and self-heating flowers or inflorescences (Thien et al., 2000) as a reward or a means of enhancing the production and dissemination of floral scents (Seymour et al., 2003). Furthermore, images could be converted to correspond to different animal visual system sensitivities (cone-catch values) (Troscianko and Stevens, 2015).

5.2.3 Experimental studies

Advancements in 3D printing technology enable printing relatively small and intricate 3D models as well as remarkably detailed colour patterns, directly (using coloured filaments) or indirectly (by applying colour on models). In addition to colour, soft and flexible resins can be used thinly to resemble the soft tissues of flowers (see Supporting Information Fig. S8 for an example of a colourless soft 3D printed artificial flower of a 3D model derived from photogrammetry). These methods should help expand the scope of experimental studies designed to test hypotheses about pollinator behaviour and flower shape, size and colour. As an example, printed artificial flowers and chimeric flowers made of artificial and natural flower parts were used to decouple and test the relative contributions of olfactory and visual signals to attract pollinators in a mimetic orchid Dracula lafleurii by Policha et al. (2016).

5.3 Natural history collections 3.0

Digitisation and archiving of information of material from natural history collections have revolutionised their current use. 3D modelling of natural history collections would further advance their value, accessibility and use. Such efforts are ongoing in entomological and ornithological collections using photogrammetry (Ströbel et al., 2018; Medina et al., 2020). Unlike zoological specimens, which generally retain their 3D shapes in collections, plant specimens are usually kept pressed in herbaria, thus losing most of their natural shape. Although morphological data can be extracted from herbarium specimens (e.g., Bilbao et al., 2021), morphological correlations between flower parts are generally lost. One strength of photogrammetry is that 2D data sets can be collected in the field to reconstruct 3D morphological features lost in herbarium specimens and can be subsequently linked to them in similar ways as other sources of information, such as genetic data or plants parts preserved separately from the specimens. Sharing such 3D models would significantly improve the quality of phenomic data obtainable from herbaria (Ströbel et al., 2018; Medina et al., 2020) or complement and extend the information on plant traits (morphological, anatomical, functional, biochemical, phenological, and physiological) that is being centralised in global databases such as TRY (Kattge et al., 2011, 2020) and PROTEUS (Sauquet, 2019). Photogrammetry could also be extended to living collections, such as botanical gardens, thus improving access to such collections by providing access to virtual plants all year round and from
anywhere in the world, creating new opportunities for scientific studies and outreach [Maschner et al., 2013]. Open access databases dedicated to natural history, cultural heritage, and scientific collections are already available for such applications [Boyer et al., 2016].

6 Conclusions

Due to its simplicity and efficacy, photogrammetry has the potential to inspire new ways to quantify flower shape and colour and explore questions and collaborations in investigations of flowering plant evolution. Combined with genomic data, phenomic information obtained from 3D models using photogrammetry will open new areas of study of floral evolution. By combining practicality, reasonable costs, portability, and user friendly applications, photogrammetry has the potential to revolutionise studies of floral evolution and ecology.

7 Data availability

The 3D model data are openly available in Morphosource at https://www.morphosource.org/, reference ID: 000369440.

8 Acknowledgments

We thank Diana Constanza Díaz for reconstructing the 3D model of Paliavana prasinata used in this study, and for testing the user-friendlyness of our detailed photogrammetry protocol. We thank Viraj Alimchandani for helping 3D-printing the 3D model of a flower belonging to Gesneria cornuta. We thank Anthony Smith for the handling and preparation for CT-scans, and Hoai-Nam Bui for the CT-scanning and and 3D model reconstruction of flowers derived from CT-scans. We also thank Janique Perreault and her team at the Montreal Botanical Garden for maintaining the Gesneriaceae collection. Finally, we acknowledge the constructive comments of Agnes Dellinger and an anonymous reviewer on a previous version of this paper. The Natural Sciences and Engineering Research Council of Canada supported this research with Discovery Grants to SJ (05027-2018) and DS (74127-2017).

9 Conflict of interests

The authors have no conflicts of interest to declare.

10 Authors contributions

DS, ML and SJ designed the research. ML, JB and SJ acquired the data. ML and SJ analysed the data. ML wrote the manuscript. ML, SJ, DS and JB edited and revised the manuscript. SJ and DS acquired funding.

References


11 Supplementary information

Table S1 Summary of the materials used to scan and reconstruct three-dimensional flower models and their approximate price in 2022. Alternative materials can be considered, and we give as an example the specific materials we used when relevant.

Table S2 Summary of the camera and turn table settings used to scan flowers.

Fig. S1 Three-dimensional models of flowers of Gesneria acaulis (75-2021) obtained from photogrammetry and CT scanning.

Fig. S2 Three-dimensional models of flowers of Rhytidophyllum exsertum (112-1991) obtained from photogrammetry and CT scanning.

Notes S1 Taxonomic groups and flower material

Table S3 Species and collection numbers associated with the Botanical Garden of Montreal database of the specimens used to reconstruct 3D models of flowers. Each species is associated with a pollination syndrome. The bibliographic reference identifying the pollination syndromes of each species is also listed. Pollination syndromes that are not confirmed were inferred.

Methods S1 3D Geometric morphometrics.

Fig. S3 Example of landmarks and semi-landmarks placement on the corolla of Gesneria cuneifolia and Gesneria cornuta.

Table S4 Identification of the landmark and semi-landmarks used for curves and surfaces.

Fig. S4 Morphospace of individual 3D floral shape variation along the second and third principal axes of the PCA.

Fig. S5 Mean floral shapes for three pollination syndromes.

Fig. S6 Principal component analysis of floral shapes of Gesneriaceae according to pollination syndromes and the
method used to reconstruct the 3D floral shapes.

**Methods S2** Phylogenetic analysis

**Methods S3** Flower colour variation analysis

**Fig. S7** Colour distance matrix heatmap and dendrogram using Ward’s distance.

**Fig. S8** Example of a 3D-printed flower of Gesneria cornuta in clear and soft resin.