Replicating the complexity of natural surfaces: technique validation and applications for biomimetics, ecology and evolution

Charchit Kumar\textsuperscript{1, 2, 5, \dagger}, Alejandro Palacios\textsuperscript{1, 2, \dagger}, Venkata A. Surapaneni\textsuperscript{2, 6}, Georg Bold\textsuperscript{2, 5}, Marc Thielen\textsuperscript{2, 6}, Erik Licht\textsuperscript{7}, Timothy E. Higham\textsuperscript{2, 3}, Thomas Speck\textsuperscript{2, 5, 6} and Vincent Le Houérou\textsuperscript{1, 4}

\textsuperscript{1}Institut Charles Sadron, CNRS UPR022, Université de Strasbourg, Strasbourg, France
\textsuperscript{2}Plant Biomechanics Group and Botanic Garden, University of Freiburg, Freiburg, Germany
\textsuperscript{3}Department of Evolution, Ecology, and Organismal Biology, University of California, Riverside, CA, USA
\textsuperscript{4}ICube, UMR7357, Université de Strasbourg, Strasbourg, France
\textsuperscript{5}FIT, Freiburg Center for Interactive Materials and Bioinspired Technologies, Freiburg, Germany
\textsuperscript{6}FMF, Freiburg Materials Research Center, Freiburg, Germany
\textsuperscript{7}Basell Deutschland GmbH, LyondellBasell Industries, Frankfurt a.M, Germany

The surfaces of animals, plants and abiotic structures are not only important for organismal survival, but they have also inspired countless biomimetic and industrial applications. Additionally, the surfaces of animals and plants exhibit an unprecedented level of diversity, and animals often move on the surface of plants. Replicating these surfaces offers a number of advantages, such as preserving a surface that is likely to degrade over time, controlling for non-structural aspects of surfaces, such as compliance and chemistry, and being able to produce large areas.

\textsuperscript{\dagger}Shared first authorship.
of a small surface. In this paper, we compare three replication techniques among a number of species of plants, a technical surface and a rock. We then use two model parameters (cross-covariance function ratio and relative topography difference) to develop a unique method for quantitatively evaluating the quality of the replication. Finally, we outline future directions that can employ highly accurate surface replications, including ecological and evolutionary studies, biomechanical experiments, industrial applications and improving haptic properties of bioinspired surfaces. The recent advances associated with surface replication and imaging technology have formed a foundation on which to incorporate surface information into biological sciences and to improve industrial and biomimetic applications.

This article is part of the theme issue ‘Bioinspired materials and surfaces for green science and technology’.

1. Introduction

Every structure, living or not, has a surface. An understanding of surfaces has inspired a myriad of applications and discoveries, incorporating disparate fields such as ecology, advanced contact mechanics, industry, biomimetics and biophysics (figure 1). The surface of an organism is a critical interface interacting with its surroundings. External surfaces are diverse and can include skin and shells (animals), but also the epidermis (with its cuticle) in plants. Internal surfaces include the gastrointestinal tract in animals or stomatal cavities in plants. From a physical point of view all surfaces of plants, animals, fungi and bacteria are interfaces between the organism and the media of its environment, which may change its aggregate state between gaseous, liquid or solid (e.g. air–water–ice), sometimes on a very short time scale (e.g. during rain). This alone shows that surfaces have to deal with highly variable physical conditions and represent very important interfaces between an organism and its environment. In addition to protective roles, the surfaces are also important for manifold types of communication between an organism and its biotic and abiotic environment. In the case of plant surfaces, the functions of epidermis and its cuticle include, among others, control of transpiration and diffusion on the one hand and of uptake of substances on the other, control of light transmission and/or reflection and of optical properties in general, wetting or anti-wetting behaviour, protection against contaminations and biotic threats (e.g. fungus or bacteria infection), cooling by increasing turbulent flow and convection, mechanical protection (against physical damage but also against feeding animals), as well as signalling for animal and host–pathogen recognition and additional control of plant–animal interaction (e.g. animals moving on slippery and non-slippery surfaces) [1–6].

(a) Plant–animal interactions

The surfaces on which animals move or hold station are inextricably linked with many behaviours that are related to fitness. For example, terrestrial animals must move over complex surfaces in order to capture prey and escape from predators. The effective attachment is, therefore, of the utmost importance. Given this, the structure of both the substrate and the animal’s propulsive structures are equally important, yet most research has focused on the animal side of the interaction. When the substrate is considered, it is often categorized as smooth or rough simply based on feel or general impression, rather than a quantitative assessment [7]. This is problematic, as it is clear that microscale differences are paramount for dictating the efficacy of animal–habitat interactions. This extends to both biotic (e.g. plants) and abiotic (e.g. rocks) surfaces, both of which are traversed by a diverse array of organisms (e.g. figure 1). Many of these organisms house an intricate microtopography on their locomotor surface, such as the adhesive systems of insects [8] and some lizards [9]. However, animals can also exhibit complex structures for holding station on rough terrain in sub-optimal environmental conditions, such as clingfish in the marine intertidal zone [10,11]. Therefore, this important interface between an animal and its habitat cannot be ignored.
Figure 1. A schematic picture showing various research areas in which surface analyses are of importance. The decagon in the middle represents: (left) original *Ludisia discolor* leaves, (top right) Confocal Laser Scanning Microscopy (CLSM) image of a fresh leaf, and (bottom right) CLSM image of its polymeric replica; (a) shows a gecko (*Rhoptropus bradfieldi*) on a dolerite surface in Namibia; (b) exhibits the incorporation of biological microstructured surfaces (replicas) to advanced (in situ) contact mechanics studies; (c) shows an injection moulded polypropylene compound demonstrator (partly covered with aluminium foil); (d) shows a surface painted with self-cleaning paint Lotusan®; and (e) shows a locomotor experiment with a Colorado potato beetle (*Leptinotarsa decemlineata*) on a smooth glass surface.

(b) Why surface matters?

From an evolutionary and ecological perspective, identifying the relationships between organismal performance and surface topography is key to understanding how animals adapt to their surroundings. This also permits an assessment of symbiotic coevolution between biotic surfaces and the animals that use them. Biotic surfaces can defend against unwanted visitors or may promote mutualistic relationships. However, without knowledge of the surface parameters relevant to an animal, we are left to qualitative categorizations that provide little utility. This is unacceptable given that the fine-scale relationships between roughness and attachment principles (e.g. adhesion) are often nonlinear [12]. Thus, one cannot simply say that rougher is worse or better for an animal, especially when roughness is not defined quantitatively [13,14].

Surfaces relevant to terrestrial animals (or benthic marine animals) can be biotic or abiotic, as noted above. Biotic surfaces are most commonly represented by plants, but small animals
may also attach to, or move on, the surfaces of larger animals. For our paper, we will focus on plant surfaces when discussing biotic substrates. Abiotic factors can include virtually anything on Earth, from rocks to human-made structures. Rocks can exhibit the same degree of microtopography as plant surfaces [15], and it is not surprising that some animals use both rocks and plants as they move through their habitat. That said, plants and rocks differ in a number of ways, including stiffness and chemistry. Comparing plants and rocks through the use of replicated surfaces is an ideal way of eliminating the variability in variables other than topography.

(c) Why we need replicates?

The natural habitats of animals are rife with complexity, and this is also true for the surfaces on which the animals move. Numerous factors can vary with abiotic and biotic surfaces, including compliance, chemistry, colour and curvature, among other things. Thus, there are often confounding factors when using natural surfaces in a laboratory experiment. To control for all of the variables described above, one must generate replicas of natural surfaces. This standardizes all of the confounding parameters and allows one to explore the consequences of variation among animals using a particular surface, but also allows one to hone in on the consequences of finer variation in surface microtopography. Finally, biotic samples often dry quickly following removal from the plant, and this can cause very quick transformations in the structure. A replicate would prolong the integrity of the surface microtopography for experiments. For these reasons, surface replicates are ideal for exploring numerous questions related to ecology, evolution and biomechanics. Identifying morphological adaptations of animals to certain types of surfaces also opens the door to biomimetic applications and a deeper understanding of evolutionary patterns.

There are situations in which replicates are the only option. The fossil record is a great example of this. With the ability to create replicas, we can take information about the surfaces of extinct plants and recreate them in order to understand how extant or extinct animals may have performed on them [16]. This will shed light on the historical patterns of plant–animal interactions, therefore opening a window into the evolution of these interactions.

(d) Biomimetic applications

In the past several decades, plant surfaces (especially leaves) have attracted great attention, not only in biology but also in other disciplines. Given their various functionalities, they have been an inspiration for technical applications, including as a transport barrier, to improve anti-fouling and/or friction reduction, for controlling surface wettability and fluid flow on surfaces, for optimizing optical properties and for controlling (anti-)adhesive properties [2,3,13,17–22]. Since the first publication of the self-cleaning mechanism of plant surfaces being based on microstructured surface roughness [23], a plethora of papers has been published describing the use of this or similar effects based on surface micro- and nano-structuring for bioinspired technical applications. Most of these deal with influencing the wettability of these surfaces [2,21,24]. The same holds for air retention under water which, after its first quantitative description in *Salvinia* [25,26], inspired many attempts of biomimetic transfer [27,28]. Others describe how periodic surface structuring can induce colour effects and how these can be transferred into biomimetic applications [29–32]. Also, the interaction of insect tarsi with plant surfaces was investigated with regard to biomimetic applications. The main aim was to understand the structural and functional aspects of this interaction on a micro-level and to create technical surfaces that are difficult or impossible for insects to adhere to and, thus, to walk on [14,33]. Such bioinspired surfaces have great potential for non-chemical protection against insect infestations [34]. Except for the wetting properties, all these properties acquired through surface structuring are relatively independent of the material used [13]. Another subject that is becoming of increasing interest for biomimetics and industrial applications is that of haptic surface properties and the perception thereof. Quantitatively dealing with the questions involved, however, is at least as complex as the aforementioned topics. On the one hand, because haptic perception is very subjective, on the other
hand, because properties such as mass, geometry and material properties (modulus of elasticity, heat capacity, etc.) also play an important role in addition to surface topology [35]. An example of a plant–animal interaction involving haptic perception is that of haptic properties of fruits—notably mass and hardness—that are presumably indicators of their ripeness for certain primates [36]. Surface roughness/topography has only scarcely been analysed in this context so far and thus might represent an interesting field of future interdisciplinary research. Other vertebrates likely assess the suitability of food plants via oral assessments of surface roughness/topography and texture, which at least for humans contribute, besides appearance and flavour, markedly to the enjoyment of eating foods [37]. A very interesting study relating surface roughness and haptic perception was conducted by [38]. They found that sliding a fingertip over rough glass surfaces generated desirable positive feelings if the surface is less rough than the fingertip, whereas surfaces rougher than the fingertip generated undesirable feelings. Roughness is perceived via two types of cutaneous receptors which differentiate between surface structures with spatial periods above and below approximately 200 µm. Relatively coarse topologies with spatial periods above 200 µm are encoded spatially and transduced by slowly adapting mechanoreceptors [35,39], while finer surface structures are perceived by so-called Pacinian corpuscles that are sensitive to vibrations generated when textured surfaces and skin move relative to each other.

(e) Surface replicas as testing tool for structure–function–relationship

Most of the functional characteristics of surfaces are owing to physico-chemical properties or micro- and nano-structuring of the surfaces [3,13,21,40,41]. Sometimes, the surface function relies on the complex interplay of all properties or of both latters. Different types of techniques have been used to transfer the structures of various plant leaf surfaces and, therefore, to emulate their properties induced by topography onto technical materials such as polymers [13,29,42–48]. Certain studies have focused on reproducing the surface super-hydrophobicity (e.g. of the Lotus leaf) [47], while others have taken advantage of the microstructure to study novel light-harvesting systems [48], just to name a few examples. Compared to other replication techniques (atomic layer deposition, electroforming, sol–gel technique and physical vapour deposition), replica moulding is relatively advantageous not only owing to its simple and inexpensive procedure, but also because it allows direct employment of an original plant leaf as the master [49]. In general, the replica moulding is performed by pouring a liquid polymeric material onto a biological surface (master) to generate a negative replica which can then be separated from the master and be used to transfer the surface structure onto a second material (positive replica) [13,43,44,47,50–54]. The easiness and accuracy of the replication method depend on the choice of materials to be used as negative and positive replicas, respectively. It will also govern the ability of the replication technique to reproduce complex and fine structures. The mutual affinity of both materials may lead to the addition of an interfacial anti-stiction layer allowing for easy demoulding when producing the positive replica [55,56]. For instance, polydimethylsiloxane (PDMS) has been used to produce both negative and positive replicas; however, this approach involves an intermediate step of an anti-stiction treatment on the negative mould by organosilane monolayer deposition [47,51,56,57]. In the same line, some studies employed the PDMS negative replica to transfer the plants’ leaf microstructures onto a hard polymer, polymethyl methacrylate (PMMA) [45,48]. Various other researchers used poly-vinylsiloxane (PVS; common imprinting material employed in dentistry) to replicate biological surface structures onto an Epoxy polymer [13,43,44,54]. Another replication approach consists in the development of the negative replica on nickel using sputtering and electroforming, and then the structures from the negative replica were further transferred to acrylonitrile–butadiene–styrène (ABS) copolymer and to a UV-curable photopolymer [50,58]. Recently, a new replication strategy suggested using an epoxy-based polymer to produce negative replicas directly from original plant leaves, before transferring the surface structure onto final positive replicas made of PDMS [53].

Much of the bio-replication research has assessed the accuracy and ability of the replication techniques by qualitatively comparing the original surface to the developed replica, using
scanning electron microscopy (SEM) or atomic force microscopy (AFM) [13,43,46,47,50,53,59]. Undoubtedly, these techniques are proven to capture high-resolution surface images, although limited to qualitative investigations and comparisons in most cases. Furthermore, SEM investigation of biological samples requires an appropriate sample preparation protocol (including chemical fixation/dehydration, critical point drier and conductive film sputter coating) which limits the further utilization of the specimen for the replication process [60–62]. As a consequence, topography comparison of the same spot on both original and replica surfaces is not feasible. With the recent advancement of high-resolution microscopy techniques over the last decade, three-dimensional laser confocal microscopy offers some beneficial advantages: three-dimensional measurement of the topography, non-contact non-destructive investigation and no pre-sample preparation requirement [63–65].

Given the need to generate an accurate way of replicating surfaces, and to assess the quality of a replicate, we employed a number of replication techniques and validation procedures on the leaves of three species of plants and one technical surface. In addition, we used a rock sample (dolerite) and a young leaf for a proof of concept. The latter is important given the sensitivity of young leaves to repeated imaging. Being able to replicate a young leaf will preserve the delicate structures to assess the functional links between them and the animals that move on them. A spot marking and tracing approach on original surfaces, using negative and positive replicas, was employed to accomplish the quantitative comparison of equivalent areas. The investigated surfaces offer distinct topographies in terms of size range, various shapes and hierarchical patterns, allowing a complete evaluation of the replication technique accuracy. Two model parameters (cross-covariance function ratio and relative topography difference) were successfully employed to evaluate the replication quality of three replication approaches applied on the surfaces investigated in this work.

2. Materials and methods

(a) Investigated surfaces

Three different plants’ leaf surfaces were studied, corresponding to Ludisia discolor (adaxial: upper side), Hevea brasiliensis (adaxial: upper side) and Litchi chinensis (abaxial: lower side). These leaves were chosen according to their surface microstructure in order to screen the various shapes and sizes of textures: L. discolor represents the coarse topography (figure 2a) with circular cone-shaped microstructures between 50 and 80 µm height and diameter, H. brasiliensis leaves have fine wrinkle-shaped microstructures (figure 2d) in the range of 0.5–2 µm wrinkle height, width and distance, and L. chinensis leaves consist of complex hierarchical structures (figure 2g) with dimensions of 15–20 µm for the main patterns [13,53]. L. chinensis shows the most complex topography from the three selected plant leaf surfaces. All leaves were cultivated and freshly collected from the Botanic Garden of the University of Freiburg. Prior to each replication, leaves were gently cleaned with distilled water and carefully dried with pressurized air. In addition, we used a rock sample (dolerite) from Namibia and young leaves of H. brasiliensis for a proof of concept. The dolerite rock is a surface on which geckos move frequently, making it a relevant surface to replicate. Given the sensitivity of young leaves to repeated imaging, being able to replicate a young leaf and to preserve the delicate structures allows for assessment of the functional links between its surface and the animals that move on them. Finally, a standard technical surface was added to the samples set: a microstructured PMMA surface organized with regularly arranged circular dimples (width of 50 µm and depth of 5 µm, figure 2j) was selected.

(b) Replication techniques

The replication techniques used in this work are based on a two-step moulding approach. The full procedure has been summarized in the sketch presented in figure 3. For all four techniques, the first step was to develop a negative replica from different natural samples (plant leaves,
rock and technical standard sample). In the second step, the resulting negative replicas were used to transfer the surface structures onto the positive replicas. An additional step during the replication procedure was needed sometimes and consisted of achieving an intermediate anti-stiction treatment allowing for detachment of the positive replica from the negative mould. For the replication of young leaves of *H. brasiliensis*, the Epoxy–PDMS method has been used for reasons discussed later. While all the three replication techniques discussed above show good potential to understand leaf surfaces in general, for the rock sample, however, we find that a PDMS–Epoxy replication method prevents stiction issues on rock and consequently appears more

**Figure 2.** Confocal laser scanning microscopy (CLSM) images: surface topography of fresh plant leaves and standard structure surface (*a,d,g,j*), negative replicas (*b,e,h,k*) and positive replicas (*c,f,i,l*), for three different plant leaves replicated by Epoxy–PDMS replica moulding. (*a–c*) *L. discolor* (adaxial), (*d–f*) *H. brasiliensis* (adaxial), (*g–i*) *L. chinensis* (abaxial) and (*j–l*) standard template. The red line crossing each image represents the location of the line profile depicted under each image. The line profiles (vertical height profiles) are located such that they establish the same section on the fresh leaf, negative replica and positive replica. All images and line profiles for the negative replicas are mirrored to facilitate the qualitative visual comparison.
suitable and advantageous for further study. The different materials for negative and positive replicas used for the replication processes, are described in detail in the following sections.

(i) Materials for replication

The first material used for negative moulding was polyvinyl siloxane (PVS, President Light Body®, Coltene Whaledent, Altstätten, Switzerland), which is normally employed in dentistry for imprinting and has also been used for replication of biological surface structures [13,43,54,66,67]. PVS has a fast polymerization time (around 10 min at room temperature), the mixing ratio of monomer and curing agent is fixed by the manufacturer as the dispenser mixes both components homogeneously when being used [43]. As the second alternative, an epoxy resin (Epoxydharz HT2, R&G Faserverbundwerkstoffe GmbH, Wandelbuch, Germany) was used to achieve the negative mould. The mixing ratio (by weight) of resin and hardener was 100:48 and the curing was performed at room temperature (25 ± 2°C) for 18 h. Once both materials are blended, the working time should not exceed 45 min as the viscosity increases rapidly. The third material used for negative moulding was PDMS (RTV 141 A & B-monomer and catalyst, Bluestar Silicones, Saint-Fons, France). The mixing ratio (by weight) of monomer to catalyst was 10:1 and the mixture was cured at room temperature for 24 h. To generate positive replicas, only PDMS was utilized in all the three processes. The PDMS elaboration procedure for the positive replicas was the same as the one used for negative replicas except for the curing which was done at 60 ± 5°C for 4 h.
(ii) Negative moulding

In order to obtain a negative replica, a small piece was cut out from a fresh plant leaf and glued onto a plastic Petri dish. The negative moulding material was slowly poured onto the leaf sample. When carrying out the process with epoxy and PDMS, a pre-degasification was done in a vacuum chamber for 20 min, to remove air bubbles trapped from the mixture. Since the plants leaf surface (master) are quite sensitive to high temperature, all the negative mouldings were conducted at room temperature. The demoulding process to obtain the negative replica was done directly after the curing was completed. The only exception was for the moulding with epoxy resin using *L. chinensis* leaves as master. Here, the complex microstructures of the leaf lead to entanglement with the negative replica (as the cured epoxy resin is highly rigid as compared with cured PDMS or PVS). In this case, the demoulding was fulfilled by treating the sample (negative replica entangled with leaf) in an aqueous solution (60 g/100 ml) of potassium hydroxide (KOH, greater than or equal to 85%, p.a., Carl Roth GmbH & Co. KG, Karlsruhe, Germany) at 60°C for 20 h. Thereafter, it was submerged in an ultrasonic bath of deionized water for 15 min to completely remove the master from the negative replica [53]. In the case of the rock sample, after the pre-degasification process, the PDMS-filled sample was kept in a heating oven for curing at 60°C for 4 h and then peeled off gently. Later on, all the negative replicas (for all three moulding materials: PVS, PDMS and epoxy resin) were used to replicate the leaf surface pattern to the PDMS surface (or Epoxy surface in the case of rock), as described in the following steps. While developing a negative replica on Epoxy from the standard structured surface, a gentle effort was needed to demould, given that both Epoxy negative replica and standard sample are made of stiff materials.

(iii) Anti-stiction coating

An intermediate step between negative and positive moulding consists in the application of an anti-stiction coating on the negative replica, which allows easy demoulding of the positive replica from the negative [55, 56, 68, 69]. The kind of anti-stiction coating depends on the nature of the materials used for negative and positive replicas. In the case of PVS/PDMS, this coating was developed by forming a thin film coating of gold. A gold sputter coater device (108 auto, Cressington Sputter Coater, UK) was used for this purpose. Coating was performed at a current of 20 mA, a pressure of 20 Pa, and a distance of 45 mm between the sputter target and the sample. An exposure time of 60 s was maintained for the *L. discolor* and *H. brasiliensis* negative replicas, and 120 s for the *L. chinensis* negative replicas. In regard to PDMS/PDMS demoulding, an anti-stiction monolayer silanization process was applied using a home-built vapour deposition set-up as described in [56]. The silane monolayer deposition was carried out by placing the PDMS negative replicas along with a few (5–6) drops of Trichloro(1H,1H,2H,2H-perfluoroocetyl)silane (FOTS, 97% Sigma-Aldrich Chemicals, USA) in a partial vacuum (40 kPa) for 5 h. In the case of Epoxy/PDMS demoulding, there was no need to apply any anti-stiction coating as the positive replica could be separated directly once it was cured.

(iv) Positive moulding

The final step to produce the positive replicas in all the three replication approaches for leaves and standard samples was done with the PDMS material. The PDMS mixture that was free from air bubbles was slowly poured onto the negative replicas (after sputtering anti-stiction coatings when needed). The samples filled with PDMS mixture were then placed in a vacuum desiccator for 1 h, to remove air bubbles formed at the interface of negative replica and PDMS mixture. The samples were kept in a heating oven for curing at 60°C for 4 h. Then the positive replicas were gently peeled off from the negative moulds. For the rock sample, the epoxy mixture after degasification for 20 min was gently poured onto the PDMS mould and was allowed to set overnight before peeling off.
(c) Surface visualization and characterization

(i) Confocal laser scanning microscopy

Surface morphology of all the fresh plant leaf surfaces, their negative replicas and final positive replicas was visualized and characterized with a confocal laser scanning microscope (LEXT OLS4000, Olympus Corporation, Japan). All measurements were performed in such a way that the same segment (spot) of the leaf surface was measured on the negative replica and the positive replica, to allow the systematic quantitative comparison of all three morphologies. For tracking the same spot from the original fresh leaf sample to negative replica and positive replica, a unique spot marking (with a tiny drop of PVS) was achieved. L. discolor samples were examined at a magnification of 20×, while the samples from H. brasiliensis (both young and adult), L. chinensis and rock were examined at 100×, 50× and 40× magnification, respectively. Confocal Laser Scanning Microscopy (CLSM) is one of the few methods which is not invasive to characterize biological samples at a consistent scale because it requires no sample preparation, the measurement causes no damage to the surface, and the leaf specimens can still be used afterwards [70].

3. Replication quality quantification models

(a) Parameters

Two model parameters, proposed by the National Institute of Standards and Technology (NIST), were used to quantitatively compare the line and surface profiles obtained from CLSM. The first parameter is called cross-covariance ratio (ACCFMAX) and it is a maximized ratio between a cross-covariance function relating two profiles and their root mean squared roughnesses (Rq) [71], as described in equation (3.1):

\[ \text{ACCFMAX} = \frac{\sum_{i,j} [(Z_A(i,j) - \bar{Z}_A)(Z_B(i,j) - \bar{Z}_B)]}{\sqrt{\sum_{i,j} (Z_A(i,j) - \bar{Z}_A)^2} \sqrt{\sum_{i,j} (Z_B(i,j) - \bar{Z}_B)^2}} \]  \hspace{1cm} (3.1)

where \( Z \) is referred to as a particular height of a profile at the point ‘\( i,j \)’ when analysing surface profiles or ‘\( i \)’ in the case of line profiles. By convention, A is the original profile and B is the replicated one (negative replica or positive replica), and \( \bar{Z} \) is the average value for each profile. ACCFMAX varies from 0 to 1: it reaches 1 when two profiles are identical and 0 when they are completely unrelated [71]. The ACCFMAX in this investigation was used in the discrete form using summations because it is adapted to resulting CLSM data, as presented in equation (3.1) [72]. ACCFMAX has a disadvantage related to scale factors; therefore, the parameter must always be calculated when the two profiles are in phase to assure that the value is maximized. Nonetheless, it is not sensitive to the differences, if one of the profiles is, for example, twice as high as the other one. Scale factors can be propagated to profiles owing to calibration problems with the equipment used for characterization [72]. As a consequence, the NIST proposed a second parameter sensitive to vertical scale differences, called relative topography difference (\( D_S \)). \( D_S \) is defined as the root mean square roughness of a virtual profile given by A–B over the mean squared roughness of a profile A (original profile), as shown in equation (3.2).

\[ D_S = \frac{\sum_{i,j} [(Z_A(i,j) - \bar{Z}_A) - (Z_B(i,j) - \bar{Z}_B)]^2}{\sum_{i,j} (Z_A(i,j) - \bar{Z}_A)^2} \]  \hspace{1cm} (3.2)

\( D_S \) also varies from 0 to 1, but it tends to 0 when two profiles are identical and to 1 when they are unrelated. Both ACCFMAX and \( D_S \) are complements to each other as the first one quantifies the similarities in the shape of two profiles, while the second one takes into account the height differences.
(b) Line profiles and surface

The LEXT microscope software (OLS400, version 2.2.3) directly allows extraction of raw data of selected line profiles. Before extracting the information, all profiles were adjusted to remove the waviness by applying a cut-off of 400 µm for \textit{L. discolor} profiles, 50 µm for \textit{L. chinensis}, 25 µm for adult \textit{H. brasiliensis}, and 80 µm for young \textit{H. brasiliensis} leaves and rock. These cut-off values were chosen manually with the help of the microscope software, which can plot primary roughness and waviness profiles when changing the input cut-off value. With the purpose of calculating average values of line profiles for ACCF MAX and \( D_S \), a total of five to six profiles distributed in two different samples of each plant leaf were analysed and then averaged. The second set of profiles associated with surface analysis was obtained from the fresh leaves and the replicas in which surface profiles (height data in two axes) were extracted. In this case, a fixed area was selected over the images and matrices of raw data were taken for analysis; the extraction also took into consideration the same cut-off values previously mentioned for analysis of line profiles. For the computation of surface profiles only one sample per leaf and replicas was selected owing to the high density of data (one single matrix could contain up to one million data points).

4. Results

Figure 2 shows a qualitative comparison of the different CLSM images (fresh leaves, negative replicas and positive replicas) obtained for the three biological morphologies investigated when replicated by the Epoxy–PDMS replication approach. Figure 4 displays some examples when the replication is done by PDMS–PDMS and PVS–PDMS moulding.

\textit{L. discolor}, having the simplest, largest and most regular microstructure out of the three plant leaves investigated, seems to show the best results in terms of accuracy of profiles and topography similarities between original leaf, negative and positive replicas (figure 2a–c). The difference in the profiles displayed for \textit{L. discolor} is rather subtle but perceptible. In \textit{H. brasiliensis}, the development of profiles shows a decrease in the height of the profile and some imperfections that can also be detected by contrasting the three images (figure 2d–f), especially when fresh leaf and positive replica are compared. The surface of \textit{L. chinensis} replicas, with the most complex morphology is notably more affected, as larger areas show visible defects and the line profile undergoes large changes in shape and height, as can be compared in (figure 2g–i). The regularly distributed coarse-size surface structures from the standard technical surface were replicated very precisely (figure 2j–l).

On the other hand, by comparing the respective images shown in figures 2 and 4, it can be noted in the case of \textit{H. brasiliensis} that the PDMS–PDMS replication approach (figure 4a,b) brings more topographic inaccuracies in the positive replica compared to the Epoxy–PDMS procedure. In the case of PVS–PDMS replica moulding, the topography of \textit{L. chinensis} is strongly damaged causing the positive replica to lose most of the fine overhanging features (hierarchical patterns), as can be seen in figure 4d. Nevertheless, a qualitative description is not sufficient to assess the accuracy of the three different methods under evaluation.

The results of the quantitative evaluation using the two parameters ACCF MAX and \( D_S \) for line profiles are plotted in figure 5 comparing negative and positive replicas against fresh leaves. For reading convenience, the relative topography difference is presented as \((1−D_S)\), so that the values corresponding to better replication would tend towards 100% instead of 0%. In the same way, the results regarding the quantitative assessment of surface profiles with ACCF MAX and \( D_S \) are plotted in figure 6. By contrasting both types of measurement, the surface profile results have greater statistical significance than the line profiles, because one surface measurement contains around 900 line profiles. Surface profiles are also significantly more sensitive when considering larger topographic defects, whereas line profiles only evaluate similarities between two fixed points. Still, one can note that results from both figures 5 and 6 are consistent and show the same ranking quality between the different samples.
Figure 4. CLSM images showing the topography of the original leaf surfaces and their positive replicas. (a,b) H. brasiliensis replicated by PDMS–PDMS moulding. (c,d) L. chinensis replicated by PDMS–PDMS. (e,f) H. brasiliensis replicated by PVS–PDMS. (g,h) L. chinensis replicated by PVS–PDMS. Line profiles were inserted as described in figure 2. (Online version in colour.)
By analysing the results shown in figures 5 and 6, it can be concluded that Epoxy–PDMS replica moulding is the most precise method to replicate the morphology of the three biological samples used in this investigation. *L. discolor* shows the microstructure with the highest topographic replicability ($\text{ACCF}_{\text{MAX}} = 88.8\%$ and $1-D_3 = 66.5\%$ in figure 6b). *H. brasiliensis* and *L. chinensis* have lower values for ACCF$_{\text{MAX}}$ and $1-D_3$, because the replicability is reduced to ACCF$_{\text{MAX}} = 79.3\%$, $1-D_3 = 60.7\%$ (for *H. brasiliensis*) and ACCF$_{\text{MAX}} = 76.3\%$, $1-D_3 = 52.6\%$ (for *L. chinensis*), respectively (figure 6b). PVS–PDMS replication is not as good as Epoxy–PDMS but it is considerably better than PDMS–PDMS. However, all three techniques were able to precisely replicate the surface structures from the standard template, resulting in high quantitative values of both parameters. For example, ACCF$_{\text{MAX}} = 98.4\%$ and $1-D_3 = 96.5\%$ were calculated for the

---

**Figure 5.** Quantitative evaluation of the surface structures using line profiles. (a) Comparison of negative replicas versus fresh leaves and standard template versus its negative replica by plotting ACCF$_{\text{MAX}}$ versus $(1-D_3)$. (b) ACCF$_{\text{MAX}}$ versus $(1-D_3)$ comparing positive replicas against fresh leaves and standard template and its positive replica. Epoxy, PVS and PDMS on the label refer to the negative moulding material used before positive moulding with PDMS. The error bar calculations were based on the accuracy to obtain the same line profile during repetitive measurements. For better graphical visualization, only the genus name is mentioned instead of the full species name: Ludisia for *Ludisia discolor*, Hevea for *Hevea brasiliensis* and Litchi for *Litchi chinensis*.

**Figure 6.** Quantitative evaluation of the surface structures using surface profiles. (a) Comparison of negative replicas versus fresh leaves by plotting ACCF$_{\text{MAX}}$ versus $(1-D_3)$. (b) ACCF$_{\text{MAX}}$ versus $(1-D_3)$ comparing positive replicas and fresh leaves. The error bars were calculated as in figure 5. For better graphical visualization, only the genus name is mentioned instead of the full species name: Ludisia for *Ludisia discolor*, Hevea for *Hevea brasiliensis* and Litchi for *Litchi chinensis*.
Epoxy–PDMS replication approach as shown in figure 5b and a similar value range was found for the other two approaches (PVS–PDMS and PDMS–PDMS).

By taking into consideration the replica moulding process, in the negative moulding step we believe that the curing time and how fast viscosity increases are the most important factors in the accuracy of the replication. Whereas PVS cures extremely fast, it might not be as precise as epoxy resin owing to diffusion limitation to completely fill the cavities on the topography of the leaf surface (epoxy resin is about 10% more precise reproducing L. chinensis microstructures in terms of $D_S$ results as compared to PVS). To increase the performance of PVS, Koch et al. [43] discussed the inclusion of cooling to increase its curing time, as well as the alternative of using a vacuum to remove air bubbles trapped in the microstructures. However, the use of lower pressures might accelerate the loss of humidity from the plant leaf and by this deteriorate the replication results.

An additional important fact is that while epoxy resin and PDMS have the same curing time, the increase in viscosity is extensively faster in epoxy resin, which reduces the working time to about 45 min, whereas in PDMS the increase in viscosity is much slower. This is relevant when working with biological samples, considering that the loss in humidity can highly affect the shape of the topographies of the leaf surface used as a master, thereby longer times of curing will lead to greater deformation of the leaves. If the negative moulding material does not harden quickly enough, the changes in the topography of the leaf master could eventually be transferred to the negative replica. This could explain why PDMS used for negative moulding shows the lowest values for $ACCF_{\text{MAX}}$ and $1-D_S$ in all three plant leaf surfaces. This issue may be confronted by including the monomer to curing agent ratio as a variable for PDMS during negative moulding to decrease the curing time of PDMS at room temperature. With regard to the PDMS positive moulding process, as all positive replicas underwent the same conditions, the reduction in accuracy can only be attributed to the complexity of the topography in each plant leaf species.

Figure 7. CLSM images. Comparison of the topography of the original and positive replicas of (a,b) young leaves of H. brasiliensis replicated by Epoxy–PDMS moulding and (c,d) dolerite rock replicated by PDMS–Epoxy moulding. Line profiles were inserted as described in figure 2.
Figure 7 shows topographical differences between originals and replicas of a young *H. brasiliensis* leaf and dolerite rock sample. As discussed earlier, the Epoxy–PDMS replication process showed better replication quality for leaf surfaces and was used to replicate young leaves of *H. brasiliensis*. The young leaves show replication quality with values $\text{ACCF}_{\text{MAX}} = 84.2\%$ and $1-D_S = 57.4\%$. These leaves as opposed to the adult ones have smooth cellular surfaces without any wrinkled microstructures. However, the relatively lower values of $1-D_S$ could be attributed to the difficulty in replicating the delicate surfaces of young leaves. Nevertheless, replication of such leaves would help in surface microstructure analysis during leaf ontogeny, as the thin and translucent cuticles of young leaves makes this difficult otherwise. On the other hand, the PDMS–Epoxy replication of rock resulted in high values of $\text{ACCF}_{\text{MAX}} = 93.7\%$ and $1-D_S = 84.6\%$, similar to the results for the standard technical samples, suggesting the effectiveness of replicating stiff and hard samples.

5. Discussion

Our study identified reliable methods for replicating the surface topologies of living (plant) and non-living (rock) surfaces and presents a method for assessing the quality of a replica. According to the results obtained in the present study, the surface topology of *L. discolor* leaves exhibits the highest replicability owing to its quasi-regularity and relatively large microstructures. The complex surface morphology of *L. chinensis* leaves makes the replication more complicated and, therefore, the negative and positive replicas exhibited a large number of defects that were correctly pointed out by the quantitative analysis. By contrast, *H. brasiliensis* surface morphology could be reproduced with higher precision than *L. chinensis*. However, a few small detail failures and imperfections were observed over the studied profiles. It is possible that the microscope measurements on *L. chinensis* surfaces suffer from some optical artifacts arising from the technique limitations, in particular when attempting to access the undercuts and overhanging structures.

Using the LEXT microscope software, we calculated the surface roughness with standard parameters such as arithmetic mean height ($R_c$), arithmetic mean deviation ($R_a$) and root mean squared deviation ($R_q$). However, the results obtained with these parameters (not reported in this paper) did not exhibit any clear tendency when negative and positive replicas were compared with fresh leaf profiles. Moreover, $\text{ACCF}_{\text{MAX}}$ and $D_S$ already take into consideration the effect of surface roughness by including $R_q$ in the calculation [71]. The use of $\text{ACCF}_{\text{MAX}}$ and $D_S$ quantification parameters revealed that the replication process is more sensitive to loss of height and depth whereas there is a good reproducibility of profile shape (particularly for *L. discolor* and *H. brasiliensis*).

Concerning the effectiveness of $\text{ACCF}_{\text{MAX}}$ and $D_S$ as quantification parameters, our data demonstrated that $\text{ACCF}_{\text{MAX}}$ shows the same tendencies as $D_S$, but the latter is more sensitive to height/depth differences (as seen in figures 5 and 6). This might indicate that the compared replication techniques are better at reproducing the shape of the structures and less effective at keeping the same height of structures over the surface. This inference is particularly notable in *H. brasiliensis* (see the line profiles in figure 2d–f). What is also interesting from this approach is that the $\text{ACCF}_{\text{MAX}}$ and $D_S$ profile comparison results obtained with fresh leaves against negative moulds and fresh leaves against positive moulds consistently show deteriorated results for positive moulds (i.e. for the second moulding step), thus validating the consistency of the quantitative analysis as well as the utility of both model parameters.

With regard to quantification of the accuracy of surface replicas, $\text{ACCF}_{\text{MAX}}$ and $D_S$ are advantageous parameters for characterizing and comparing the surface morphologies. For our study, both values were appropriate to use (with a relatively small margin of error), given the fact that standard parameters (e.g. surface roughness) are not descriptive enough when comparing two different surface morphologies, especially when the replication is done with biological samples.

Now that we can quantify the accuracy of surface replicas, we must determine what level of accuracy is necessary to answer different scientific questions related to biological surfaces and
for using the replicates for bioinspired applications. The answer is not straightforward and is completely dependent on the desired outcome of a study or application. For example, it is difficult to know what a value of 90% for ACCFMAX and DS means for an animal. If adhesive performance is measured on the original and the replicate in this scenario, the outcome will dictate the level of accuracy needed. If the same adhesive performance is achieved, then one would conclude that 90% is perfectly reasonable. However, future work must quantitatively test the importance of accuracy in real-world situations. This study attempted to cover surfaces with a broad range in terms of size, shape and complexity of their surface topography; however, surfaces with extreme structures, such as long hairy trichome structures or fragile wax crystal structures, still need further investigation.

(a) Using surface replicas to study evolution, ecology and biomechanics of plant–animal interactions and for improving haptic properties of bioinspired technical surfaces

We have presented a reliable way of replicating natural surfaces and for assessing the quality of the replicas quantitatively. Keeping in mind that these surfaces are instrumental in shaping the evolution of the animals that move on them, the question that arises is how to implement the replication procedures to explore evolutionary, ecological and biomechanical phenomena. We outline potential avenues of research below.

When we think of animals adhering to surfaces, geckos are likely to spring to mind. The adhesive apparatus of geckos has been intensely investigated over the past two decades, and has been characterized as being ‘overbuilt’. However, this idea was challenged when the microtopography of the natural surfaces on which geckos move was examined in concert with the adhesive morphology [15,73]. These studies examined the morphology of geckos from the genus Rhoptropus in Namibia and compared this to the topography of different types of rocks from their natural habitat. When considering the available contact area between the setal fields of the gecko toe and the surface roughness, it is clear that geckos can encounter situations in which contact is dramatically reduced, which in turns bring the safety factor down considerably. A separate study found that different populations of this species exhibit differences in their adhesive microstructure in relation to habitat structure [74], highlighting how these interactions can influence the evolution of animal morphology. This also highlights the importance of considering ecology when measuring adhesive performance, and signals that replicating surfaces across different habitat types will help investigators determine which factors cause a shift in adhesive performance. Although geckos are highlighted here as an example, this idea applies to any animal that uses an adhesive system to attach to surfaces, as is the case for many invertebrates.

(i) Some like it smooth, some like it rough

The type of surface that is ideal for an animal depends on the type of attachment employed. In both benthic aquatic habitats and terrestrial environments, some species will attach more effectively on smooth surfaces whereas others will benefit from rougher surfaces. For example, intertidal marine organisms that rely on attachment mechanisms for holding station under high-force wave action will be impacted by the microtopography of the substrate. Among fishes, this is clearly important for clingfish [10] and sculpin [75]. These two groups also reflect the differences observed among terrestrial animals. Clingfish will do better on smooth surfaces, much like geckos [12]. Sculpin rely on their hooked pectoral fins to interdigitate with the rough rocky substrate [75], much like lizards that rely on claws for attachment [76]. Thus, surface roughness matters, and qualitative lumping of surfaces into smooth and rough categories is not sufficient as has been also proven recently for the attachment of leeches [77].

Several studies have examined the impact of surface topography and microstructuring on the ability of animals to cling. This includes, for example, studies of beetles [13,78], aquatic insect larvae [79], other insects [80], geckos and other lizards [7,12], and fishes [11]. All of these studies find that smoother surfaces are generally better for the animal if adhesion is being used. For those
animals that use claws, rougher substrates are beneficial. For example, claw removal in dock beetles (*Gastrophysa viridula*) resulted in a reduction in attachment force on rough surfaces [78]. Given that many terrestrial vertebrates and invertebrates have both claws and adhesive systems, they might exhibit a shift in reliance on a given system as the substrate type changes.

Using replications with defined surface roughness, a recent study examined the ability of mayfly larvae to attach to surfaces using their tarsal claws [79]. They identified a minimum roughness value that is needed before the claws can grip the substrate. Thus, some animals that use adhesion require a certain degree of smoothness, whereas other animals may require a certain degree of roughness in order to grip rough surfaces with their claws. This highlights the divergent attachment mechanisms that have evolved, and the potential for coevolution between animals and the biotic surfaces on which they attach.

(ii) Surface topography and locomotor biomechanics

Although much of the focus of attachment studies is on the ability to grip or adhere, it is clear that many animals are moving around on surfaces in their natural habitat. Therefore, locomotor experiments are necessary for determining the impact of surface topography on organismal performance. Replicated surfaces can provide flat and possibly enlarged areas that facilitate accurate assessments of locomotor ability. Without replication, many studies simply use manufactured surfaces to mimic natural surfaces in order to provide flat running trackways. For example, sandpaper, cork and mesh wire are often used for running experiments in lizards [81–83]. Sandpaper is thought to mimic rocky surfaces, but the effective similarity depends on the microtopography and the specific type of rock and grit of sandpaper. We propose that future studies should standardize the running trackways by either replicating the surfaces of interest or at least quantifying the topography and roughness of the surfaces used.

(iii) Improving haptic properties of bioinspired technical surfaces

Over the last two decades, plant surfaces have been used widely as inspiration for the development of novel biomorphic, bioinspired and biomimetic demonstrators and products by applying different process sequences of biomimetic research [84,85]. Examples include so-called ‘Lotus-Effect-Surfaces’ and ‘Salvinia-Effect-Surfaces’ [2,21,28] and biomimetic surfaces with optical properties inspired by plant surfaces [30], which have been briefly exemplified in §1. Haptics is a field of application in which inspiration from plant surfaces is of increasing interest. Potential fields of applications are wide-ranging, examples of which include the interiors of automotives and aeroplanes, medical devices, touch-pads of computers and entertainment electronics, and interfaces of soft-robotics and controls of kitchen appliances. The need for novel and adaptive haptic surfaces becomes even more evident in an ageing community if one keeps in mind that tactile spatial acuity drastically decreases with age, as does visual capacity. Spatial resolving capacity of cutaneous receptors, measured by the two-point touch threshold (i.e. spatial separation between two stimuli to the skin that can be detected), not only varies significantly across the body surface (approx. 1 mm–several cm) but also changes with age. From the age of 12 to 85 it declines by about 1% per year [35]. This seems to be a potential target for age-adapted biomimetic products with self-explanatory haptic properties.

(iv) Industrial applications

In industry, thermoplastics have widely been used to produce finished parts for a number of things (e.g. automotive interiors) for decades. This stems from the ability to produce complex parts using cost-effective injection moulding processes, the low density (compared to metal, for example), low costs of thermoplastics and their recyclability. The most important thermoplastic is polypropylene (PP, for example, homopolymers and copolymers), which has customizable properties like stiffness and impact performance. These properties are modified by blending different kinds of PP as well as adding reinforcing mineral fillers like talc, colouring pigments...
and stabilizing additives in a compounding process. The final PP compound, in this way, can be tailored for a specific task. In order to get to a finished part that is both functional and has an optically attractive surface with good haptics, a steel surface from an injection moulding tool is structured with the negative image of a certain grain to produce, for example, an instrument panel. By melting and injecting the PP compound into the mould, the finished part with the positive grain is produced. Interestingly, ‘natural’ based leather grains have been common for ‘technical’ interior trim parts for many years, mimicking the surface optics and haptics of the natural leather product. In recent years, a trend to use technical and geometrical grains for additional applications has been observed. Regardless of the type of grain, currently only a few examples of functional grains are known. The most popular example for functionality is the low wettability of ‘Lotus-Effect-Surfaces’. As many kinds of features suitable for a biomimetic transfer are known in nature, it seems to be desirable to use these more efficiently for industrially produced finished parts in the future.

In order to structure the steel surface of a tool for the production of finished parts, etching and, more recently, laser graining techniques are applied as industrial processes. There is an imaging quality limitation of these techniques caused by the layer-wise shaping process of a grain leading to a stair-like tool surface structure. This limits the resolution to dimensions between 1 mm and 100 µm, this typically being much coarser than many (functional) structures found on leaf surfaces (figure 8). A novel technique called Cerashibo, developed by the company Eschmann Textures together with its Japanese partner some years ago, overcomes the limitations of the techniques described above and enables an exact image of nearly every kind of grain without such stair-like effects. As Cerashibo is allowing grains into injection-moulding tools in an industrially effective manner, this technique is opening up new possibilities, including the imaging and transfer of bioinspired surfaces in the low µm-range into injection-moulding tools.

LyondellBasell, Eschmann Textures and the University of Freiburg have started to work together to explore the potential of transferring surface structures from plant to bioinspired injection-moulded thermoplastic plaques. With this method, we will not only be able to develop new biomimetic products, but also to create as reference surfaces for basic research, for example, large surfaces with identical topography but different mechanical properties that can be used in locomotor experiments.
In order to evaluate the surface quality of the injected test specimen, it is essential to use and potentially further develop complementary techniques for a comprehensive surface characterization as presented in this paper. This will be the basis of ensuring the effective transfer and subsequent well-performing bioinspired functionality on finished parts at a high and well-defined level of quality.

### 6. Conclusion

In terms of the accuracy of the three different replication techniques presented in this work, Epoxy–PDMS appeared to be the most precise technique to replicate the three biological surfaces with the highest cross-covariance ratio and lowest relative topography difference. As for PVS–PDMS, the process was slightly less accurate, attributed to the fast curing time of PVS, which might have caused loss of replicability of the finest and more complex structures. Lastly, PDMS–PDMS replica moulding was the least accurate method studied. The result was ascribed to the long curing time and slow increase in viscosity (compared with epoxy resin) during the negative moulding process supposedly resulting in a change of topography by drying of the fresh leaf. By quantifying the surface structure of biological and other natural templates (e.g. plant leaves and rock surfaces) and of their replicas, it becomes possible not only to test plant–animal interactions on highly defined surface structures, but also to change parameters other than surface roughness by keeping the latter constant. This will allow one to quantify the impact of various surface parameters like surface chemistry, humidity and surface flooding much more accurately as to their impact on moving animals. For biomimetic applications, it will help to transfer exactly these parameters to the bioinspired technical products that are necessary for a given desirable property and/or function. Additionally, it will become possible to quantify which kind of accuracy of a surface (micro-)topography is needed to achieve a given property which will be of special importance for haptics where subjective ‘feelings’ may play a major role on the side of the human user. The precise level of accuracy needed for a specific application will largely depend on the type of application. Future work is necessary for defining these thresholds. One may also consider exploring some advanced materials that address the issue concerning the robustness of replicas for the direct industrial applications. Regardless, surface replication is a promising method for exploring biological diversity and for creating novel applications.

**Data accessibility.** The supplementary material and data can be viewed at: doi:10.6094/UNIFR/16790.

**Authors' contributions.** V.L.H., T.S. and C.K. designed the study and supervised it together with G.B. and M.T. Data collection, data assessment and statistical analyses were carried out by A.P., C.K. and V.A.S. Data evaluation and discussion of the results was a joint effort by all authors (C.K., A.P., V.A.S., G.B., M.T., E.L., T.E.H., T.S. and V.L.H.), who also contributed equally to the first draft of the manuscript and improved further versions. All authors gave final approval for publication.

**Competing interests.** We declare no competing interests exist.

**Funding.** We acknowledge funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skodowska-Curie grant agreement No. 722842 (ITN Plant-inspired Materials and Surfaces—PlaMatSu) to V.A.S., M.T., G.B. and T.S. We are also grateful to the German Research Foundation (Deutsche Forschungsgemeinschaft: DFG), for funding support under the framework of International Research Training Group (IRTG) ‘Soft Matter Science—1642’ to C.K., A.P., T.S. and V.L.H. T.H. gratefully acknowledges funding by the Humboldt Foundation for a one-year research stay in the laboratory of T.S.

**Acknowledgements.** We would like to thank the gardeners of the Botanic Garden Freiburg for cultivating the plants used in this investigation.

**References**


