Exposure to rainfall inhibits the reflectiveness of glossy buttercup petals

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Abstract

Ranunculus flower petals possess a multilayered structure that enhances the reflectance of ultraviolet (UV) and visible light. This study investigated how water affects the structural colouration in *Ranunculus graniticola* flowers, using spectrophotometry to compare the reflectance of petals exposed to dry versus wet weather conditions. Petals recently exposed to rainfall showed lower reflectance in the visible spectrum and almost no UV reflectance; when wet petals returned to dry conditions, their UV reflectance increased at a linear rate. The glossy surface of the petals appeared to be unchanged by exposure to water, indicating that the gloss is not the cause of the remarkably high UV reflectance seen in *Ranunculus* flowers. The significance of these results is discussed in relation to two prominent theories about the adaptive purpose of *Ranunculus* structural colouration.

Running Title: Rainfall exposure inhibits buttercup petal reflectance

Key Words: *Ranunculus graniticola*, structural colouration, pigmentary colouration, ultraviolet reflectance

Introduction

Having evolved alongside colour vision in pollinating insects, flower colour in angiosperms is an important visual signal for attracting pollinators. Pollinating insects use flower colour to locate flowers and as an indicator of flower quality, which allows them to discriminate between food sources (Chittka & Menzel 1992). In response, flower colour has coevolved with insect colour vision as a way for angiosperms to advertise themselves and compete with other species for pollinators (Chittka & Menzel 1992; Rudall 2020). Most pollinating insects possess photoreceptors that can detect ultraviolet (UV) light as well as light in the visible spectrum (Briscoe & Chittka 2001). On top of displaying a diverse range of colours within the visible spectrum, some angiosperms have been found to reflect and absorb UV light (Schulte *et al.* 2019), creating colours and patterns that are invisible to the human eye but are conspicuous to pollinating insects. This is theorised to be a targeted form of visual signalling that can only be detected by certain species with the matching photoreceptors. The distribution of UV-absorbing pigment molecules is typically the cause of patterns in UV reflectance and absorption in flowers, although the role of petal surface structures in creating these patterns has also been recognised in some species (Schulte *et al.* 2019; van der Kooi *et al.* 2014).

The most common source of colouration in flowers is pigmentary colouration, where plastids produce specialised pigment molecules that absorb and reflect specific wavelengths of light (Brett & Sommerard 1986). Structural colouration is considerably rarer in nature, and its role in the light reflectance patterns of flowering plants has not been extensively studied (van der Kooi *et al.* 2017). Instead of using chemical pigments to reflect light, structural colouration utilises the physical behaviour of light. As light passes through the air spaces of complex nanostructures, it can be reflected, diffracted, refracted or scattered in a way that deflects certain wavelengths and allows the passage of others (Kinoshita *et al.* 2008). Naturally occurring structural colouration in animals is often made up of either multilayered structures or photonic crystals (Kinoshita *et al.* 2008), which often appear to be iridescent and remarkably vibrant compared to their pigmentary counterparts (Galsterer *et al.* 1999).

The buttercup genus *Ranunculus* (Ranunculaceae) is one of the few cases where structural colouration is found in plants (van der Kooi *et al.* 2017). *Ranunculus* consists of approximately 600 perennial herb species, which are distributed in environments ranging from sea level to the arctic zone in most parts of the world (Hoffmann *et al.* 2010). For many *Ranunculus* species, the adaxial surface of the petals exhibit a distinct gloss and are highly reflective of both visible and ultraviolet light (Galsterer *et al.* 1999; van der Kooi *et al.* 2017; Vignolini *et al.* 2011). This phenomenon mostly appears in yellow-flowered *Ranunculus* species, although has also been identified in some white-flowered species that have evolved from glossy yellow flowers (Parkin 1928).

The species selected for this study, *Ranunculus graniticola* (common name: granite buttercup), is endemic to the alpine environment of the Australian Alps, which includes Kosciuszko National Park and the Victorian high country. *R. graniticola* is predominantly found in tall alpine herbfields and sod tussock grasslands where there is a high availability of water, and often coexists alongside other alpine *Ranunculus* species such as *R. dissectifolius* and *R. gunnianus* (Costin *et al.* 1982). The flowers of *R. graniticola* consist of five rounded petals with a vibrant yellow colour (Figure 1), where the outer surface of the petal displays the distinct gloss and reflective capabilities common to the *Ranunculus* genus.

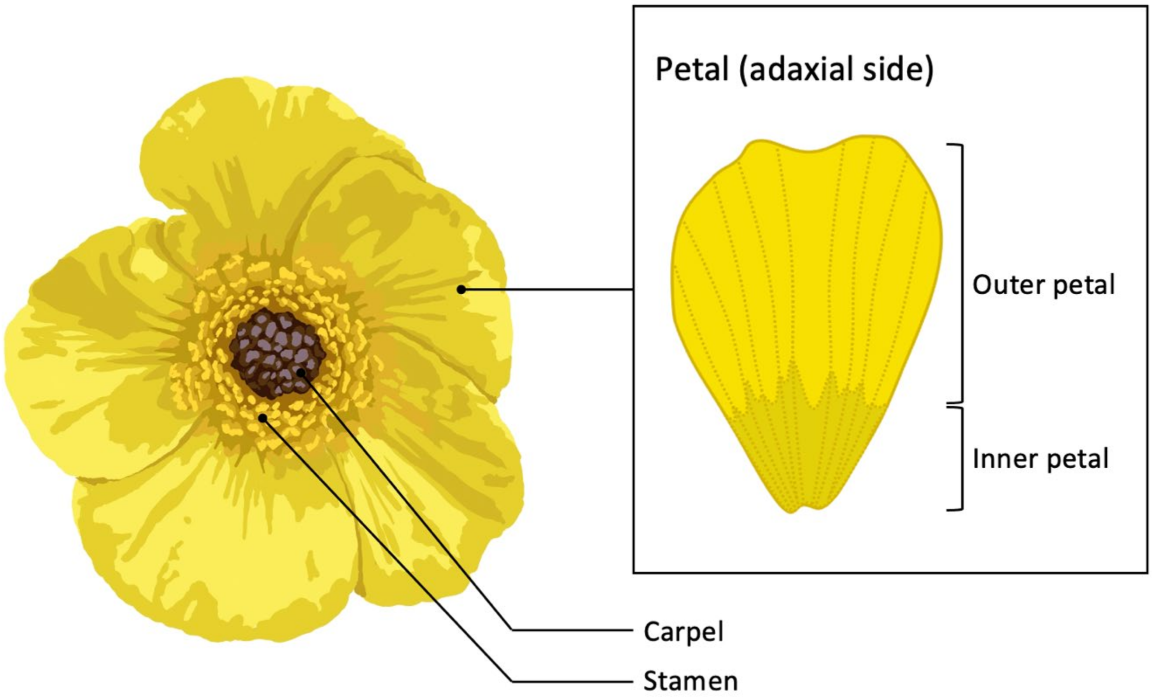

Figure 1. *A* Ranunculus graniticola *flower as viewed from above, and the layout of the adaxial (upper) surface of an* R. graniticola *petal. The outer petal possesses a glossy surface and layered structural colouration, while the inner petal lacks structural colouration and is matte in appearance.*

The unique appearance of the outer surface of buttercup petals is created by combination of pigmentary and structural colouration, which are arranged in a layered mirror-like petal structure (Parkin 1928). The upper epidermis contains a smooth cuticle, and an underlying layer of epidermal cells that contain a transparent oily solution of yellow-reflecting carotenoid pigment molecules (Brett & Sommerard 1986; Galsterer *et al.* 1999; Parkin 1928; Vignolini *et al.* 2011). This oily solution is produced by specialised plastids in the epidermal cells during the early developmental stages of the flower. As the flower reaches maturity, the plastids, nuclei and other organelles break down so that the cells are entirely filled with the carotenoid solution (Brett & Sommerard 1986). A discontinuous air gap separates the epidermis from the mesophyll. The top layer of cells of the mesophyll under the air gap are specialised starch cells. These cells are opaque and colourless, and are slanted at different angles to create an uneven surface (Parkin 1931, 1935). As light hits the petal, 20% of this light is either reflected off the epidermis or is absorbed by the carotenoid molecules in the epidermis, giving the petal its yellow colour (Brett & Sommerard 1986; Galsterer *et al.* 1999; van der Kooi *et al.* 2017). The remaining 80% of the incoming light is transmitted through the epidermis into the air gap underneath (Galsterer *et al.* 1999). The uneven layer of starch cells scatters the incident light, redirecting it out of the epidermis at different angles to those at which it entered. This increases the amount of reflected light, creating the iconic glowing appearance of the buttercup (Galsterer *et al.* 1999; Parkin 1931; van der Kooi *et al.* 2017).

Galsterer *et al.* (1999) found that the structure of *Ranunculus* petals considerably increases their UV reflectance compared to that of non-glossy flower species. The adaptive reasons for high UV reflectance in *Ranunculus* have been speculated upon in the literature, where two theories have emerged. The first proposes that the enhanced UV reflectance is a method of visual signalling to flying pollinating insects that can detect this wavelength, where the scattering effect of the starch layer causes the reflected UV light to appear as a bright flash from a long distance relative to the insects' movement (Galsterer *et al.* 1999; Parkin 1928). The second theory argues that the reflectance is a mechanism used by the flower to maintain its reproductive organs at an optimal temperature. The outer petals are arranged in a parabolic shape to reflect UV light into the centre of the flower, where it is absorbed by the non-glossy inner surfaces of the petals. This adaptation would allow *Ranunculus* to survive in cold alpine environments by mitigating the impacts of low temperatures on pollen maturation, and could have the additional benefit of generating a desirable heat source for pollinators (van der Kooi *et al.* 2017). Studies investigating the temperature regulation hypothesis have confirmed that the reflection of light off the petals affects the temperature of the reproductive organs (Stanton & Galen 1989). Flower temperature has been reported to impact both seed production and insect visitation in *Ranunculus* species (Luzar & Gottsberger 2001; Stanton & Galen 1989).

Some naturally occurring structural colours will change or lose their reflectance when soaked in a liquid. The blue wings of *Morpho* butterflies will lose their iridescence and turn a dull green colour upon contact with ethanol (Kinoshita *et al.* 2008), and the feathers of the Himalayan monal will lose their iridescence when soaked in water (Rashid *et al.* 2020). As the liquid evaporates out of the structures, their original colours and iridescence are restored. Although *Ranunculus* structural colouration has been investigated in the literature, there has been little exploration of its interaction with water. Brett and Sommerard (1986) found that petals soaked in water or ethanol maintain their shiny surface but appear more translucent, and van der Kooi *et al.* (2017) observed that the reflectance of petals would decrease by approximately half when saturated with water. Aside from these two observations, there have been no dedicated studies exploring the effect of rainfall on *Ranunculus* structural colouration under natural environmental conditions. Investigating how exposure to rainfall impacts the reflectance of *Ranunculus* flowers may provide some insight into the adaptive purpose of the structural colouration.

This would have implications for the visual signalling hypothesis (Galsterer *et al.* 1999; Parkin 1928), in which a reduction in reflectance during rainfall may reduce the visual attractiveness of the flower to pollinating insects. If thermoregulation is the main function of the petal reflectance (van der Kooi *et al.* 2017), a reduction in this reflection would imply that rainfall inhibits the ability of *Ranunculus* flowers to maintain pollen at an optimal temperature. A reduction in thermoregulation may also alter interactions with pollinating insects that would otherwise take refuge in the flowers for their heat.

The original aim of this experiment was to study differences in petal reflectance between *Ranunculus* species in Kosciuszko National Park. However, what started as an unfortunate turn in the weather lead to a chance discovery that *Ranunculus* UV reflectance was sizeably lower immediately after rainfall. We decided to change the focus of our experiment to further explore this exciting discovery. As a result, the goal of this study was to investigate the effects of water on the structural colouration of *Ranunculus* flowers, and to identify how long these effects persist. We ask: how does UV and visible light reflectiveness differ between *R. graniticola* flowers exposed to precipitation versus those exposed to dry conditions? We hypothesised that the outer petals in wet weather conditions would have a lower UV reflectance than in the outer petals in dry conditions. We expected to see a similar pattern in the visible spectrum but to a lesser extent, due to the additional contribution of pigmentation to the overall reflectance of yellow light. In comparison, we expected to see no difference in reflectance between the inner petals in wet and dry conditions, considering that they lack the gloss and starch layer present in the outer petals. We expected that upon a wet petal's return to dry conditions, the UV reflectance of the outer petal would increase over time until it reached a maximum reflectance.

Methods

Sample collection

The samples from wet conditions were collected in the morning from alongside Spencers Creek in Charlotte Pass (-36.4336, 148.3361) on the 8th of December 2021. During collection there was a heavy fog and light to moderate rainfall. For the 'wet' treatment group, nine *R. graniticola* flowers were picked, leaving a stem length of approximately 5 cm, and immediately placed into sealed vials with the stem ends submerged in water. Samples for the 'dry' treatment group were picked from whole *R. graniticola* plants from the same location that had been uprooted the previous day and maintained indoors. Nine flowers were picked from these plants as required, leaving a stem length of approximately 5 cm.

Spectroscopic measurement of reflectance

Twenty-seven petals from nine flowers were measured for each treatment group – a total of fifty-four petals and eighteen flowers. The order in which the flowers were measured was blocked into groups of three, so that three wet flowers were measured, followed by three dry flowers, followed by three wet flowers from a different vial. Once flowers were selected either from a vial or picked from a 'dry' plant, they were labelled, and three adjacent petals were removed, placed flat on a white sheet of paper, and numbered 1 to 3. To maintain a constant humidity for the 'wet' flowers, they were only removed from the vials as needed, and prior to measurement petals were lightly dried with a paper towel to remove any water from the surface.

A Jaz spectrophotometer equipped with a deuterium-tungsten halogen light source for UV-VIS measurement (Ocean Insight, Orlando) was calibrated with a fused silica reflectance standard (Ocean Insight, Orlando) once before the first measurement, and was recalibrated once every three flowers (nine petals). Reflectance spectra were recorded from 300–700 nm at a 45° angle using the dedicated measurement probe tip. The outer surface of each petal was recorded, followed by the inner surface at the base of the petal. The file name of the reflectance spectrum for each petal was recorded for future reference.

Data analysis

The 'pavo' package (Maia *et al.* 2019) in RStudio (RStudio Team 2021) was used to plot the spectrophotometry data as light reflectance spectra for the 'wet' versus 'dry' treatments. The data were then separated into two separate datasets for visible light reflectance and UV reflectance values. The highest % UV reflectance and highest % visible light reflectance values were calculated for the inner and outer surface of each petal. A two-way analysis of variance (ANOVA) was used to test the difference in the mean peak % UV reflectance between petal conditions (wet versus dry) and the position on the petal (inner versus outer surface), as well as the relationship between the two. A Tukey HSD test was conducted to highlight the differences between specific variables. A two-way ANOVA and Tukey HSD test was repeated for the visible light wavelength, testing the difference in the mean peak % visible light reflectance between petal conditions and petal position.

UV reflectance of wet petals over time

A petal was removed from a 'wet' flower and gently dried with a paper towel, then placed on a white sheet of paper glossy side up. The spectrophotometer was calibrated, and the narrow pen tip was used to periodically record the absorption spectra of the glossy surface of the petal over the span of 15 minutes. The first five recordings were spaced 30 seconds apart, and the remaining thirteen were spaced 60 seconds apart. Throughout this process, the spectrophotometer was recalibrated once every five minutes. The maximum percentage absorption in the UV range was recorded from each spectra and converted to reflectance values. A linear model was constructed to illustrate the change in maximum percentage UV reflectance over time (in minutes).

Results

The UV reflectance of the dry outer petals was substantially higher than the UV reflectance of the wet outer petals, in which the mean % reflectance for the wet outer petals was 89.5% lower than for the dry outer petals. The UV reflectance of the dry outer petals was also higher than the reflectance of the inner petals for both wet and dry conditions (Figure 2, Figure 3A). Using a two-way ANOVA test (α = 0.05), there was a significant difference in mean UV reflectance between petals from different weather conditions (df = 1, F = 98.90, $p = 2 \times 10^{-16}$), as well as between outer versus inner positions on the petals (df = 1, F = 72.65, $p = 1.23 \times 10^{-13}$). The UV reflectance of the inner petals was similar in both wet and dry conditions (Figure 3A) with a Tukey HSD test yielding a p-value of 0.27 for the difference between them. There was no significant difference in UV reflectance between the wet outer petals, and the inner petals from either wet or dry conditions. The dry outer petals showed the most variation in UV reflectance values (Figure 2) with a standard deviation of 45.99 (4 significant figures), whereas the standard deviations of the dry inner, wet inner and wet outer petal UV reflectance were 15.85, 3.787 and 9.477 respectively.

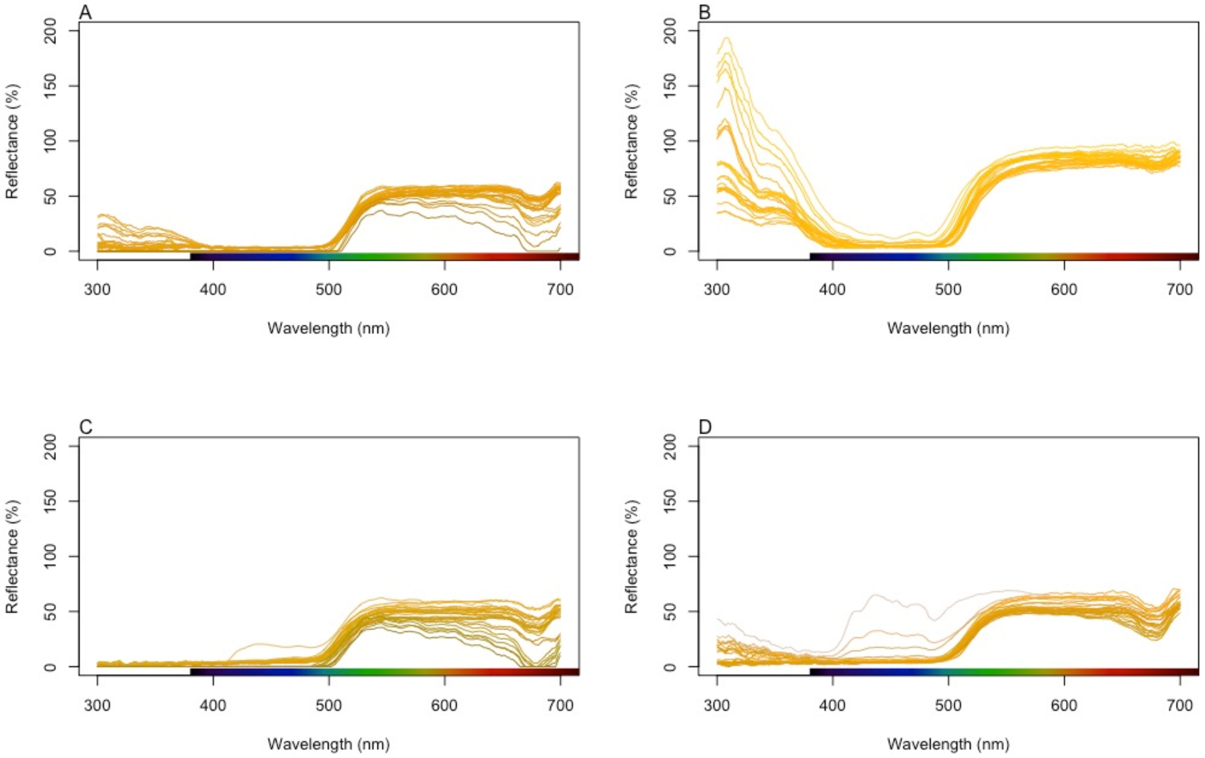

Figure 2. *The light reflectance spectra of* R. graniticola *petals from different conditions, where each panel shows 27 spectra. (A) Outer surface of petals from wet conditions. (B) Outer surface of petals from dry conditions. (C) Inner surface of petals from wet conditions. (D) Inner surface of petals from dry conditions. The yellow colour of the lines indicates the colour of the petal to the human eye.*

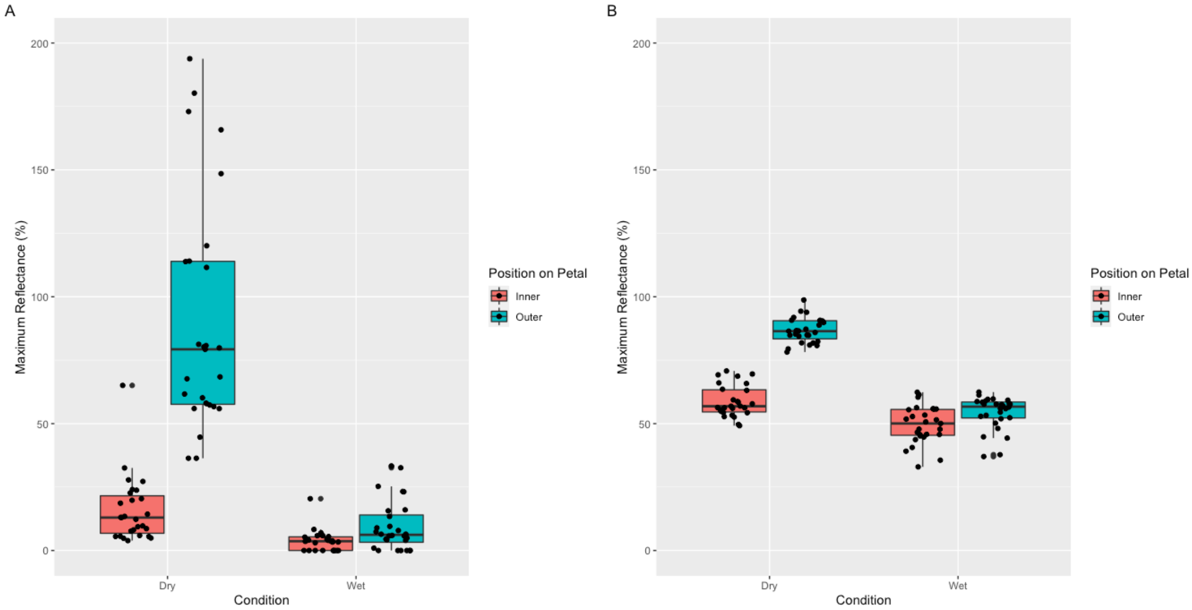

Figure 3. *The maximum reflectance values of the inner and outer petal surfaces from wet versus dry conditions, for (A) the UV spectrum (wavelength = 300–450 nm) and (B) the visible spectrum (wavelength = 451–700 nm).*

Reflectance of the visible spectra was 37.5% lower in the wet outer petals than in the dry outer petals (3 significant figures) (Figure 3B). The difference in reflectance of the visible spectra between outer petals from wet versus dry conditions is smaller than the difference for UV reflectance, and in the wet outer petals the visible reflectance is not as low as the UV reflectance (Figure 3). The reflectance of the inner petals from wet conditions was also slightly lower than it was for the dry inner petals by 15.8% ($p = 2.6 \times 10^{-6}$). Compared to UV reflectance, there was substantially less variation in visible reflectance, and this was more consistent between treatment groups (Figure 3). The standard deviations for dry inner, dry outer, wet inner and wet outer petal visible reflectance were 6.180, 4.938, 7.469 and 6.550 respectively (4 significant figures).

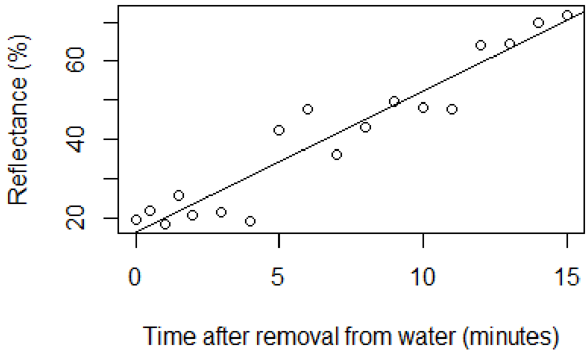

Figure 4. *Maximum % UV reflectance over time (in minutes) for the outer surface of a* R. graniticola *petal from wet conditions.*

When the outer surface of a petal from wet conditions was monitored for 15 minutes, its UV reflectance increased at a strongly linear rate (R = 0.91) (Figure 4). Approximately every five minutes, there appears to be a sharp upwards spike in % reflectance – it was at these times that the spectrophotometer was recalibrated.

Discussion

For both the visible and UV spectra, the % reflectance in the outer petals was lower when the flowers had been recently exposed to rain. This supports the initial hypothesis of this study and aligns with previous observations made by Brett and Sommerard (1986) and van der Kooi *et al.* (2017), who found that soaking *Ranunculus* petals in water decreased their reflectiveness. The multilayered structural colouration found in *Ranunculus* flowers only occurs on the adaxial side of the outer petal, where a distinct gloss can be seen on the surface. Likewise, the only part of the flower where rain exposure affected reflectance was the outer petals. Our results suggest that the effects of the structural colouration were absent in the wet outer petals, giving them reflectance values approximately equal to those of the inner petals from wet and dry conditions. This result confirms that the reflectance-enhancing ability of structural colouration in *R. graniticola* is affected by exposure to water under natural conditions.

Ranunculus structural colouration contains a discontinuous air layer separating the transparent epidermal layer from the opaque starch layer, which enhances the scattering effect by refracting incident light as it passes between layers (Vignolini *et al.* 2011). A possible explanation for the outcome of this study is that water infiltrates this air layer during rainy conditions, changing the angle of refraction and reducing the scattering of incident light. This was proposed by Brett and Sommerard (1986) to explain their observations on *Ranunculus* petals, and a similar phenomenon also occurs in the structural colour of some butterfly wings and bird feathers (Rashid *et al.* 2020). In these animals, structural colouration consists of air pockets that diffract light, causing some wavelengths to be redirected out of the structures and other wavelengths to interfere with themselves and cancel out (Kinoshita *et al.* 2008; Rashid *et al.* 2020). The velocity of light as it travels through air versus a liquid is different, so as liquid fills the air pockets inside colour-reflecting nanostructures, the light is refracted at an angle as it passes between air and water (Kinoshita *et al.* 2008). This interferes with the angles and velocities at which the light travels and prevents the movement of certain wavelengths that would normally be reflected, which changes the appearance and reflectance of the surface.

While exposure to rain decreases petal reflectance of both visible and UV wavelengths, the time series experiment (Figure 3) showed that UV reflectance gradually recovers over time when flowers return to dry conditions, supporting our initial hypothesis. We also expected that the rate of increase would reach a plateau as the reflectance returned to its pre-rain levels, but this could not be seen in the results. The most likely explanation is that the water took longer than 15 minutes to completely clear out of the air pockets. A repeat of this experiment with a longer time period of measurement would show the petal's full recovery, and recording the reflectance prior to rain exposure would reveal whether the petals partially or fully recover their reflectance upon returning to dry conditions. The accuracy of the data could be improved by calibrating the spectrophotometer more frequently, and the reliability of the results could be improved with more repetitions on multiple flowers. It cannot be concluded whether the interfering water is passively evaporating out of the petals or is being actively transported out of the air pockets via some other mechanism. Since the flowers are detached from the plant, it is most likely passive transport; however, confirming this theory would require close observation of the petal structures over time.

The calibration issues with the spectrophotometer can be seen in the data, where sharp increases in reflectance occur every five minutes after each recalibration as opposed to a constant rate of increase. It is unclear why the spectrophotometer only responded this way in the UV region; nor is it known why it needed to be recalibrated so often. However, it may be that the underlying technological issues with the spectrophotometer have lowered the accuracy of the specific UV reflectance values. Considering that the datapoints immediately after calibration were recorded when the spectrophotometer had the same UV sensitivity, it is still reasonable to compare them with one another to identify the presence of a trend. Ideally, a repeat of this experiment would use a more accurate spectrophotometer to confirm the results, and would measure the UV reflectance of a petal from dry conditions over time as a control.

While the buttercup flowers from wet conditions were picked from plants in the field, the flowers from dry conditions were picked from plants that had been uprooted and stored indoors overnight. We cannot completely eliminate the possibility that this difference between the two treatment groups affected the results, although this is not likely to be the case. The uprooted plants showed no signs of wilting or poor health at the time of plucking the flowers. It is unlikely that uprooting these plants the day before the experiment had a substantial effect on petal structure in a way that would have influenced the outcome of this experiment. Nonetheless, we recognise that it would have been more ideal if the flowers from both wet and dry conditions had been taken from plants in the field.

When comparing the appearance of petals from wet and dry conditions, they were equally glossy and could not be easily distinguished from each other. A similar observation was made by Brett and Sommerard (1986), who noticed that when wet, *Ranunculus* petals appeared slightly duller in colour but no less glossy. Considering that exposure to water impacted reflectance but had no effect on gloss, the enhanced petal reflectance under dry conditions would seem not to be caused by gloss. This deduction aligns with the findings of Vignolini *et al.* (2011), who observed that when the epidermis was isolated from the outer petal, it maintained a glossy appearance but showed significantly less scattering and almost no reflectance in the UV range. In comparison, the isolated starch layer showed levels of scattering and UV reflectance closer to that of the whole petal. The glossy appearance of the petals is a product of the smooth cuticle combined with the translucent layer of epidermal cells underneath (Brett & Sommerard 1986; Parkin 1928; Vignolini *et al.* 2011); the UV reflectance and scattering effect is separately caused by the starch layer, and is enhanced by the multilayered petal structure (Galsterer *et al.* 1999; van der Kooi *et al.* 2017; Vignolini *et al.* 2011).

Assuming that the enhanced reflectance of *Ranunculus* petals is important to the reproductive success of the plant, losing this reflectance in the rain could have some deleterious effects. The purpose of the structural colouration in *Ranunculus* has not been described. It has been suggested that the enhanced UV reflectance acts as a long-distance visual signal to pollinating insects, in which it appears as a sudden bright flash at a certain angle in relation to the pollinator's movement (Galsterer *et al.* 1999; Parkin 1928). If this were the case, *Ranunculus* flowers may become less attractive to pollinators during the rain when they lose their UV reflectance, since this would be perceived by insects as a complete change in petal colour. Flying pollinators in the alpine zone are usually less active in the rain than during clear weather (Goodwin *et al.* 2021), so, considering that *Ranunculus* flowers are already less likely to encounter pollinators when it rains, a temporary colour change may not be a major detriment. This study showed that upon entering dry conditions, the time it takes the petals to recover their reflectance is longer than 15 minutes. However, this could vary in the field depending on duration and amount of rainfall, as well as ambient temperature and humidity once the rain stops. A future study could investigate whether recovery speed varies between and within species, since this might imply that this is a trait subject to selection. As pollinators begin to emerge after a period of rain ends, buttercups that recover their reflectance faster than their neighbours of a different species could have an advantage by being able to attract more pollinator activity.

Another theory proposes that the UV light reflected by the outer petals is absorbed at the centre of the flower, and this maintains the reproductive organs at an ideal temperature (van der Kooi *et al.* 2017). The ability to maintain reproductive success amid low temperatures is important for alpine species such as *R. graniticola*, so it seems counterproductive that flowers should lose the ability to heat themselves during rain when ambient temperature drops. If this were the case, *R. graniticola* could potentially show a decrease in reproductive success over long periods of rainfall. *R. graniticola* flowers partially close up during rain, which could be an adaptation with the function of decreasing the amount of water that comes into contact with the outer surfaces of the petals. An extension of this theory suggests that the heating provided by the reflective petals may have the additional function of creating a refuge for insects during cooler ambient temperatures, and this would act as an incentive for pollinators to spend a longer amount of time on a single flower (Luzar & Gottsberger 2001; Stanton & Galen 1989; van der Kooi *et al.* 2017).

This experiment found that the petals of *R. graniticola* lose a substantial amount of their UV reflectance after prolonged exposure to water, which would appear as a change in flower colour when viewed through the eyes of an insect. Why *Ranunculus* flowers change colour in the rain and whether this phenomenon has any adaptive function remains unknown. Further investigations into the effects of rainfall on plant–pollinator interactions and flower temperature regulation may eventually lead to identification of the purpose of the buttercup's unique petal structure.

Conclusions

This study found that exposure to rainfall inhibits the reflectiveness of *R. graniticola* petals for both visible and UV wavelengths, and conjectured that interference with the air layer in the outer petal is the cause of these results. The petals demonstrated an ability to recover their UV reflectance at a linear rate upon returning to dry conditions. The interaction between weather patterns and reflectance in *Ranunculus* has potential implications for pollinator interactions and temperature regulation, which both impact reproductive success.

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References

Brett DW, Sommerard AP (1986). Ultrastructural development of plastids in the epidermis and starch layer of glossy *Ranunculus* petals. *Annals of Botany* **58**, 903–910.

Briscoe AD, Chittka L (2001) The evolution of colour vision in insects. *Annual Review of Entomology* **46**, 471–510.

Chittka L, Menzel R (1992) The evolutionary adaptation of flower colours and the insect pollinators' colour vision. *Journal of Comparative Physiology A* **171**, 171–181.

Costin AB, Gray M, Totterdell CJ, Wimbush DJ (1982). 'Kosciusko alpine flora'. (CSIRO & Collins: Melbourne & Sydney, Australia)

Galsterer S, Musso M, Asenbaum A, Fürnkranz D (1999). Reflectance measurements of glossy petals of *Ranunculus lingua* (Ranunculaceae) and of non-glossy petals of *Heliopsis helianthoides* (Asteraceae). *Plant Biology*, **1**, 670–678. https://doi.org/10.1111/J.1438-8677.1999.TB00279.X

Goodwin EK, Rader R, Encinas-Viso F, Saunders ME (2021) Weather conditions affect the visitation frequency, richness and detectability of insect flower visitors in the Australian alpine zone. *Environmental Entomology* **50**, 348–358. https://doi.org/10.1093/ee/nvaa180

Hoffmann MH, von Hagen KB, Hörandl E, Röser M, Tkach NV (2010). Sources of the Arctic flora: origins of Arctic species in *Ranunculus* and related genera. *International Journal of Plant Sciences* **171**, 90–106. https://doi.org/10.1086/647918

Kinoshita S, Yoshioka S, Miyazaki J (2008) Physics of structural colors. *Reports on Progress in Physics* **71**. https://doi.org/10.1088/0034-4885/71/7/076401

Luzar N, Gottsberger G (2001) Flower heliotropism and floral heating of five alpine plant species and the effect on flower visiting in *Ranunculus montanus* in the Austrian Alps. *Arctic, Antarctic, and Alpine Research* **33**, 93–99. https://doi.org/10.1080/15230430.2001.12003409

Maia R, Gruson H, Endler JA, White TE (2019) pavo 2: new tools for the spectral and spatial analysis of colour in R. *Methods in Ecology and Evolution* **10**, 1097–1107. https://doi.org/10.1111/2041-210X.13174

Parkin J (1928). The glossy petal of *Ranunculus. Annals of Botany* **42**, 739–755.

Parkin J (1931). The structure of the starch layer in the glossy petal of *Ranunculus*. *Annals of Botany* **45**, 201–205.

Parkin J (1935). The structure of the starch layer in the glossy petal of *Ranunculus*: II. the British species examined. *Annals of Botany* **49**, 283–289.

Rashid I, Hassan MU, Nazim M, Elsherif M, Dou Q, Hu D, Kamran M, Dai Q, Butt H (2020). Structural colouration in the Himalayan monal, hydrophobicity and refractive index modulated sensing. *Nanoscale* **12**, 21409–21419. https://doi.org/10.1039/D0NR06382G

RStudio Team (2021). RStudio: integrated development for R. (RStudio PBC: Boston, USA). http://www.rstudio.com/

Rudall PJ (2020) Colourful cones: how did flower colour first evolve? *Journal of Experimental Botany* **71**, 759–767. https://doi.org/10.1093/jxb/erz479

Schulte AJ, Mail M, Hahn LA, Barthlott W (2019) Ultraviolet patterns of flowers revealed in polymer replica – caused by surface architecture. *Beilstein Journal of Nanotechnology* **10**, 459. https://doi.org/10.3762/BJNANO.10.45

Stanton ML, Galen C (1989) Consequences of flower heliotropism for reproduction in an alpine buttercup (*Ranunculus adoneus*). *Oecologia* **78**, 477–485. https://doi.org/10.1007/BF00378737

van der Kooi CJ, Stavenga DG, Theo J, Elzenga M, Dijksterhuis J (2017). Functional optics of glossy buttercup flowers. *Journal of the Royal Society Interface* **14**. https://doi.org/10.1098/rsif.2016.0933

van der Kooi CJ, Wilts BD, Leertouwer HL, Staal M, Elzenga JTM, Stavenga DG (2014) Iridescent flowers? Contribution of surface structures to optical signaling. *New Phytologist* **203**, 667–673. https://doi.org/10.1111/NPH.12808

Vignolini S, Thomas MM, Kolle M, Wenzel T, Rowland A, Rudall PJ, Baumberg JJ, Glover BJ, Steiner U (2011) Directional scattering from the glossy flower of *Ranunculus*: how the buttercup lights up your chin. *Journal of the Royal Society Interface* **9**, 1295–1301. https://doi.org/10.1098/rsif.2011.0759