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DISENTANGLING EFFECTS OF FLOWER SYMMETRY AND ORIENTATION ON POLLINATION – *SAXIFRAGA* AS A STUDY CASE

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Abstract—Some *Saxifraga* species possess an intraindividual variation of flower symmetry and orientation: flowers with horizontally oriented corolla planes are radially symmetrical, whereas flowers with vertically oriented corolla planes are bilaterally symmetrical. Petal size, petal colouration, sequence of stamen movement, and interpetal angles are induced by gravity and thus correlate with symmetry and orientation. The flower visitors' responses to flower symmetry and orientation were tested in the field with fly-pollinated *Saxifraga stellaris* and *S. cuneifolia*. Video analysis of approach and landing behaviour showed that the landing site on the flowers was independent of flower symmetry and orientation. The flower visitors' body axis, however, was aligned with the flowers' vertical axis in most cases, since most flies land with their heads facing upwards. The flies' movement on the flower is more constrained in vertically oriented flowers: instead of walking around the protruding carpels, they walk over them. In a lab-based experiment, the deposition of pollen surrogate on stigmas of *S. fortunei* was tested using *Episyrphus balteatus* hoverflies that were dusted with pigment particles before a single flower visit. The hoverflies deposited more pigment particles on vertically oriented flowers, irrespective of flower symmetry. It is discussed whether vertically oriented bilaterally symmetrical *Saxifraga* flowers benefit from the avoidance of self-pollination and reduced pollen clogging. The role of differences in bilateral symmetry among *Saxifraga* species with regard to the display of floral guides in the upper petals, initiation of stamen movement in the lower stamens, petal length and interpetal angles is discussed in the context of pollination efficiency.

Keywords—Flower symmetry, Flower orientation, Pollen placement, Bilateral symmetry, Radial symmetry, *Saxifraga*

INTRODUCTION

Flower colour and shape, as well as other visual stimuli, are essential in shaping pollinators' flower approach and landing (Giurfa & Lehrer 2001; Horridge 2005; Chittka & Raine 2006). Understanding the relationship between form and function of floral features in the context of pollination efficiency is key to comprehending the evolution of these floral traits (also referred to as 'floral specialisation', sensu Armbruster and Muchhala (2009)). Long-term trends of floral trait evolution are an outcome of pollinator-mediated selection associated with the evolution of precise placement of pollen (Stebbins 1970; Fenster et al. 2004; Citerne et al. 2010). Floral traits that affect the precision of pollen placement include floral colouration, floral symmetry and flower orientation, all tailored to guide or restrict the movements of pollinators consistently to increase the pollinators' contact with sexual organs (Stewart et al. 2022). Guiding pollinators to a specific position on flowers to ensure optimal pollen placement on the pollinator's body, as well as effective withdrawal of pollen from stigmas, represents a significant challenge for plants (Macior 1974; Thorp 2000; Westerkamp & Claßen-Bockhoff 2007). For instance, the pollination success of flowers can be enhanced by the movement of stamens into a position that is equivalent to the stigma of a conspecific flower (Barrett 2002; Armbruster et al. 2009). This mechanism is present in protandrous species of the genus *Saxifraga*, where stamens progressively bend towards the centre of the flower during the male flowering phase, maturing when positioned above the stigmas. Thus, the position of pollinators is crucial and optimal when similar movements of pollinators occur in both the male and female flowering phases. This synchrony can be facilitated by colour patterns of bilaterally symmetrical flowers, which emphasise their symmetry at close range and promote consistent landing behaviours (Lunau 1992; Lunau et al. 2009).

The evolution of flower bilaterality comes at a pollination cost, since bilaterally symmetrical flowers receive less pollen deposited on the stigmas. The possible overcompensation of reduced pollen deposition might be the avoidance of self-pollination, which might be a strong benefit in self-compatible species such as saxifrages (Cunha & Aizen 2023).

The visual appearance of flowers – encompassing colour pattern, shape, number and arrangement of floral organs – is considered to be mostly species-specific or morph-specific (Weberling 1981; Endress 1999, 2001b). Exceptions to the concept of a species-specific or morph-specific flower morphology are for example the disc and ray florets of Asteraceae and the staminate and pistillate flowers in monoecious plants. The genus *Saxifraga* represents another exception by exhibiting a unique intraindividual variation in floral symmetry, caused by several coherent floral traits (Fig. 1). These characteristics of *Saxifraga* flowers include variations between vertical and horizontal orientation of corolla planes, the start of stamen movement with downward-facing stamens, and an incipient bilateral symmetry of flowers, by displaying smaller interpetal angles, shorter petal length, and more prominent floral guides in the upper half of flowers – suggesting an evolutionary trend towards a comprehensive bilateral symmetry. Previous studies have indicated that gravity, and consequently orientation of flowers relative to the gravitational axis, influences the development of bilateral symmetry in *Saxifraga* (Koethe et al. 2017).

Flower symmetry is primarily classified in two fundamental types: radially symmetrical flowers (also referred to as ‘regular’, ‘polysymmetrical’ or ‘actinomorphic’) and bilaterally symmetrical flowers (also known as ‘irregular’, ‘monosymmetrical’ or ‘zygomorphic’) (Coen et al. 1995; Endress 1999, 2001a; Luo et al. 1996). Flower symmetry is usually described by the entire flower structure with all its constituent organs, however, the classification of flowers as radially symmetrical or bilaterally symmetrical is often based on the form of the perianth, particularly when symmetry varies among floral organs (Citerne et al. 2010; Endress 2012). For instance, many species within the genus *Saxifraga* are classified as radially symmetrical (Soltis 2003), although the orientation of the solitary septum and intraspecific variations in petal colouration do not align with the radial symmetry observed in other floral organs (Wiegel & Lunau 2023).

Flower orientation can be defined from the flowers’ perspective itself, with the two-dimensional display area presented horizontally (i. e., upright) or vertically (i. e., sideways). Bilaterally symmetrical flowers are typically presented vertically, while radially symmetrical flowers are mostly oriented horizontally, influencing how flower visitors perceive floral cues (Neal et al. 1998; Fenster et al. 2009; Arnon et al. 2009). For instance, radially symmetrical flowers that are horizontally oriented exhibit a uniform appearance, allowing flower visitors to approach from multiple directions in a similar manner. In contrast, the vertical presentation of bilaterally symmetrical flowers reveals a distinct bilateral symmetry, which can impose directional consistency in pollinator movements towards and within the flowers (Willmer 2011). Similarly, radially symmetrical flowers may attain a bilateral appearance when oriented sideways due to gravitational bending of petals, stamens and styles (see Vöchting 1886) as e. g. in *Epilobium angustifolium*. This ‘positional monosymmetry’ may represent an early stage in the development of bilateral symmetry (Endress 1999). The evolutionary transition from radial to bilateral symmetry could have been facilitated in inflorescences (i. e. racemes, spikes, or thyrses) by a gravitropic physiological response, which was further shaped by selection via insect pollination (Robertson 1888; Coen et al. 1995; Endress 1999). In the genus *Saxifraga*, the inflorescence structures are typically thyrses and racemes, where the orientation of flowers along the main axis and sub-axes naturally varies between horizontal and vertical orientation.

Numerous studies have focused on visual pattern recognition and foraging choices of flower visitors (Lunau et al. 1996, 2006; Lehrer 1999; Rodríguez et al. 2004; Arnon et al. 2009; Hempel de Ibarra et al. 2015), suggesting a preference among pollinators for symmetrical shapes; although both radial and bilateral flower shapes have been reported as favoured in the literature (West & Laverty 1998; Wignall et al. 2006; Lázaro et al. 2008). Some studies propose that bilaterally symmetrical flowers, particularly those with a vertical symmetry plane, are especially preferred by a variety of pollinators (Lehrer et al. 1995; Fenster et al. 2004; Gómez et al. 2006; Ushimaru et al. 2009). Radially symmetrical flowers typically attract generalist pollinators from diverse, unrelated taxa, whereas bilaterally symmetrical flowers tend to be visited by a more restricted set of specialised pollinators (Neal et al. 1998; Cubas 2004).

To investigate the relative importance of floral symmetry and orientation on the consistency of flower visitors' approach behaviour and pollen placement efficiency, we compared video-taped visits to suitable *Saxifraga* flowers with different symmetries and different orientations and quantified the body orientation of landing flower visitors and duration of flower visits (see Methods). First, this study aims to assess the effect of flower symmetry and flower orientation on the approach behaviour of flower visitors in two *Saxifraga* species. Do the orientation of flowers to the gravitational axis and the flower symmetry affect the landing directionality and position of flower visitors on the flowers, as well as the visitation time? To investigate this, two species – *Saxifraga stellaris* and *S. cuneifolia* – were studied under natural conditions. Second, this study aims to quantify pollen surrogate placement efficiency in response to symmetry and orientation experimentally. Do the orientation of flowers to the gravitational axis and the flower symmetry affect the amount of pollen surrogate deposited on stigmas? To investigate this, stigmatic pollen surrogate deposition to flowers of *Saxifraga fortunei* by *Episyrphus balteatus* was studied in an experimental setting. The role of differences in bilateral symmetry among *Saxifraga* species with regard to display of floral guides in the upper petals, initiation of stamen movement in the lower stamens, petal length and interpetal angles is discussed in the context of pollination efficiency.

MATERIALS AND METHODS

PLANT SPECIES AND STUDY SITE IN FIELD OBSERVATIONS

Two species – *Saxifraga stellaris* and *Saxifraga cuneifolia* – were studied under natural conditions in the Beverin Nature Park (Graubünden, Switzerland). Both species are abundant in the park area. *Saxifraga cuneifolia* was found in moist and shady areas with bushes and trees of montane habitat along the river Avers (46°33'54.7"N 9°26'17.0"E). The study population was flowering in small clusters along the riverside. The fieldwork was carried out in June and July of 2021. *Saxifraga stellaris* was studied on the southeast mountain slope of Piz Beverin (46°38'11.3"N 9°21'51.3"E, 2,189 m a. s. l.) above the village Mathon. The study population covered an area of approximately 100 m^2, situated in a wet spring meadow of subalpine habitat. The data acquisition was performed during two flowering seasons from June to July 2021 and 2022.

The Starry Saxifrage, *Saxifraga stellaris* L. belongs to the section *Micranthes* (Haw.) D. Don within the genus *Saxifraga* (Saxifragaceae) (Conti et al. 1999; Deng et al. 2015; Tkach et al. 2015). The closed thyrsoid inflorescences with 3–15 flowers bear five white star-shaped spreading petals with two yellow pollen-mimicking spots at the base. Ten stamens with white filaments, red anthers and pollen, divided into two whorls, are arranged around the reddish bicarpellate gynoecium (Heß 2001; Lunau 2006; Lauber et al. 2018). A previous study demonstrated a significant difference in floral symmetry between horizontally oriented terminal flowers and vertically oriented flowers on sub-axes of the inflorescences (Wiegel & Lunau 2023). Terminal flowers have a radially symmetrical flower structure with larger petals, an even distribution of interpetal angles and yellow spots of similar size on each petal. Vertically oriented flowers on partial inflorescences of the thyrses are bilaterally symmetrical with variation of interpetal angles, and larger yellow spots on the petals of the upper half of the flower (Fig. 1A–B).

The Shield-Leaved Saxifrage, *Saxifraga cuneifolia* L. is included in the section *Gymnopera*

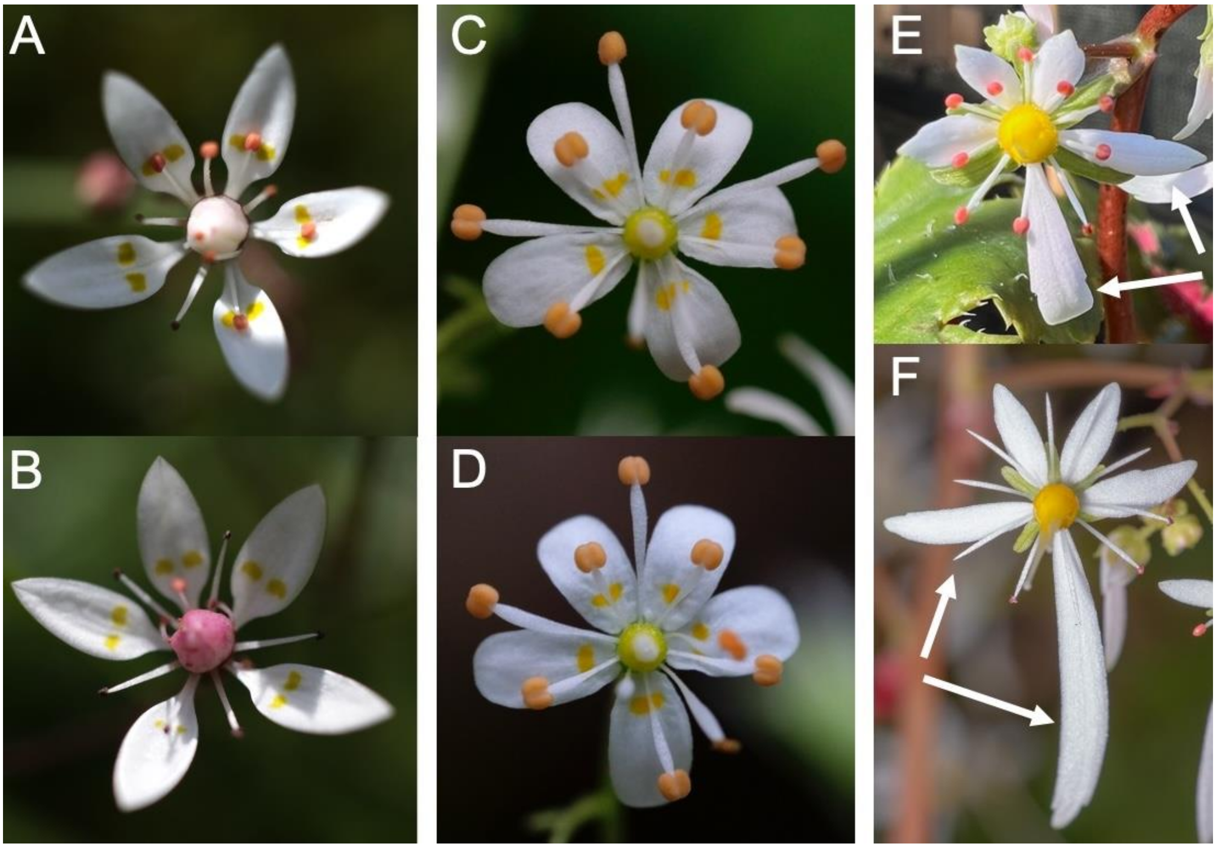

Figure 1. Floral dissimilarity among three *Saxifraga* species. (A) *Saxifraga stellaris* with radial symmetry in petal colour pattern (uniform yellow dot guides) and petal arrangement. (B) *S. stellaris* showing bilateral symmetry in petal colour pattern (due to variation in size of yellow dot guides) and variation in interpetal angles. (C) *Saxifraga cuneifolia* with radially symmetrical petal arrangement (minimal variation in interpetal angles). (D) *S. cuneifolia* showing bilateral symmetry due to pronounced variation in interpetal angles. (E) *Saxifraga fortunei* with bilateral symmetry and stronger elongation of the left one of the two elongated lower petals. (F) *S. fortunei* with bilateral symmetry and stronger elongation of the right one of the two elongated lower petals.

D. Don within the genus *Saxifraga* (Saxifragaceae) (Conti et al. 1999; Deng et al. 2015; Tkach et al. 2015). The lax panicles with 5–15 flowers bear five white petals with small yellow pollen-mimicking spots at the base. Ten stamens with white filaments, orange anthers and pollen, divided into two whorls, are arranged around the yellow bicarpellate gynoecium (Köhlein 1995; Heß 2001; Lauber et al. 2018). Most flowers in the inflorescence have a bilaterally symmetrical flower shape due to the petal arrangement (Heß 2001; Koethe et al. 2017) (Fig. 1D).

For the identification of species, Lauber et al. (2018), Schmeil et al. (2019), Gerhardt and Gerhardt (2021) and Chinery et al. (1984) were used. Voucher specimens were not collected.

Data collection and processing

The approach behaviour of flower visitors was studied using a high-speed camera system (Panasonic Lumix GH5 with an Olympus 60 mm f/2.8 macro lens). The activity of flower visiting insects on *S. stellaris* and *S. cuneifolia* was recorded at daytime between 10:00 a.m. to 5:00 p.m. CEST on each observation day, when the weather was appropriate. The camera was mounted on a tripod, placed about 40 cm from the focal flowers. At this distance high-definition videos with a full HD resolution of 1920 x 1080 pixels could be obtained, while minimising the disturbance for flower visitors. Plants of *S. stellaris* were selected with inflorescences showing a maximum of two flowers at anthesis (as determined by the presence of open, vibrantly coloured flowers). Observation of flower visitors' approach behaviour in response to radially symmetrical flower shapes was performed on terminal flowers, and in response to flowers with bilaterally symmetrical flower shapes, bilateral flowers of partial inflorescences were selected.

Most flowers in *S. cuneifolia* are bilaterally symmetrical and oriented vertically. Here, inflorescences were selected that were showing one to five bilateral flowers at anthesis. The orientation was manipulated in some cases with flower wire to ensure the even distribution of orientations in the dataset, and to ensure that all flowers faced the camera.

The following floral features of the investigated flowers were included to estimate flower symmetry in advance: angles between petals and petal colouration, which are known to mainly contribute to floral symmetry in saxifrages in experimental settings (Koethe et al. 2017) and observational studies (Wiegel & Lunau 2023). The angle of the flower relative to the gravitational axis was measured for each flower in the field using a clinometer attached to a sounding lead. A colour photograph was taken of each flower from an *en face* view, with a horizontally aligned camera position (x-axis). The internal electronic spirit level was used to ensure a horizontal alignment of the camera. The photographs were used to measure the angle between each petal and the gravitational axis, that is congruent with the vertical (y-axis) of the photograph. The flower petals were consecutively named beginning with the lower left petal, P1, in a clockwise manner to P5 (Fig. 2A). The measurements were conducted using the computer program Image J/Fiji (Schindelin et al. 2012; Schneider et al. 2012). Insect visitation on the measured flowers was recorded and analysed with respect to four shape-angle combinations. The shape-angle combinations result from two flower shapes (radial and bilateral symmetry) (Fig. 1A–B, Fig. 2B) and two angles (horizontal and vertical orientation) (Fig. 2C): horizontal x radial (h x r; 1°–30° degrees from the gravitational axis, radially symmetrical shape), horizontal x bilateral (h x b; 1°–30° degrees from the gravitational axis, bilaterally symmetrical shape), vertical x radial (v x r; 61°–90° degrees from the gravitational axis, radially symmetrical shape) and vertical x bilateral (v x b; 61°–90° degrees from the gravitational axis, bilaterally symmetrical shape). Since no flower was presented perfectly horizontal (0°), the alignment of the landing visitor's body axis with the gravitational axis of the flower could be quantified.

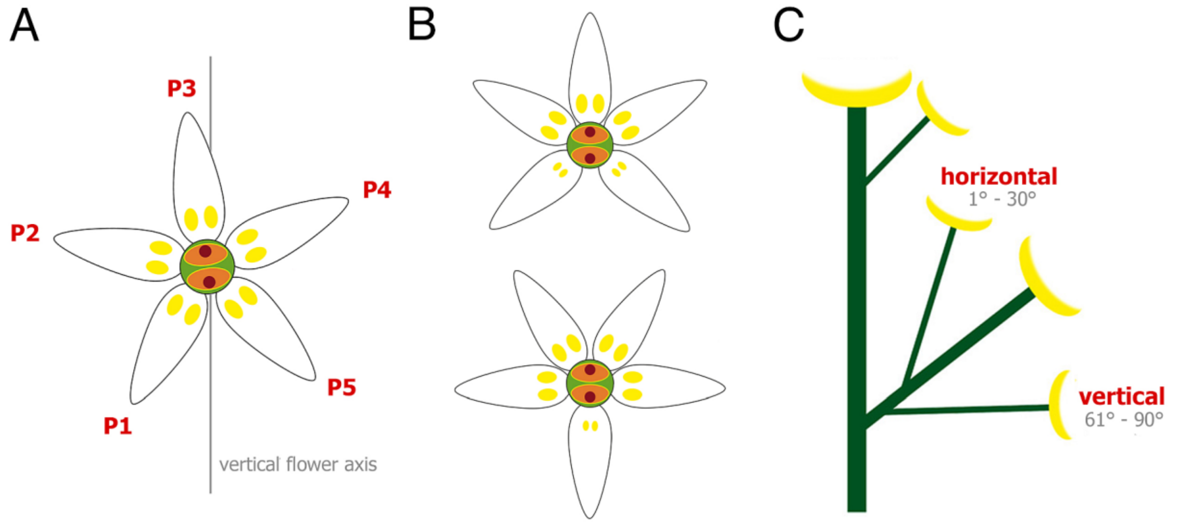

Figure 2. Petal terminology and common symmetry types. (A) Flower petals were labelled P1–P5, with P1 defined as the first petal to the left of the vertical flower axis. Grey line indicates the principal vertical axis, which aligns with the gravitational axis. The illustration represents the typical radially symmetrical petal arrangement and colour pattern of *S. stellaris*. (B) Two abundant colour patterns and arrangements of petals around the floral disc observed in bilaterally symmetrical flowers of *S. stellaris*. The two petal arrangements are also present in *S. cuneifolia*, although the floral guides around the floral disc are more uniform in size. (C) Illustration of the inclination of flowers in an inflorescence of *S. stellaris* and *S. cuneifolia* with the two categories of flower orientation that were investigated. Angles refer to degrees from gravitational axis.

The video footage was analysed by observing each video multiple times to ensure replicability of display evaluation and to avoid missing observations. The quantification of the data on directionality of the body of landing flower visitors and the duration of flower visits aimed to identify differences linked to the orientation and symmetry of the flowers. Flower-visiting insects' activity was monitored from the arrival on the first flower until the visitor left the field of view. As it was not possible to assign individual identities to flower-visiting insects, every individual entering the video frame was considered as a new subject. A single flower could be visited by multiple individuals. Therefore, if an individual insect visited multiple flowers, only the first visit of the insect of each category (horizontal x radial, horizontal x bilateral, vertical x radial, and vertical x bilateral) was used to avoid pseudo-replication. A successful flower approach was defined as the flower visitors' posture being stopped on a flower with its proboscis extended. The directionality of the landing flower visitors' body was measured from a video screenshot. The angle was measured between two lines extending from the centre of the flower, i. e. one which goes vertically down to the middle of the flower and another that goes through the midline of the flower visitors' body. The vertical line was defined as zero degrees and the clockwise angle of the midline of the insects' body was measured on a 0°–360° scale as the directionality of the landing angle (Fig. 3A). Each flower approach was categorised to four categories of landing angles, each encompassing an angle of 90°, named top, right, bottom, and left (Fig. 3B). The landing position of the flower visitor was categorised to five landing positions (arrays), named top, right, bottom, left and centre (Fig. 3C). Fine-scale data (exact degrees) of landing angles were processed as well (see Appendix I). Furthermore, the time spent on the flowers by flower visitors was measured and compared with respect to flower symmetry and orientation.

Statistical analysis

All statistical analyses were performed using the R statistical computing software (version 4.3.2; R Core Team (2023)) and a significance level of $\alpha < 0.05$ (5% significance) was set as standard. To assess differences between means, post-hoc tests were performed using pairwise comparisons, if necessary. Comparisons of categorised landing positions and angles were performed using the Chi-square test with the null hypothesis assuming that landing positions and landing angles were identical across all categories. If the sample size was less than 20 or counts of a group were less than 5, Monte Carlo Correction or Yates' correction was applied, or alternatively Fisher's Exact test was used. For comparison of data with multiple groups (e. g. four shape-angle combinations in flowers of *S. stellaris*), such as the duration of flower visits in response to flower orientations and flower symmetry, one-way ANOVA was used for normally distributed data and the Kruskal-Wallis test for non-normally distributed data; the corresponding null hypothesis assumed that the response was identical across all four categories. Comparisons between two groups (e. g. two orientations in flowers of *S. cuneifolia*) were performed using a t-test for normally distributed data and Mann-Whitney-U test for non-normally distributed data; the corresponding null hypothesis assumed that the response was identical across both categories. Tukey HSD or Dunn-Bonferroni post-hoc tests were applied to make further differentiation among groups if necessary.

POLLEN SURROGATE PLACEMENT EFFICIENCY

The transfer efficiency of pigments, used as pollen analogues, by the marmalade hoverfly *Episyrphus balteatus* to flowers of *Saxifraga fortunei* in response to flower symmetry and orientation was studied under laboratory conditions in Düsseldorf (Germany) in October 2023 (Fig. S1). The experiment was conducted using potted plants of *Saxifraga fortunei* and flower-naïve freshly emerged *Episyrphus balteatus* hoverflies (Fig. S2).

The Fortune's Saxifrage, *Saxifraga fortunei* Hook. belongs to the section *Irregulares* Haw. (Saxifragaceae) (Zhang et al. 2020). The inflorescences are characterised by lax panicles with numerous pure white flowers. Each flower has five narrow, unspotted petals of which the bottom one (or two) are always three or four times larger than their fellows. Ten stamens with white filaments, reddish anthers and pollen, divided into two whorls, are arranged around the yellow bicarpellate gynoecium. The floral symmetry is bilateral by the elongation of one (or two) of the lower petals (Fujii et al. 2020; Shin et al. 2022).

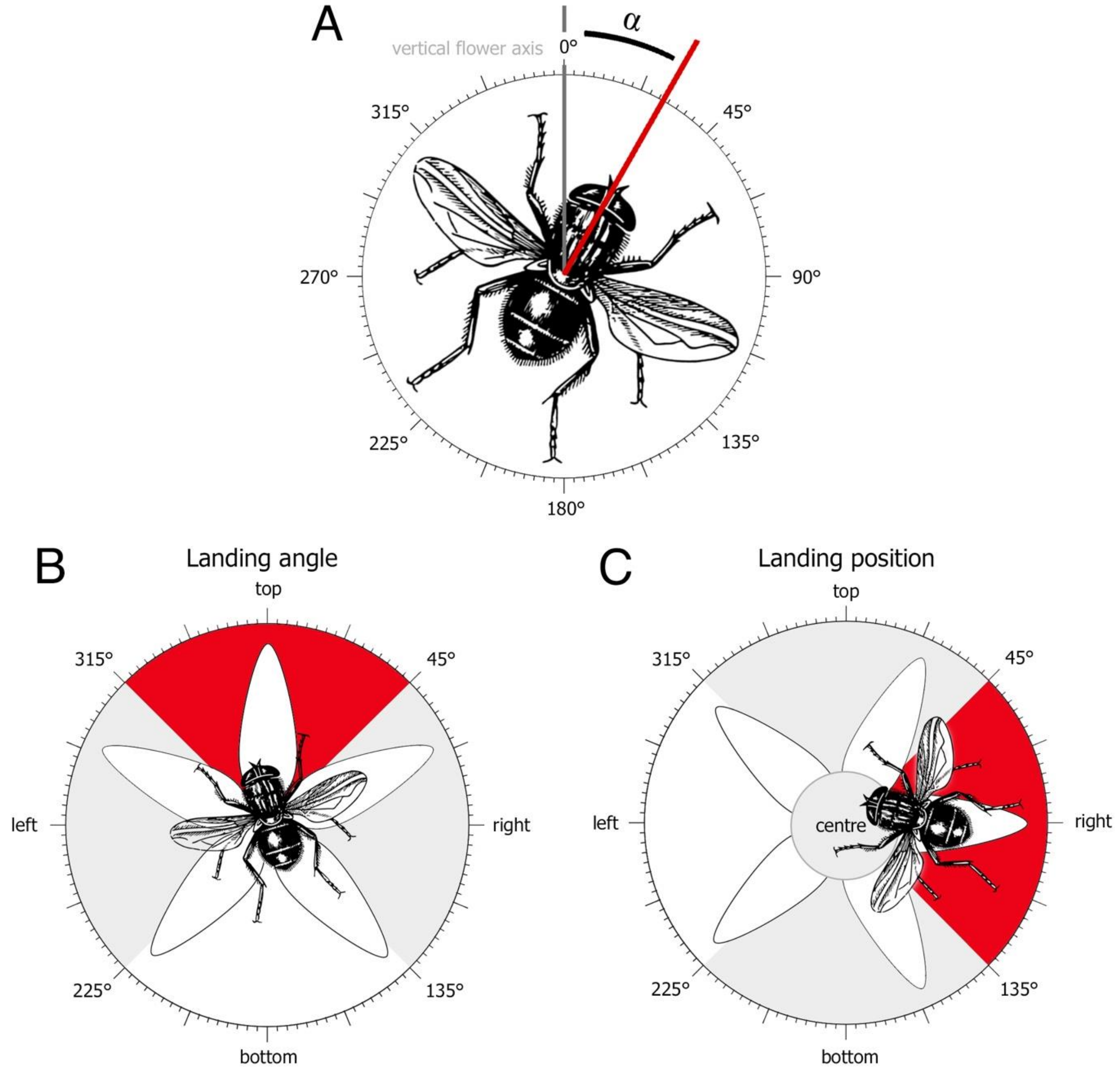

Figure 3. Measurement and categorisation of flower visitor body orientation and landing position. (A) Schematic representation of the method used to measure the landing direction (landing angle) of flower visitors. Two lines were drawn from the centre of the flower: one aligned with the principal vertical flower axis (dark grey), which corresponds to the gravitational axis (0°), and a second through the midline of the insect's body (red). The angle α was measured clockwise between these lines on a 0°–360° scale to define the flower visitor's landing angle. (B) The four sectors used to categorise the flower visitors body orientation (i. e., top, left, bottom, and right). The red sector indicates the selected category in correspondence to the direction the fly was facing. (C) The five sectors used to categorise the landing position of the flower visitors on the flower (i. e., top, left, bottom, right, and centre). The red sector indicates the selected category in correspondence to the fly's landing position (landing array).

The Marmalade hoverfly, *Episyrphus balteatus* De Geer belongs to the Syrphidae family (Diptera), and is considered the most abundant hoverfly in Central Europe (Gerhardt & Gerhardt 2021).

Data collection and processing

Thirty plants of *Saxifraga fortunei* var. *fortunei* were obtained prior to flowering at a commercial perennial nursery Staudenkulturen Stade (Borken, Germany) in May 2023 and raised in the following months. The plants were kept under an insect-proofed net (L = 180 cm, W = 150 cm, H = 100 cm) to prevent any contact between plants and insects. At an early stage of flowering, all plants were checked daily for flower buds at anthesis to remove anthers to avoid selfing and any contamination with pollen. *Saxifraga* flowers are protandrous. We carefully checked each flower prior to use to ensure that no pollen contamination had occurred.

Pupae of *Episyrphus balteatus* hoverflies were supplied by Katz Biotech AG (Baruth, Germany) and Sautter & Stepper GmbH (Ammerbuch, Germany). The insects were kept at room temperature (21 ± 2°C), and natural light (16:8 h light:dark photoperiod) until adults emerged. Pupae of hoverflies were individually stored on a layer of vermiculite in flight-cages (H = 66 cm, W = 42 cm, L = 42 cm) sealed with a net to allow a sufficient airflow. Only freshly emerged flower-naïve hoverflies were used for the experiments, since they are sexually immature in the first days, and are thus assumed not to be attracted by sexual pheromones, as reported for many Diptera species (Jeavons et al. 2022). Individuals were fed with a 70% solution of sucrose, as they are likely to die after 24 hours when they are not offered sugar intake. Each individual was therefore caught with an acrylic glass tube and presented with the sugar solution (a small piece of cotton was soaked in the solution, placed in a 0.5 ml microcentrifuge and offered to the fly. Individuals that did not feed on the cotton ball with sugar were not used for the test. The feeding occurred at 6:00 p.m. to 1–24 h old individuals. The experiment was carried out 15–24h after the preliminary sucrose feeding to stimulate the foraging behaviour of the tested insects.

Flowers showing clear evidence of the female flowering phase were selected and cut before the experiments. The female flowering phase was visually assessed by stigma receptivity, based on the presence of liquid exudates on the stigmatic surface indicating a receptive, wet stigma, which is typical for high-mountain *Saxifraga* species (Steinacher and Wagner 2010; Guerrina et al. 2022). In half of the flowers, the elongated petals were trimmed to the same length as corresponding petals to achieve radial symmetry. The flowers were placed into an acrylic glass cage (H = 10 cm, W = 10 cm, L = 10 cm). Four cages, each representing one of the four flower categories (horizontal x radial, horizontal x bilateral, vertical x radial and vertical x bilateral), were placed in a grey flight arena with natural illumination. The experiment was monitored using a high-speed camera system (Panasonic Lumix GH5 with an Olympus 60 mm f/2.8 macro lens) to allow review of time measurements if necessary. The experiment was conducted at daytime between 9:00 a.m. and 6:00 p.m. Active individuals of the hoverfly population were selected and transferred into a transparent polystyrene vessel (Mini-Life vessel obtained from bioform Dr. J. Schmidl e.K. (Nürnberg, Germany)). The vessel was filled with a low quantity of red burnt ferrous oxide pigments as pollen analogues (no. 10, red #1675410), obtained from Kremer Pigmente GmbH & Co.KG (Aichstetten, Germany). The pigment particles are detectable with 'macro-stigmagraphy', a method to quantify the deposit of pollen (or pollen analogues) onto a flower's stigma via macro-photography (MacInnis & Forrest 2017). Each pigment particle has a size of 27 µm, equivalently to the average size of pollen grains of *Saxifraga* species (15.4–44.4 µm in the polar axis and 11.4–34.6 µm in the equatorial axis (Yifeng et al. 2014)). Maximum application of the pigments to the insect body was achieved by a careful rotation of the tube (Fig. S3A). After full coverage of the insect's body the hoverfly was released into the acrylic glass test arena (Fig. S3B). Individuals that did not forage within 15 minutes after entering the acrylic glass cage were removed and tested again later or the next day. Each individual was given a maximum of three trials, after which it was discarded from the experiment. The acrylic glass cages were washed with distilled water between each test, and with ethanol at the end of each day to prevent the presence of olfactory marks deposited by the individual previously tested.

Once an individual successfully approached a flower, the point of time was noted, and the behaviour of the flower visitor was carefully observed until the visitor left the flower. The end point of the flower visit was noted, and the duration was calculated. The hoverfly was immediately removed from the cage to ensure no further flower approach. Following this, a macro-photograph with focus on the stigmas was taken with a Canon EOS 70D and a LAOWA 60 mm f/2.8 Ultra-Macro 2:1 lens (Fig. S3C–D). Each macro-stigmagraph was taken at the same angle, lighting, and camera aperture setting to ensure consistency during image analysis. All photographs were further processed using Image J/Fiji (Schindelin et al. 2012; Schneider et al. 2012). Pigment particles were counted using the Cell Counter tool (MacInnis & Forrest 2017).

Statistical analysis

All statistical analyses were performed using the R statistical computing software (version 4.3.2; R Core Team (2023)) and a significance level of $\alpha < 0.05$ (5% significance) was set as standard. Count data of pigment particles were statistically assessed using a two-way (factorial) analysis of variance (ANOVA) to determine whether and how floral symmetry and orientation affected the number of pigment particles, transferred by flower visitors to the stigmas; the corresponding null hypothesis assumed that neither flower symmetry nor orientation affected pollen transfer. The pigment count was used as the response variable. Floral symmetry and orientation were considered as fixed effects, in combination with an interaction term between symmetry and orientation. Levene's test for homogeneity of variance and Shapiro-Wilk test for normality were applied to verify statistical test assumptions. As a visual assessment of the QQ-plot of the residuals and Shapiro-Wilk test indicated that the dataset followed a non-normal distribution, the data were transformed using Yeo-Johnson transformation. The Yeo-Johnson is a Box-Cox transformation that can handle zeroes and negative values (Yeo 2000), hence was necessary to deal with flower visits without any deposition of pigment particles. To assess differences between means, post-hoc tests were performed using Tukey HSD. The effect of the duration of flower visits in response to flower symmetry and orientation was likewise analysed using a two-way (factorial) analysis of variance (ANOVA) (see above); the corresponding null hypothesis assumed that the duration of flower visits was identical across all combinations of flower symmetry and orientation.

RESULTS

FLOWER VISITORS' APPROACH BEHAVIOUR

Video analysis of approach and landing behaviour showed that most flower visitors on flowers of *S. stellaris* belong to the group of non-syrphid Diptera, and mainly to calyptrate muscoid flies (64%). A diverse set of species of the family of Syrphidae (22%) was also frequent in the video observations. Additionally, a few insects from other families and orders could be documented (14%). Flower visitor composition on flowers of *S. cuneifolia* in contrast was characterised by a higher number of species belonging to the family of Syrphidae (58%) than non-syrphid Diptera with species of calyptrate muscoid flies (27%). Additionally, a few insects from other families and orders could be documented (15%). As the identification of flower visitors was conducted by analysing the video footage, identification to lower taxonomic levels was not possible. Identification was mainly used to categorise flower visitors into functional groups of foraging behaviour by discriminating syrphid and non-syrphid Diptera.

Measurements of flower visit duration showed that the time spent on flowers of *S. stellaris* is highly variable (Fig. S4). Flower visitors spent significantly more time on radially symmetrical flowers compared to bilaterally symmetrical flowers (between symmetries: $W = 4{,}096$, $P = 0.001$, Mann-Whitney-U test). The orientation of flowers did not significantly impact the duration of flower visits (between orientations: $W = 5{,}322$, $P = 0.393$, Mann-Whitney-U test). The duration of flower visits on *S. cuneifolia* corresponded with the results for *S. stellaris*, with no significant difference regarding flower orientation (between orientations: $W = 340$, $P = 0.794$, Mann-Whitney-U test).

Flower visitors showed a generally consistent approach behaviour in response to flowers of *S. stellaris* and *S. cuneifolia* (Fig. 4). A preferential directionality of landings (landing angles) could be shown in almost all shape-angle categories by a higher frequency of landings to the upward-facing category (except for vertical x bilateral in *S. stellaris*). Neither flower symmetry, nor flower orientation had a statistically significant effect on pollinator landing directionalities in *S. stellaris* (between orientations: $\chi^2 = 2.645$, $P = 0.916$, Chi-Square test; between symmetries: $\chi^2 = 7.687$, $P = 0.361$, Chi-Square test), as well as flower orientation in *S. cuneifolia* (between orientations: $\chi^2 = 4.649$, $P = 0.199$, Chi-Square test). The tendency of the insect's alignment with the flowers' vertical axis is present within each combination – most flies land with their heads facing upwards. This is much more distinct in *S. cuneifolia* compared to *S. stellaris* (between species: $\chi^2 = 17.607$, $P = 5.301 \times 10^{-4}$, Chi-Square test).

The landing position (arrays) on the flowers was independent of flower symmetry and

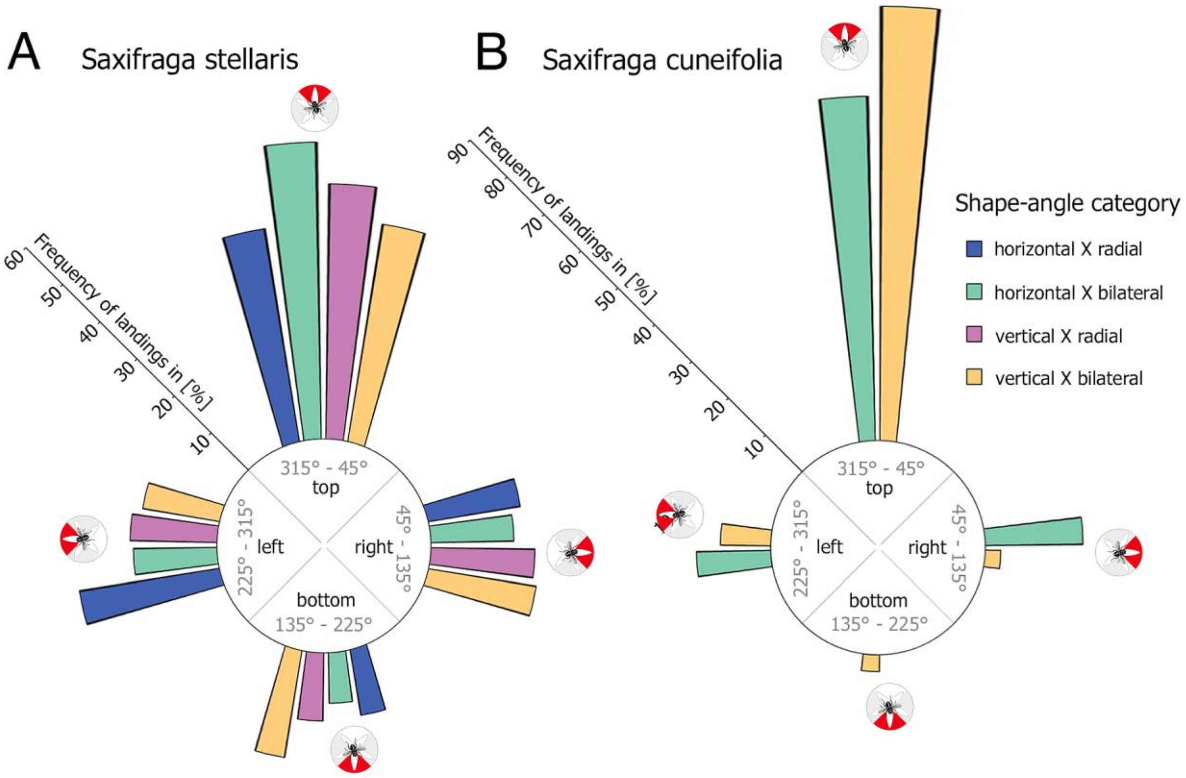

Figure 4. Directionality of flower visitors' body orientation when landing on flowers of (A) *Saxifraga stellaris* and (B) *S. cuneifolia*. Circular bar plots display the frequency (in %) of landings within the four categories of landing angles (top, left, bottom, and right), each corresponding to a 90° sector of the circle. Each coloured plot represents one of four shape-angle combinations: horizontal x radially symmetrical, horizontal x bilaterally symmetrical, vertical x radially symmetrical, and vertical x bilaterally symmetrical. Bars indicate the percentage of landing events assigned to each category of landing angles. No significant differences in landing angles were found between the different shape–angle combinations, although variation was observed within each combination.

orientation. Frequencies of landing positions vary between the four shape-angle combinations in flowers of *S. stellaris* and between horizontal and vertical orientation in flowers of *S. cuneifolia* (between shape-angle combinations in *S. stellaris*: $\chi^2 = 25.449$, $P = 0.013$, Chi-Square test; between orientations in *S. cuneifolia*: $P = 0.043$, Fisher's Exact test). Flower visitors did show a clear preference for a specific landing position in horizontal flowers of *S. stellaris*, irrespective of flower symmetry (Fig. 5A) (between positions in horizontal x radial: $\chi^2 = 16.312$, $P = 0.003$, Chi-Square test; between positions in horizontal x bilateral: $\chi^2 = 23.959$, $P = 8.139*10^{-5}$, Chi-Square test). In vertically oriented flowers no such preference could be measured (between positions in vertical x bilateral: $\chi^2 = 9.22$, $P = 0.055$, Chi-Square test; between positions in vertical x radial: $\chi^2 = 3.515$, $P = 0.476$, Chi-Square test). However, all categories were dominated by more frequent landings on the lower flower quarter and the flower centre. Contrastingly, flower visitors on flowers of *S. cuneifolia* did not show a distinct preference for a specific landing position (Fig. 5B). Horizontal and vertical orientations are significantly different to each other by the difference in more landings towards the lower quarter in horizontal flowers and centred landings in vertical flowers (between orientations: $P = 0.043$, Fisher's Exact test).

POLLEN PLACEMENT EFFICIENCY

The experimental approach to quantify pollen surrogate deposition on stigmas of flowers of *Saxifraga fortunei* in response to different shape-angle combinations revealed significant differences in the mean number of transferred pigment particles (Fig. 6). Pigment particle deposition was significantly impacted by flower orientation, whereas the effect of symmetry and the interaction of both factors were not statistically significant (between symmetries: $F = 3.643$, $P = 0.059$, two-way ANOVA; between orientations:

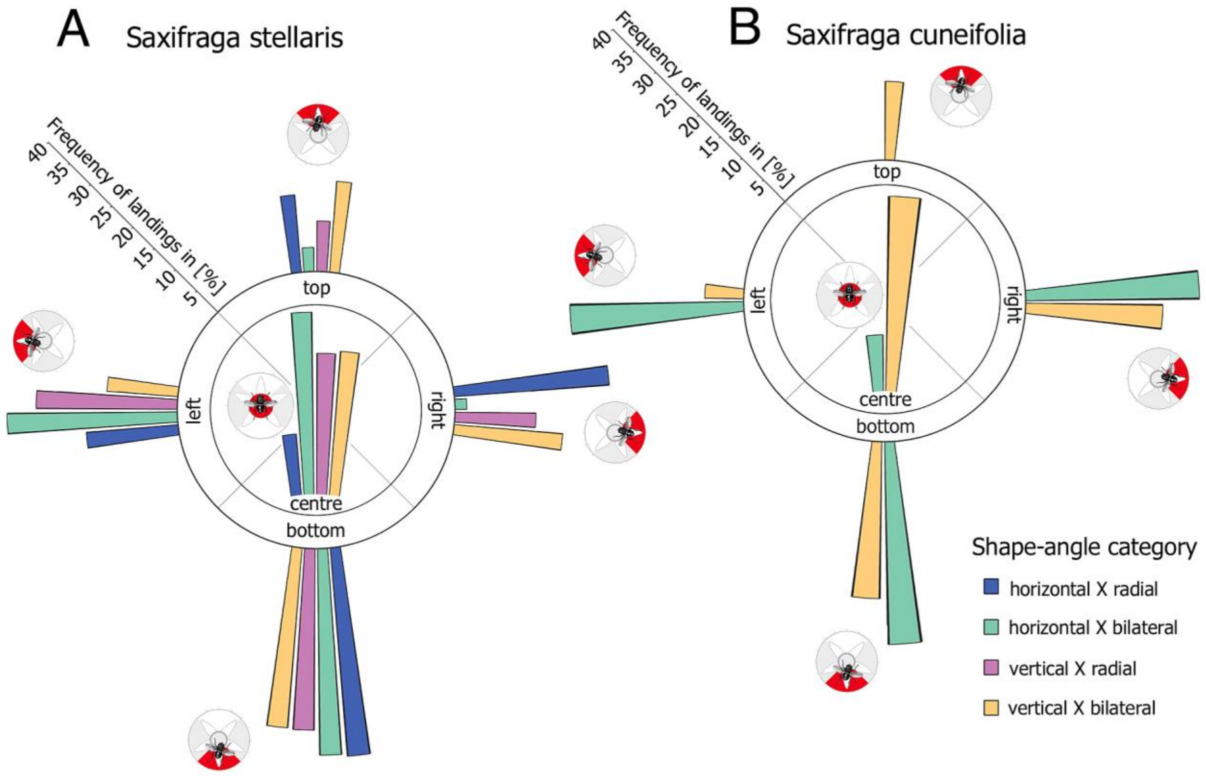

Figure 5. Landing position of flower visitors on flowers of (A) *Saxifraga stellaris* and (B) *S. cuneifolia*. Circular bar plots show the frequency (in %) of landings across five categories of landing positions (top, left, bottom, right, and centre). Each coloured plot represents one of four shape-angle combinations: horizontal x radially symmetrical, horizontal x bilaterally symmetrical, vertical x radially symmetrical, and vertical x bilaterally symmetrical and contains five coloured bars, each corresponding to the proportion of landings in a specific position category. (A) In *S. stellaris*, significant differences in landing position were found within horizontal flowers and between the four shape-angle combinations. (B) In *S. cuneifolia*, no significant differences were found between flower orientations, although variation in landing position was observed within each shape-angle combination.

$F = 17.266$, $P = 6.24*10^{-5}$, two-way ANOVA; interaction of symmetry*orientation: $F = 0.811$, $P = 0.369$, two-way ANOVA). Vertically oriented flowers received a higher number of pigment particles on the stigmas compared to horizontally oriented flowers, irrespective of flower symmetry.

Measurements of flower visitation time in relation to the orientation and the symmetry of flowers showed that the time spent on flowers was similar in all shape-angle combinations. Flower shape and flower orientation had no significant impact (between symmetries: $F = 0.174$, $P = 0.677$, two-way ANOVA; between orientation: $F = 0.000$, $P = 0.992$, two-way ANOVA; interaction of symmetry*orientation: $F = 0.006$, $P = 0.938$, two-way ANOVA). A longer visitation time did not lead to a higher pigment particle deposition in any of the tested shape-angle combinations ($R^2 = -0.008$, $F = 0.065$, $P = 0.799$, linear regression). The time prior to flower approaches was used by individuals of *Episyrphus balteatus* to groom themselves and thus to reduce potential pigment particles for deposition onto the stigmas. However, a longer timespan preceding the flower approach had no measurable effect on pigment particle deposition in any of the tested shape-angle combinations ($R^2 = 0.019$, $F = 3.272$, $P = 0.073$, linear regression).

DISCUSSION

FLOWER VISITORS' APPROACH BEHAVIOUR

In the species studied, gravity, flower symmetry and orientation are intrinsically connected and influence the directionality of flower visitors' landings. This study reveals that the dorso-ventral body axis of approaching flower visitors is generally aligned with the flowers' vertical axis, regardless of the flowers' symmetry and orientation. It is reasonable to assume that body orientation and landing behaviour of flower

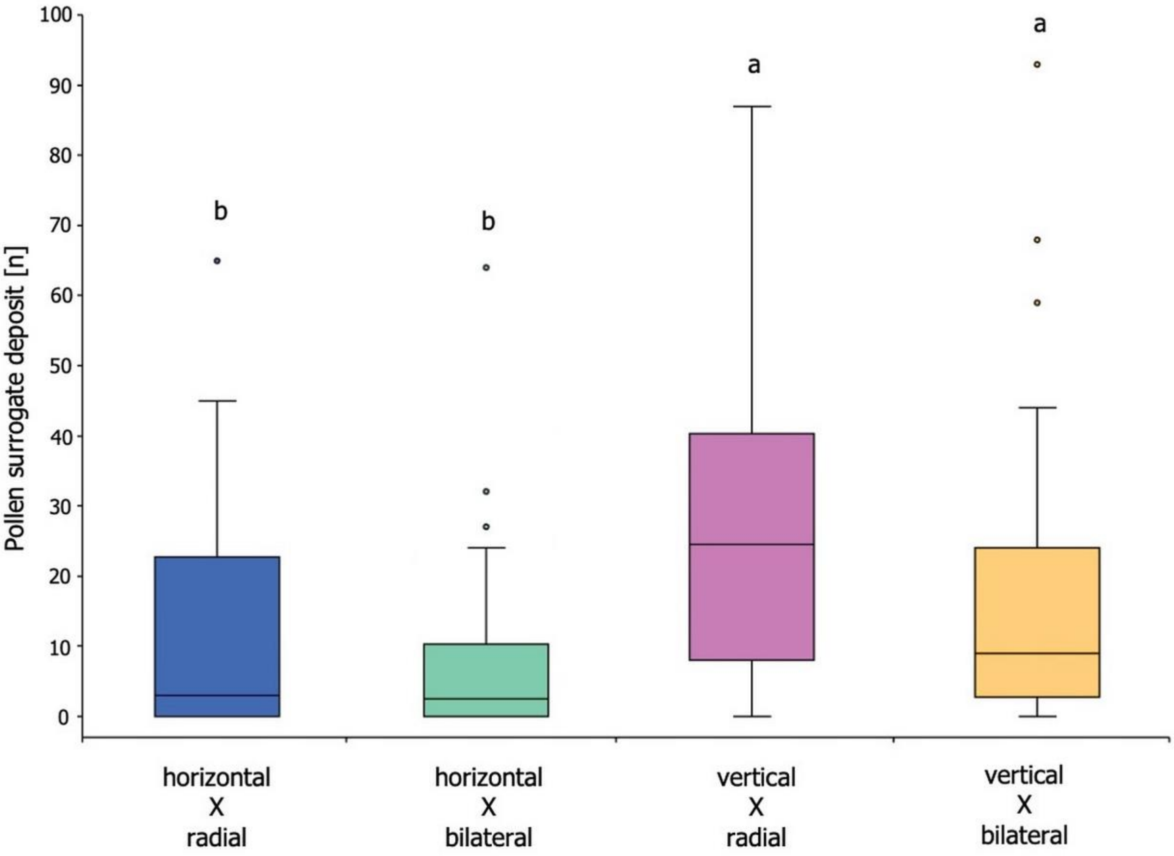

Figure 6. Pollen surrogate deposition on flowers of *Saxifraga fortunei* in regard to shape-angle combinations. The y-axis indicates the number of pigment particles (pollen surrogates) deposited on the stigmas, while the x-axis shows the four shape–angle combinations: horizontal x radial, horizontal x bilateral, vertical x radial, and vertical x bilateral. Flower orientation had a statistically significant effect on pollen surrogate deposition. Combinations that share the same letter are not significantly different (*P* < 0.05).

visitors are strongly influenced by the pull of gravity. Aerodynamic constraints, particularly those resulting from the need to counteract gravity, limit the range of motion of flying insects to a predominantly horizontal body orientation (Fenster et al. 2009). Observations in the field, by contrast, showed an enormous variety of landing manoeuvres, especially in non-syrphid flies, suggesting a less stringent constraint for insects that are more manoeuvrable (Taylor 2001; Dacke & Srinivasan 2007). For instance, due to our non-quantified observations in the field many flies performed rotations around their body axis in the coronal plane and head-down landing manoeuvres on flowers of *S. stellaris*. Furthermore, we noticed that syrphid flies seem to inspect the flower longer than muscoid flies before choosing the landing position.

Insects perceive flowers in a fundamentally different way from humans, often leading to a significant overestimation of the signalling distance range and the function of floral displays (Lunau & Maier 1995; Dafni & Kevan 1996; Lunau 2014; Hempel de Ibarra et al. 2015; Lunau & Verhoeven 2017). Flower visitors perceive a contorted image of radially symmetrical flowers, which are presented horizontally, as they are mostly approaching from the side rather than from above, resulting in the perception of a slightly bilaterally symmetrical shape (Fig. S5). The results of fine-scale analysis of the landing angles (see Appendix I) indicate that the orientation of bilateral flowers of *S. cuneifolia* affected the landing behaviour of flower visitors at close range on 0–180° scale (Fig. S7–8). The vertical display of a bilateral pattern appeared to align pollinators' approach direction significantly towards a more consistent alignment with the vertical symmetry axis, as demonstrated by flower visitors approaching horizontally versus vertically oriented flowers of *S. cuneifolia*. This is consistent with the results of Jirgal and Ohashi (2023), who observed that flower orientation, particularly vertical orientation, had the greatest impact on the variance of pollinator landing angle because pollinators typically fly with the ventral side of their body facing in the direction of gravity. Jirgal and Ohashi (2023) concluded that the stabilisation effect does not stem from the visual guidance of the corolla shape or the presence of landing platforms on bilateral flowers, but rather from the forced directionality imposed by vertical orientation. Fenster et al. (2009) also provided evidence that a simple change in flower orientation from horizontal to vertical led to more consistent and predictable approach behaviour.

The flower symmetry of *S. cuneifolia* and *S. stellaris* mainly differs in how bilateral symmetry is achieved. In *S. cuneifolia* the bilateral symmetry is established solely through the petal arrangement and in *S. stellaris* through both, petal colouration and arrangement (Fig. 1A–D). The variations in interpetal angles might not be detectable from all sides, making vertical orientation crucial for perceiving the symmetry of these flowers. In contrast, the yellow floral guides, which differ in size and shape, are much more prominent in all flower orientations and all sides. It is therefore conceivable that a development towards the vertical presentation of flowers accompanied by the development of a bilateral symmetry axis through petal colouration is favoured through natural selection, if a pollinators' movement and approach directionality is more consistent. Ushimaru and Hyodo (2005) showed that the number of legitimate landings on bilaterally symmetrical flowers of *Commelina communis* is higher when they are presented vertically and that plants benefit from bilaterally symmetrical flowers because pollen transfer is enhanced. Some species of the genus *Saxifraga*, like *S. stolonifera* and *S. fortunei* exhibit a distinct bilateral flower symmetry, supporting the hypothesis of an evolutionary adaptive advantage of clearly bilateral flowers by a more elaborate development which is independent of environmental factors (Koethe et al. 2017). The importance of floral orientation for the display of bilateral flowers is further emphasised by our field observations, where flowers of *S. cuneifolia* reorient after mechanical bending for restoring their predominantly vertical orientation. This mechanism of quick corrective reorientation of bilateral flowers has already been observed in an experimental study on *Aconitum delphinifolium* and *Stylidium ciliatum*, but has not been observed in radially symmetrical flowers (Armbruster & Muchhala 2020).

Studies on landing behaviour have focused on the visual stimuli that trigger and regulate landing manoeuvres in various insects including fruit flies, house flies, but mainly bees (Braitenberg & Ferretti 1966; Eckert 1983; Egelhaaf & Borst 1993; Evangelista et al. 2010; Srinivasan et al. 2000; van Breugel & Dickinson 2012; Wehrhahn et al. 1981); however, the mechanisms underlying the decision-making of non-syrphid flies to approach flowers (i. e. innate preferences for flower symmetry and orientation, as well as perception of other visual cues) remain less understood. Gong and Huang (2011) provided some evidence for preferences related to symmetry and floral characteristics in muscid flies. Our non-quantified field observations showed that most hovering flower visitors (syrphid flies) initially hovered in front of the centre of the flower before landing slightly lower. More specialised pollinators usually land on flowers after they assess pollen and/or nectar availability (Lunau 2000). As visual patterns help pollinators to assess flowers and facilitate accurate landing and reward localisation, the perceived value of the reward is enhanced, improving learning (Lunau 1992; Wilmsen et al. 2017). The flower visitors of *S. stellaris* are mainly non-syrphid dipterans, specifically muscoid flies, which, according to our observations, are much more driven by frantic activity and did not hover in front of the flowers to assess flower quality (Fig. S6). This could explain the less consistent landing position on flowers of *S. stellaris*, which are generally more towards the margin (Fig. S9-10). A general trend in landing positions on flowers of *S. stellaris* and *S. cuneifolia* was observed towards the centre and lower half of flowers, irrespective of the symmetry patterns. The flower orientation, however, influenced the exact landing position along the flowers' vertical axis, insofar that an increasing inclination to the gravitational axis relates to a shift in the landing position of pollinators towards the centre of the flower. Bilateral symmetry in *S. stellaris* is frequently expressed by an increase of the interpetal angle of the two lower petals of flowers (Wiegel & Lunau 2023), reducing the landing surface in the lower part of bilaterally symmetrical flowers and by the pronounced yellow pollen-mimicking dot guides (Lunau et al. 2014) in the upper three petals of the flowers which might have led to the shift of landings to the flower centre (An et al. 2018) or to the left and right. Due to our observations, in *S. cuneifolia* most flower visitors of the family of syrphids were larger, which could also have led to a more centred landing position than that of smaller non-syrphid flies in *S. stellaris*.

Flower visitors that consume pollen have innate search images for stamens, and flower-naïve individuals respond to yellow stamens as well as to stamen-mimicking structures with characteristic proboscis extension (Lunau & Wacht 1994; Dinkel & Lunau 2001; Pohl et al. 2008; Lunau et al. 2009, 2024; An et al. 2018; Lunau 2014). In this context the floral signalling features, by means of coloured anthers and pollen and floral dot guides, act in concert in the two investigated species. Both *S. stellaris* and *S. cuneifolia* have reddish to orange anthers and pollen that are less conspicuous to bees and flies than the yellow floral dot guides, since they are insensitive to red light (Lunau & Maier 1995). Bilateral symmetry in *S. cuneifolia*, through the arrangement of petals around the floral disc, does provide a denser arrangement in the upper flower half (Fig. 1D) that might guide insects' attention and navigate them towards them directly over the flower centre, whereas the radial distribution in radially symmetrical flowers of *S. stellaris* might lead to a more inconsistent movement pattern around the floral disc. A bilaterally symmetrical shape of flowers in *S. stellaris* led to a decrease of the duration of flower visits. Since the pollinator composition did not vary in frequencies between the shape-angle combinations, the decrease might be based on the shorter handling time of the flowers through the guidance of floral colouration and the reduced feeding on pollen. Interestingly, the duration of flower visits on bilaterally symmetrical flowers of *S. cuneifolia* is longer compared to bilaterally symmetrical flowers of *S. stellaris*. This might be caused by the difference in flower visitor composition.

The discrepancy between the consistency with which flower visitors approached flowers of *S. cuneifolia* in contrast to those of *S. stellaris* might be explained by the composition of pollinator communities of the two species. *Saxifraga cuneifolia* is visited by almost three times more syrphid flies compared to non-syrphid Diptera, the main pollinators of *S. stellaris*. Much focus has been devoted to the flowers' efficiency in the placement of visitors on the flowers to achieve consistency in flower visits so that pollen transfer is enhanced. However, successful reproduction is mostly dependent on the transfer of pollen to a conspecific stigma. Effective pollinators are characterised by the deposition of a sufficient amount of viable and conspecific pollen grains on receptive stigmas (Ne'eman et al. 2010; King et al. 2013; Gorenflo et al. 2017). Single-visit pollination efficacy is a best practice method to investigate pollinator effectiveness and is known to be similar for honeybees, *Bombus spp.*, and *Osmia* spp. (Andrikopoulos & Cane 2018; for review Page et al. 2021). Nonetheless, among muscid flies, many species have been identified as being functionally efficient *en masse* through high abundance and high visitation rates (Orford et al. 2015) and even on the percentage of individuals carrying pollen (Kevan 1972).

POLLEN PLACEMENT EFFICIENCY

Flower visitors' behaviour is the main factor that influences pollen deposition. In our experiment the varying behaviour of *E. balteatus* in respect to flower orientation has affected the pollen surrogate deposition. Behavioural variations with respect to flower orientations may be a consequence of mechanical constraints. Maintaining a stable position on vertically oriented flowers appears to be more difficult. It might be reasonable that the flexibility of petals and consequently the mechanical bending under the weight of the visiting insect (that especially accounts for heavier bees and syrphids) should lead to a more secure position for flower visitors at the centre of the flower and with more force that is applied through actively clinging to the floral structures with the legs (Fig. S6).

A change in movement patterns, from rotating around the floral disc in horizontal flowers towards crawling over the centre of flowers in vertical flowers, irrespective of symmetry (Fig. 7) was observed, yet not quantified in the experimental tests highlighting the mechanical constraint described above. These findings correspond especially with the analysis of landing positions on flowers of *S. cuneifolia*, where the vertical inclination of flowers was related to the shift in landing position towards the centre of flowers. The effect of symmetry, by means of the length of petals in *S. fortunei*, did not influence pollen surrogate deposition in the experiment. The effect of petal elongation might be more important in the frequent recruitment of flower visitors from a distance by the extension of the visual signalling apparatus. Many species of the genus *Saxifraga*, like *S. stolonifera* and *S. fortunei*, but also vertically oriented flowers of the investigated species *S. stellaris*, achieve bilateral flower symmetry by the elongation of the lower petals, in addition to variation in interpetal angle and petal colouration (Wiegel & Lunau 2023). *Episyrphus balteatus* visits flowers for nectar but pollen is also an important component of their diet. If they are visiting flowers primarily to feed on pollen, it is possible that they may not attend to petals but focus directly on anthers or pollen-mimicking dot guides (Golding et al. 1999). *Saxifraga fortunei* does not possess a petal colour pattern but has an enlarged yellow floral disc which might display similar signals to the yellow floral dot guides.

The evolutionary transitions from radial to bilateral flower symmetry in the genus *Saxifraga* have been related to behavioural changes of pollinators through flower orientation, the flowers' advancement in precise placement of pollinators and gravity through orientation in the developmental stages hitherto. A more specialised, fine-tuned alliance between plant and pollinators

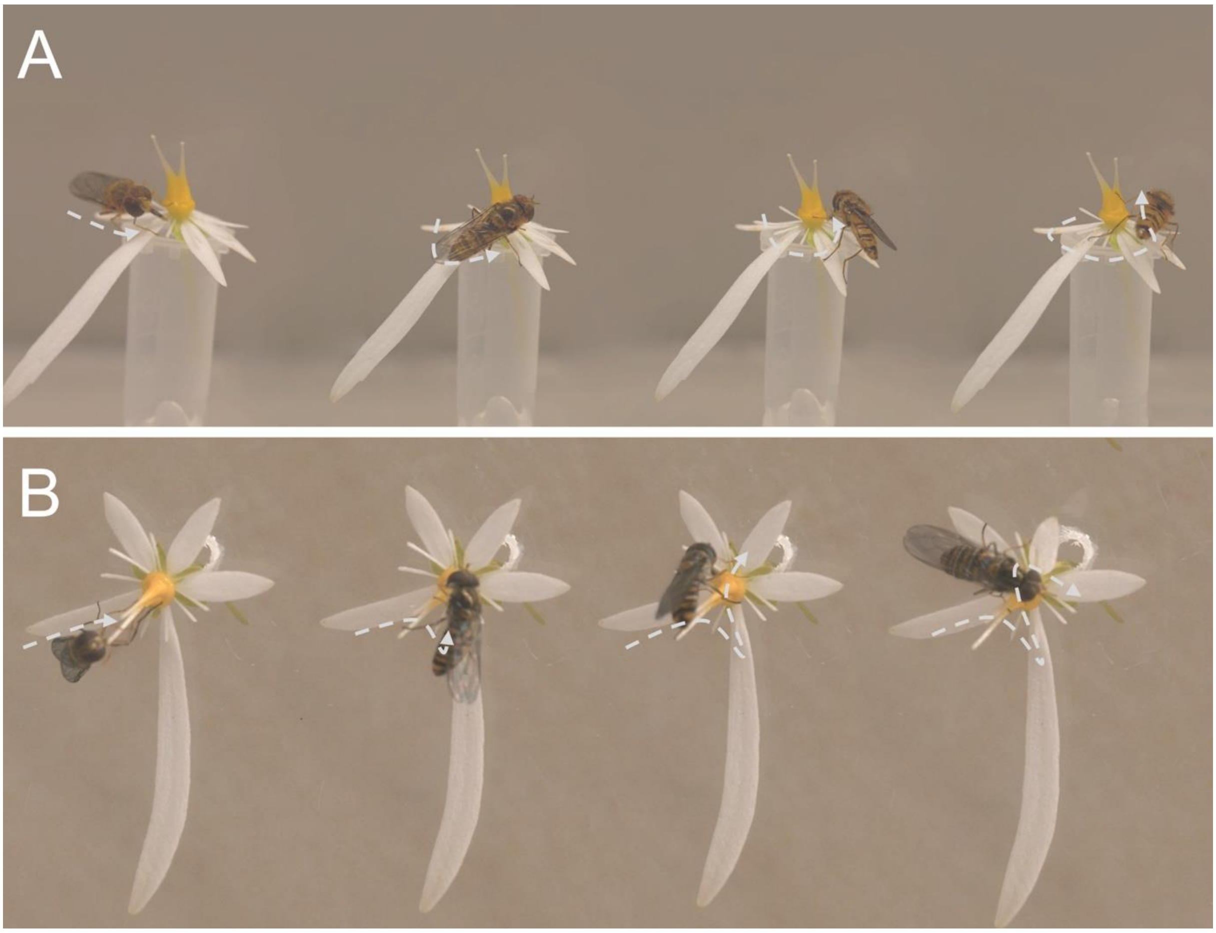

Figure 7. Behavioural variation in pollinator movement patterns relative to flower orientation. Dashed white lines indicate abundant movement patterns. (A) *Episyrphus balteatus* approaching a horizontally oriented flower by revolving around the floral disc. (B) Vertical orientation changes the movement pattern to approach the flower from a central position by moving over the floral disc.

can put plants at risk of pollinator failure. As a result of pollinator activities, pollen loss during the process of pollen transfer is always very high, and typically less than 1% of the pollen grains removed from the anthers reach conspecific stigmas (Holsinger & Thomson 1994; Harder & Wilson 1998). Longer visits by unspecialised pollinators, in contrast, may lead to increased self-pollen deposition (Kudo 2003). Usually, incoming outcross pollen is far less abundant than a flower's own pollen production, often by several orders of magnitude (Harder & Routley 2006). A comprehensive literature review and pollination studies conducted by Cunha and Aizen (2023), examining 171 angiosperm species, revealed a notable trend: bilateral flowers tended to exhibit lower pollen receipt on their stigmas compared to radially symmetrical flowers. This reduction in pollen receipt was particularly pronounced among self-compatible species. Mechanisms to avoid self-pollination seem to be more elaborate in self-compatible species. Cunha and Aizen (2023) suggested that the evolution of flower bilaterality is strongly linked to self-compatibility, giving a novel perspective on the functional significance of bilateral floral symmetry.

For successful outcross pollination in self-compatible species (as tested for *S. stellaris*; unpublished), it might be beneficial to have a temporal gap between anther dehiscence and stigma receptivity, i. e. dichogamy, to avoid self-interference in sexual function and to minimise self-pollen transfer (Lloyd & Webb 1986). In fact, dichogamy and particularly protandry can be found commonly in saxifrages. Moreover, this phenomenon appears to be inherently linked with the evolution of bilateral symmetry, as these two traits seem to be connected through shared regulatory genes (Kalisz et al. 2006). In the tested saxifrages, insects are limited in their position on the flowers through mechanical constraints imposed by verticality and partly visually by bilaterality, resulting in a tighter morphological fit between the flowers and their pollinators. It can be assumed that this enhances the transfer of incoming pollen while preventing autonomous or even pollinator-mediated self-pollination through the association with dichogamy. Self-pollination can also be prevented by increased distances between anthers and stigmas. The sequential movement of stamens in *Saxifraga* species to a position on top of the stigmas encompasses several aspects, for instance, spatial separation of stamen and stigma and the progressive presentation of pollen, which is considered a solution to the herkogamy dilemma and furthermore, a back and forth transfer of pollen if the pollinator movement is uniform in both male and female flowering phases (Armbruster et al. 2014). Stamen movements have been observed in several families, including Loasaceae, Rutaceae, Celastraceae/Parnassiaceae and Tropaeolaceae (Henning & Weigend 2012; Ren & Tang 2012), but its association with bilateral symmetry of flowers is unique for *Saxifraga* (Wiegel & Lunau 2023).

EVOLUTION OF FLOWER SYMMETRY

Fossil records suggest that bilateral symmetry in angiosperm flowers evolved independently in multiple lineages from ancestral radially symmetrical forms, coinciding with the diversification of specialised insect pollinators (Neal et al. 1998; Endress 2001b; Armbruster & Muchhala 2009; Citerne et al. 2010; Hileman 2014; Reyes et al. 2016). Bilateral symmetry seems to confer advantages for flowers that facilitate more rapid diversification than their radially symmetrical counterparts (Sargent 2004; Gómez et al. 2006). Reversions of bilateral symmetry to radial symmetry are relatively rare (Donoghue et al. 1998; Spencer & Kim 2018).

Variations in flower morphology and visual cues can enhance precision in pollen placement, thereby improving cross-pollination through restriction and control of pollinator movements within flowers (Citerne et al. 2010). In particular, floral traits such as symmetry, corolla fusion, floral orientation, and stamen number and position are thought to constrain how pollinators approach and interact with flowers (Stebbins 1951; Ushimaru & Hyodo 2005; Herrera et al. 2008; Fenster et al. 2009; Culbert & Forrest 2016; O'Meara et al. 2016; Yu et al. 2021; Stewart et al. 2022). Flowers with fewer stamens are expected to accomplish more precise pollen placement compared to flowers with higher numbers of stamens, which provide more opportunities for pollinators to make contact with different areas of their bodies (Stebbins 1951; O'Meara et al. 2016). Consequently, traits such as bilateral symmetry, sympetaly, vertical or semi-pendant orientation, reduced stamen number, and defined position of open anthers are predicted to offer fitness advantages by improving conspecific pollen transfer while minimising pollen loss.

Although the incipient bilateral symmetry of flowers in the tested *Saxifraga* species might provide benefits in terms of pollination success, the evolution of bilaterally symmetrical flowers of the genus *Saxifraga* seems to be hampered by the orientation of the two carpels in the pentameric flowers and other constraints resulting in an evolutionary and developmental lability of floral symmetry (Naghiloo 2020). Further studies are required to bridge the gap between floral orientation, symmetry and developmental constraints in the genus *Saxifraga*, and how cross-pollination of plants with bilaterally symmetrical, complex flowers is important for their evolution.

CONCLUSION

This study demonstrates that the pattern of floral symmetry and flower orientation are important factors influencing flower visitor approach behaviour and more research is necessary to disentangle the various and complex relationships of floral traits in the evolution of plant-pollinator interactions in saxifrages. Flower orientation, in particular vertical flower orientation, was shown to be of significant importance influencing flower visitors' approach and landing behaviour and pollen placement efficiency by single-visit pollen surrogate deposition. The reinforcement of vertical orientation and bilateral symmetry of flowers, the start of stamen movement with downward facing stamens, and more conspicuous floral guides on the upper half of the flower are in correspondence with the hypothesis of Cunha and Aizen (2023) on the functional significance of bilateral floral symmetry as a mechanism for avoiding self-pollination and adds a novel aspect to the perspective on the evolutionary constraints of flower symmetry in saxifrages. In this context, it is decisive that *Episyrphus* hoverflies deposited more pollen surrogate grains on vertically oriented flowers. In *Saxifraga*, where male and female flowering phases overlap due to protandry, at the end of the male flowering phase the downward-bending stamens have already shed their anthers. Consequently, a flower visitor moving upwards cannot transfer self-pollen to the stigmas, but can deposit outcross pollen on the stigma instead and may later receive pollen from the upper stamens. The successive placement of stamens with open anthers on top of the stigmas ensures optimal transfer of pollen from and to the pollinators, if their movements on the vertically oriented flowers are consistent from the bottom to the top.

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AUTHOR CONTRIBUTION

The authors confirm contribution to the paper as follows: Conceptualisation: K. Lunau and M. Wiegel; Field work M. Wiegel; Writing: K. Lunau and M. Wiegel. All authors contributed critically to the manuscript review and editing.

DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

GENERATIVE AI DISCLOSURE STATEMENT

We declare that no generative AI tools were used in the design, execution, analysis, or writing of this study.

DATA AVAILABILITY STATEMENT

The data used to write this article are available as Appendix III.

APPENDICES

Additional supporting information may be found in the online version of this article:

Figure S1. Experimental design of the pollen transfer quantification test.

Figure S2. Single flower with *Episyrphus balteatus* and inflorescences of *Saxifraga fortunei*.

Figure S3. Experimental approach to quantify pollen placement efficiency with pigments as pollen analogues and macro-stigmagraphy.

Figure S4. Duration of flower visits to *Saxifraga stellaris* flowers in relation to shape-angle combinations.

Figure S5. Optical contortion of a radially symmetrical flower of *S. umbrosa*.

Figure S6. Behavioural variations of pollinators through mechanical constraints.

Appendix I. Fine-scale analysis of landing angles.

Figure S7. Fine-scale data of flower visitors landing angles in *S. cuneifolia* using angles ranging from 0°–360°.

Figure S8. Fine-scale data of flower visitors landing angles in *S. cuneifolia* using angles ranging from 0°–180° (landing angles >180° were translated to corresponding landing angles <180°).

Figure S9. Fine-scale data of flower visitors landing angles in *S. stellaris* using angles ranging from 0°–360°.

Figure S10. Fine-scale data of flower visitors landing angles in *S. stellaris* using angles ranging from 0°–180° (landing angles >180° were translated to corresponding landing angles <180°).

Appendix II. Statistical test results including post-hoc tests.

Appendix III. Raw data.

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