“Quarter to daisy”

The complex world of floral movements.

Bachelor thesis at the Institute of

Plant Sciences

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Written by

Sarah Gauss

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Supervisor:

Dr. Katja Rembold, Prof. Dr. Markus Fischer,

Institute of Plant Sciences

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1. Abstract

Carl Linnaeus was the first botanist to describe the idea of a clock based on the opening and closing time of plant flowers, almost 300 years ago. In the meantime, several molecular based studies have been made, linking the circadian cycle of plants as well as the influence of temperature, light intensity and air humidity to the flower opening and closing patterns. This observational study focuses on the opening times of the individual species on the first floral clock in Bern which was installed in the Botanical Garden of the University of Bern (BOGA) during summer 2022. Precise opening and closing times have been found for the selected plant species. Furthermore, at least one influence of abiotic factors such as temperature, light intensity and relative air humidity on opening and closing times, has been found for most of the species. Over two thirds of the day-flowering species closed their flowers when standing in the shade.

2. Introduction

Most plants are thought to be static, object like things, rather than being considered a live species. Plant movements can be rather slow, but there are also some examples where it easily can be observed. An example for this is the reaction of the leaves, known as thigmonastic movements, of *Mimosa pudica* to environmental stimuli, such as being touched or triggered by wind, as well as vibrations (Volkov et al. 2010). Not only can plants move their leaves, but they are also able to close and open their flower petal. Carl Linneaus was one of the first botanists to discover that plants were able to open and close their flowers according to a regular temporal pattern (Linnaeus 1763), which could allow us to read the time of the day, according to which plant species shows opened or closed flowers. However, Linnaeus never realized a floral clock. There is much more importance behind the reason why plants open and close their flowers, not just so humans could assemble a floral clock. The plants are in synchronization with the insects that pollinate them (Van Doorn & Van Meeteren 2003). Not only are flowers in sync with their pollinators, but some species, such as *Ipomoea purpurea* are even able to self-pollinate if no pollinator visited them. With this mechanism, the plant can assure that it is able to produce seeds and to propagate itself (Liu et al. 2020). Other experiments were conducted with *Eustoma grandiflorum*, where it has been shown that the light intensity influences the opening and closing time by the diurnal rhythms (Bai & Kawabata 2015). It has been shown that *Arabidopsis thaliana* have an inner circadian clock which is controlled by different transcription factors being expressed either in the morning or in the evening (Muroya et al. 2021). An assembly of proteins, which were named “evening complex”, are part of the functioning of the circadian cycle found in *A. thaliana*. Studies have shown that the pathways of the “evening complex” proteins are regulated by the light signalling pathway as well as temperature perception of the plants (Huang & Nusinow 2016). However, it remains unclear if exact opening and closing times in different plant species can be observed. There is also not much information about triggers of said openings and closings, as for example if light intensity, relative air humidity and temperature influence the plant’s floral movement.

2.1 Floral clock

As mentioned above, the first idea and species list were published by Carl Linnaeus, but he never realised the project. The idea of a floral clock installation was born. But the flowering times of the species used by Linnaeus were not suitable for a floral clock in Bern, as it was based on the species of Sweden and the daylength differs from Switzerland. An intensive literature research was done by Dr. Katja Rembold, Dr. Sylvain Aubrey, Claudia Huber and Adrian Möhl in 2020 which resulted in information of the flower petal movement of over 180 species. Unfortunately, the data obtained from the literature research showed missing and some contradicting results, due to which a test phase with 56 species was conducted in the BOGA, where plants suitable for the Bernese climate were selected. With the data obtained from this experiment, 23 species were chosen based on their flowering duration, reliability, and differences in the opening/closing times of the different species. Not all the 23 species were used in the installation. Some of them were planned as backup species if a planned species was not ready and lacking flowers. Dr. Sylvain Aubrey designed the floral clock that was installed at the BOGA as a living exhibition from May 13, 2022 to October 02, 2022. For each month between May and end of September suitable plants were chosen and the floral clock was changed according to this (Figure 1). To read the floral clock, all of the seven day-flowering species must be considered, as the night-flowering species all bloom the same time during night.

2.2 Hypotheses

For these reasons, further investigations into the nature of flower opening and closing are of relevance on selected species. Against this background, the following research question shall be answered in this thesis: Do the selected species show a clear pattern in their opening and closing times and is it influenced by weather and shade?

In order to investigate this question, the following hypotheses will be tested:

- There is a pattern for the opening and closing times of the selected species.
- Individuals of species standing in the shade often react to it by closing their flowers.
- Weather has an influence on the opening of the species flowers. Weather is defined by the following abiotic factors: light intensity, temperature and relative air humidity.

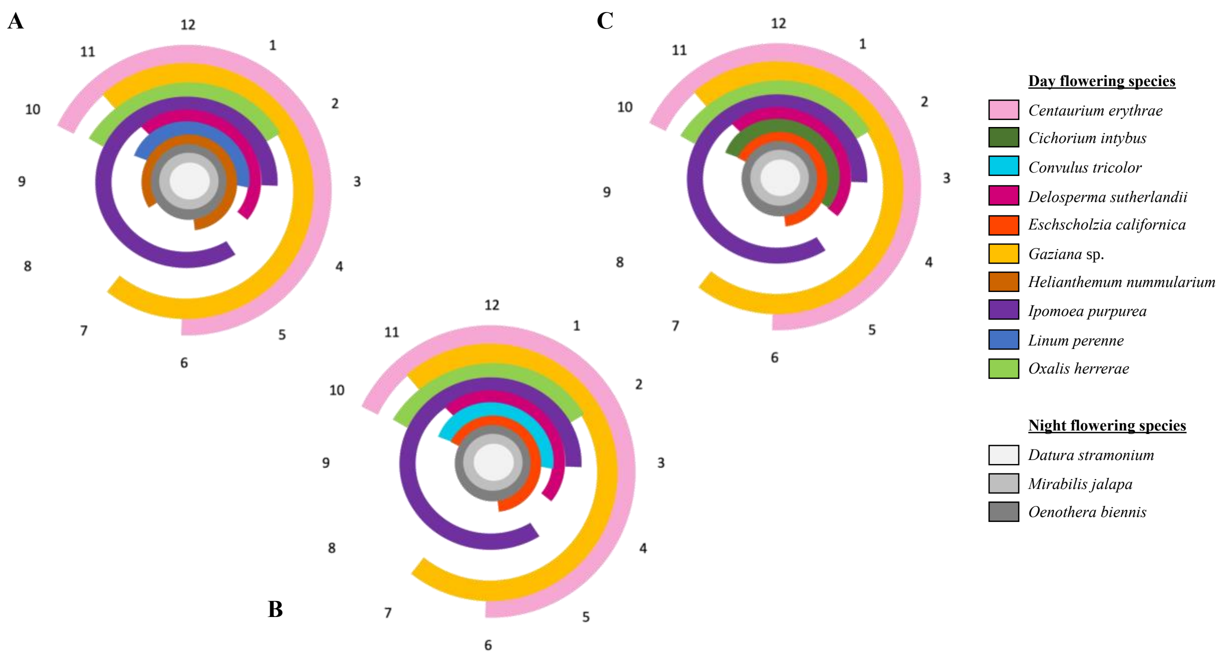

Figure 1. Overview of the installed floral clocks, showing the expected opening times of the individual species. In the middle the three night-flowering species are shown as gray scale circles. Figure A shows the setup at the beginning of the measurements. In B, *H. nummularium* and *L. perenne* were exchanged by *E. californica* and *C. tricolor*. C is at the end of the measurements on the floral clock where *C. tricolor* was exchanged by *C. intybus*.

3. Methods

From the 8th of June to the 31st of July, I collected data on 26 individual days. As the flower beds contained up to 180 individuals per species, 10 flowering individuals were marked per species that were present (Table 1). During the study there were always ten species present in the floral clock, seven day-flowering and three night-flowering species. Three species had to be exchanged, due to them being done with flowering (Figure 1). The plants replacing them had a similar flower opening time. Individuals of each species were marked by attaching a label with numbers from one to ten.

Table 1. List of the 13 plant species included in the present study and the time span in which I measured their floral movement.

Species	***Start of measurement***	***End of measurement***
Centaurium erythrae	8th of June	31st of July
Cichorium intybus	22nd of July	31st of July
Convulus tricolor	20th of June	20th of July
Datura stramonium	8th of June	31st of July
Delosperma sutherlandii	8th of June	31st of July
Eschscholzia californica	16th of June	31st of July
Gazania sp.	8th of June	31st of July
Helianthemum nummularium	8th of June	15th of June
Ipomoea purpurea	8th of June	31st of July
Linum perenne	8th of June	16th of June
Mirabilis jalapa	8th of June	31st of July
Oenothera biennis	15th of June	31st of July
Oxalis herrerae	6th of June	31st of July

As I was interested in the influence of weather and shade on the selected species, the following measurements were taken for sun as well as shade: temperature (°C), light intensity (Lux) and relative air humidity (%). The measurements were always done on the hour. From 9 am to 9 pm I collected data on the present species seven times for each hour on different days. From 10 pm to 6 am three measurements were done for each hour on three nights. The measurement in the shade was only done if there were any of the ten selected individuals standing in the shade. I measured three times at the hour I was measuring, with an interval of about 10 minutes between the measurements, for sun and if needed shade to have a mean value for temperature (°C), light intensity (Lux) and relative air humidity (%) for that hour. To ensure that the mean value of the measurements per hour was sufficient, I measured all the above measurements for each individual separately for a day and did not find a significant difference between the measurements. The light intensity was measured with a MS-200 LED luxmeter from Voltcraft the temperature and relative air humidity was measured with Greisinger GFTB 200. Additionally, the weather was observed and included in the measurements by differentiating between sunny, lightly cloudy, cloudy and rain. All the individuals were examined for their opening status as well as if the individual was standing in the sun or in the shade. At the end the dataset contained the species, day, hour, individual, number of open species, temperature in the sun, temperature in shade, humidity in the sun, humidity in shade, standing in the shade, light intensity in the sun (lux), light intensity in shade and weather.

3.1 Statistical analysis

The dataset containing all the data mentioned above was used for the statistical analysis in R (R Core Team 2022). Firstly, the data was checked for correlation between the variables using the cor function. The dataset was then divided by species and the percentage of open individuals per hour was calculated and plotted using ggplot2. Each species was then statistically analysed with a fitting generalized linear mixed-effect model using the glmer function (Bates et al. 2015). As the opening measurements were binary, the model used was set to binomial. Individuals as well as the hour, nested into day, were specified as random effects.

4. Results

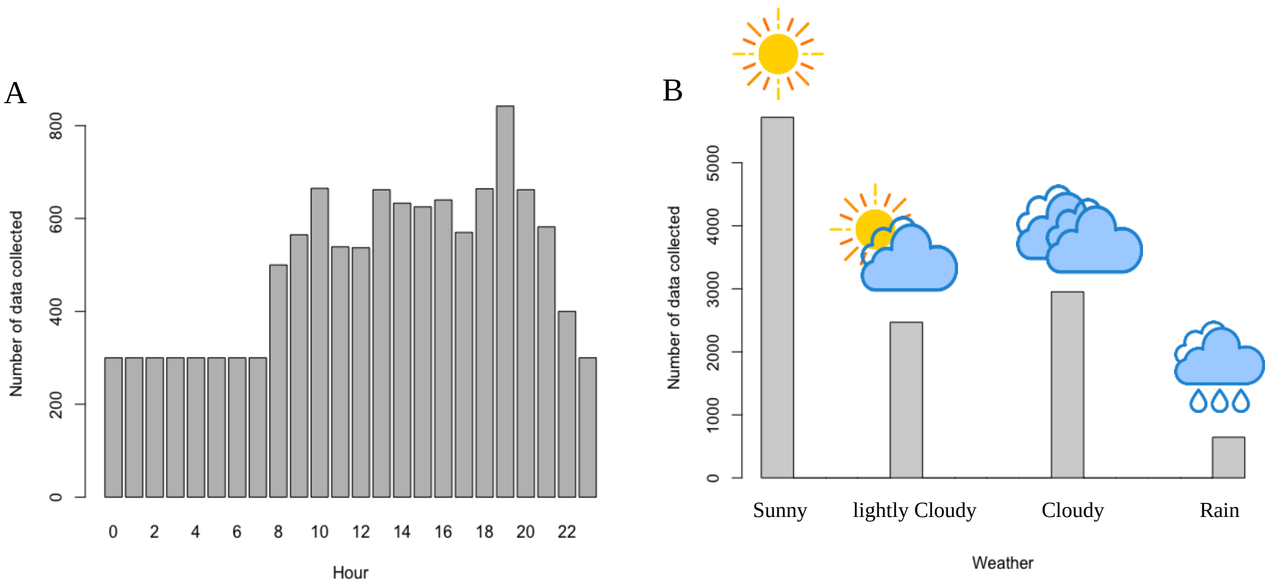

Figure 2. In A the total number of data collected, over all the species counting the individuals, is presented per hour. On the left side the plot shows the number of data collected for the four different weathers.

Over the time span of 26 days, I collected 11’787 measurements. Figure 2A shows, that the whole 24h cycle was covered, with more data collected during the day than at night. In Figure 2B the data collected for the weather is presented with more data collected when it was sunny, as the summer of 2022 was quite dry and sunny and did not have a lot of rainy or cloudy days. For the species the flowers were counted as open if more than 25% of the present flowers of the selected individuals were open. For instance *M. jalapa* was counted as open between 7 pm and 10 am (Figure 3B) Day-flowering species open their flowers between 9 am and 1 pm and close them again between 1 pm and 7 pm. Whereas night-flowering species opened their flowers between 7 pm and 10 pm and closed them again at 10 am (Table 2).

Table 2. Showing the measured opening times of the species. (>25% flower is open) And the number of data collected in the sun as well as shade, by species

Day-flowering Species	**Opens at**	**Closes at**	**Number of data collected**
Centaurium erythrae	9 am	7 pm	879
Cichorium intybus	9 am	1 pm	632
Convulus tricolor	9 am	2 pm	117
Delosperma sutherlandii	11 am	7 pm	1053
Eschscholzia californica	9 am	5 pm	523
Gazania sp.	1 pm	5 pm	869
Helianthemum nummularium	9 am	3 pm	106
Ipomoea purpurea	7 am	2 pm	1075
Linum perenne	9 am	4pm	30
Oxalis herrerae	10 am	2 pm	1220
Night-flowering Species			
Datura stramonium	7 pm	10 am	592
Oenothera biennis	10 pm	10 am	443
Mirabilis jalapa	7 pm	10 am	1089

In Figure 3 all of the night-flowering species show a similar opening and closing schedule. It can especially be seen in Figure 3A and B, with *D. stramonium* and *M. jalapa* as they both open at 7 pm and close at 10 am (Table 2). Day-flowering species show more variability with their opening and closing times, compared to the night-flowering species, especially for their closing times. For *C. intybus, C. tricolo*r and *L. perenne* there are missing values in the bar plots as I was not able to collect data at that time, due to the plants being replaced as they were done flowering (Figure 4B, C and I). These can be explained by missing measurements at certain timepoints, as they only were present for a short period of time in the flower clock (Table 1).

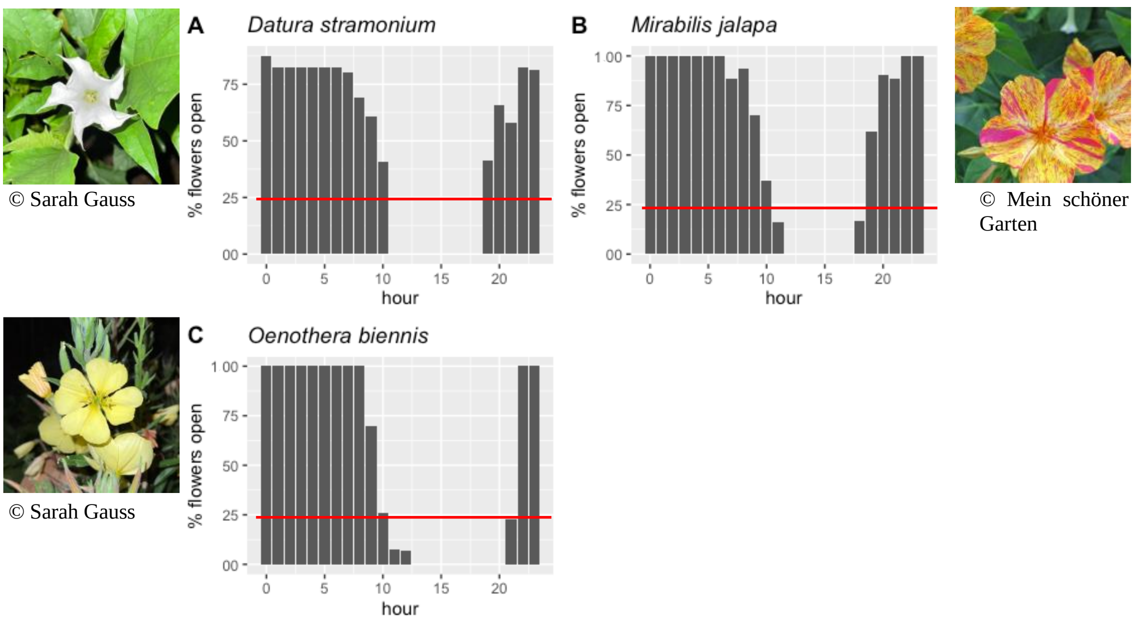

Figure 3. Bar plots of the night-flowering species showing the percentage of open flowers per hour with the red line highlighting the 25% margin.

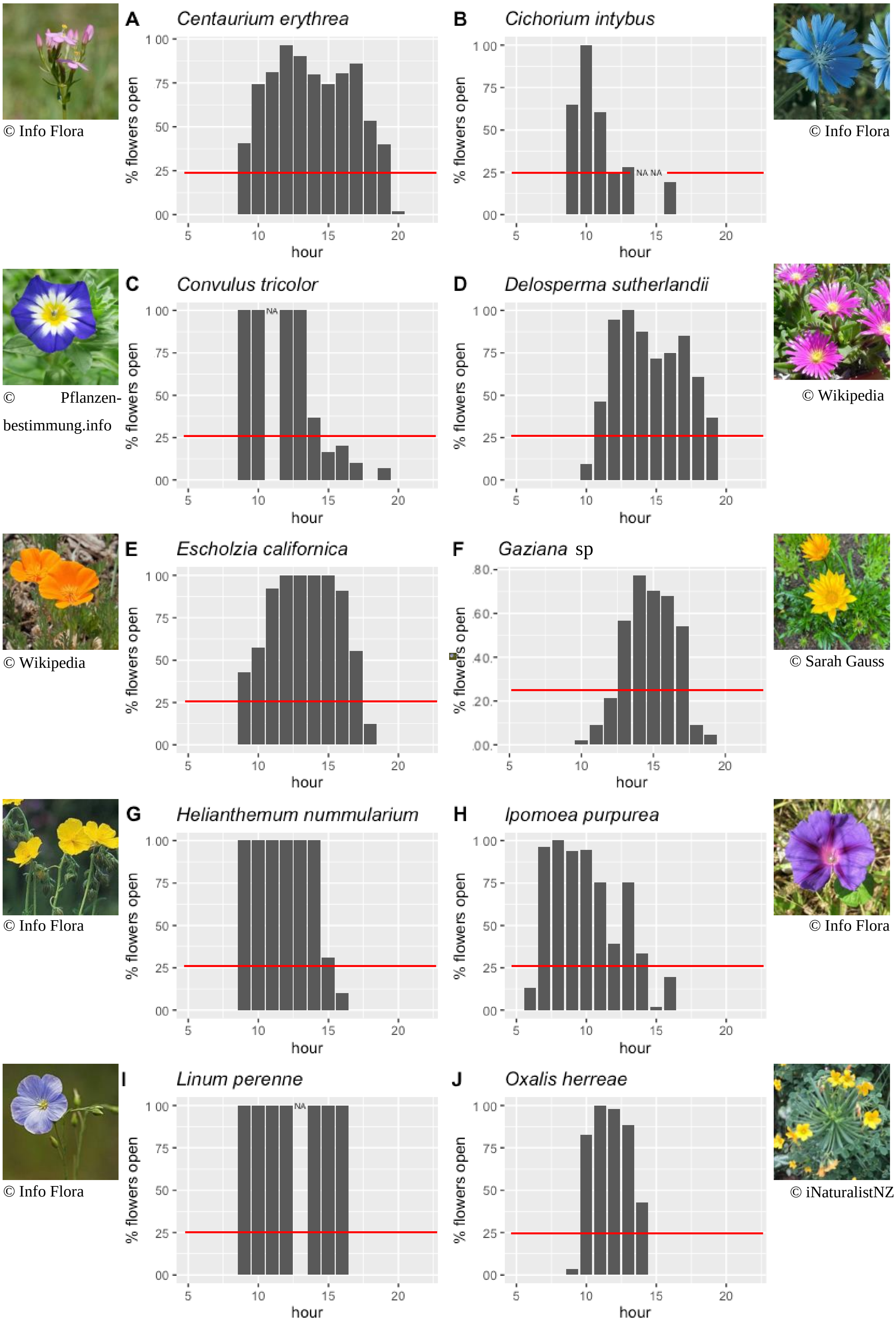

Figure 4. Bar plots of the day-flowering species showing the percentage of open flowers per hour and the red line highlighting the 25% margin.

Table 3. Odds ratios, standard errors (SE) and p-values found from the glmer analysis for the interaction of the different variables for the flower opening of the different species. With "shade" referring to individuals standing in shade.

Species	***Explanatory variables***	***Odds Ratios***	***SE***	***P-Value***
Centaurium erythrea	Temperature	2.21	1.05	0.093
	Light intensity	1	0	**<0.001**
	Humidity	0.98	0.07	0.828
	Shade	0.56	0.42	0.435
	Temperature shade	3.5	1.79	**0.014**
	Light intensity shade	1	0	0.184
	Humidity shade	1	0.15	0.495
Cichorium intybus	Temperature	0.98	1.25	0.985
	Light intensity	1	0	0.428
	Humidity	1.13	0.26	0.605
	Shade	0	0	**<0.001**
	Temperature shade	0	0	**<0.001**
	Light intensity shade	0.82	0	**<0.001**
	Humidity shade	0	0	**<0.001**
Convulus tricolor	Temperature	2.01	0	**<0.001**
	Light intensity	1	0	**<0.001**
	Humidity	14.13	0.03	**<0.001**
	Shade	0.06	0	**<0.001**
	Temperature shade	1291.04	378046.2	0.98
	Light intensity shade	1	0.01	0.643
	Humidity shade	427.15	34037.96	0.939
Datura stramonium	Temperature	0.53	0.14	**0.018**
	Light intensity	1	0	**0.001**
	Humidity	0.98	0.04	**0.016**
	Shade	16.89	0.007	**0.0007**
	Temperature shade	0.81	0.27	0.527
	Light intensity shade	1	0	0.273
	Humidity shade	1.06	0.11	0.576
Delosperma sutherlandii	Temperature	5.7	4.39	**0.024**
	Light intensity	1	0	**0.006**
	Humidity	0.96	0.11	0.748
	Shade	0.01	0.01	**0.001**
	Temperature shade	1.98	1.18	0.255
	Light intensity shade	1	0	0.972
	Humidity shade	0.63	0.14	0.039
Eschscholzia califonica	Temperature	3.85	0.01	**<0.001**
	Light intensity	1	0	**<0.001**
	Humidity	1.3	0	**<0.001**
	Shade	0.14	0	**<0.001**

	Temperature shade	3.19	0	**<0.001**
	Light intensity shade	1	0	**<0.001**
	Humidity shade	1.21	0	**<0.001**
Gazania sp.	Temperature	1.57	0.36	0.051
	Light intensity	1	0	**0.006**
	Humidity	0.98	0.06	0.748
	Shade	0.52	0.31	0.276
	Temperature shade	1.27	0.39	0.438
	Light intensity shade	1	0	0.43
	Humidity shade	0.79	0.08	**0.023**
Helianthemum nummularium	Temperature	0.1	0.06	0.074
	Light intensity	0	0	0.302
	Humidity	0.01	0.01	0.61
Ipomoea purpurea	Temperature	0.64	0.49	0.558
	Light intensity	1	0	0.053
	Humidity	1.8	0.66	0.111
	Shade	27.47	45.88	**0.047**
	Temperature shade	0.29	0.25	0.156
	Light intensity shade	1	0	0.598
	Humidity shade	1.29	0.36	0.356
Mirabilis jalapa	Temperature	0.26	0.12	**0.003**
	Light intensity	1	0	**<0.001**
	Humidity	0.83	0.09	0.065
	Shade	2.89	4.34	0.479
	Temperature shade	0.46	0.28	0.205
	Light intensity shade	1	0	0.054
	Humidity shade	1.21	0.25	0.355
Oenothera biennis	Temperature	0.09	0.17	0.214
	Light intensity	1	0	0.535
	Humidity	1.45	0.51	0.288
	Shade	0.03	0.24	0.644
	Temperature shade	0	0	**<0.001**
	Light intensity shade	0.99	0	0.114
	Humidity shade	1	0.48	0.993
Oxalis herrerae	Temperature	0.96	0	**<0.001**
	Light intensity	1	0	0.124
	Humidity	1.02	0	**<0.001**
	Shade	0	0	**<0.001**
	Temperature shade	182.91	244.6	**<0.001**
	Light intensity shade	1	0	0.391
	Humidity shade	4.38	1.81	**<0.001**

All species except for *H. nummularium* had a significant effect with at least one environmental factor (light intensity, temperature, relative air humidity, standing in the shade) (Table 3). For *H. nummularium* there were not enough measurements, which explains the exclusion of the results from the shade (Table 3). *L. perenne* could not be analysed, as there were a lot of NAs as well as not enough data overall (Table 2).

5. Discussion

In general, a clear pattern for the opening times of the species, with quite a precise opening and closing time can be observed. Those patterns are species specific and the day-flowering species differ in their opening times. When comparing the expected opening times from 2021 to the measured opening times in 2022, there could be seen some differences such as in the opening time of *Gazania* sp. (Figure 1 and Table 3). With the species *L. perenne* one should be careful regarding the flowers, they get blown away by wind, which means the data collected will not be accurate. It is interesting to see that *I. purpurea* did not show any significances regarding the influence on the opening of light intensity, relative air humidity and temperature, as I was able to observe during the data collection that they closed their flowers around 15'o'clock, which is when the temperature was the highest. The closing of the flowers at the highest temperature could be a protection against losing to much water, as it has been shown in previous studies that stress signalling impacts the flower openings and closings (Seo & Mas 2015). On the other hand, in *C. tricolor* all the abiotic factors were significant. A similar reaction in *C. tricolor* to high temperatures/light intensity as with *I. purpurea* was observed while collecting the data, where blue coloured petals closed and white petals stayed open. One can observe that the lighter colours did not close, even though the darker ones closed up. This phenomenon could be due to lighter colours reflecting more light and with this staying cooler than the darker ones. Overall, the species, with exception for *H. nummularium,* at least one of the weather measurements consisting of temperature, relative air humidity and light intensity had a significant impact on the flower petal movements. But the significance of the influence of the three different weather measurements on the opening behaviour of the species is species dependent. For seven of the thirteen species, standing in the shade showed a significance regarding the opening/closing of the flowers. By this it can be assumed that plants are able to sense if they are in the shade and react to it by closing their flowers. Depending on the species, difference was quite extreme and could also be observed while collecting the data. An example for this is *D. sutherlandii,* whose flowers started to close again while standing in front of them and by that creating a shade on them. Within the framework of my bachelor thesis, I was not able to consider the data as a time series in my statistical analysis, so this should be considered in further analysis of the data collected. Furthermore, more experiments could be made, such as a greenhouse experiment, where the measured abiotic variables of this observational study could be controlled. Doing this could help to further understand the significances found in the abiotic factors an if they overpower the inner circadian clock of the species.

6. Conclusion

This observational study showed that there is a species-specific opening and closing pattern of the flowers. In seven out of ten day-flowering species, individuals reacted to standing in the shade by closing their flowers. Generally, there was a significant influence by at least one of the abiotic factors measured. This study has showed that not all species react the same way to the same abiotic factors. Which means, that further studies are needed to get a better understanding of the complex world of the floral movements, as there are many factors that seem to influence species.

7. Acknowledgements

First, I would like to thank Dr. Katja Rembold for all her help and support. For the statistical analysis I would like to thank Dr. Caterina Penone as well as Noëlle Schenk for helping me figure out how to handle and analyse the data collected. For taking care of the floral clock I would like to thank Anna Thöni. And lastly, I would like to thank Fabienne Aebersold and the BOGA cat for staying up with me the whole night to collect the data.

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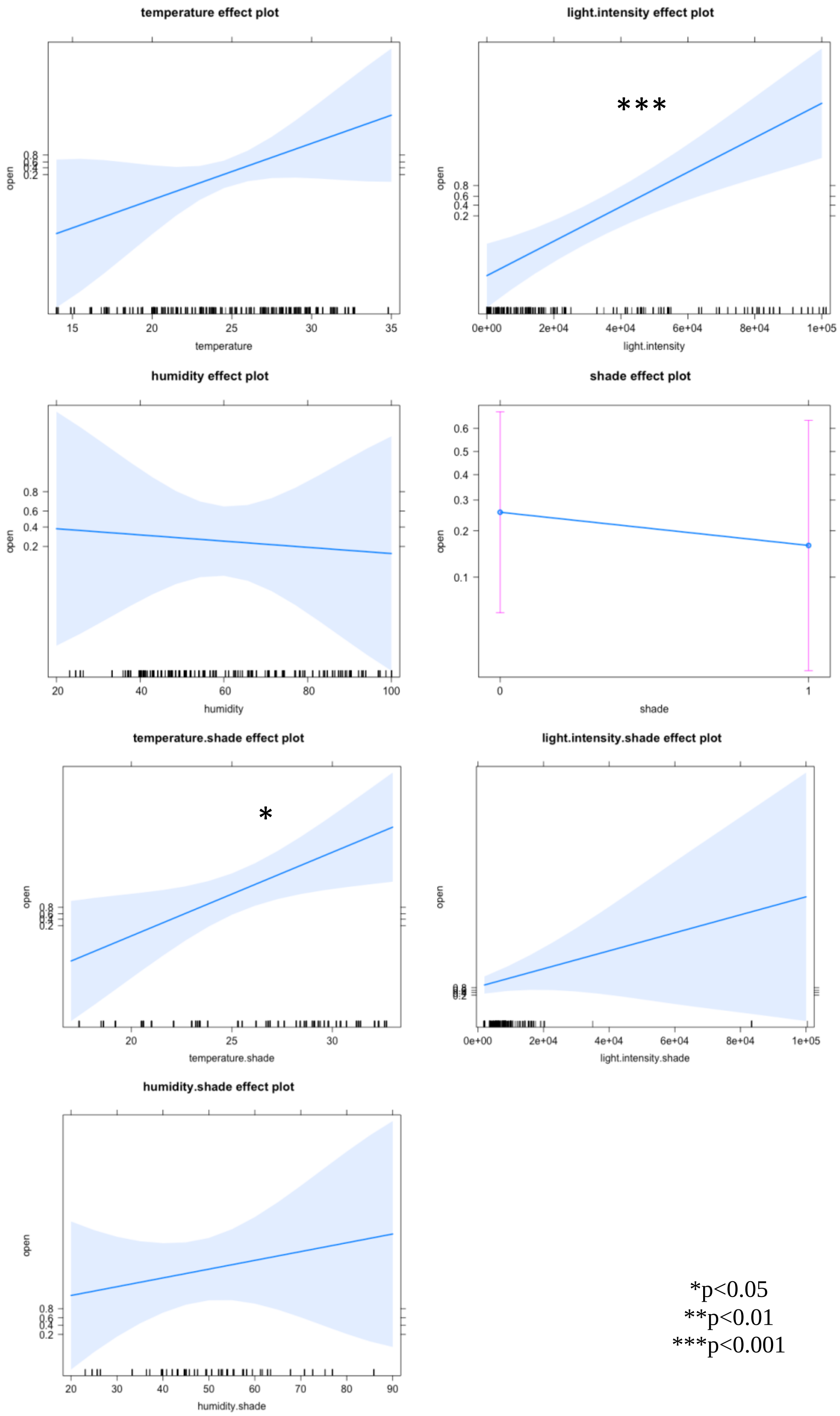
9. Appendix

<u>*Centaurium erythrae*</u>



*p<0.05
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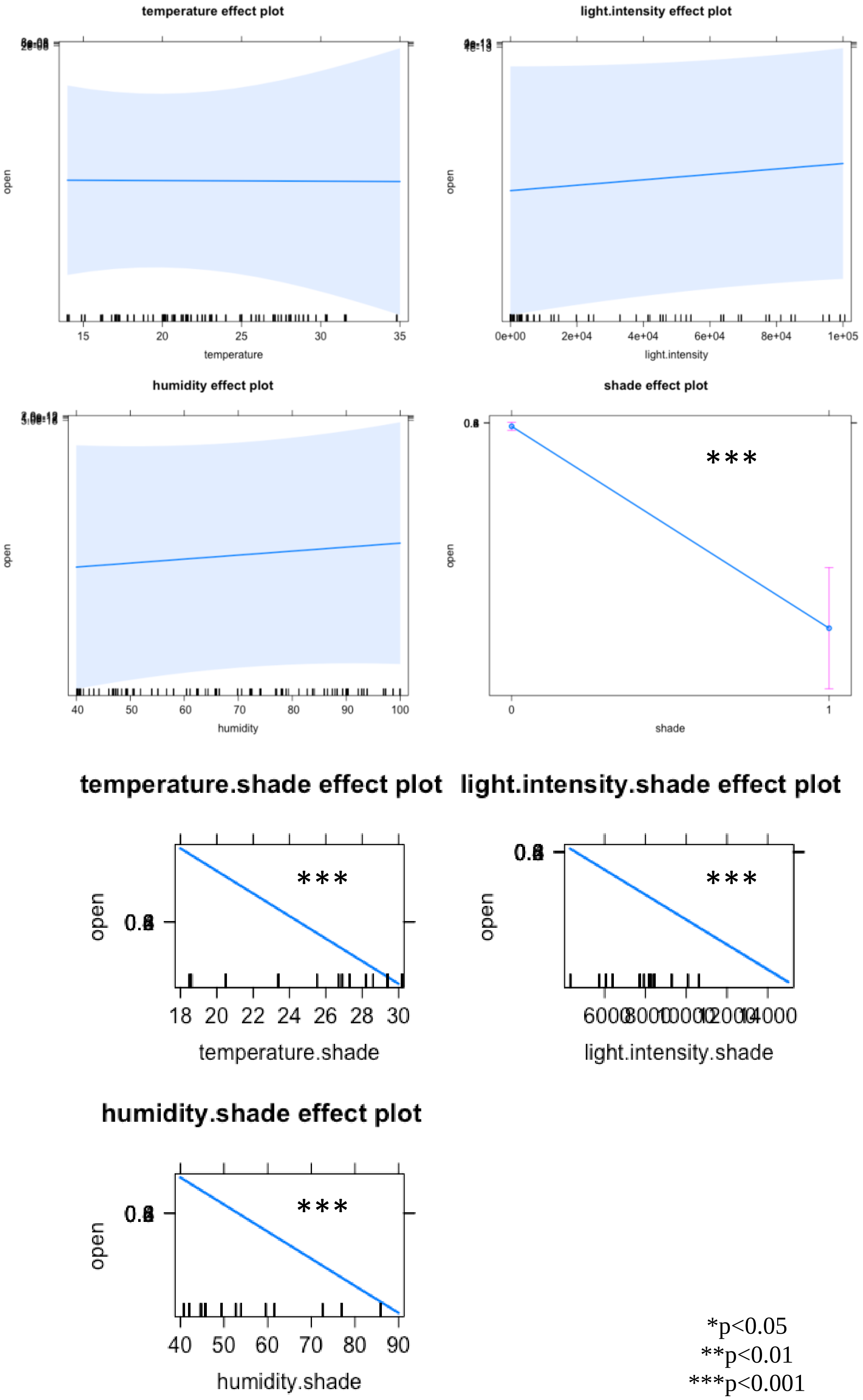
Cichorium intybus



*p<0.05
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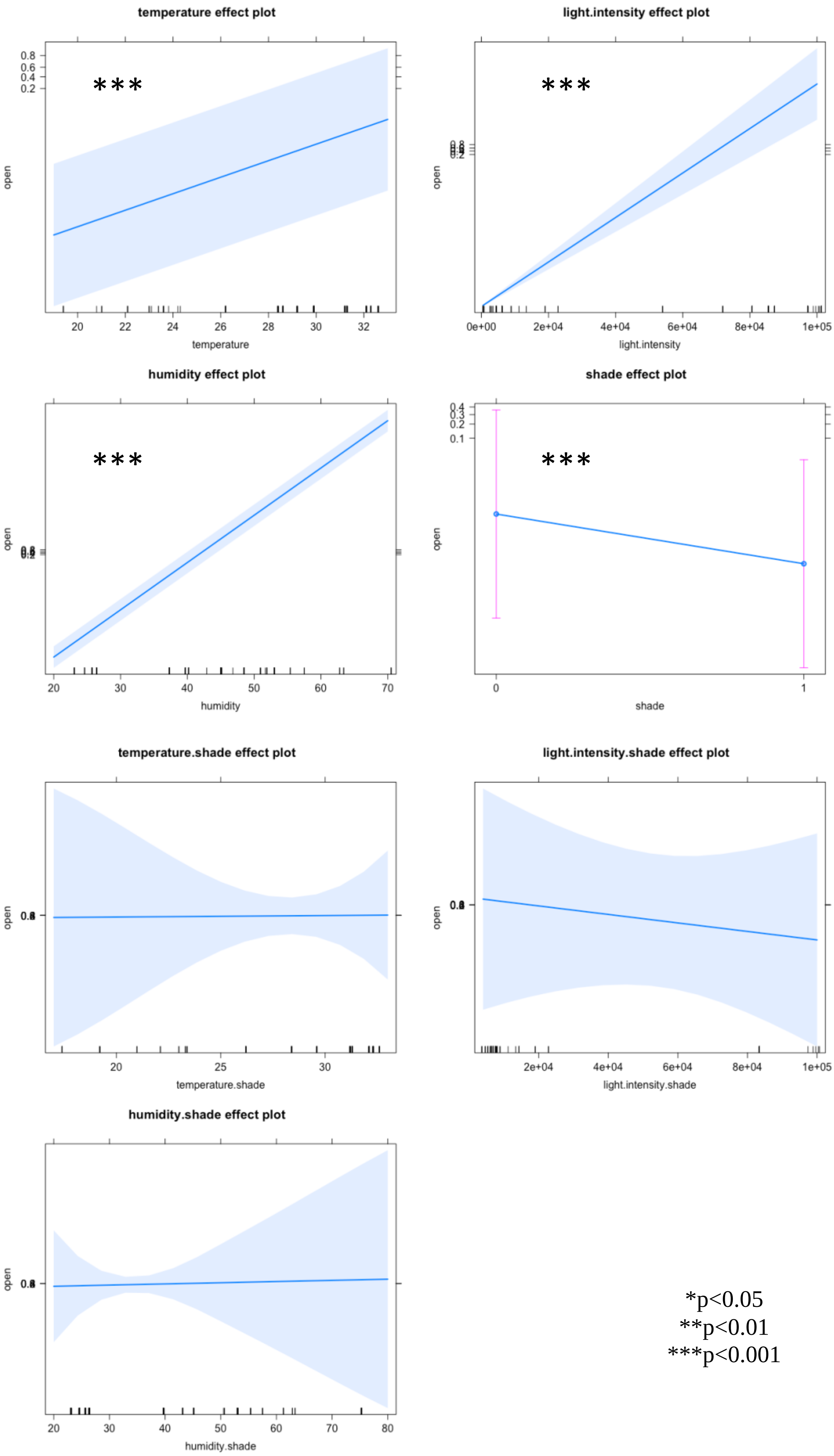
Convulus tricolor



*p<0.05
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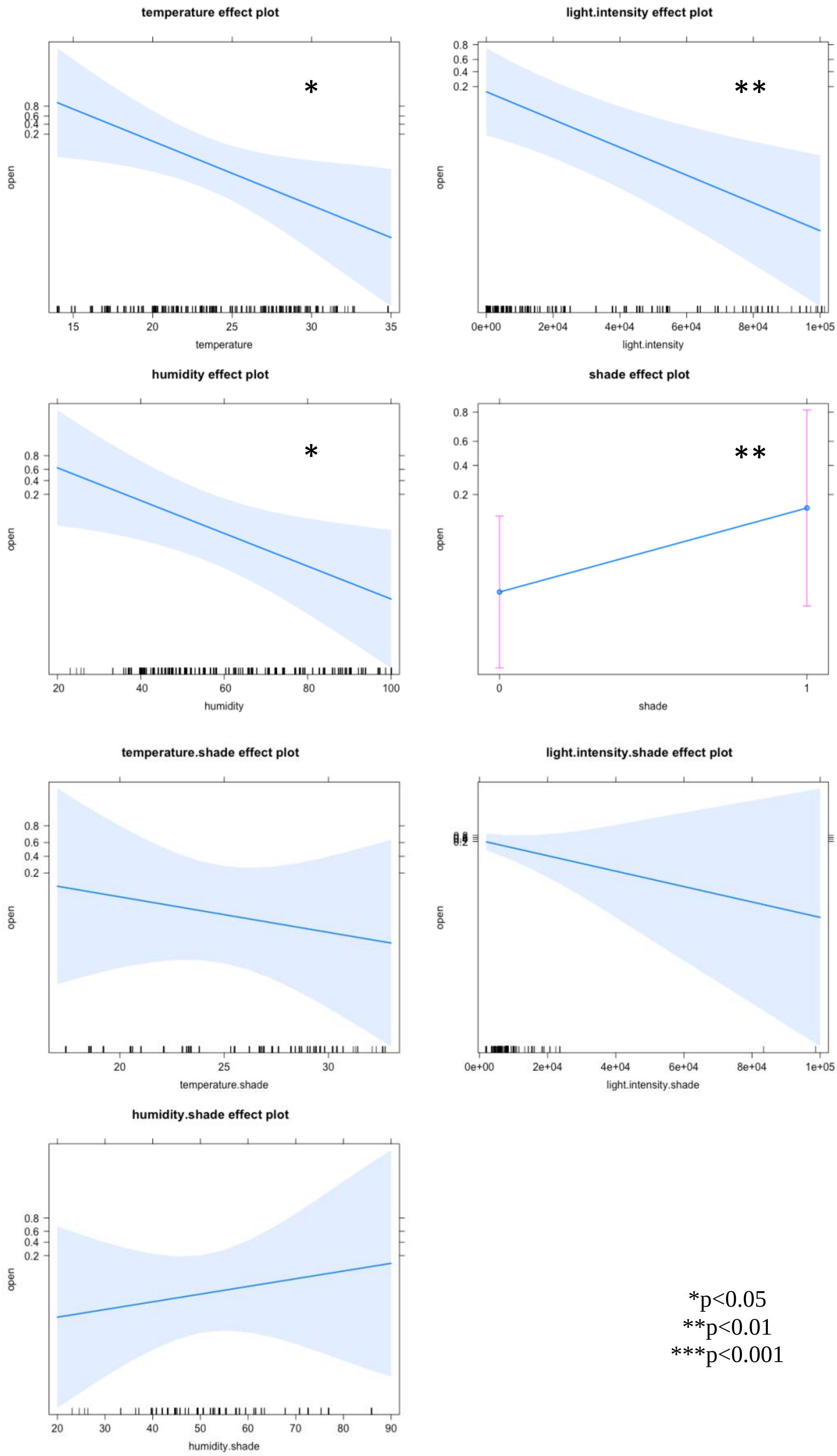
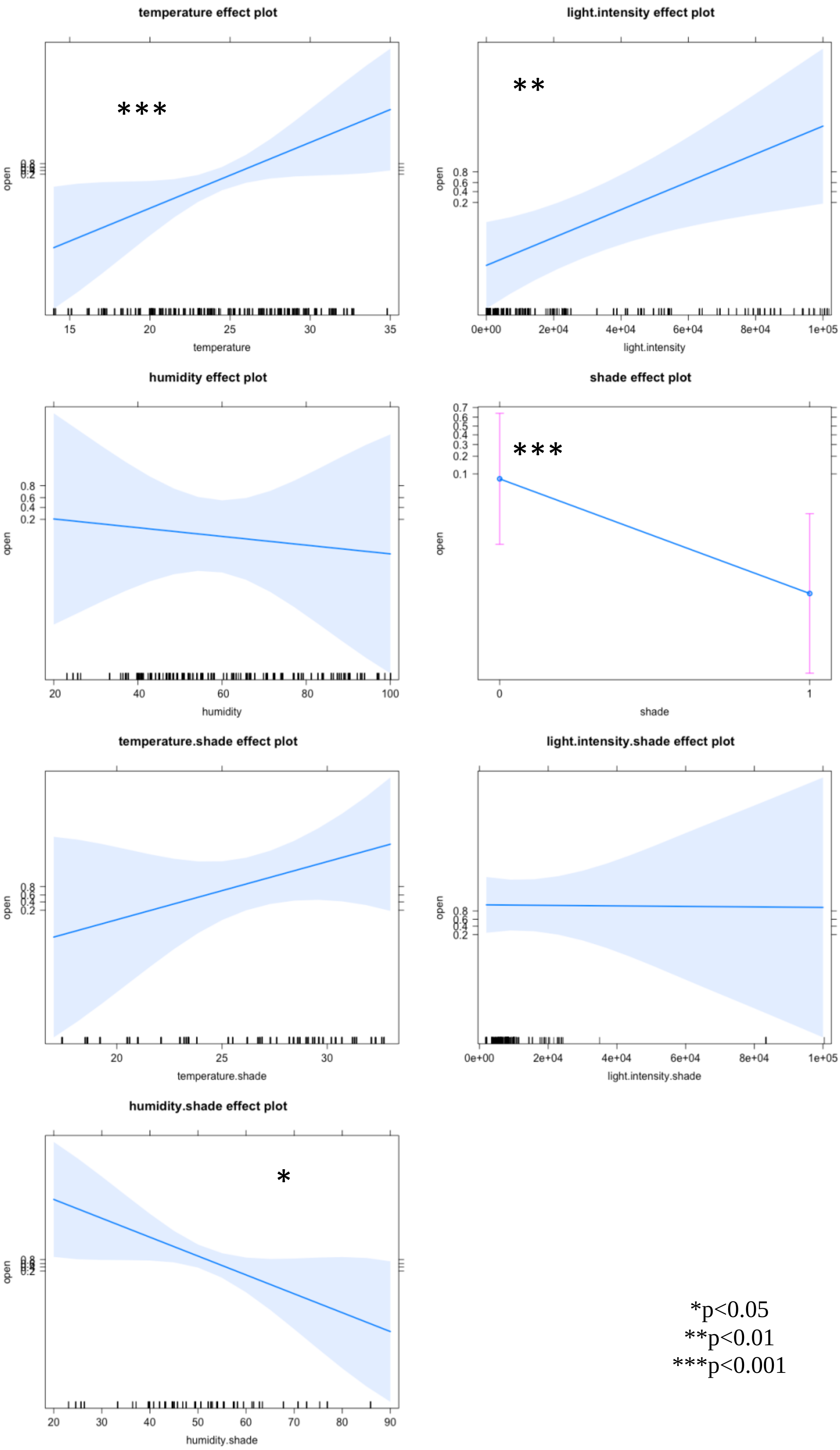
Datura stramonium



*p<0.05
**p<0.01
***p<0.001

<u>*Delosperma sutherlandii*</u>



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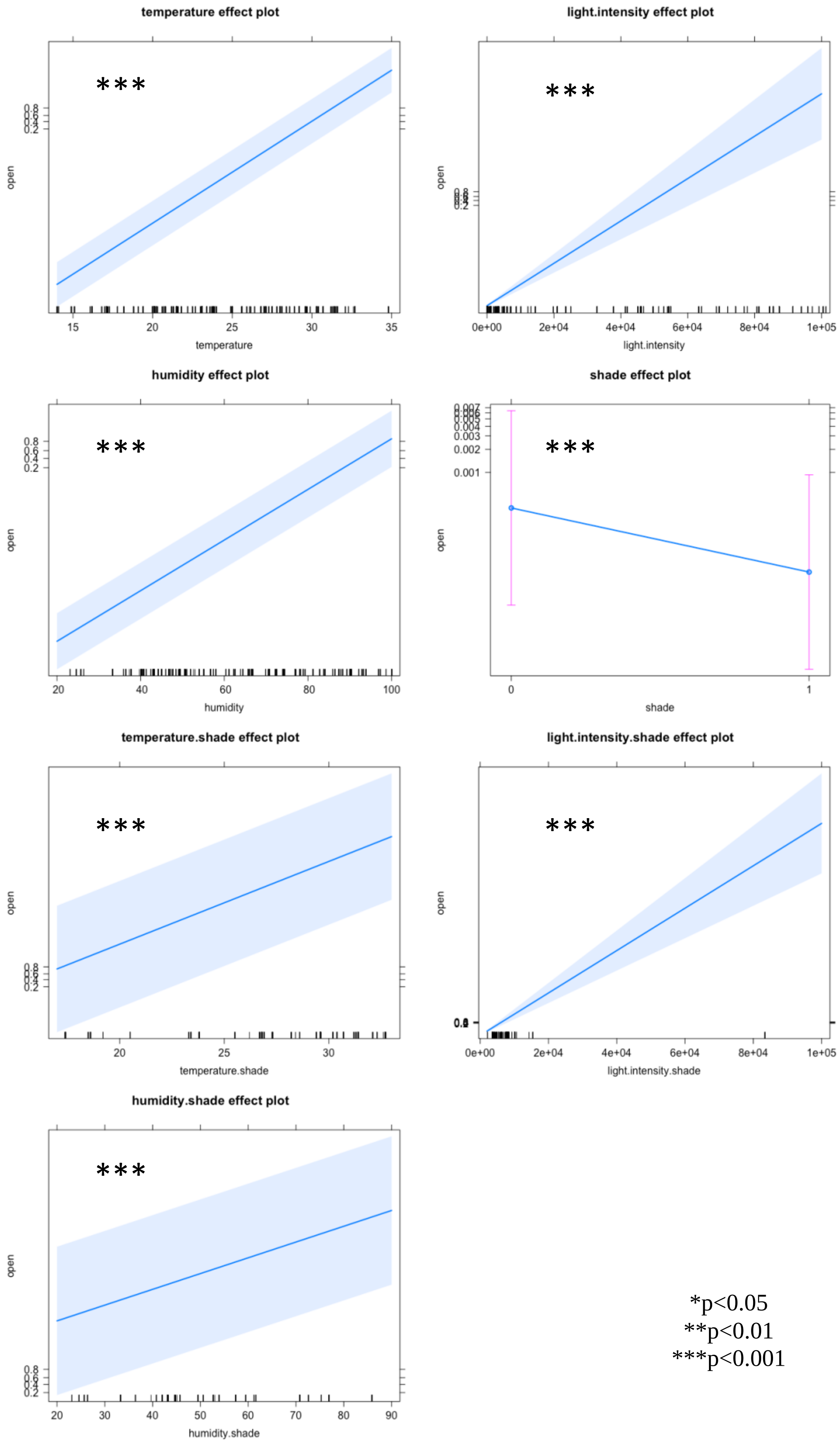
**p<0.01
***p<0.001

Eschscholzia californica



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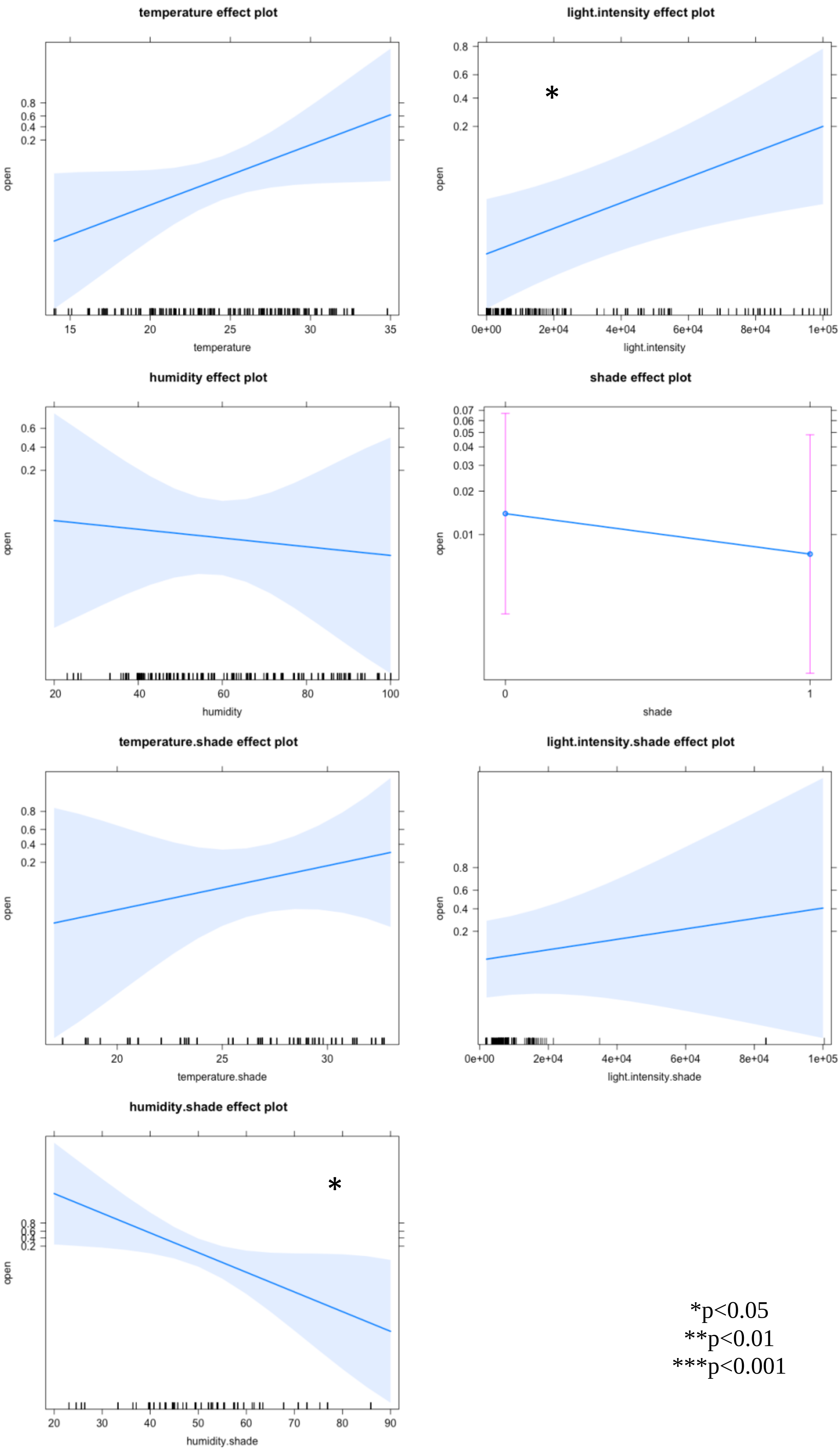
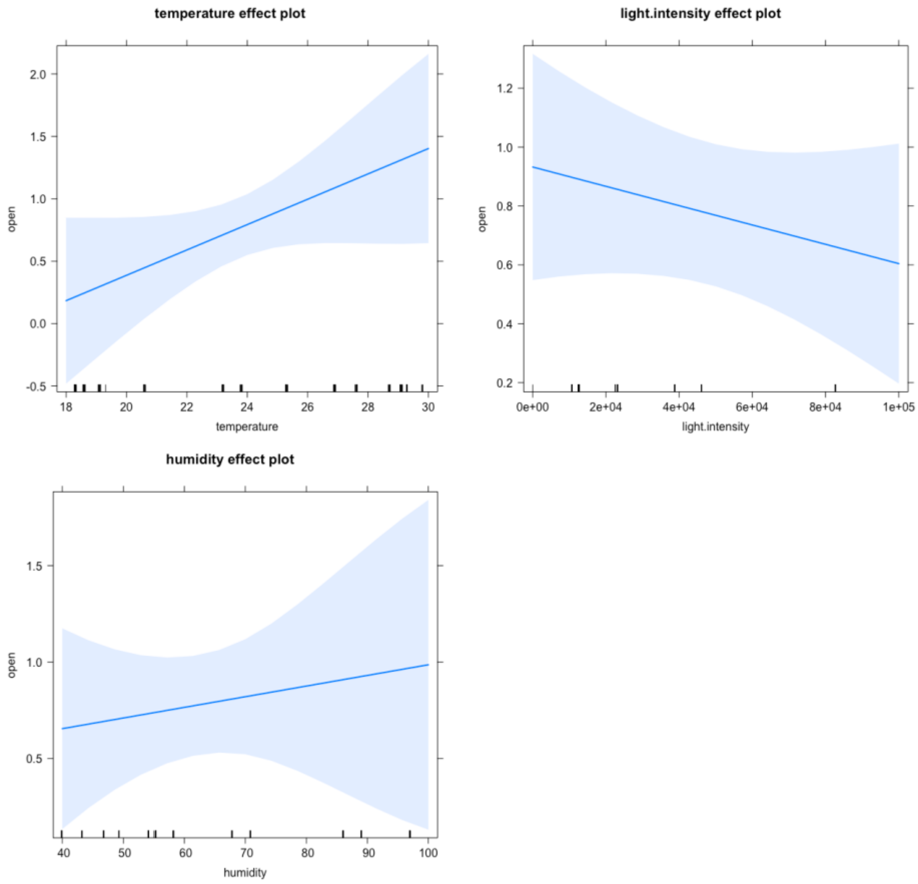
Gazania sp.



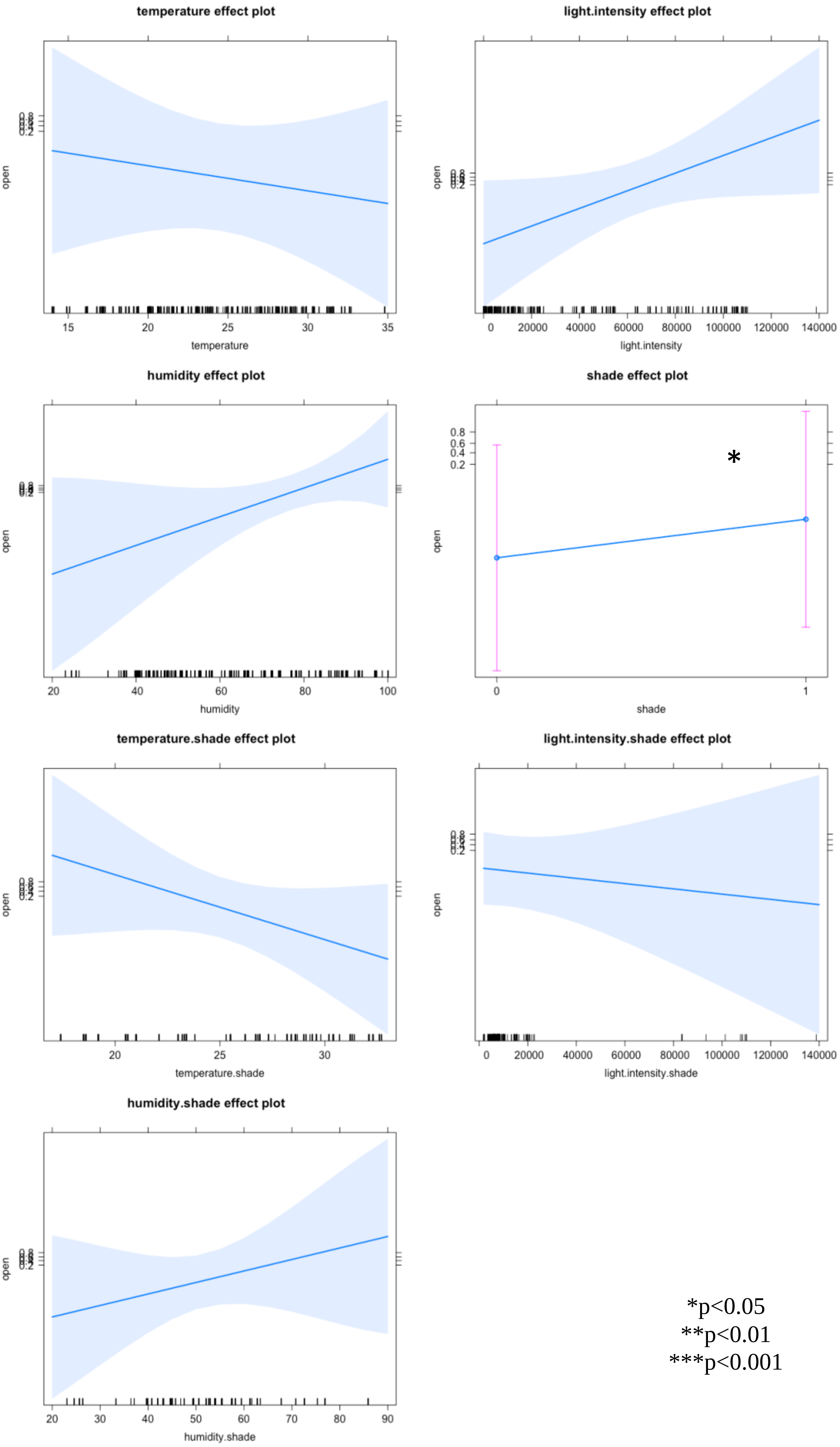
*p<0.05
**p<0.01
***p<0.001

Helianthemum nummularium



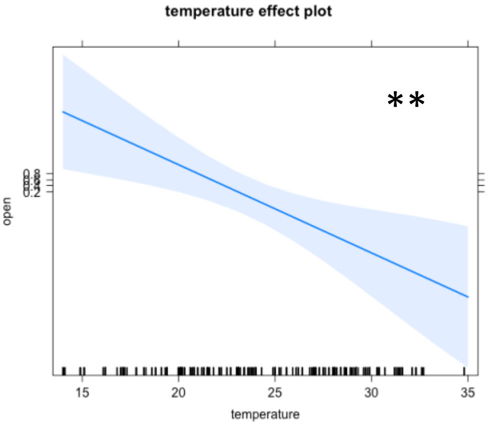
*p<0.05
**p<0.01
***p<0.001

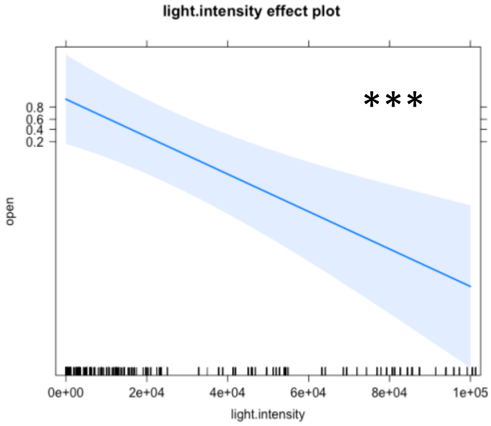
Ipomoea purpurea

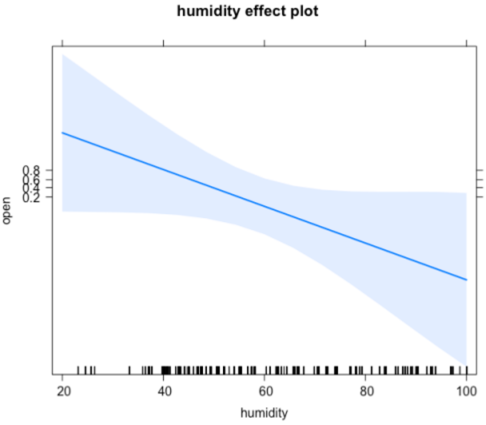


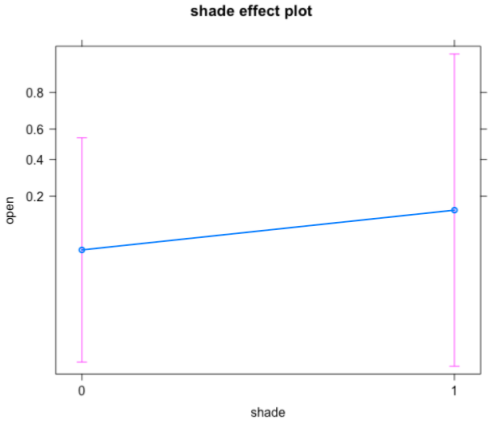
*p<0.05
**p<0.01
***p<0.001

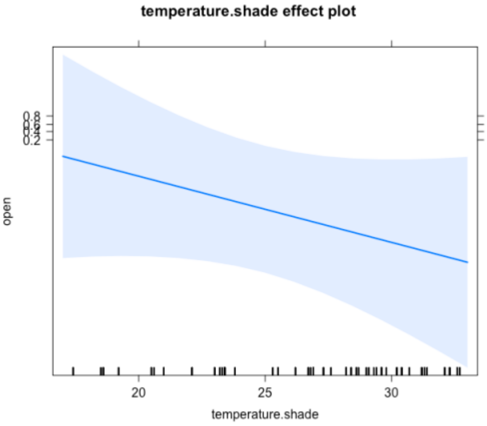
Mirabilis jalapa

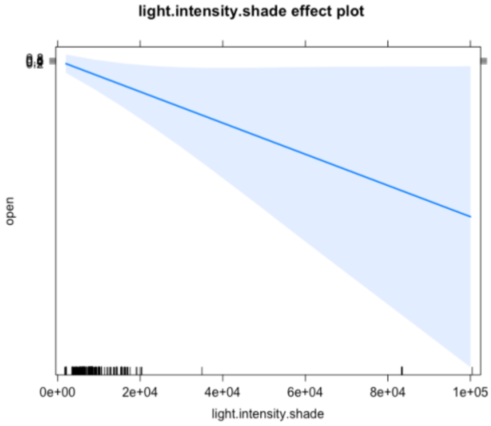


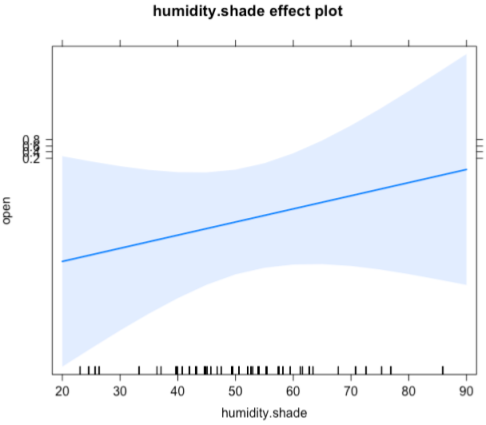






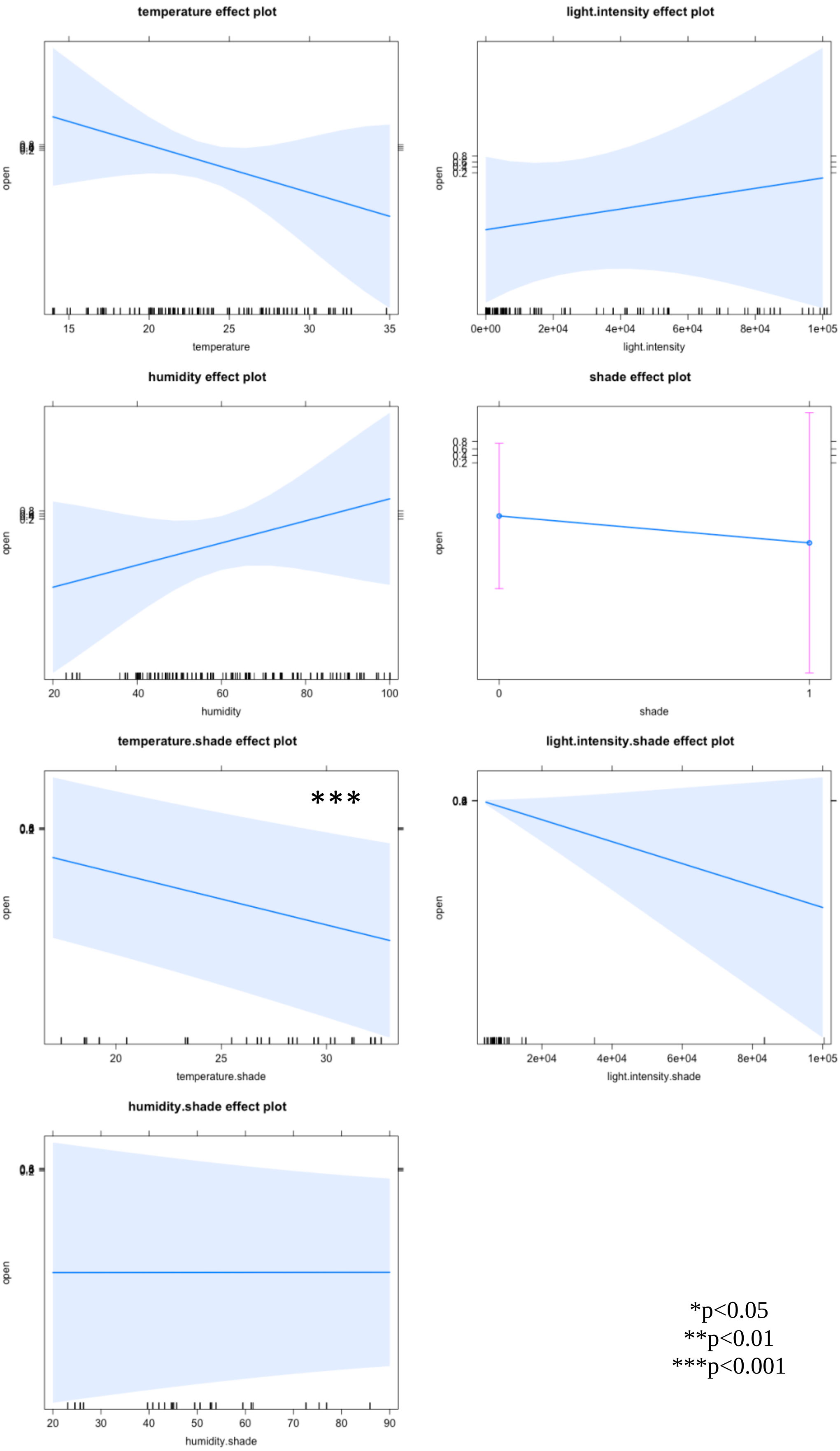






*p<0.05
**p<0.01
***p<0.001

Oenothera biennis



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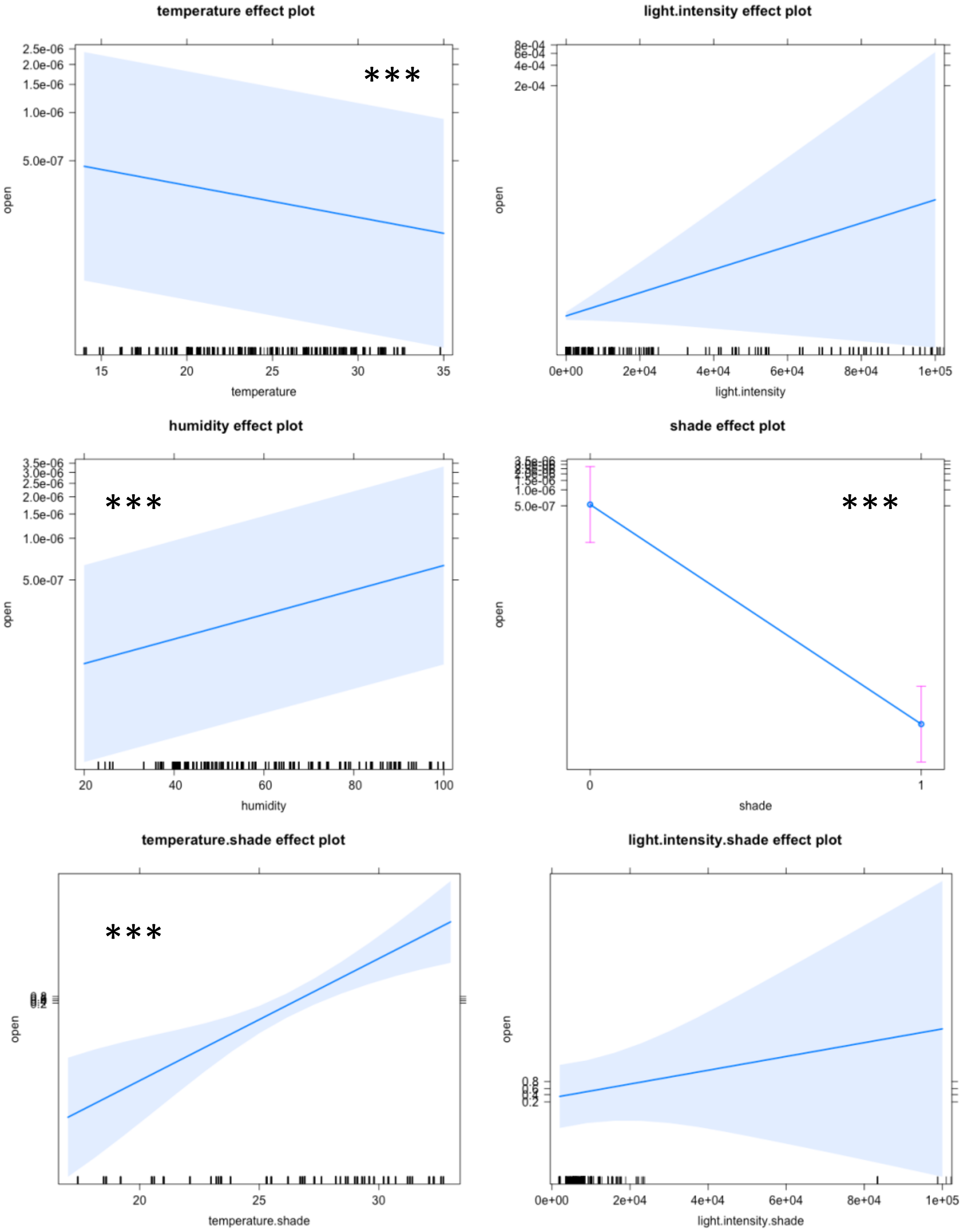
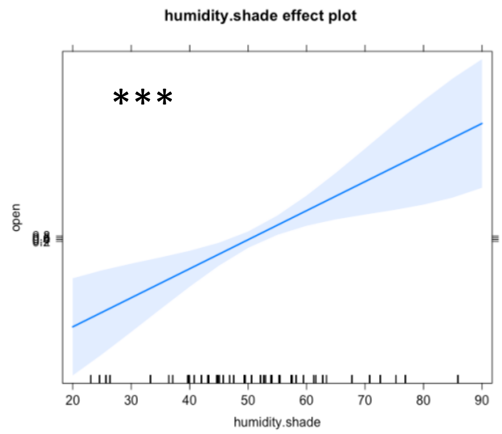
***p<0.001

<u>*Oxalis herrerae*</u>





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Erklärung

gemäss Art. 30 RSL Phil.-nat.18

Name/Vorname: Gauss Sarah

Matrikelnummer: 17-418-716

Studiengang: Bachelor of Science in Biology

Bachelor ☑ Master ☐ Dissertation ☐

Titel der Arbeit: "Quarter to daisy"
the complex world of floral movements.

LeiterIn der Arbeit: Dr. Katja Rembold
Prof. Dr. Markus Fischer

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Ort/Datum

Bern der 27. März 2023

Unterschrift Sarah Gauss