Pollen-placement and pollen-portioning in diverse *Salvia*-species

Dissertation zur Erlangung des Grades
„Doktor der Naturwissenschaften"

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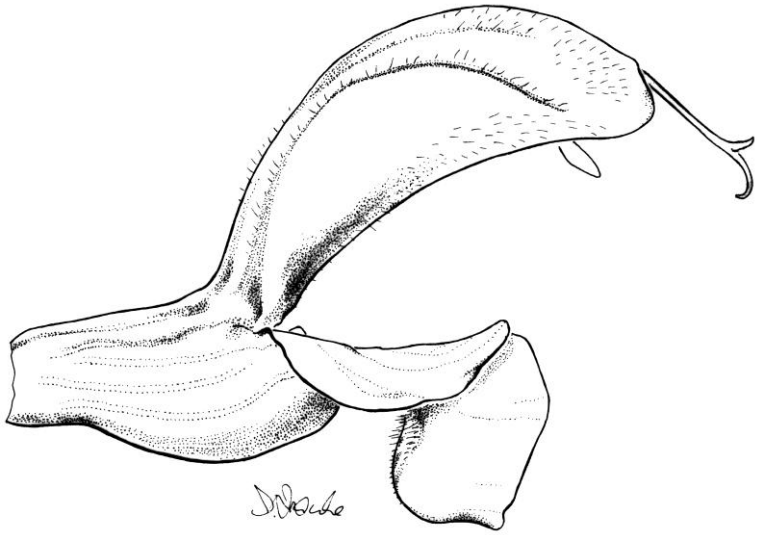

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Zusammenfassung

Die vorliegende Dissertation befaßt sich mit dem Staubblatthebelmechanismus der Gattung *Salvia*. Verschiedene Hypothesen zu seiner genauen Funktion und seiner Funktionsweise werden geprüft und erläutert. Die Hypothese, daß der Hebelmechanismus eine mechanische Barriere darstellt, die der Bestäuberselektion dient, indem sie schwache Bestäuber aus der Blüte ausschließt, wird widerlegt. Hierzu werden die Ergebnisse von Kraftmessungen und morphologischen Untersuchungen, die an den Staubblatthebeln und Blüten 8 bienenbestäubter (melittophiler) und 6 vogelbestäubter (ornithophiler) Salbeiarten durchgeführt wurden, dargelegt, statistisch ausgewertet und diskutiert. An einer weiteren melittophilen Art, ohne funktionierenden Staubblatthebel, wurden zum Vergleich Kraftmessungen an anderen Blütenstrukturen vorgenommen, welche ein Hindernis für einen Blütenbesucher darstellen könnten. Die Hypothesen nach denen der Staubblatthebel der Pollenportionierung dient und eine wiederholte, exakte und artspezifische Pollenplazierung auf diversen Bestäubern, vollführen kann und somit das Risiko von Pollenverlust und Hybridisierung mindert, werden hingegen bestätigt. Untersuchungen zur Pollenportionierung wurden an 13 Salbeiarten durchgeführt. Der Hebelmechanismus kann mehrfach hintereinander ausgelöst werden. Dabei geben die Pollensäcke genau plazierte Pollenportionen an den Bestäuber ab. Pollenplazierung wurde an 12 Salbeiarten untersucht. In sympatrisch vorkommenden Salbeiarten sind Hebellänge und Pollenablageplatz von besonderem Interesse. Auf einem gemeinsamen Bestäuber finden sich so artspezifische Areale der Pollenablage für die einzelnen Salbeiarten. Die genaue Pollenplazierung sorgt hierbei für eine effiziente Bestäubung. Die Frage nach der genauen Funktionsweise des Hebelmechanismus hingegen kann in dieser Arbeit nicht zweifelsfrei geklärt werden. Das Zurückschwingen des Hebels wird nicht durch den adaxialen Hebelarm verursacht. Um als Gegengewicht zum abaxialen Hebelarm zu dienen und diesen in seine ursprüngliche Position zu bewegen, ist der adaxiale Arm zu leicht und zu kurz. Es konnten dagegen Hinweise auf eine Art Federmechanismus, der im Filament auf zellulärer Ebene arbeitet, gefunden werden. Dies scheint nach momentanem Erkenntnisstand, die plausibelste Erklärung für die Hebelbewegung zu sein. Um dies jedoch eindeutig zu klären, müssen weitere histologische Untersuchungen zum Gelenkkomplex des Hebelmechanismus durchgeführt werden

Summary of the thesis

This dissertation addresses the staminal lever mechanism of the genus *Salvia*. Various hypotheses referring to its purpose and function are tested and elucidated. The first hypothesis maintains that the lever is a mechanical selection mechanism which excludes weak pollinators from the flower. This hypothesis is refuted and the respective results of force measurements and morphological investigations are presented, statistically evaluated and discussed. The force measurements and morphological investigations were conducted on the staminal levers and flowers of 8 bee pollinated (melittophilous) and 6 bird pollinated (ornithophilous) species. For comparison a ninth melittophilous species that lacks the staminal lever was investigated. In this species the force measurements were conducted on floral structures that were suspected to hinder a flower visitor. The hypotheses, which state that the staminal lever is a tool for pollen portioning and reduces the risk of pollen loss as well as hybridisation due to its ability to perform a repeatable, accurate and species-specific pollen placement on a wide range of diverse pollinators, are confirmed. Investigations with respect to pollen portioning were carried out on 13 sages. The lever mechanism can be released several times in a row, while the pollen sacs leave a dosed pollen portion on a well defined spot on the pollinator's body. Pollen placement was investigated for 12 sages. In sympatric sages, lever length and the area of pollen placement are of particular interest. A shared pollinator bears species-specific areas of pollen placement for different sages. The accurate pollen placement ensures an efficient pollination. However, the question of the functionality of the lever mechanism can not be answered with absolute certainty. The lever's backswing is not caused by the adaxial lever arm; the adaxial lever arm is too light and too short to be an adequate counterweight to the abaxial lever arm. Therefore, the adaxial lever arm can not pull the abaxial lever arm to return it to its neutral position. But there are indications of a cellular mainspring in the filament. According to the current state of knowledge, this is the most plausible explanation for the lever's backswing, but further histological investigations on the joint of the lever mechanism are necessary to confirm this assumption.

General Introduction

The genus *Salvia* with its over 900 species [ALZIAR 1988-1993] is characterized by a modification of the androeceum. Contrary to usual Lamiaceae only two stamina are developed in *Salvia*. A staminal lever mechanism deposits pollen on the pollinator's body. The morphology of this lever was first described by HILDEBRAND (1865), but SPRENGEL (1793) already mentioned the nototribic pollination mechanism. In the two remaining stamens, the connectives are elongated and a lever is formed. A joint, located at the point of contact of filament and connective, divides the connective in an adaxial and an abaxial arm and guarantees mobility like a seesaw [CORRENS 1891]. The core piece of this joint is a tiny ligament which connects filament and connective. Usually, the adaxial arm is sterile and approximately widened like a palm. In the majority of cases, only the abaxial arm bears pollen sacs. The two connectives of the neighbouring stamens, which can be clotted or fused, form a functional unit. Animals looking for nectar have to push back the adaxial arms, which impede admittance to the nectar by obstructing access to or even sealing the tube. While this barrier is pushed back with head or beak, the abaxial lever arm sinks until the pollen sacs hit the pollinator's body and pollen is placed on the animal. When the pollen-loaded animal approaches another flower, the pollen is deposited on the stigma, which is positioned such that it can grab the pollen off the animal's body [MÜLLER 1873, CORRENS 1891, TROLL 1929, HRUBÝ 1934, TRAPP 1956, WERTH 1956, CLAßEN-BOCKHOFF et al. 2003, 2004a]. Furthermore, the pollen positioning not always has to work in a vertical way, causing nototribic pollination. Several modified staminal levers are known which offer sternotribic or plagiotribic pollination or even species which entirely lack the lever mechanism [HILDEBRAND 1865, CLAßEN-BOCKHOFF et al. 2004b, WESTER & CLAßEN-BOCKHOFF 2007].

The lever mechanism has been the subject of many investigations and much research, but it is still not known, which purpose this unique lever serves and how it works. It was hypothesised whether the lever mechanism is a selection tool which excludes certain pollinators [CLAßEN-BOCKHOFF et al. 2003, THIMM et al. 2003] or whether it widens the range of pollinators [CLAßEN-BOCKHOFF et al. 2004a, WESTER & CLAßEN-BOCKHOFF 2006a, 2006b, KUSCHEWITZ 2004]. Other hypotheses assumed the lever mechanism to be an instrument for controlled pollen portioning [WESTER & CLAßEN-BOCKHOFF 2007] or pollen positioning [FAEGRI & VAN DER PIJL 1971, Grant 1994a, CLAßEN-BOCKHOFF et al. 2003].

This doctoral thesis joins a series of studies dealing with the morphology and the function of the staminal lever mechanism of the genus *Salvia* [TWERASER 2000, CLAßEN-BOCKHOFF et al. 2003, 2004b, THIMM et al. 2003, KUSCHEWITZ 2004, REITH et al. 2006, 2007, WESTER & CLAßEN-BOCKHOFF 2006a, 2006b, 2007]. The first chapter investigates the questions referring to the hypothesis that the lever could serve as a tool for pollinator selection. The second chapter demonstrates the lever's efficiency and accuracy in the pollination process and furthermore its possible meaning as a key innovation promoting adaptive radiation. The third chapter addresses the question how lever movement is managed. Histological investigations of the ligament shall reveal the mechanism that enables the repeatable lever movement. The only comparable investigations referring to this topic date back to the 19th century [CORRENS 1891]. The hypothesis that the ligament is responsible for the levers back swing [CORRENS 1891] has not been investigated until now.

1. Quantitative force measurements in diverse *Salvia*-species

1.1 Abstract

The motivation for force measurements on the staminal lever of *Salvia* was the question if the staminal lever of the genus *Salvia* serves as a selection tool which excludes weak and small pollinators. In 15 diverse *Salvia*-species from different sections and regions the forces necessary to move the pivoting stamen were measured by means of a special force measuring device. Ornithophilous as well as melittophilous species were investigated. The morphological data of flowers and staminal levers were recorded. The measured forces range from 1.08mN ± 0.6mN in *S. thermarum* to 21.34mN ± 21.88mN in *S. patens*. After analysis, the gathered data showed no correlation between morphometric data and the measured force. Also, no minimum effort exerted by pollinators or species-specific forces to move the levers were identified. *S. verticillata* lacks the staminal lever mechanism. In order to test whether they might restrict access to the nectar, floral structures like the upper lip and the hairy ring inside the corolla tube of *S. verticillata* were compared with the lever mechanism of the other investigated species. Regarding the forces needed to overcome these supposed obstacles, there were no essential differences detectable. The results of these investigations lead to the conjecture that the lever mechanism's primary function is to ensure an exact and repeatable pollen positioning and pollen portioning and not pollinator selection.

1.2 Introduction

The interaction between plants and pollinators is mostly physical. While pollinators seek food or sexual intercourse [KULLENBERG 1950], plants are looking for a pollinator that ensures fertilisation with as little loss of pollen as possible [WESTERKAMP 1993, 1997]. At any rate, physical interaction between plant and pollinator is unavoidable in the transfer of pollen from stamen to pollinator and further to the stigma. This includes that animals and plants have to muster, resist or demand certain forces or physical, mechanical load. At last some kind of physical contact is always necessary. So, certain specialized structures must have evolved to make it possible for plants and pollinators to interact.

A multiplicity of structures dealing with physical contact or rather enabling it can be found all over the plant kingdom. Buzz-pollination is an obvious example for physical interaction as the investigations of BUCHMANN & HURLEY (1978) show. In *Fabaceae*, stigma

and stamina are hidden in the carina, which has to be moved demanding a certain physical force by the pollinator [ETCHEVERRY et al. 2005**,** FAEGRI & VAN DER PIJL 1971, WESTERKAMP 1993, 1997]. *Marantaceae* developed a special tensed stylus, which catapults the pollen onto the pollinators' body [DELPINO 1869, CLAßEN-BOCKHOFF 1991, KENNEDY 1978, 2000, PISCHTSCHAN 2007, PISCHTSCHAN & CLAßEN-BOCKHOFF 2008, CLAßEN-BOCKHOFF & HELLER 2008, LEY 2008]. In *Zingiberaceae* even a lever mechanism evolved [TROLL 1929]. Some Orchids fire their pollen sacs onto an approaching pollinator [SIMONS 1992]. As these examples show, plants have found their own highly inventive structures and methods to interact with their pollinators.

The *Salvia*-lever and its existence have been described in detail several times [MÜLLER 1873, CORRENS 1891, TROLL 1929, HIMMELBAUR & STIBAL 1933-1935, HRUBÝ 1934, TRAPP 1956, WERTH 1956, CLAßEN-BOCKHOFF et al. 2003, 2004a, 2004b, THIMM et al. 2003, REITH et al. 2006], but it is still unclear what purpose it serves. There have been several hypotheses that it is a device for pollinator-selection, a barrier, which might exclude insects which are too weak to overcome it [CLAßEN-BOCKHOFF et al. 2003, THIMM et al. 2003]. Such assumptions of flowers excluding weaker pollinators by demanding a minimum force to gain access to the nectar have been made for other genera, too [EDWARDS et al. 2005]. Alternatively it could be a mechanism for controlled pollen portioning [WESTER & CLAßEN-BOCKHOFF 2007]. It also could be an instrument of pollen positioning [FAEGRI & VAN DER PIJL 1971] adjusting for different body sizes of different pollinators, leading to a higher diversity of pollinators [CLAßEN-BOCKHOFF et al. 2004a, WESTER & CLAßEN-BOCKHOFF 2006a, 2006b, KUSCHEWITZ 2004]. A further purpose of the lever could be a mechanical isolation mechanism in sympatric species [GRANT 1994a, CLAßEN-BOCKHOFF et al. 2004b, WESTER & CLAßEN-BOCKHOFF 2006a, RAMAMOORTHY & ELLIOTT 1998].

The barrier hypothesis has recently been questioned [THIMM et al. 2003, CLAßEN-BOCKHOFF et al. 2004a, REITH et al. 2006]. Earlier investigations including force measurements on *Salvia*-levers were not comprehensive, though. They dealt with fewer measurements, fewer species and less diverse species. Reith (2006) measured only melittophilous species and the sample as well as the number of force measurements per species was low (n=49 or less) [see REITH et al. 2006]. Walter (2000) investigated in a wider range of species, including ornithophilous species, but did not finish the investigations. The hypothesis that the required forces to move the *Salvia*-lever are no match for insects or hummingbirds had not been tested sufficiently. Questions whether the forces are species specific or whether ornithophilous and melittophilous species or species from the Old World

and species from the New World differ had been ignored. Furthermore, it is an interesting question, which morphological structures influence the levers mobility and whether there are other floral structures, like for example hairy rings, which might be obstacles.

The present study deals with these questions. Therefore more and especially more diverse species than in the earlier studies were investigated. The forces measured to move the levers in 14 different species are presented: melittophilous as well as ornithophilous species; and species from the New World as well as from the Old World. A *Salvia*-species without a lever was investigated, too (*Salvia verticillata* fig. 1.1P & 1.2D). Statistical tests were made to clarify whether there are significant differences between the forces required by the species and whether these forces are influenced by pollinators, origin of the species or morphology of the flowers.

Indeed the lever mechanism could serve a different purpose than originally thought. Keeping in mind that different sympatric species of *Salvia* share the same pollinators, a mechanism to avoid hybridisation could be quite ingenious. The staminal lever mechanism of *Salvia* could be an effective tool to place pollen precisely and individually for each species on a pollinator's body [FAEGRI & VAN DER PIJL 1979, Grant 1994a, CLAßEN-BOCKHOFF et al. 2003].

1.3 Material and Methods

1.3.1 Material

Fifteen morphologically and ecologically diverse *Salvia* species from different systematic sections and geographical regions were selected for the force measurements and morphometric investigations: *S. aethiopis* L., *S. africana-lutea* L., *S. austriaca* Jacq., *S. canariensis* L., *S. exserta* Griseb., *S. forskahlii* L., *S. glutinosa* L., *S. involucrata* Cav., *S. mexicana* L., *S. patens* Cav., *S. pratensis* L., *S. sclarea* L., *S. thermarum* Van Jaarsv., *S. uliginosa* Benth., *S. verticillata* L. (figs. 1.1 &. 1.2, tab. 1.1). All species are cultivated at the Botanical Garden of the University of Mainz. Fresh flowers from the Botanical Garden were used for investigations.

Five sages, namely *S. aethiopis*, *S. austriaca, S. glutinosa*, *S. pratensis* and *S. verticillata* occur sympatrically in Austria [TWERASER 2000]. Most of the species are bee pollinated, but six are bird pollinated (tab. 1.1). The flowers differ in size, length, height as well as in shape, construction and function of their staminal lever mechanism (figs. 1.1 & 1.2, tab. 1.1 & 1.2). While most species deposit their pollen by just sinking the staminal lever like

in *S. pratensis* (fig. 1.2A), three further variations of this mechanism can be found among the species which are investigated in this doctoral thesis (fig. 1.2B-C). In ornithophilous *S. exserta*, the movement of the lever is managed by a combination of joint-movement and the elastic filament (fig. 1.2B) [WESTER & CLAßEN-BOCKHOFF 2007]. In melittophilous *S. austriaca* the pollen is positioned lateral on the insect's body by shutting the stamina like a pair of scissors (fig. 1.2C) [CLAßEN-BOCKHOFF et al. 2004b]. *S. verticillata* does not possess a staminal lever mechanism. The pollen sacs are hidden in the upper petals, which have to be flapped to reach the pollen sacs [HILDEBRAND 1865]. Further a hairy ring in the flower-tube restricts access to the nectar. It has to be penetrated to obtain nectar (fig. 1.2D).

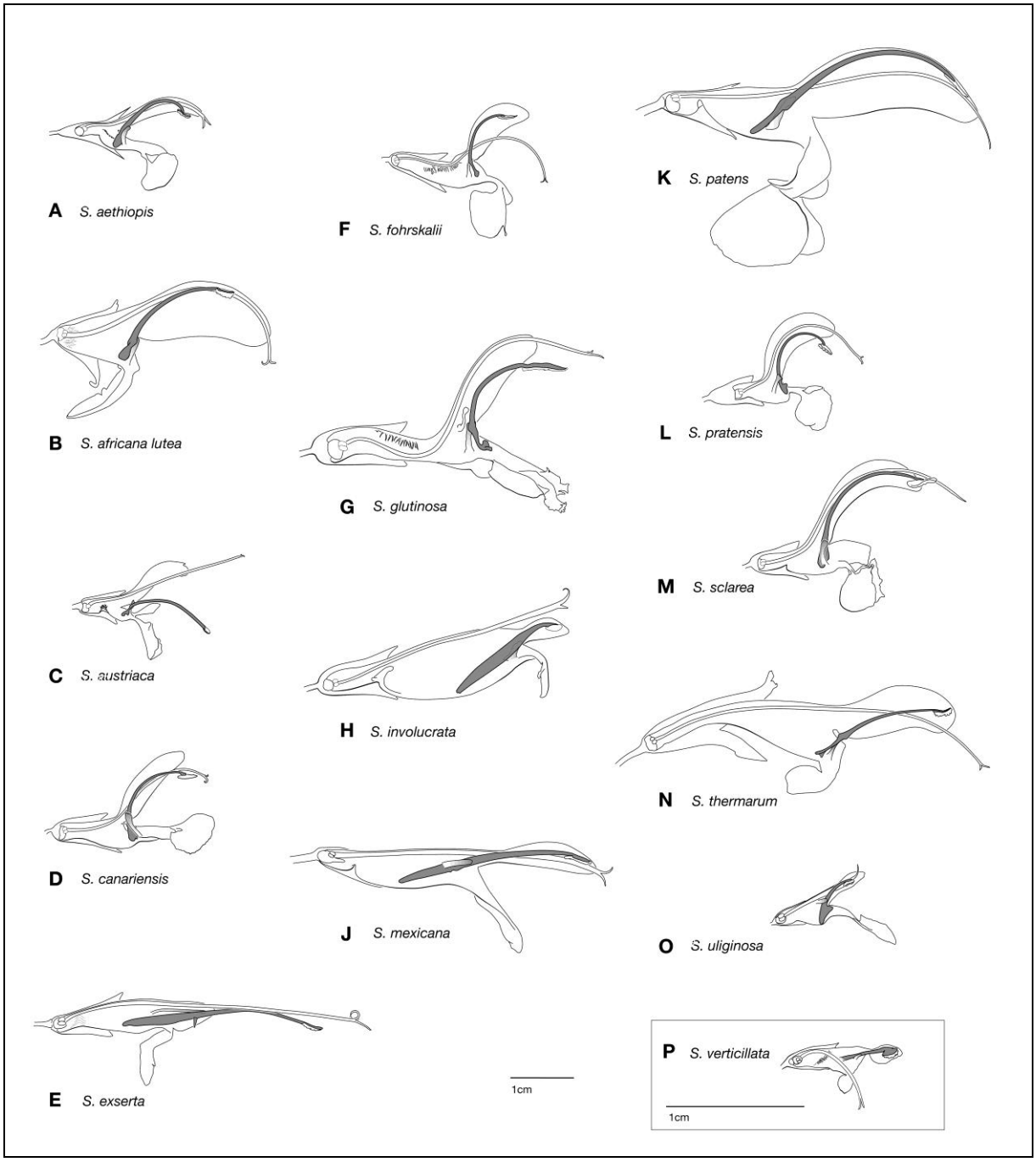


Fig. 1.1: Fifteen highly diverse *Salvia* species were selected for force measurements and morphological investigations. The species' flowers differ in size, shape and especially in lever construction (see Tab. 2).

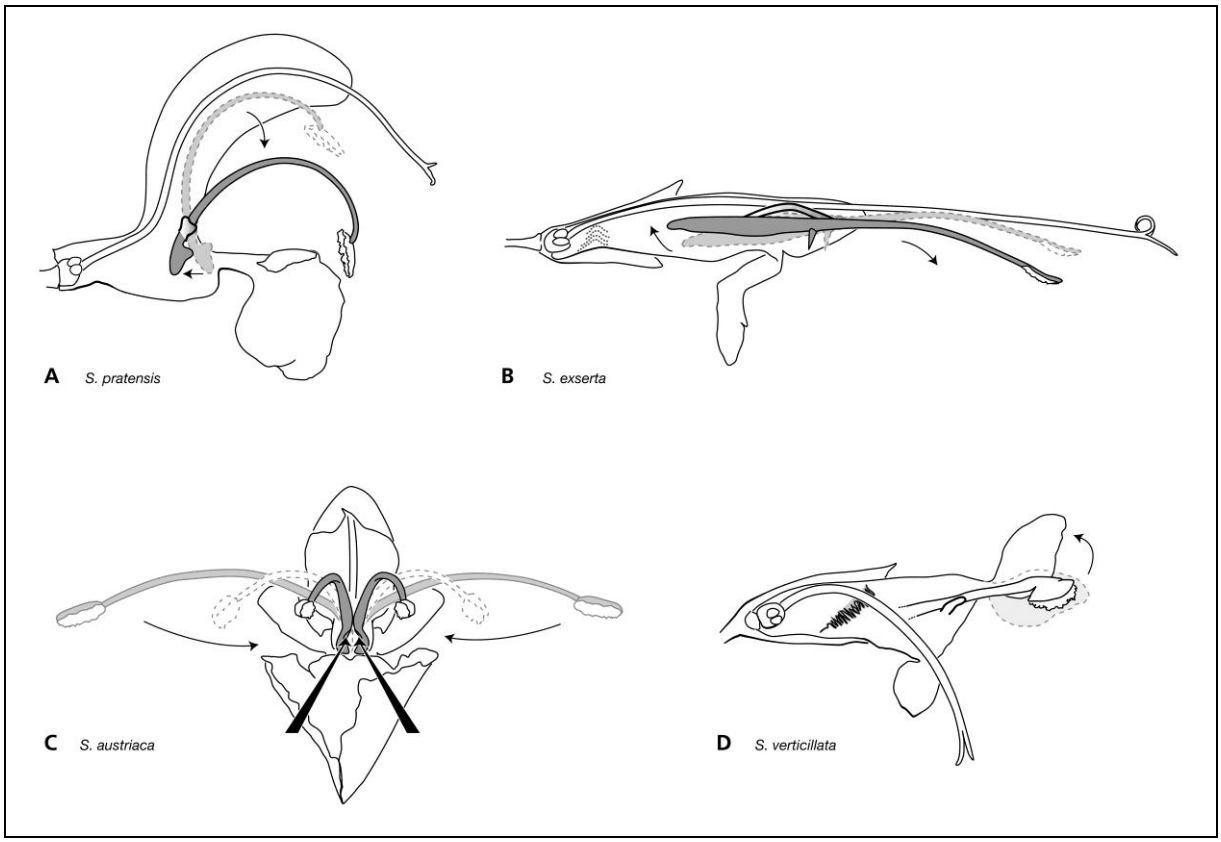


Fig. 1.2: Among the selected species, four different schemes of lever movement occur. **A:** Most species feature a vertical lever movement with a ligament between connective and filament as centre of rotation. **B:** The lever movement of *S. exserta* involves an elastic bending of the filament. **C:** In *S. austriaca* the stamens move horizontally and close like pincers. **D:** *S. verticillata* lacks a lever mechanism. The only moving part of the flower is the upper lip, which has to be flipped upwards to release the pollen sacs.

Tab 1.1: The selected species were highly diverse. Species with different origin, different pollinators and different variations of the pollination mechanism were measured. ([1]Bentham 1848, [2]Epling 1938)

species	growth form	pollinator guild	pollen deposition and transfer mechanism	section	origin
S. aethiopis	annual	bees	dorsal, lever mech.	Aethiopis[1]	Europe /SW-Asia
S. africana-lutea	shrub	birds	dorsal, lever mech.	Hymenosphace[1]	South Africa
S. austriaca	perennial	bees	lateral, scissor mech.	Plethiospace[1]	Europe
S. canariensis	shrub	bees	dorsal, lever mech.	Hymenosphace[1]	Canaries
S. exserta	annual	birds	dorsal, lever mech.	Mineatae[2]	Bolivia
S. forskahlii	annual	bees	dorsal, lever mech.	Horminum[1]	South-East Europe
S. glutinosa	perennial	bees	dorsal, lever mech.	Drymosphace[1]	Europe
S. involucrata	perennial	birds	dorsal, lever mech.	Calosphace[1]	Mexico
S. mexicana	perennial	birds	dorsal, lever mech.	Calosphace[1]	Mexico
S. patens	perennial	birds	dorsal, lever mech.	Calosphace[1]	Mexico
S. pratensis	perennial	bees	dorsal, lever mech.	Plethiosphace[1]	Central Europe
S. sclarea	perennial	bees	dorsal, lever mech.	Aethiopis[1]	Europe/North-Africa
S. thermarum	perennial	birds	dorsal, lever mech.	no section	South Africa
S. uliginosa	perennial	bees	dorsal, lever mech.	Calosphace[1]	South America
S. verticillata	perennial	bees	frontal, no lever mech.	Hemisphace[1]	Europe - Asia

1.3.2 Methods

1.3.2.1 Floral force measurements

The force needed to move the lever was measured in all species except *S. verticillata,* which lacks the staminal lever mechanism. In this particular species, the forces needed to move the upper lip and the forces to foraminate the hairy ring within the flower tube, was measured instead.

To measure the forces, a measuring device, which was custom-built at the University of Freiburg, Institut für Biologie II, Technischer Bereich by Jürgen Schmidt (fig. 1.3, see also SPECK et al. 2003) was used. This force measuring device can be equipped with a probe, which is attached to a force sensor (Type 8510-5001, Burster, Gernsbach, Germany). Measuring range of the force sensor is 0-400mN. The measurement accuracy of the force sensor is 0.25% F.S.. Because of the diverse flower structures, several specific probes had to be designed to fit each of the individual corolla and stamen shapes (fig. 1.4).

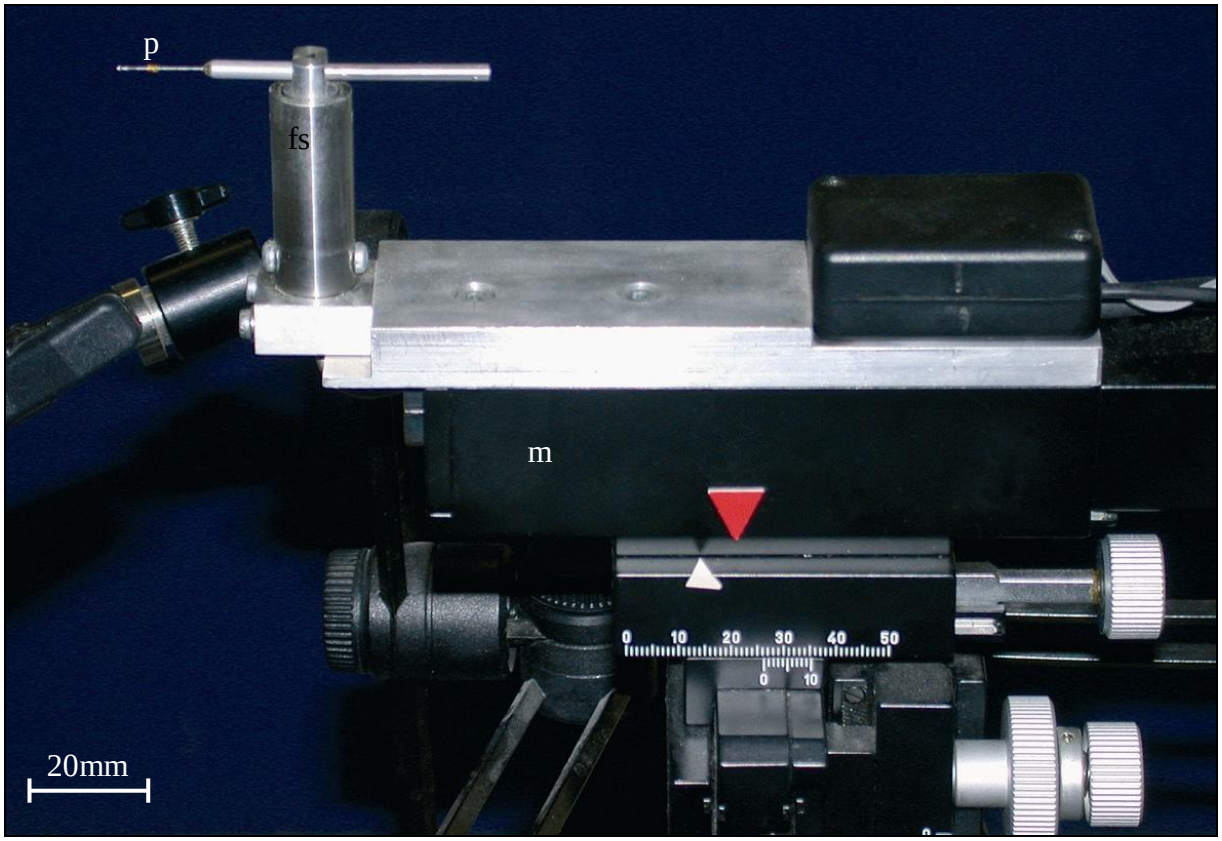


Fig. 1.3: The measuring device bears a probe (p) connected to the force sensor (fs). Both are moved by a microtome motor (m). The whole apparatus is mounted on a tripod.

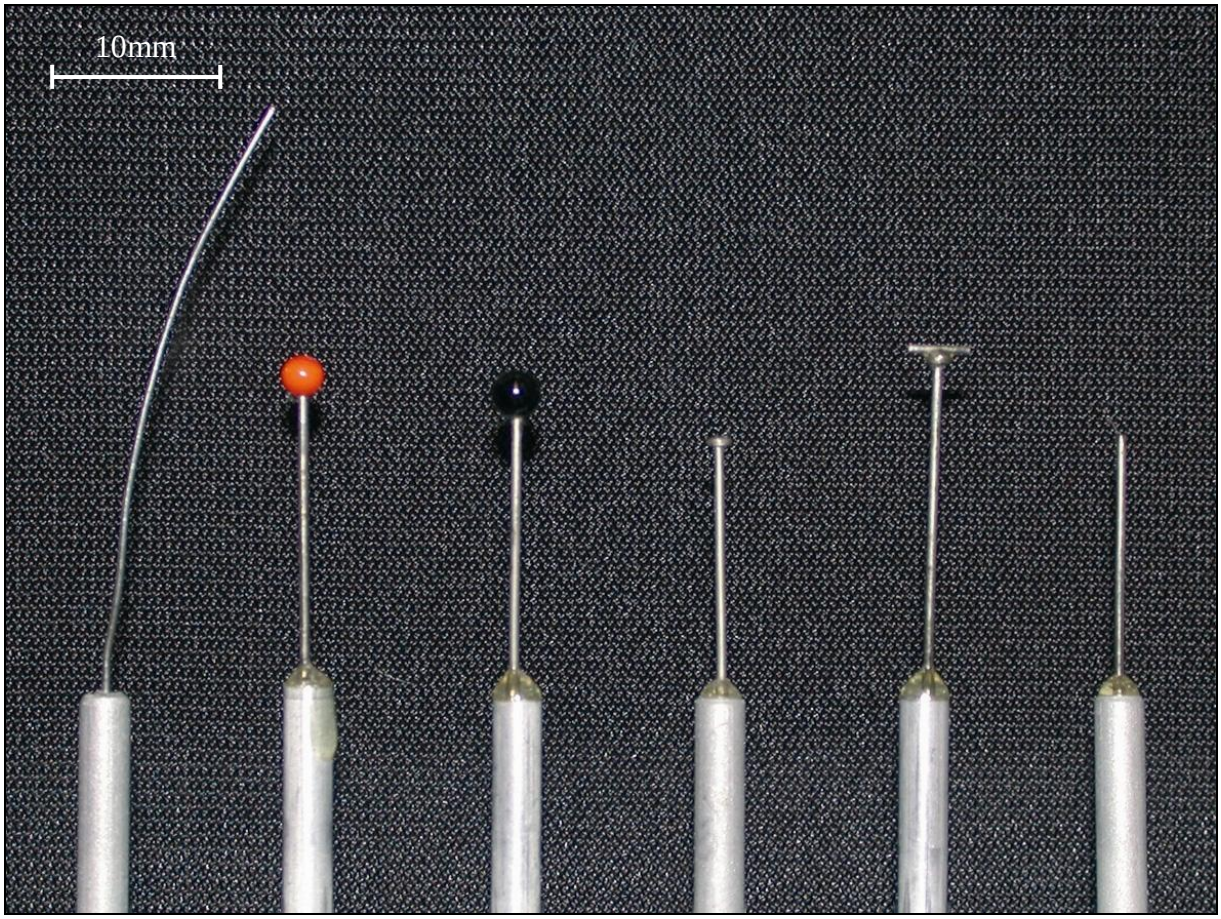


Fig 1.4: Differently shaped probes were used to invade the flowers and to move the lever. A special T-shaped probe (2nd from the right) was used to move the intractable lever of *S. patens.*

Very challenging objects were the species with non-fused lever arms, because the probes easily glided off the adaxial lever arms or pushed them aside to slip through them. Especially *S. patens* was difficult to investigate. Its connective levers are not fused and due to the flowers shape it was difficult to reach the adaxial lever arm with the probe. In *S. patens* and *S. involucrata* a hole had to be cut into the corolla or the lower lip had to be removed to reach the adaxial lever arm and to enable successful measurements.

The force sensor and the probe again are mounted on a sledge that can be moved forwards and backwards by a microtome motor (Type 1624T012S, 1,5W, Faulhaber, Schöneich, Germany). During the measurements the sledge was moved with a constant speed of 0,4mm/s. To arrange the probe properly in front of the flower's entrance (fig. 1.5), a micromanipulator (MM33, Märzhäuser, Wetzlar, Germany) was installed underneath the sledge. The whole apparatus is affixed on a tripod.

Until the measurements, the plants were bagged with a finely woven net (length of mesh side <0.5mm), which kept any possible visitor from the plants. This ensured that the investigated flowers were fully intact and not harmed by any flower visitors and guaranteed

that the lever had not been moved before. These dispositions were necessary, because earlier measurements had already shown that differences between the first and subsequent measurements were not unusual.

Usually each flower was successively measured 20 times with interim pauses of maximally 3s. Variations of this procedure sometimes were necessary due to technical problems or fatigue of the lever. The measuring process was steered and controlled by a computer program, which also registered the detected forces. The program was written by Jürgen Schmidt (Freiburg).

The data were analysed in Microsoft Excel and statistically tested using SPSS 13.0 for Windows, Microsoft Excel and JMP IN 5.1. To test whether the forces are species specific or at least differ significantly, an Analysis of Variance (Bonferroni-test) was performed (see tab. A.1). To visualize the relations between the species and their force range, a boxplot was made (see fig. A.1).

The differences of forces between the single species raised the question about possible differences between certain groups. Therefore the forces of ornithophilous and melittophilous species were compared in a t-test. Furthermore ornithophilous species of the Old World and ornithophilous species of the New World were compared in a t-test. Because of the extreme values of *S. patens* and the difficulties that had occurred during the measurements of this species, both aforementioned tests were repeated without *S. patens*.

To ensure the picking of the flowers and the status of not being attached to the plant anymore did not influence the results, force measurements on flowers left on the plant were performed, too. Flowers of *S. pratensis* were chosen as the representative. Three flowers of one individual were left on the plant for force measurements and three flowers of the same individual were picked and measured. The measuring results of flowers left on the plant and of picked flowers were compared using a t-test.

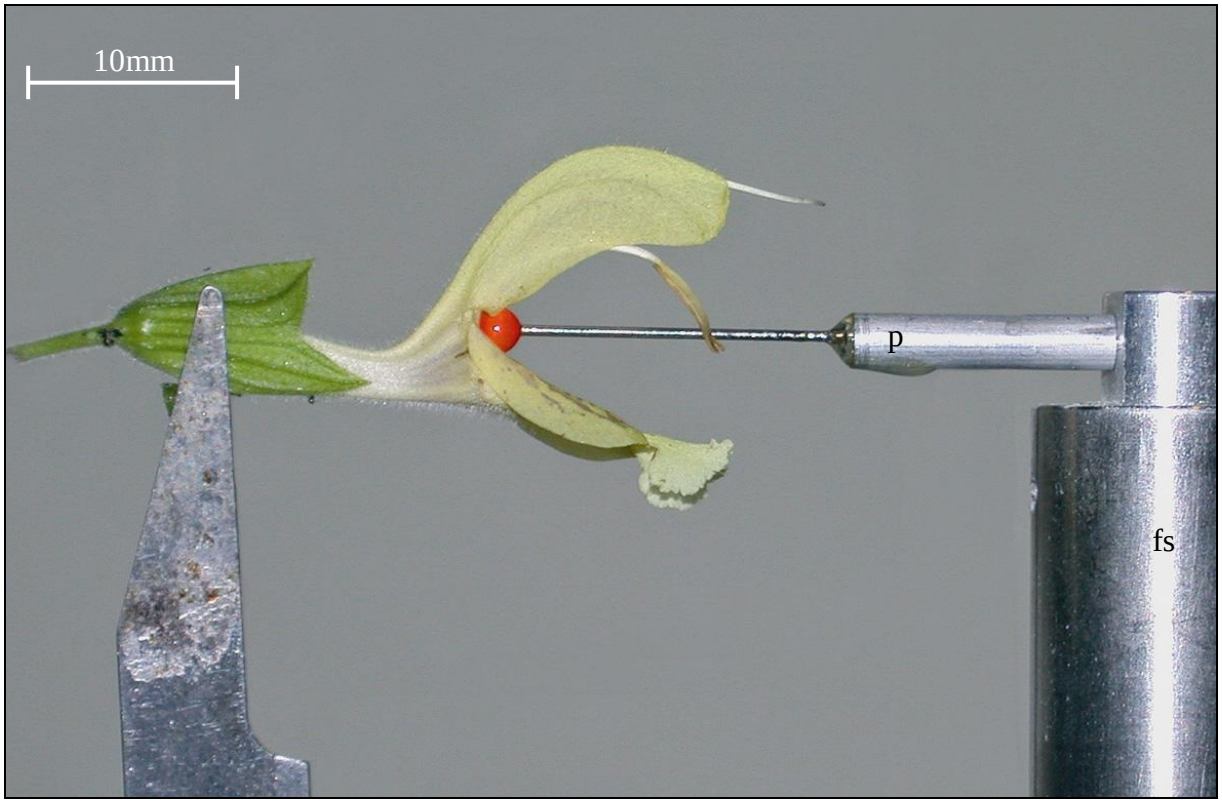


Fig 1.5: The probes released the lever of the flower that was arranged in front of the measuring device (flower of *S. glutinosa*, fs: force sensor, p: probe).

1.3.2.2 Floral diversity and floral structures

Floral construction was analysed by artificially releasing the lever and by reconstructing the process of pollen transfer. From each measured flower, morphometric data were collected (fig. 1.6) after the force measurements. Flower height and flower length were measured by means of a sliding calliper. The height was taken from the lower flower ground to the apex of the upper lip. The length was taken from the flower ground to the distal point of the upper lip. The lower lip was not involved in these measurements because it can hang down in several ways and does not give any indication to the flower's dimensions.

The length of the connective was measured by means of a sliding calliper. The absolute length of the adaxial lever arm (fig. 1.7c) was measured as well as the absolute length of the abaxial lever arm (fig. 1.7a). The straight distance between the joint and the pollen sacs was measured (fig. 1.7b) to determine whether this distance is constant in each species or maybe characteristic in some way.

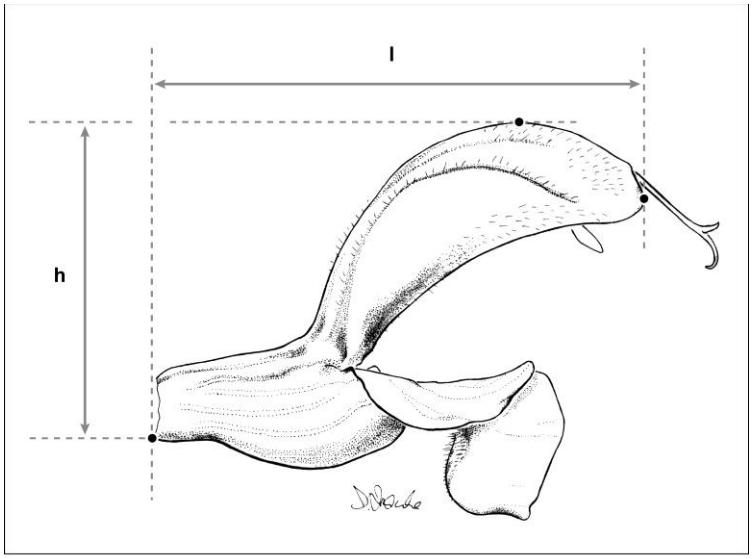


Fig. 1.6: The outer dimensions, height (h) and length (l) were determined.

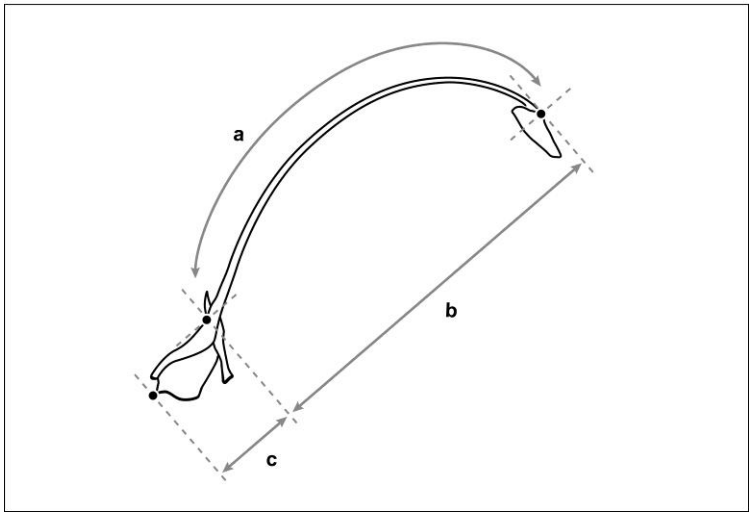


Fig. 1.7: The morphometric data of the staminal levers.
a: absolute length of the abaxial lever arm;
b: distance between joint and pollen-sacs;
c: absolute length of the adaxial lever arm.

To determine if the mass of the lever influences the demanded force, connective lever arm was weighed on a scale (Sartorius *basic* BA210S, Sartorius, Göttingen, Germany). For these weighings each connective was cut out of the flower and weighed. To avoid wilting, flowers and the connectives were stored in a humid chamber. Preceding tests confirmed this method to be most effective. The weight of the connectives varied extremely, if stored in water or a dry chamber.

Morphometric and weight data were related to each other as well as to the results of the force measurements. An analysis of correlation and a linear regression were performed to test whether morphometric structure or lever weight and construction influence the required forces. To test if these results are falsified by the extreme values of *S. patens*, the tests were repeated without *S. patens*.

1.3.2.3 Weight of pollinators

For comparison with pollinator data from Westerkamp (1993, 1997) and to augment data of that topic, 16 randomly chosen individuals of *Bombus terrestris,* which is a common pollinator to melittophilous species, were weighed. The animals were weighed alive on a scale type Sartorius *basic* BA210S (Sartorius, Göttingen, Germany). The animals had been provided by Professor Dr. Christa Neumeyer (Institut für Zoologie III – Neurobiologie, Universität Mainz).

1.4 Results

1.4.1 Floral force measurements

Even though in exceptional cases the required forces to move the lever were so low that the measuring device could not detect them and 0mN were measured, generally the forces of the fifteen investigated *Salvia* species were at least measurable. They range from 1.08mN ± 0.6mN in *S. thermarum* to 21.34mN ± 21.88mN in *S. patens* (tab. 1.2). The high deviations of the average values measured in the force measurements are caused by both differences among and within the individual flowers. In *S. pratensis* for instance 91 flowers were measured. The maximal forces among the flowers of this species range from 0.14mN to 15.61mN showing an absolute difference of 15.47mN. Each flower was measured 20 times in succession. The maximal and minimal forces within the same flower range from 1.2mN to 14.99mN, covering a margin of 13.79mN.

Tab. 1.2: The forces needed to move the lever differ from species to species.

species	average maximum force [mN]	dev	max. force ever measured [mN]	number of measured flowers	number of measurements
S. aethiopis	5.04	2.62	17.27	21	210
S. africana-lutea	6.44	2.72	12.17	3	50
S. austriaca	6.21	3.49	15.63	24	295
S. canariensis	4.35	2.35	11.77	16	64
S. forskahlii	3.32	3.42	31.54	24	435
S. glutinosa	1.34	0.92	4.25	38	760
S. involucrata	9.37	0.34	49.35	15	150
S. mexicana	9.62	2.15	47.95	9	120
S. patens	21.34	21.88	72.73	21	255
S. exserta	1.25	1.64	12.97	20	200
S. pratensis	2.93	2.37	15.61	91	1820
S. sclarea	8.49	4.74	24.35	21	210
S. thermarum	1.08	0.6	2.08	4	40
S. uliginosa	3.40	2.90	13.79	32	640
S. verticillata upper lip	0.16	0.37	1.07	13	123
S. verticillata tube	3.58	7.18	42.01	20	130

The force-distance diagrams of the individual flowers clearly illustrate that the curves of repeated measurements show the same pattern. While measuring the flowers, each one of them up to 20 times in a row, often an abatement of the maximum force was noticed with every repetition.

In some species, the connectives do not always swing back exactly to their original position. Then the connective lever is a little bit inclined, which leads to a longer distance that has to be traversed by the probe, until it reaches the adaxial arm. This becomes visible in the graphs by a later ascent of the curve.

Sometimes the pollen sacs were surrounded by the upper lip and held back. In these cases initially higher forces had to be applied to free the pollen sacs. The upper lip was the only morphological structure which clearly influenced the measured force. This blockage caused by the upper lip was an exceptional and irregular phenomenon, though.

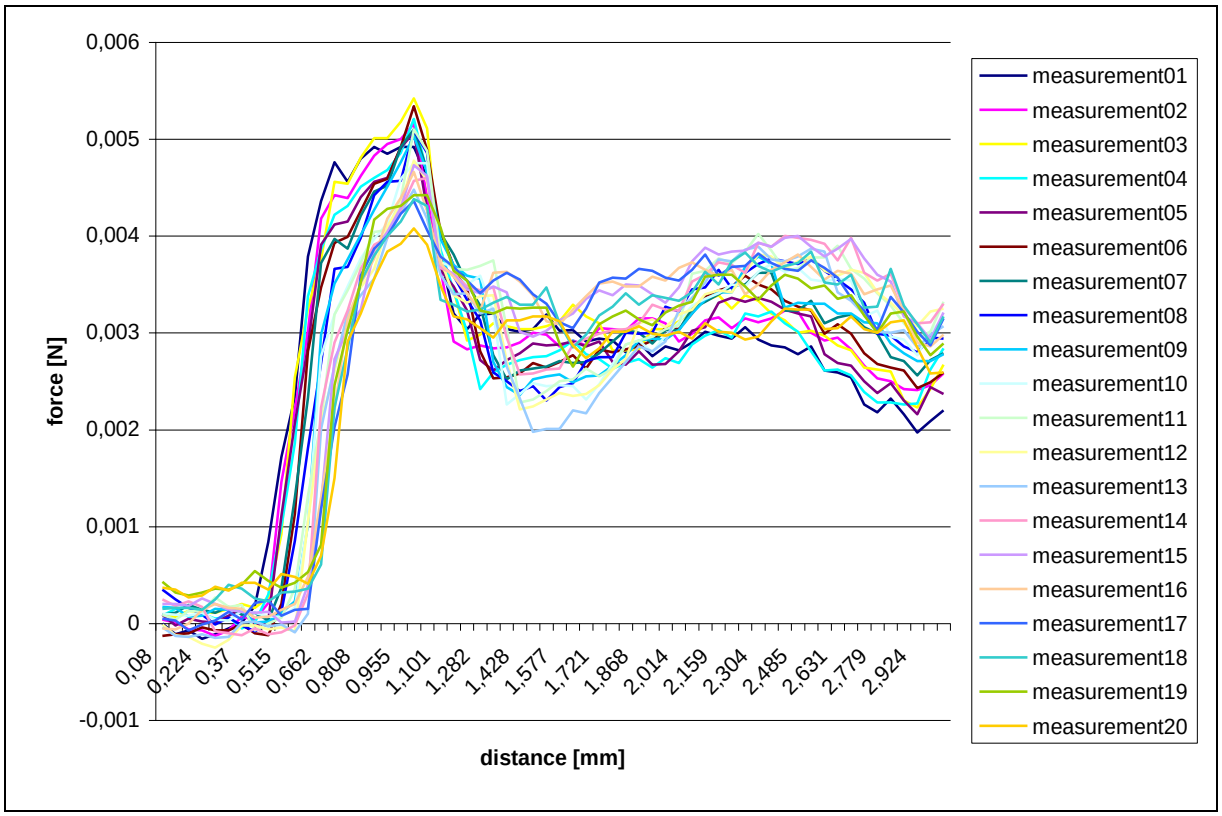


Fig 1.8: *S. pratensis*, force-distance diagram. The upper lip can cause a recurring peak when it constantly holds back the pollen sacs.

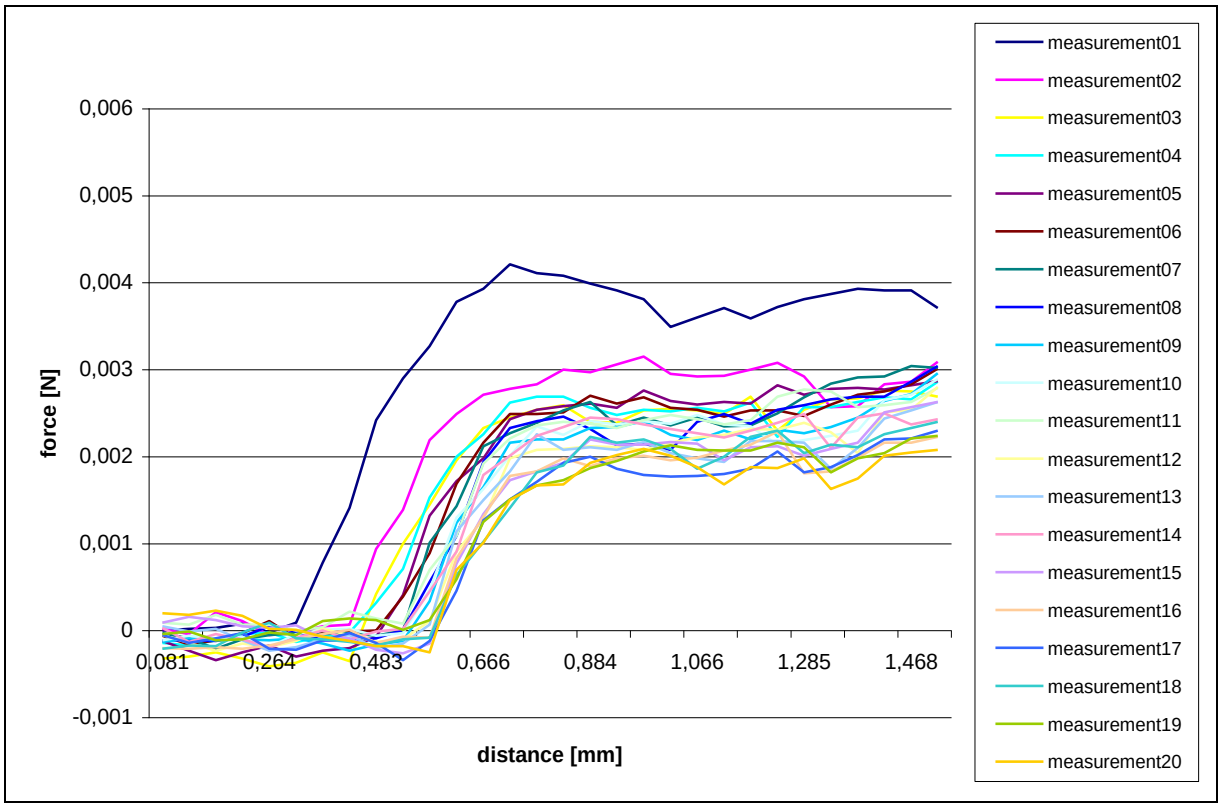


Fig 1.9: *S. pratensis*, force-distance diagram. No peak appears when the pollen sacs are not trapped in the upper lip. Only the first release of the lever demands slightly higher forces than the following.

The graphs of ***S. pratensis*** (figs.1.8 & 1.9) are very typical. In this species, the peak caused by the upper lip was recognized for the first time (fig. 1.8). The two petals forming the upper lip of *S. pratensis* can be clotted very tightly. There are several scenarios that may unfold after the first release of the lever.

First, the lever swings back into its original position and the pollen sacs are covered by the upper lip again. If the connective swings back into the original position, two further possibilities present themselves: Either the petals close tightly every time, then the pollen sacs have to be pushed out of the upper lip every time the lever is released and every curve of the graph offers a peak (fig. 1.8), or the petals sag and do not hold back the pollen sacs anymore. The latter entails that no additional force is needed to retrieve the pollen sacs out of the upper lip. Only the first measured curve displays a peak.

Second, the upper lip closes behind the pollen sacs and bars them from taking their original position. This leads to a slight deflection of the lever. In subsequent measurements the probe has to cover a longer distance to reach the lever. The second and following measurement curves of the graph rise later.

In some flowers, however, the pollen sacs already are hanging out of the upper lip or the upper lip is loose (fig. 1.9). In these flowers, no additional force has to be applied to release the lever and no peak appears. Simply, the first release requires a little more force than the following ones. Further, the graph reveals that the connective lever did not swing back properly after the first release. The second and the following curves rise later (fig. 1.9). The pliability of the lever is not affected by this.

In ornithophilous ***S. patens*** the highest forces of all *Salvia*-species were measured (fig. 1.10, tab. 1.2). The lines of the second measurement and the following measurements rise later than the ones of the first measurement. As already mentioned for *S. pratensis*, it is caused by the fact that the connective lever does not always swing back exactly to its neutral position. Often, the chubby pollen sacs can not re-enter the shelter of the upper lip, or the joint loses tension. This leads to a connective lever which is not in its original position and the adaxial arm is moved back a bit. This leads to a longer distance which has to be travelled by the probe. *S. patens* is the only species which force values showed no equality to any other species in an analysis of variance ($p = 0.00$, ANOVA) referring to the forces (annex tab. A.1).

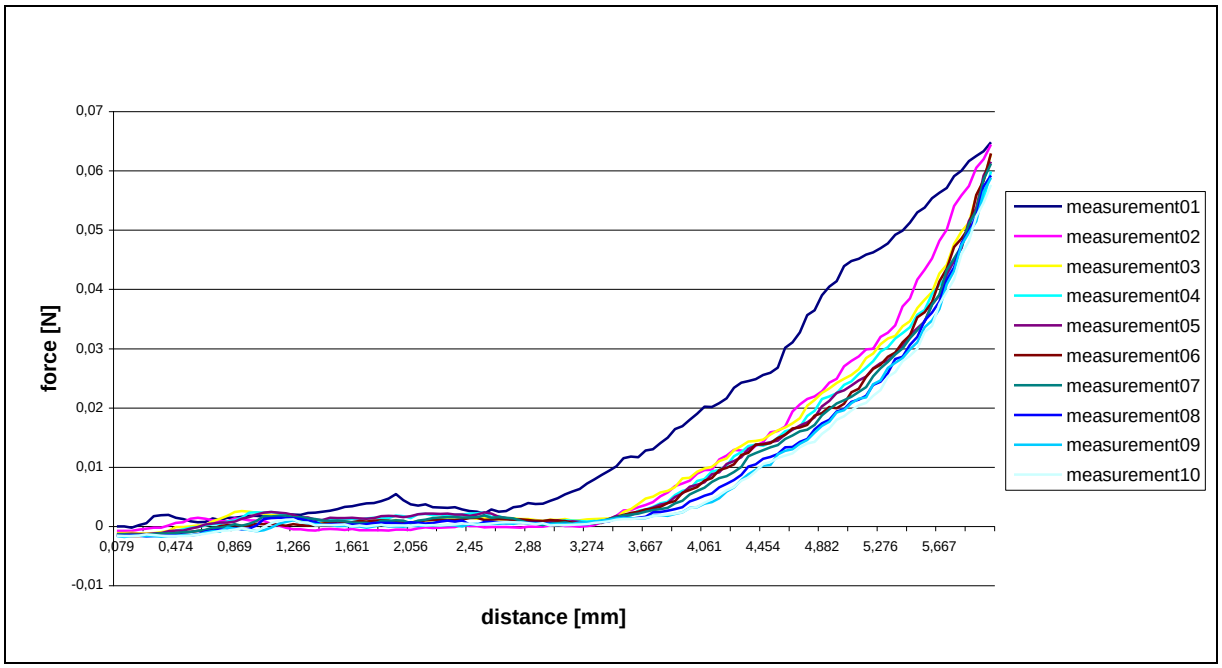


Fig. 1.10: *S. patens* force-distance diagram. The line of the first measurement rises earlier than the others. This is caused by an imperfect back-swing of the lever.

S. thermarum is the species with the lowest forces measured in this investigation (tab. 1.2, fig. 1.11). This species is ornithophilous, too, but unlike *S. patens*, from the Old World. The graph has a peak as well, which is caused by the upper lip. The two petals of the upper lip enclose the pollen sacs and, as already mentioned for *S. patens* and *S. pratensis*, require more effort. This phenomenon can occur with variable intensity in other flowers of this and other species, e.g. *S. pratensis*. The intensity of this peak depends on the petals and how tightly they enclose the pollen sacs.

The analysis of variance (Bonferroni-test) of the forces needed to move the staminal lever revealed that *S. thermarum* does not differ from *S. forskahlii*, *S. glutinosa*, *S. exserta*, *S. pratensis* and *S. verticillata's* upper lip (p=1). Furthermore, *S. thermarum's* values do not differ significantly form *S. canariensis's* values (p=0.156). And with a marginal significance (p=0.058) *S. thermarum* and *S. uliginosa* are not different (annex tab. A.1).

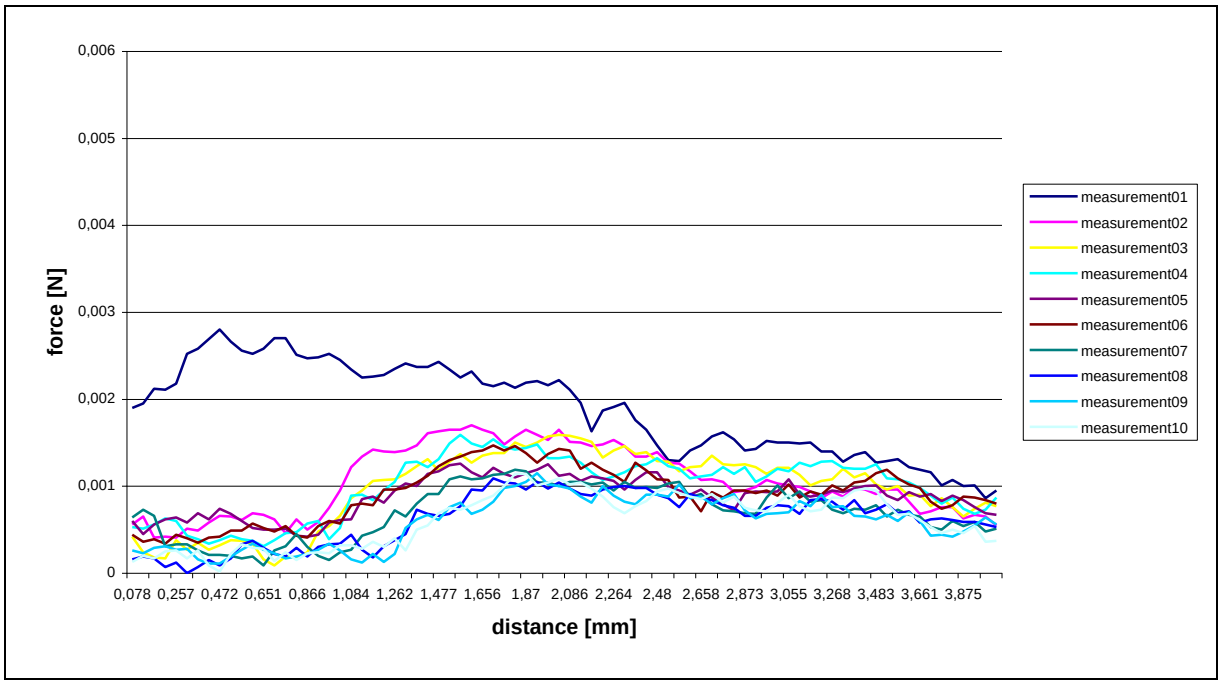


Fig 1.11: *S. thermarum* sometimes shows a small peak originating from the pollen sacs which are held back in the upper lip. Later the lever does not swing back completely and the pollen sacs are not covered by the petals.

In ***S. verticillata*** the force to move the upper lip was measured (fig. 1.12), as well as the force to penetrate the hairy ring in the flower tube (fig. 1.13).

The force to move the upper lip is barely worth mentioning; quite contrary to the hairy ring (tab. 1.2, fig. 1.13). The force data of the hairy ring do not lie above or below the forces of the lever movement of other flowers. Therefore *S. verticillata* is in line with the other species for obstruction.

Interestingly, a graph of *S. verticillata* can display a peak, too (fig. 1.13). This peak is caused by the hairy ring which is located in the corolla tube. It takes an average force of 3.58 +/-7.18mN to penetrate this hairy ring.

The statistical analysis of variance (Bonferroni) of the averagely measured forces showed that the values of the **upper lip** of *S. verticillata* do not differ from the values of the levers of *S. glutinosa*, *S. exserta* and *S. thermarum* (p=1.0). If the **hairy ring** of *S. verticillata* is subject to an analysis of variance, it turns out that the forces needed to penetrate this structure are comparable to the forces of *S. aethiopis, S. africana-lutea*, *S. austriaca* and *S. canariensis* (p=1, annex tab. A.1).

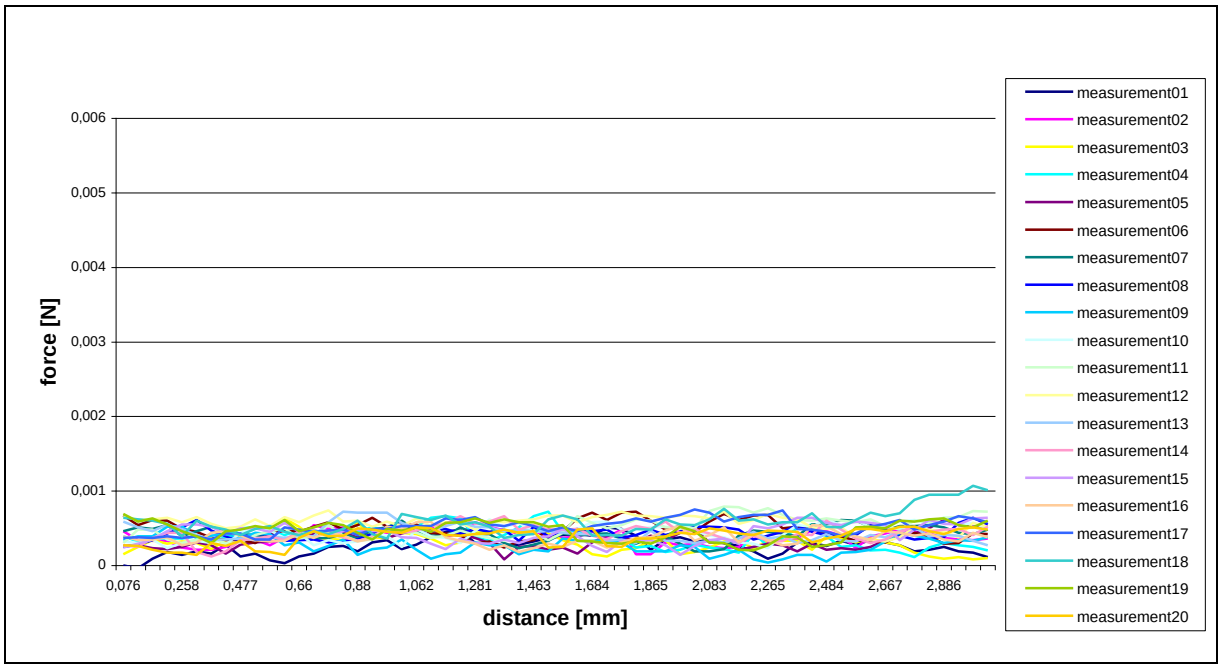


Fig 1.12: The **upper lip** of *S. verticillata* does not demand remarkable forces. In this example the measured forces hardly reach 1mN. The graph seems to fluctuate considerably, but these fluctuations are less than 0.5mN.

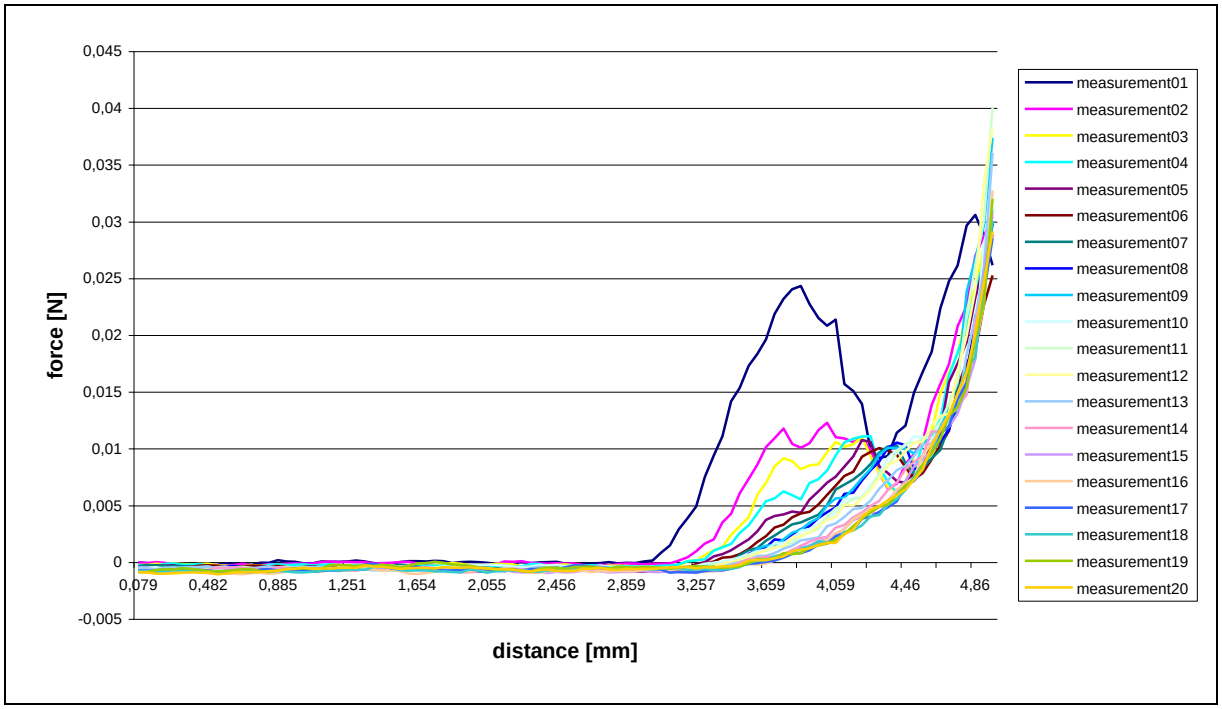


Fig 1.13: In the flower tube of *S. verticillata*, the probe first has to pass a distance of about 3mm before it reaches the **hairy ring**. The hairs become increasingly penetrable with each time the ring is penetrated.

To test whether these forces needed to move the pollination mechanism and to reach the nectar, respectively are comparable among the species, an analysis of variance was conducted (annex tab. A.1). It revealed that only one species consistently differs from all

others. The values of *S. patens* exhibit significant difference to all other species. For every other species exist at least two species to which they are comparable, referring to the forces. So, only *S. patens's* forces stand out.

A t-test shows that melittophilous and ornithophilous species do significantly differ in forces. The average force of the ornithophilous species is 9.03mN +/-12.9mN. The average forces of the melittophilous species is 3.27mN +/-2.99mN ($T=-25.265$, $df=4834$, $p<0.001$, annex tab. A.2a). A second t-test without *S. patens* confirms these results. Without *S. patens,* the average forces of the ornithophilous species are considerably lower (5.42mN +/-8.31mN), but they are still significantly different to the forces of the melittophilous species ($T=-9.835$, $df=4469$, $p<0.001$, annex tab. A.2b). Though the melittophilous and ornithophilous species show significant difference, single species of these groups do not when compared.

A second t-test was applied to compare ornithophilous Old World species and ornithophilous New World species (annex tab. A.3a). With an average force of 10.04mN +/-15.45mN ornithophilous species of the New World differ significantly from the Old World-species, which average force is 4.0mN +/-3.37mN ($T=-3.66$, $df=651$, $p<0.001$, annex tab. A.3a). In the repetition of this test without *S. patens* the average force of the New World-species is 5.74mN +/-9.01mN. The values are not significantly different to the Old World-species ($T=1.81$, $df=494$, $p=0.07$, annex tab. A.3b).

The t-test comparing the measured forces of picked flowers and flowers left on the plant showed that there was no difference between the forces measured picked flowers and flowers left on the plant ($T=-0.05$, $df=152$, $p=0.960$, annex tab. A.4). During the force measurements, a decline of forces was detected in each flower and throughout all species (figs. 1.8-1.13). The flowers always demanded a little bit more force in the first measurements than they did in the later measurements. As already mentioned, in some cases this difference obviously was caused by the upper lip, which sometimes held back the pollen sacs. But even when this disruptive element did not occur or had been neutralized there was still a discernable difference between the first and later measurements (annex tab. A.5).

To determine a gradient in this loss of force, different successive measurements were compared. The average loss of force between the first and the fifth measurement was about 29%. Between the first and the 10^{th} measurement, a difference of about 39% was detected. To exclude the phenomenon of the upper lip holding back the abaxial arm, the second and fifth measurements were compared as well as second and 10^{th} measurements. This still showed an average difference of 12% respectively 27%. After 20 releases, the difference between first and last measurement was about 53%, between second and last measurement about 45%.

1.4.2 Floral diversity and floral structures

Just as the species differ in shape and operating mode of the lever, they also differ in their flowers' shapes and dimensions. *S. patens* has the biggest flower, while *S. verticillata* has the smallest flower (fig. 1.1 & 1.2, tab. 1.3).

Deviations of the levers' lengths are low within the species. Bird pollinated *S. involucrata* has the longest adaxial lever arm (11.68 +/-0.87mm) and the shortest abaxial lever arm (5.09 +/-0.54mm). As its abaxial lever is not curved, the joint–pollen sac distance is identical with the abaxial arm's length. The longest abaxial arm can be found in *S. patens* which also covers the longest joint–pollen sac distance (tab. 1.3).

Apart from their dimensions and the way of function (fig. 1.2) the levers of the investigated species show different construction criteria. Investigations of the internal floral structures revealed that the neighbouring connectives which form the lever can be unconnected or partially fused or clotted. *S. africana-lutea, S. austriaca, S. forskahlii, S. patens* and *S. thermarum* have free and unfused connectives. Though the connectives form a functionally unit, they can be moved independently. The non-fused connectives of *S. thermarum* sometimes seemed to be attached very lightly to the filament. In *S. aethiopis, S. canariensis, S. glutinosa, S. pratensis* and *S. sclarea,* the two connectives are connected only at a single point at the lower tip of the adaxial lever. Apart from this connection point the connectives are unconnected. In *S. exserta, S. involucrata, S. mexicana* and *S. uliginosa* the adaxial arms of the connectives are clotted over a longer distance. The connectives are disconnected near the joint area. In all investigated species the adaxial arms and the anthers were not fused or clotted.

Inside the corolla tube no internal structures that would hinder the levers movement were identified. The only structure that limited the levers movement was the corolla itself. The levers could be moved without any problems.

Tab. 1.3: Morphometric data of floral structures (see fig 1.6 & 1.7; all data in mm; n = number of flowers analysed; n.l. = no lever; dev = deviation).

species	flower height [mm]	dev [mm]	flower length [mm]	dev [mm]	length of adaxial lever arm [mm]	dev [mm]	length of abaxial lever arm [mm]	dev [mm]	joint pollen sacs -distance [mm]	dev [mm]	n
S. aethiopis	7.49	0.77	19.07	0.83	2.96	0.14	10.92	1.18	8.92	0.56	21
S. africana-lutea	14.10	2.88	33.9	5.7	3.91	0.16	17.54	4.22	17.26	3.18	11
S. austriaca	8.34	1.10	16.87	0.6	2.75	0.16	14.88	1.05	12.50	0.41	24
S. canariensis	12.10	1.28	19.87	5.13	3.29	0.29	9.48	2.94	11.04	1.06	16
S. forskahlii	11.98	0.98	21.08	1.82	3.10	0.25	13.05	0.85	11.12	0.79	24
S. glutinosa	15.10	1.61	32.53	2.81	4.99	0.26	12.03	0.57	11.36	0.21	39
S. involucrata	4.75	0.80	38.06	1.93	11.68	0.87	5.09	0.54	5.09	0.54	15
S. mexicana	4.28	0.57	26.00	0.43	7.33	0.90	7.78	0.67	7.29	0.81	10
S. patens	13.82	1.60	48.89	2.18	5.96	0.14	30.65	3.29	27.85	1.72	21
S. exserta	4.87	0.50	25.31	2.21	10.38	0.89	15.75	1.61	15.56	1.14	20
S. pratensis	9.81	0.90	16.81	2.26	3.09	0.31	12.99	2.10	10.34	0.10	90
S. sclarea	12.67	1.59	26.23	1.38	4.10	0.20	20.33	1.28	15.07	1.11	21
S. thermarum	12.01	2.10	48.75	4.55	5.50	0.41	14.38	0.48	14.21	2.93	4
S. uliginosa	3.31	0.45	13.52	0.65	4.29	0.49	4.21	0.25	4.04	0.38	32
S. verticillata upper lip	2.80	0.29	8.61	1.06	n.l.	n.l.	n.l.	n.l.	n.l.	n.l.	10
S. verticillata tube	2.80	0.29	8.61	1.06	n.l.	n.l.	n.l.	n.l.	n.l.	n.l.	10

Tab 1.4: The weight of lever was measured.

	average mass of the connective lever [mg]	dev [mg]	n
S. aethiopis	1.65	0.11	8
S. africana-lutea	7.4	1.10	7
S. austriaca	1.23	0.05	14
S. canariensis	2.16	0.39	14
S. exserta	3.73	0.41	14
S. forskahlii	3.25	1.02	14
S. glutinosa	2.17	0.59	14
S. involucrata	15.59	0.44	14
S. mexicana	24.82	1.25	18
S. patens	2.32	0.22	14
S. pratensis	1.99	0.72	17
S. sclarea	5.61	0.59	10
S. thermarum	3.95	0.07	2
S. uliginosa	2.41	0.27	14
S. verticillata UL	no lever	no lever	-
S. verticillata tube	no lever	no lever	-

Bigger flowers and ornithophilous flowers tend to have heavier levers (tab. 1.4).

To test the possibility that forces may be influenced by inner or outer floral structures, an analysis of correlation and a linear regression on flower size and the size of the staminal lever was performed (annex tab. A.6, figs. A.2-A.7).

It turned out that both the flower height and flower length (see fig. 1.6) do influence the required forces (r=0.68, p=0.00; r=0.5, p=0.05; respectively). The absolute length of the staminal lever (fig.1.7a) (r=0.68, p=0.01), the distance covered by the lever (fig. 1.7b+c) (r=0.61, p=0.03) and the joint pollen sac distance (fig. 1.7b) (r=0.62, p=0.02) have an influence on the required forces, too. Despite this, it could not be ascertained that the mass of the staminal lever (r=0.29, p=0.33) has an influence on the force. An "r" close to 0 insinuates no correlation but the p-value of 0.33 invalidates this conclusion (annex tab. A.6a).

As aforementioned, the same tests were repeated without *S. patens*, to test if its extreme values falsified the results. This time no morphological structure except the lever's mass had influence on the required forces. Flower height and flower length (see fig. 1.6) do not influence the required forces (r=0.23, p=0.45; r=0.21, p=0.49; respectively). Neither the absolute length of the staminal lever (fig.1.7a) (r=0.36; p= 0.23), nor the distance covered by the lever (fig. 1.7b+c) (r=0.33; p=0.3), nor the joint pollen sac distance (fig. 1.7b) (r=0.01; p=0.97) have an appreciable influence on the required forces. But the mass of the staminal lever (r=0.76; p=0.00) influences the force.

1.4.3 Weight of pollinators

The 16 individuals of *Bombus terrestris* weighed an average of 0.18g +/-0.07g. The weights of the animals ranged from a minimum of 0.10g to a maximum 0.27g. Assuming a rate of fall of 9.81m/s², this corresponds with an average force of 1.76mN up to a maximum of 2.67mN, which the bumble-bees can perform solely by their weight.

1.5 Discussion

If the lever should be a tool for pollinator-selection, one first may have a look at the forces it demands and then see what forces can be exerted by the pollinators. As the high deviations show, the needed forces to move the lever differ considerably within the species as well as between the species. High deviation of the forces also could be caused by an error in the experimental setup. But no error in the accomplishment of the force measurement is visible. It seems more likely that the high deviation is an indicator that no specific force is necessary to move the lever.

The average force for *S. pratensis* is 2.93mN +/-2.37mN (tab. 1.2). Within *S. pratensis*, the measured forces range from 0.14mN up to 15.61mN, which is a difference by two orders of magnitude. Even in one and the same flower forces ranging from 1.2mN up to 14.99mN were measured. A constant or specific demand of force obviously is not required. Also, the required forces are not high compared to other *Salvia*-species. The highest forces were detected in ornithophilous *Salvia patens.* On average 21.34mN +/-21.88mN were needed to move the lever in this species. Hardest melittophilous species was Eastern-Mediterranean *S. sclarea.* 8.49mN +/-4.74mN were necessary to move the lever in this species. The lever restricting access to the nectar effectively is an obstacle which leads to a longer dwelling time of the insects in the flower [OHASHI 2002]. But one may doubt that the lever mechanism really is an insuperable barrier, which is meant to hinder an animal to access the flower [THIMM et al. 2003, CLAßEN-BOCKHOFF et al. 2004a]. There are assumptions that smaller pollinators are excluded, even if low forces are required for flower opening (0.1-0.5mN in *Cornus canadensis*) [EDWARDS et al. 2005]. But workers of the bumble bee *Bombus terrestris* weigh about 0.18g +/-0.07 (n=16) and can exert forces up to 59mN [REITH et al. 2006]. They apply 1.76mN just by their mass and so in some flowers they would release the lever by simply leaning on it. Workers of *Apis mellifera* can apply up to 29mN [REITH et al. 2006]. In all investigated *Salvia*-species the average maximum force is lower than the forces that can be exerted by these bees (see tab. 1.2). Even most of the maximal forces ever measured in melittophilous species are still manageable for the weaker *Apis mellifera*. So the usual pollinators definitely can apply the necessary forces and move the lever. Other species and other genera demand substantially higher forces from their visitors. Pollinators of *Vigna caracalla* (Fabaceae) have to muster 53mN +/-6mN to reach the nectar [ETCHEVERRY et al. 2005]. *Lathyrus sylvestris* (Fabaceae) demands 25mN +/-10mN on average and at maximum

up to 40mN [BARRY unpubl.]. Its visitors are *Bombus sylvarum, Bombus pascuorum* and not exactly determined species of the genus *Megachile* [BARRY unpubl.]. *Desmodium canadense* (Fabaceae) demands 26mN up to 55mN [KUTZMANN unpubl.]. In Middle-Europe, proven pollinators of *Desmodium canadense* are *Bombus pascuorum, Bombus hortorum* and *Apis mellifera* [KUTZMANN unpubl.]. Even animals which are smaller than e.g. *Bombus terrestris*, a common *Salvia*-pollinator, can exert such forces. *Megachile ericetorum* weighs only 0.085g and has to apply forces up to 100mN to fulfil its obligation as pollinator of *Lathyrus latifolius* [WESTERKAMP 1993, 1997]. So if small insects can muster these forces easily, the reason for the staminal lever can not be to select pollinators by demanding high forces from the insects.

The selected species are highly diverse (tab. 1.3, fig. 1.1). They serve different pollinators (tab. 1.1), differ in flower shape and construction (fig. 1.1) as well as in shape, way of function and construction of the lever (fig. 1.2). The measured forces do correlate with the flowers' sizes and the connectives' lengths, if *S. patens* is involved in this analysis (annex tab. A.6a). But the lowest forces were measured in *S. thermarum*, which is among the big-flowered species. Furthermore, *S. sclarea* though being the hardest melittophilous flower is still neither an extremely big nor a small flower and ranks in the upper third regarding the forces. It seems incomprehensible that forces should be influenced by the size of flower or lever. Furthermore, *S. patens* is a very special case. Its investigation was always very difficult and this species features extreme values and might falsify the results. So the same analysis was performed without *S. patens* and the results considerably changed. The new results show no correlation between inner or outer flower structures and the forces. So neither flower size nor lever construction is an indicator for the fluidity of the lever mechanism. But the analysis of correlation between the required force and the mass of the lever shows that there is a correlation ($r=0.76$; $p=0.00$). The mass of the lever does have a significant influence on the mechanism's fluidity. According to Newton's Second Law of Motion (force = mass x acceleration), the more mass an object has, the more it resists any change in its motion. So, if the same force is applied to an object of a mass of 2kg and to an object of a mass of 1kg, the acceleration of the 2kg mass will be less than that of the 1kg mass. Although the masses we are dealing with are very weak, they influence the lever's mobility.

The ANOVA referring to the forces showed that every species at least has two other species it is comparable with. The only outstanding species, which is different to all other species, is *S. patens*. But even here the range of the forces overlaps with other species (annex fig. A.1). The ANOVA and the boxplot (annex tab. A.1, fig. A.1) show that no species has a specific or species-specific range of force. This becomes even more evident when the high

deviation is observed. Although there is an ornithophilous species as the most pliable (*S. thermarum*) and an ornithophilous species as the most rough-running (*S. patens*), ornithophilous flowers turned out to be significantly different from melittophilous flowers with respect to the force measurements. Even if *S. patens* is ignored in the t-test. Furthermore within the group of ornithophilous flowers, the Old World species differed significantly from the New World species. This result changes slightly when *S. patens* is ignored again. But the significance is only marginal (p=0.07) and considering the small sample involving 3 New Wold-species and 2 Old World-species the result might not be meaningful.

As already mentioned, an eye-catching fact is the outstanding position of *S. patens.* The high forces demanded by its lever mechanism are difficult to explain. As the adaxial arms of the connective lever are not fused, they tend to be pushed aside and let the probe slip through between them. To avoid this phenomenon, a probe shaped like a T (fig. 1.4) that could not slip through the adaxial arms had been designed. But with the benefit of hindsight, this might have been a mistake. For it could be possible that by using the newly designed probe, the lever had been triggered in the wrong way. The adaxial arms possibly are meant to be pushed aside. This might lead to a lever movement and pollen positioning comparable to *S. austriaca* when a beak invades the flower. At the moment, this is mere speculation, as none of the beak-shaped probes could release the lever of *S. patens* properly. Furthermore the joint of *S. patens* is arranged vertically and not twisted or inclined at all. This arrangement does not favour a diagonal or even a horizontal movement like in *S. austriaca* at all.

Throughout all species for nearly all individuals the curves of measurements within one flower always followed the same track. The force progression in one flower always was of relatively consistent quality. But the curves also showed that the absolute forces dropped off with repeated measurements.

This decline of force during the repeated release of the lever could hint at the mechanism that causes the levers' upswing and guarantees its reliability. The assumption that some elastic tissue is involved stands to reason. The drop of forces could be a sign of atony [KÖHLER unpubl.]. This backswing-mechanism will be the subject of the third chapter of this dissertation, but it is worth mentioning that the decline of forces during the repeated lever movement did not affect the reliability of the lever mechanism.

A further mentionable finding is the fact that in species like *S. pratensis* the upper lip is responsible for high average-forces. Without the upper lip holding back the pollen sacs and boosting the demanded lever-moving-force, especially the first time of release, the average-forces would be lower by far (compare fig. 1.9). This is a further hint that the lever

mechanism itself might not be a mechanism to select pollinators by force. A selection mechanism which is trumped by a second structure is invalid. Besides the forces required to penetrate the hairy ring of *S. verticillata* are comparable to the forces required to move the levers (see annex tab. A.1 and fig. A.1). The hairy ring of other *Salvia*-Species such as *S. glutinosa* can be penetrated with comparable ease to *S. verticillata* [see REITH et al. 2006]. So the hairy ring of *S. verticillata* is not special in this respect. It is a structure that can be found in other species of that genus, too. The lever mechanism is not harder to overcome than the hairy ring, a common structure of the *Salvia*-flower. This again makes it unlikely that the lever is some kind of obstacle. And as e.g. *S. pratensis* can be visited by at least 13 different pollinators [TWERASER 2000], the lever mechanism does not seem to exclude pollinators but to be able to serve a wide rage of pollinators.

So the hypothesis that the lever might be a barrier to select pollinators and to limit access to the flower has to be refuted. I presume it to be an instrument to deposit pollen exactly on a visiting insect and to ensure pollen portioning (see chapter 2). It has already been shown that insects visiting different species of sympatric sages are loaded with pollen on different spots which are typical for the species [CLAßEN-BOCKHOFF et al. 2004b]. This minimizes the risk of hybridisation and squandering of pollen in sympatrically occurring populations. Investigations on the lever as a tool to portion pollen and to deposit pollen precisely and species-specific on an insect's body are subject of the second chapter.

2. Pollen-placement and pollen-portioning in diverse Salvia-species

2.1. Abstract

The genus *Salvia* is characterized by a specific modification of the androeceum. A highly evolved lever mechanism is formed by the stamens. Force measurements on this staminal lever mechanism and on pollinators recently refuted the hypothesis that weak flower visitors are excluded by this mechanism. In fact the staminal lever is considered as a highly effective and accurate tool to portion and place pollen on the pollinator's body. Morphological data of the flower and the stamen in 14 highly diverse *Salvia*-species were collected and the levers abilities for exact pollen placement and pollen portioning were tested in 13 diverse *Salvia*-species. The data support the hypothesis that the staminal lever mechanism is a tool of highly accurate pollen placement and pollen portioning. Each species has species specific lever-lengths and sympatric species have individual and well defined spots of pollen placement on the pollinator. Furthermore, the lever serves as an excellent pollen portioning device. The pollen/ovule-ratios of 280.8:1 (*S. aethiopis*) up to 1047.5:1 (*S. forskahlii*) indicate a highly effective pollination system. The size and dimension of the area of pollen deposition are constant and species specific. Therefore, the lever is a precise instrument of precygotic isolation.

2.2. Introduction

The safe and efficient transfer of pollen from anthers to stigma is essential for the reproductive success of a plant. Plants have to ensure that as little pollen as possible gets lost or wasted [WESTERKAMP 1993, 1997]. Anemophily is often seen as less effective than zoophily [CRUDEN 1977, 2000, ACKERMAN 2000] because in zoophilous plants pollen is delivered directly to the next stigma and is not just spread into the environment [FAEGRI & VAN DER PIJL 1971]. In anemophilous species pollen usually has to reach the stigma accidentally. Anemophilous plants have to bargain for a high loss of pollen as pollen concentration declines with distance from the source [MCCARTNEY & LACEY 1991, WILKINSON et al. 2003 JAROSZ et al. 2003]. Therefore anemophilous plants produce high amounts of pollen, which is one reason for high pollen/ovule-ratio.

Efficiency of pollination can be up to seven times better when animals are involved [HAYTER & CRESSWELL 2006]**.** Zoophilous species can influence transfer of pollen to a certain extent. Though animal pollinators can be influenced by several biotic and abiotic factors and

sometimes harvest pollen to raise their offspring [WESTERKAMP 1993, 1997] or even infect plants with various diseases [ALEXANDER et al. 1993, THRALL & JAROSZ 1994, BIERE & ANTONOVICS 1996] they still ensure a minimum of pollen transfer [HAYTER & CRESSWELL 2006] and therefore are a more effective pollen vector than wind [CRUDEN 1977, 2000, ACKERMAN 2000, HAYTER & CRESSWELL 2006, FAEGRI & VAN DER PIJL 1971].

The genus *Salvia* features a unique staminal lever mechanism, which deposits pollen on the pollinator's body. Its explicit function and meaning still could not be uncloaked.

There have been several hypotheses, whether it is a barrier, which might exclude insects which are too weak to get over it [CLAßEN-BOCKHOFF et al. 2003, THIMM 2003, chapter one].

The barrier hypothesis, which proposed the lever as an instrument to exclude weak pollinators has been recently refused [THIMM et al. 2003, chapter one, CLAßEN-BOCKHOFF et al. 2004a, REITH et al. 2006]. The required forces are no match for insects or hummingbirds [THIMM et al. 2003, chapter one].

So it is likely that the lever mechanism must serve another purpose [see chapter one]. The lever can be released several times in a row [THIMM et al. 2003, chapter one]. Keeping in mind that different sympatric species of *Salvia* share the same pollinators, a mechanism to avoid hybridisation could be quite ingenious. It seems that the lever of *Salvia* always hits the same spot on a pollinator, when pollen is released [TWERASER 2000, CLAßEN-BOCKHOFF et al. 2004b]. Different species have different lever sizes and forms. So a mechanism that repeatedly gives pollen away to a defined spot on a pollinator averting hybridisation and pollen waste would be the very key innovation [see CLAßEN-BOCKHOFF et al. 2004b] emerging all aforementioned features and promoting speciation.

In the present chapter, diverse species and their morphometric flower data are compared in order to find differences and similarities between the species and their staminal lever. The goal of this chapter is to illuminate the purpose of the complex lever mechanism in *Salvia*. This issue was investigated by measuring the length of the levers and acquiring the pollen portions given to the pollinators. To test the hypothesis, to which the lever could serve as a mechanical isolation in sympatric species, four sympatric Austrian species were included in the investigations. *S. austriaca*, *S. glutinosa*, *S. pratensis* and *S. aethiopis* occur sympatrically in Austria and share the same pollinators [TWERASER 2000, CLAßEN-BOCKHOFF et al. 2004b]. The efficiency of a pollination mechanism can be derived from the pollen/ovule-ratio [CRUDEN 1977]. But until now P/O-ratios for only three *Salvia*-species (*S. cardinalis*, *S. chiapensis* and *S. coccinea*), which are all ornithophilous species from Mexico

have been published [CRUDEN 1977, GRASES & RAMIREZ 1998]. Literature data were compared with recent results and further, for reason of better comparison, the P/O-ratio of *S. coccinea* was determined once again.

2.3. Material and Methods

2.3.1 Species

Of the fifteen species, which had been investigated for force measurements (chapter one), twelve were chosen for experiments on pollen portioning: *S. aethiopis* L, *S. africana-lutea* L., *S. canariensis* L., *S. exserta* Griseb., *S. forskahlii* L., *S. glutinosa* L., *S. involucrata* Cav., *S. patens* Cav., *S. pratensis* L., *S. sclarea* L., *S. thermarum* Van Jaarsv., *S. uliginosa* Benth. (fig. 1.1). In addition and to verify literature data *S. coccinea* was investigated.

The morphometric data on the staminal lever were taken in fourteen morphologically and ecologically diverse *Salvia* species from different systematic sections and geographical regions: *S. aethiopis* L., *S. austriaca, S. africana-lutea* L., *S. canariensis* L., *S. exserta* Griseb., *S. forskahlii* L., *S. glutinosa* L., *S. involucrata* Cav., *S. mexicana* L., *S. patens* Cav., *S. pratensis* L., *S. sclarea* L., *S. thermarum* Van Jaarsv., *S. uliginosa* Benth. (fig. 1.1 & 1.2, tab.1.1).

2.3.2 Morphometry / Pollen placement

The lever's dimension is an important factor for the pollen placement. Data of the absolute length of the adaxial (=sterile) lever arm (fig. 1.7c) as well as the absolute length of the abaxial (=fertile) lever arm (fig. 1.7a) and the straight distance between the joint and the pollen-sacs (fig. 1.7b) were taken from the preceding study (chapter one).

2.3.3 Pollen portioning

The following investigations do not claim to be a perfect imitation of nature but standardized laboratory conditions had to be established to guarantee comparable and reproducible results. To test portioning and constant positioning of the pollen, the measuring device described in chapter one (fig. 1.3) was used. Originally designed to measure forces to move the lever, it was now used to ensure steady and controllable conditions. Self-made bee

dummies were used and a special probe, which could carry the dummy and also release the lever (fig. 2.1), was designed. In order to create dummies, which in matter of size are comparable to real pollinators, the thorax height of 10 randomly chosen individuals of *Bombus terrestris* was measured and dummies whose dimensions lay within the measured parameters were designed. The 10 individuals of *Bombus terrestris* were dead and had been had been provided by Enikö Tweraser (Institut für Spezielle Botanik und Botanischer Garten, Universität Mainz). The bee dummies were covered with fleecy cloth to imitate the furry thorax of a flower visitor like a bee or bumble-bee. The cloth ensured that the pollen adhered to the fake bee. The dummy then was positioned on the special probe. While the probe released the lever, the dummy was hit by the pollen sacs and pollen was left on the dummy (fig 2.2). During this artificial pollination, the force needed to move the lever did not exceed the maximum forces available by pollinators nor the maximum forces needed to release the lever in this particular species (chapter one). After each release and pollen deposition a new clean dummy was put onto the dummy-probe.

The pollen on the bee dummies was counted under a binocular eyepiece (Leitz-Wetzlar, Germany)**.** The tests ended when no pollen came out of the pollen sacs anymore. The pollen still remaining in the pollen sacs was also counted. The number of required bee dummies, which would be the number of portions per flower, was registered. The plants had been bagged to keep pollinators off the plants and to hinder them from taking pollen.

Pollen sacs did not open properly on humid or rainy days and pollen grains used to agglomerate when humidity was too high. Under dry and warm conditions the pollen sacs were wide open and the pollen was dry and grainy. Optimal conditions for pollen release were at a relative humidity of about 40% and a temperature between 20-25°C. The collecting of the flowers always had to be managed very carefully to avoid loss of pollen by tremor. Sometimes the slightest touch could cause the pollen to fall out of the pollen sacs.

Fig. 2.1: A special probe, capable to carry a pollinator-dummy and to release the lever approaches a flower of *S. pratensis*.

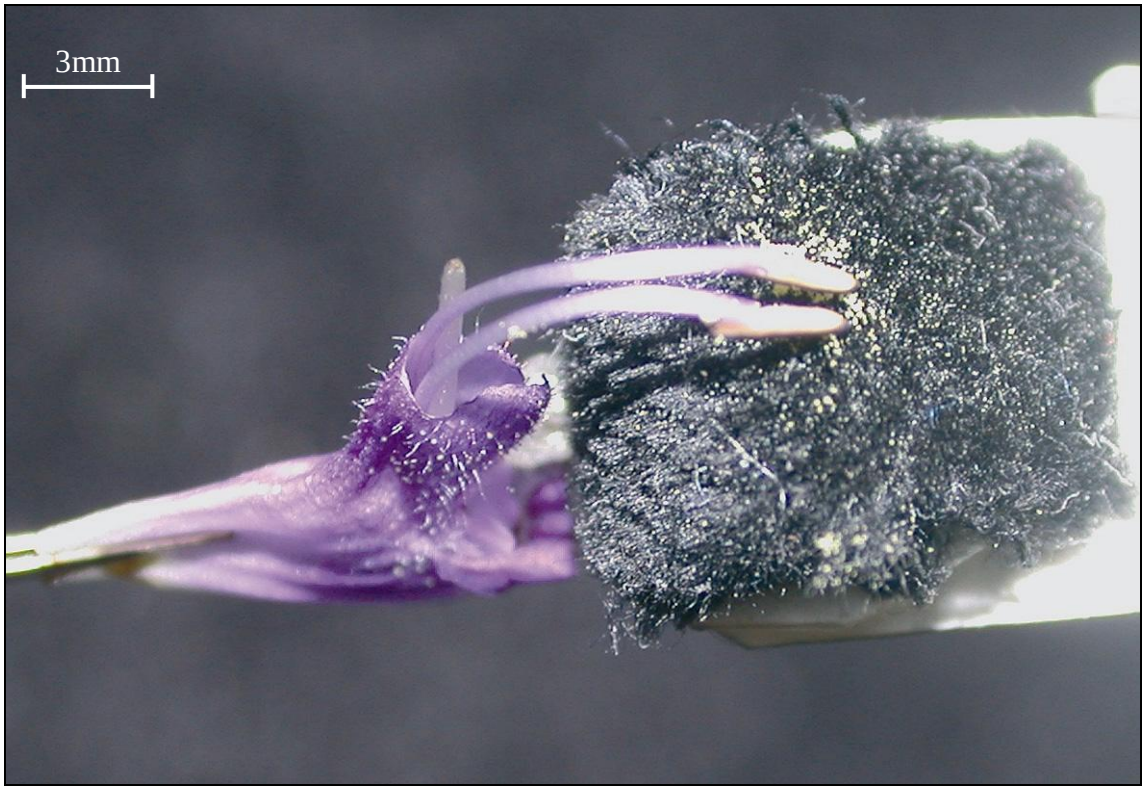


Fig. 2.3: *S. pratensis* placing the pollen the dummy. Afterwards the pollen was counted.

2.3.4 Comparison with literature

To verify the method of pollen determination described in literature and to align current results with data from literature, the method of determine pollen by counting pollen deposited on bee dummies was applied to *S. coccinea* L.. *S. coccinea* was chosen because P/O-data are already known and it is cultivated in the Botanic Garden of Mainz. To double-check the method on *S. coccinea* a second method for pollen counting was used. Buds were collected just prior to flowering and preserved in 70% ethyl alcohol solution. To determine the pollen count, the anthers were transferred into plastic tubes containing 100µl of a glycerol-toluidine-solution. The anthers were ground in order to release the pollen. The solution was subsequently homogenized. Three 5µl samples were taken out of each tube and placed on microscope slides. Each sample was enumerated at 20-40x magnification under a binocular eyepiece (Leitz-Wetzlar, Germany). Then the average sum of pollen grains per sample was extrapolated to the total number of pollen grains per anther.

2.3.5 Arena of pollen deposition

On each dummy the size of the area that had been covered with pollen was determined, by means of a sliding calliper. This should answer the question, whether the area where pollen is placed on the pollinator is of constant size and circumference.

2.4. Results

The species are highly diverse both in dimension of flowers and dimension of levers (tab. 1.3). Deviation in both cases is low, especially in the levers' dimensions. Ornithophilous *S. patens* is the biggest flower while melittophilous *S. verticillata* is the smallest flower (fig.1.1). Bird pollinated *S. involucrata* has the longest adaxial lever arm (11.68 +/-0.87mm) and the shortest abaxial lever arm (5.09 +/-0.54mm). As its abaxial lever is not curved, the joint–pollen sac distance is equal to the abaxial arm's length. The longest abaxial arm can be found in *S. patens,* which also covers the longest joint–pollen sac distance.

2.4.1 Pollen placement

Throughout all species the lever's dimension is subject to close tolerance. Especially deviation of the joint-pollen sac distance (fig. 1.7b) is low (tab. 1.3).

An analysis of variance of the joint-pollen sac distance showed that there are several species, which do not differ significantly or are even equal in the joint-pollen sac distance (tab. A.7). Most of them do not occur sympatrically. Only two cases have occurred in which sympatric species are significantly equal in their joint-pollen sac distance. These are *S. aethiopis* with *S. pratensis* and *S. austriaca* with *S. glutinosa.*

2.4.2 Pollen deposition

Apart from lever length (pollen placement), pollen portioning and P/O-ratio, the pollen depositioning was investigated. The deposition areas are well defined and constant in shape and size (tab. 2.1). Only slight variances were recognized. In some cases the length of the areas diminished due to declining portions of pollen. The deviation in the area of pollen placement is caused by this phenomenon of shrinking pollen portions and not by imprecise pollen placement. Some species had an interfacial chasm due to pollen sacs that were separated even on impact. An important point was that no smearing was detected. Pollen was placed on the exact point where the pollen sacs hit the dummy. Even when the dummy was moved further into the flower the pollen sacs held their position due to the elastic and arcuated connective lever arm.

Tab 2.1: Precision of pollen deposition: The area of pollen deposition on the pollinators' bodies is well defined and deviation is low. Some species leave a gap or chasm in this area, which can be traced back to the fact that these species' pollen sacs still are separated when they hit the pollinator.

	area of pollen-placement [mm²]	**dev**	**length of area [mm]**	**dev**	**width of area [mm]**	**dev**	**of chasm [mm]**	**dev**	**n (flowers)**
S. aethiopis	2.32	1.01	1.95	0.83	1.19	0.03	no chasm	--	6
S. africana-lutea	4.13	0.53	2.55	0.25	1.61	0.10	no chasm	--	6
S. canariensis	3.03	0.47	2.43	0.08	1.24	0.17	no chasm	--	8
S. exserta	8.17	1.78	3.08	0.39	2.68	0.39	1.09	0.33	8
S. forskahlii	7.00	0.87	2.33	0.29	3.00	0.00	1.00	0.00	7
S. glutinosa	6.08	1.12	5.27	0.64	1.20	0.19	no chasm	--	7
S. involucrata	2.70	0.30	1.70	0.09	1.59	0.13	no chasm	--	4
S. patens	3.55	0.33	3.11	0.06	1.14	0.10	no chasm	--	6
S. pratensis	2.11	0.23	2.49	0.14	0.85	0.08	no chasm	--	4
S. sclarea	2.78	0.08	2.25	0.06	1.23	0.03	no chasm	--	9
S. thermarum	8.99	1.71	2.97	0.62	3.06	0.30	0.99	0.17	5
S. uliginosa	5.08	0.38	1.64	0.08	3.10	0.25	1.27	0.14	6

2.4.3. Pollen portioning

The pollen portioning-test (tab. 2.2 & 2.3) showed that there was always the same pattern in releasing the pollen though different amounts of pollen were released. The pattern merely varied for each species. In the beginning the pollen load was quite high but on proceeding releases it distinctly dropped off, until after up to 17 releases (*S. africana-lutea)* no pollen was detected on the dummies any more (tab. 2.3). The last counts always were between 10-50 pollen grains. However, the pollen sacs were not empty, yet. A number of about 100-200 pollen grains, which did not come out, always remained in the pollen sacs.

Tab 2.2: Pollen load, pollen portioning and P/O-ratio in the selected *Salvia* species. *S. coccinea* was tested to verify the method of pollen-count. A second test (*S. coccinea* 2nd*)* with a more common method should double-check the results

	sum of pollen grains released	dev	pollen grains remaining in ps	dev	average sum of pollen grains per flower	dev	average pollen portion per release	dev	average number of portions = av. number of releases	dev	pollen-ovule-ratio	log (p/o)-ratio	n
S. aethiopis	1023.3	230.6	120.0	51.5	1143.3	266.1	165.9	214.7	6.2	1.3	285.8	2.4	6
S. africana-lutea	2803.3	169.1	241.7	80.1	3045.0	124.4	215.6	184.5	13.0	3.3	761.3	2.9	6
S. canariensis	1677.8	573.8	550.0	160.7	2227.8	552.5	227.8	227.7	7.3	1.7	556,95	2.7	8
S. exserta	1572.0	293.4	525.0	108.7	2097.0	236.6	230.4	196.8	6.2	1.1	524.3	2.7	10
S. forskahlii	3848.3	1298.7	410.0	201.2	4190.0	1307.6	355.2	277.6	10.8	2.6	1047.5	3.0	7
S. glutinosa	3411.4	315.1	492.9	354.1	3904.3	380.4	497.5	480.2	6.9	2.0	976.1	3.0	7
S. involucrata	3677.5	263.7	112.5	65.0	3790.0	229.3	408.6	437.9	9.0	2.2	947.5	3.0	4
S. patens	2756.7	731.5	166.7	81.6	2923.3	663.3	300.7	358.1	9.2	2.6	730.8	2.9	6
S. pratensis	3413.3	300.9	516.7	425.2	3930.0	710.4	284.4	193.2	12.0	0.0	982.5	3.0	4
S. sclarea	3614.4	765.5	125.6	113.5	3740.0	716.4	361.7	352.9	10.8	2.4	935.0	3.0	9
S. thermarum	2670.0	805.1	210.0	114.5	2880.0	849.9	256.7	185.4	11.5	0.6	720.0	2.9	5
S. uliginosa	2555.0	453.0	733.3	367.0	3288.3	315.4	300.6	252.1	8.6	3.5	822.1	2.9	6
S. coccinea	2215.3	588.4	235.0	85.7	2450.3	566.4	250.8	164.1	8.8	1.9	612.6	2.8	7
S coccinea 2nd					3631.1	210.1					907.8	3.0	3

Tab 2.3: The amount of pollen per portion constantly decreased with proceeding lever releases.

	number of portion	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
average amount of pollen grains per portion	*S. aethiopis* (n=6)	500.0	295.0	78.3	40.0	60.0	73.3	23.3	10.0									
	dev	176.1	258.7	66.2	27.6	93.6	68.1	23.1	0.0									
	S. africana-lutea (n=6)	575.0	450.0	408.3	250.0	230.0	168.3	198.3	153.3	105.0	55.0	65.0	86.7	56.7	110.0	75.0	60.0	40.0
	dev	384.4	181.7	139.3	104.9	86.0	86.6	83.8	137.4	79.7	49.3	50.0	51.3	45.1	28.3	21.2	0.0	14.1
	S. canariensis (n=8)	518.8	456.3	227.5	181.3	123.8	110.0	78.0	36.7	45.0	2.0							
	dev	295.1	280.9	88.1	84.3	72.3	49.7	25.9	23.1	7.1	0.0							
	S. exserta (n=10)	625.0	285.0	210.0	175.0	157.0	116.7	90.0	50.0									
	dev	190.4	115.6	39.4	48.6	41.4	51.6	22.4	0.0									
	S. forskahlii (n=7)	525.0	500.0	608.3	475.0	383.3	326.7	380.0	244.0	290.0	176.0	87.5	80.0	70.0				
	dev	379.1	212.1	290.5	125.5	377.7	335.4	251.5	142.9	163.6	121.0	25.0	26.5	84.9				
	S. glutinosa (n=7)	1050.0	1158.3	416.7	383.3	280.0	237.5	100.0	90.0	30.0	50.0							
	dev	295.0	672.6	172.2	238.0	90.9	103.1	0.0	14.1	28.3	0.0							
	S. involucrata (n=4)	1375.0	625.0	650.0	300.0	200.0	142.5	162.5	93.3	105.0	150.0							
	dev	505.8	189.3	238.0	40.8	91.3	15.0	47.9	51.3	91.9	0.0							
	S. patens (n=6)	800.0	808.3	403.3	241.7	191.7	130.0	70.0	75.0	56.7	40.0	35.0	40.0					
	dev	352.1	443.2	317.9	142.9	163.0	114.0	30.8	50.0	20.8	26.5	7.1	28.3					
	S. pratensis (n=9)	550.0	500.0	500.0	450.0	283.3	266.7	283.3	200.0	156.7	116.7	60.0	46.7					
	dev	50.0	200.0	100.0	150.0	76.4	76.4	189.3	0.0	51.3	57.7	17.3	5.8					
	S. sclarea (n=5)	1044.4	605.6	583.3	266.7	268.9	222.2	168.9	125.0	141.4	136.7	142.0	88.0	60.0	10.0			
	dev	495.9	282.2	333.5	109.0	140.0	166.0	104.7	53.5	69.9	80.4	68.0	44.4	56.6	0.0			
	S. thermarum (n=5)	444.0	444.0	400.0	250.0	290.0	286.0	192.0	175.0	107.5	137.5	60.0						
	dev	211.4	248.4	183.7	100.0	119.4	155.7	109.9	119.0	72.3	85.4	27.1						
	S. uliginosa (n=6)	691.7	550.0	458.3	245.0	241.7	175.0	117.5	75.0	70.0	100.0	100.0	65.0					
	dev	201.0	273.9	180.0	101.7	66.5	95.7	62.4	28.9	52.0	0.0	70.7	21.2					
	S. coccinea (n=7)	425.0	391.7	283.3	321.7	258.3	213.3	144.0	86.4	77.5	400.0	50.0	20.0					
	dev	121.6	105.7	98.6	101.7	113.3	179.5	84.5	65.7	72.6	0.0	0.0	0.0					

2.5. Discussion

2.5.1. Floral diversity and pollen transfer mechanism

2.5.1.1. Length of levers constant – area of pollen placement well defined

As pollen is a rare and precious good (tab. 2.2), loss of pollen has to be prevented. An efficient and precise pollination mechanism or an isolation mechanism that avoids hybridisation could save pollen. A common and highly effective precygotic isolation mechanism is temporal isolation [GERARD et al. 2006, LEVIN 1971]. Just by flowering at different times sympatric species can eliminate the possibility of pollen waste caused by hybridisation [YANG et al. 2007]. Another most effective type of (mechanical) isolation is to adapt to different pollinators with different body sizes and behaviour [GRANT 1993, 1994a,b]. Some species of the genus *Salvia* happen to share the same pollinators as well as flowering time [TWERASER 2000, KUSCHEWITZ 2004]. An association of simultaneously flowering species can help to attract pollinators, which would ameliorate reproductive success [KUNIN 1993, 1997, KWAK 1988, LAVERTY 1992, OHASHI & YAHARA 1998, PETANIDOU et al. 1995, RATHCKE 1983]. However, an accumulation of closely related species flowering at the same time and sharing the same pollinator bears the danger of pollinator competition [LEVIN & ANDERSON 1970] and furthermore interspecific pollen transfer [GARDNER & MACNAIR 2000]. The latter leads to undesirable obstructions in the process of reproduction [YANG et al. 2007]: such as blocked stigmas, reduced seed set [GALEN & NEWPORT, 1988, KWAK & JENNERSTEN, 1991, PETANIDOU et al. 1995, WASER 1978, WELLER 1979], disabled seeds or sterile hybrids [CHARLESWORTH 1989, GARDNER & MACNAIR 2000]. Therefore the strategy of using different places of pollen placement on one common pollinator has been noticed for some sympatric and closely related species [GRANT 1994b].

But this requires a species-specific, precise and reliable tool for pollen placement. Such mechanisms of precise pollen placement have been recognized in several other genera like *Pedicularis* [MACIOR 1982; GRANT 1994b], *Rhinanthus* [KWAK 1978], *Polygala* [BRANTJES 1982], *Stylidium* [ARMBRUSTER et al.1994], *Heliconia* [STILES 1975] or Fabaceae [FAEGRI & VAN DER PIJL 1971, WESTERKAMP 1993, 1997] and orchids [VAN DER PIJL & DODSON 1966, DRESSLER 1968, 1981, SIMONS 1992].

The staminal lever in the genus *Salvia* is directly involved in the process of pollination. Without the lever no pollen would be placed on the pollinator let alone that any

pollen would be transferred to another stigma. In this connection the length of the lever or to be more precisely the distance covered by the lever inheres in an important key role.

The lever lengths of the investigated *Salvia*-species were relatively constant and the length measurements had a low deviation. It turned out that the absolute length of the abaxial lever arm (fig. 1.7a, tab. 1.3) could vary but the distance covered by it, the joint-pollen sac distance, (fig. 1.7b, tab. 1.3) was highly constant. Deviation of the joint-pollen sac distance (fig. 1.7b, tab. 1.3) mostly was smaller than deviation of the abaxial lever arm (fig. 1.7a). So even if the absolute length of the abaxial lever arm varies, the distance covered by it (the joint-pollen sac-distance) is always the same. The constancy of this distance seems to be an important point for the plant as it determines the space of pollen placement on the pollinator's body. Significant differences between the joint-pollen sac distances in several sympatric species were detected (tab. A.7). Few species have a comparable joint-pollen sac distance. Even less similar joint-pollen sac distances are found in sympatric species. So each species has a more or less specific distance between joint and pollen sacs. The pollen sac's length, as well, is constant (data not shown). Constant and species specific distances covered by the lever leads to specifiable and species specific areas on the insects body for each species to deposit pollen. Furthermore, it could be proved that these areas of pollen deposition are well defined indeed (tab. 2.1). Their shape and dimensions may differ slightly due to shrinking portions of pollen, but generally their position and dimension stay constant. A less accurate pollen placement and smearing is reported especially for ornithophilous species with long corolla-tubes and reduced lever mechanism [WESTER & CLAßEN-BOCKHOFF 2006, WESTER & CLAßEN-BOCKHOFF 2007]. However, melittophilous species possessing a working lever mechanism are thought to avoid smearing [WESTER & CLAßEN-BOCKHOFF 2007]. As a matter of fact no smearing was recognized during the experiments of pollen placement, as the flexible lever keeps the position of the pollinator's body even if it is engaging the flower. These results match with earlier observations in which different and species-specific areas of pollen placement on bees serving as pollinators for different *Salvia*-species had been described [CLAßEN-BOCKHOFF et al. 2004b]. Such species-specific areas of pollen positioning can avoid hybridisation and enhance pollination and reproduction. As all floral parameters are constant and species-specific, at last the pollinator itself has to be locked into a special position, which guarantees the exact and species-specific pollen placement. Specially formed flower parts such as corolla tube and the adaxial lever arms could guide the pollinators way ensuring a fixed position for pollination [WESTER & CLAßEN-BOCKHOFF 2007, see also REITH et al. 2007].

So the lever mechanism obviously enables *Salvia* to place the pollen on species-specific and well defined areas on the insect's body. In sympatric species each *Salvia* species would have its own and species-specific place on the pollinator to deposit its pollen, enhancing its efficiency. The efficiency of a pollinator is a contribution to the plant's fitness [HERRERA 1987, 1989, KEARNS & INOUYE 1993, FISHBEIN & VENABLE 1996, WASER et al. 1996, TRAVESET & SÁEZ 1997, GÓMEZ 2000]. The more pollen manages to reach the receptive stigma of the own species, the better the male fitness [YANG & GUO 2007, CLAßEN-BOCKHOFF et al. 2004b, MOLLER & ERIKSSON 1994].

2.5.1.2. Sympatric species with similar abaxial lever arms

There are two cases of sympatric species with comparable or even equal joint-pollen sac distance and that in addition share the same pollinator (tab. A.7): *S. austriaca* and *S. glutinosa* as well as *S. aethiopis* and *S. pratensis* [TWERASER 2000, CLAßEN-BOCKHOFF et al. 2004b]. Different flowering-time could at least minimise the possibility of interspecific pollen transfer and avoid pollen waste and hybridisation [GRANT 1992, 1994b]. But this case is not given. *S. austriaca* and *S. glutinosa* have successive flowering periods [TWERASER 2000] which does not eliminate the possibility of overlapping flowering time. But *S. austriaca* and *S. glutinosa* have a quite elegant and simple solution. Though their joint-pollen sac distance does not differ (tab. A.7), they still have different areas to deposit the pollen. *S. austriaca* places the pollen lateral (fig 1.2C) and *S. glutinosa* places its pollen nototribic on the insects dorsal side (see also CLAßEN-BOCKHOFF et al. 2004b).

The other two species, *S. aethiopis* and *S. pratensis*, both leave the pollen on the insects back. They both bloom between May and August and share the same habitat [KRÜNITZ 1773-1858, KUSCHEWITZ 2004, TWERASER 2000, ROTHMALER 2002]. So no temporal or any other precygotic isolation is visible. Hybridisation occurs in the genus *Salvia* [EPLING 1947, GRANT &GRANT 1964, HAQUE & GHOSHAL 1981, PALOMINO et al. 1986, HUCK 1992, OWENS & UBERA-JIMÉNEZ 1992, RAMAMOORTHY & ELLIOTT 1998]. Waste of pollen and hybridisation can be direct consequences of this lack of difference. This truly shatters the hypothesis of the lever being an instrument for mechanical isolation. Nevertheless, it has been reported that if *S. aethiopis* and *S. pratensis* occur sympatrically, they do have different anthesis [TWERASER pers. conversation]. Furthermore, an ethological isolation mechanism occurs as pollinators collecting nectar in a population of a certain species are known not to switch to flowers of a different species, even if the plants occur sympatrically and blossom simultaneously [TWERASER 2000]. Finally, due to the fact that *S. aethiopis* has been

introduced to Germany in the 17th century [BESLER 1613, ROTHMALER 2002] this might be a quite new and unforeseen problem in evolutionary history of *Salvia.*

2.5.2 P/O-ratios

The pollen/ovule-ratio favoured by CRUDEN (1977) is a common method to determine the breeding system of plants. [CRUDEN 1977, DAMGAARD & ABBOTT 1995, HUANG et al. 2002, JÜRGENS et al. 2002, MIONE & ANDERSON 1992, NIETO-FELINER 1991, PRESTON 1986, RITLAND & RITLAND 1989, WYATT et al. 2000, YASHIRO et al. 1999]. The species can easily be assigned to one of the five breeding systems (xenogamy, facultative xenogamy, facultative autogamy, obligate autogamy, cleistogamy) [CRUDEN 1977]. But P/O-ratio bears certain dangers of misinterpretation [BENNETT 1999]. Several factors, such as the pollen vectors, pollination mechanisms, flower morphology [MCDADE 1985, WYATT et al. 2000, WANG et al. 2004] and ecological factors [CRUDEN 2000, GALLARDO et al. 1994, JÜRGENS et al. 2002, RAMIREZ & SERES 1994, SMALL 1988], affect the number of pollen and ovules and thus also the P/O-ratio [CHOUTEAU et al. 2006, JÜRGENS et al. 2002]. Significant variations in P/O-ratios within one species can also be caused by the habitat. Pollination systems change increasingly from pollination by insects to self-pollination as they are found farther north [e.g. *Anthyllis vulneraria*, see COUDERC 1978, KLOTZ et al. 2002, NAVARRO 1999]. An accompanying change is to be expected for the P/O-ratios [e.g. *Petrorhagia prolifera*: Mediterranean: P/O = 241, Central-European: P/O = 69, THOMAS & MURRAY 1981]. These facts have to be applied cautiously when discussing Central-European populations [KLOTZ et al. 2002]. The sorting just by P/O-ratio seems risky and imprecise, according to this phenomenon. Even studies that tend to be opposite to Cruden's data [1977, 2000] are known [CHOUTEAU et al. 2006]. Nevertheless, several studies affirm the P/O-ratio as a reliable indicator of the breeding system [CRUDEN 2000, GALLARDO et al. 1994, JÜRGENS et al. 2002, MCDADE 1985, LOPEZ et al. 1999, WYATT et al. 2000].

Cruden stated that "the more efficient the transfer of pollen is, the lower the P/O ratio should be" [CRUDEN 1977, 2000]. The pollen sacs of the investigated *Salvia*-species bore a constant amount of pollen, varying only slightly from flower to flower. The P/O-ratio of the investigated species ranges from 280.8:1 to 1047.5:1 (tab. 2.2). According to CRUDEN (1977) these results suggest a very effective pollination system. They seem to be too high for autogamous [P/O-ratio ~ 27.7:1, CRUDEN 1977] or facultatively autogamous [P/O-ratio ~ 168.8:1, CRUDEN 1977] species and too low for xenogamous species [P/O-ratio ~ 5859.2:1,

CRUDEN 1977]. Literature offers P/O-ratios for only three ornithophilous Mexican species: *S. cardinalis* Kunth. with a P/O-ratio of 19525:1, *S. chiapensis* Fern. with a P/O-ratio of 11687:1 [Cruden 1977] and *S. coccinea*, which has a P/O-ratio of 11343.36:1 [GRASES & RAMIREZ 1998]. As these P/O-ratios are very much higher than the current findings (tab. 2.2), I had serious doubts about the correctness of my results and the accuracy of my method of determining the pollen amount by counting the pollen left on the bee dummies. Several sources of error came into consideration: (1) pollen could have been stolen somehow though the flowers were sealed off from any considerable pollinator or thief. (2) Pollen could have inwardly been lost during harvesting, transporting or investigating the flowers. (3) A simple miscount. But it is quite unlikely that an error of this magnitude prevails through all investigated species and individuals. Nevertheless, *S. coccinea* was examined to verify the method of pollen counting. In addition to the method of pollen counting, a second method to double-check these results was tried out. Again the P/O-ratios did not match with the literature data nor did they match with each other (tab. 2.2). Apart from individual variation, the latter could be due to the fact that the second one of both results is only calculated from samples. Miscalculations can not be ruled out and, furthermore, the method involving this glycerine-toluidine-solution is known to be vague. The correct volume of glycerine has to be determined by try and error [KEARNS & INOUYE 1993]. As a matter of fact, literature data were determined by similarly uncertain methods. They are extrapolations from samples [Cruden 1977, GRASES & RAMIREZ 1998]. An obvious miscount occurred in the work of GRASES & RAMIREZ (1998). They determined the number of pollen per flower as 5360 +/- 1334.06 but stated a P/O-ratio of 11343.36:1 which would require about 0.47 ovules per flower. So the literature data referring to the P/O-ratios of other *Salvia*-species do not seem be accurate.

P/O-ratios of other Lamiaceae tend to be slightly higher than postulated for their breeding system [RAJU 1989, OLSEN et al. 1998]. Primarily autogamous *Ocimum americanum* and *Ocimum basilicum* have P/O-ratios of 350:1 and 330:1 [RAJU 1989]. A comparison of P/O-ratios of different genera is not suitable without reservation as P/O-ratios of single species within one family can differ though their breeding systems do not [CRUDEN 2000]. But these higher P/O-ratios in other Lamiaceae could indicate that in the genus *Salvia,* P/O-ratios generally are a little bit higher, too. *S. pratensis* is supposed to be xenogamous in the first instance [KLOTZ et al 2002]. But as some Lamiaceae seem to tend to higher P/O-ratios than suggested for their breeding systems, the reproduction of *Salvia* might be even more effective than the P/O-ratios suggest. Furthermore, latest insights revealed that *S. aethiopis*, *S.*

austriaca, S. glutinosa, S. pratensis and *S. verticillata* are facultatively autogam [TWERASER pers. conversation] and in New-World ornithophilous species potential selfing is assumed, too [e.g. *S. exserta*, WESTER pers. conversation].

2.5.3. Constant amount of pollen in pollen sacs / Pollen portioning

Plants can adjust to pollinators e.g. change the calyx length to serve different visitors with different proboscis lengths [DOHZONO & SUZUKI 2002]. Several plants adjust their pollen presentation and pollen release to their pollinators [HARDER & THOMSON 1989, HARDER & WILSON 1994, 1998, LEBUHN & HOLSINGER 1998, THOMSON 2003]. The lever leaves small portions of pollen on the pollinator. In the investigations on the pollen portioning up to 17 portions per flower (*S. africana-lutea*) were determined. These findings fit with former investigations on *S. pratensis* [CLAßEN-BOCKHOFF et al. 2004b]. The portioning of pollen is a common method [HARDER & THOMSON 1989] and increases the male fitness [CASTELLANOS et al. 2006]. By portioning the pollen the plant responds to potential unreliability of the pollinators [CASTELLANOS et al. 2006]. A comparable phenomenon has been described for the animal kingdom as well. Some fish e.g. dose their sperm in order to increase reproductive success [WARNER et al. 1995, WEDELL et al. 2002]. But deviation of the pollen portions is high for all investigated species (tab. 2.2). This can be explained by the fact that the first portions are relatively opulent and the successional portions represent less and less pollen. The first large portions ensure that as much as possible pollen is spread at once. Smaller successional portions are insurance for pollen spread, just in case the first portions are lost.

There are two methods of metering pollen in the plant kingdom: packaging (timing of the opening of anthers, flowers or inflorescences) and dispensing (portioning the pollen for every single visitor) [CASTELLANOS et al. 2006, HARDER & THOMSON 1989]. So a single *Salvia*-flower obviously is dispersing the pollen instead of packaging it. The whole plant is packaging the pollen, as the flowers open successively.

The pollen sacs were not fully emptied. About 3-22% of the pollen remains in the pollen sacs (compare tab. 2.2). Pollen remaining in pollen sacs at the end of anthesis is not unusual [SCHLINDWEIN et al. 2005]. But compared to other species as *Campanula* the *Salvia*-values are quite high [SCHLINDWEIN et al. 2005]. But these few high values of pollen remaining in the pollen sacs can probably be attributed to the youth of some flowers and the humidity. Nevertheless, the investigated *Salvia*-species manage to get a high percentage of their pollen onto the pollinators while e.g. in *Campanula* 95.5% of the plant's pollen is

harvested by bees [SCHLINDWEIN et al. 2005]. This can be seen as a selection advantage and an increased male fitness for the genus *Salvia*.

3. Anatomy and functionality of the staminal lever in the genus *Salvia*

3.1 Abstract

The staminal lever in the genus *Salvia* is a very special pollination- and pollen-portioning mechanism and a robust and highly precise tool which can endure much more strain than actually needed in daily use. But it is still unclear which design-feature ensures the repeatable pivoting and the swing-back of the lever into the neutral position. The only investigations referring to this topic date back more than one hundred years. Correns (1891) conjectured the driving force for the levers backswing in the joint-ligament itself. The results of this thesis confirm Correns' findings referring to the joint's durability and capacity. But it seems unlikely that solely the ligament is responsible for the re-erecting of the lever after being released. Surely some kind of spring-mechanism as Correns already stated has to be involved. However, present histological investigations indicate that the ligament itself is not the spring. It remains stiff and transmits the power of gyration onto the tissue of the filament next to the joint. The real cause of the re-erection of the lever has to be located in the tissue of the connective surrounding the joint. This very tissue absorbs the powers of the rotation and by releasing the tension it re-erects the lever.

3.2 Introduction

Though plants are quite stationary, the capability to perform movement is essential in the plant kingdom [HAUPT 1977, SIMMONS et al. 1995, UEDA & NAKAMURA 2006]. By moving single organs plants respond to internal or external impulses [DARWIN 1881, HAUPT 1977, HART 1990]. Most movements are slow and irreversible due to the fact that they are growing movements. But some movements are repeatable and can occur more rapidly [BYNUM 2001]. Such movements can be performed to protect organs [DARWIN 1881, BÜNNING & MOSER 1969, EISNER 1981, BRAAM 2005], to regulate water supply [LANGE et al. 1971], to promote seed spread [KERNER VON MARILAUN 1902, MASUDA & YAHARA 1994] or to ensure or enhance pollination [CLAßEN-BOCKHOFF 1991, SIMONS 1992, KENNEDY 2000, chapter two]. However, few plants are able to perform repetitive and reversible movements of their reproductive structures [BYNUM 2001]. The staminal lever mechanism of the genus *Salvia* is able to perform such a repetitive and reversible movement [SPRENGEL 1793, HILDEBRAND 1865, MÜLLER 1873, CORRENS 1891, TROLL 1929, HRUBÝ 1934, TRAPP 1956, WERTH 1956, CLAßEN-BOCKHOFF et al. 2003, 2004b]. The lever is initially moved by the

foraging pollinator but the backswing into the neutral position is managed by the plant itself [CORRENS 1891, CLAßEN-BOCKHOFF et al. 2003, 2004b]. How this backswing is managed, is still unclear. The only investigations concerning the movement itself date back over a hundred years.

CORRENS (1891) investigated the internal joint and the cell structures for the first time. No other investigations referring to the joint and the movement itself have been made since then. According to CORRENS (1891) so-called elastic cell-fibres within the ligament are likely to manage the re-erection of the lever. The fibres would be twisted and stretched when the lever is deflected by a pollinator. When the fibres de-twist and contract again, the lever is re-erected (fig. 3.1) [CORRENS 1891]. The motivation for this chapter was, to find out if these findings can be confirmed: Is the ligament the driving force of the lever's re-erection or is the backswing of the lever managed in some other way?

The backswing could be caused by the adaxial (=sterile) lever arm functioning as a counterweight. Otherwise turgor or the twisting cells in the joint could work like some kind of mainspring to re-erect the lever.

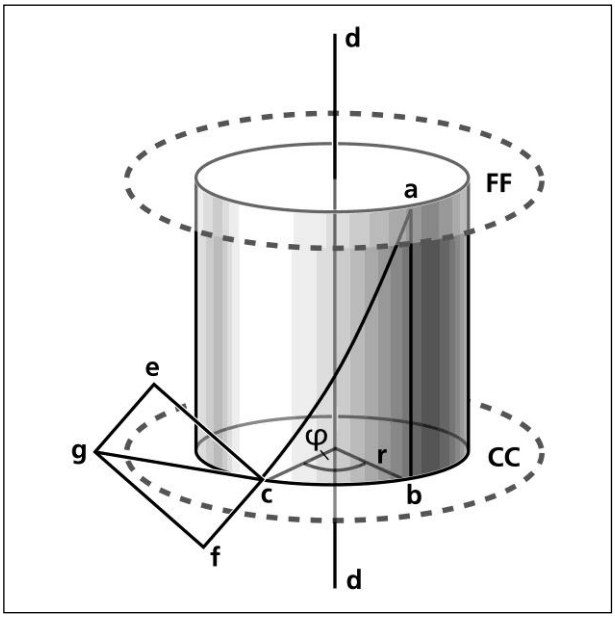


Fig. 3.1: Schematic drawing of the joint of the staminal lever in *Salvia* after Correns 1891. (CC: connective; FF: filament; a-b: cell fibre idle state; a-c: cell fibre deflected state; d: axis of rotation; r: radius of ligament, φ: angle of deflexion; c,e,f,g: are the corners of a parallelogram of forces.)

3.3 Material & Methods

3.3.1 The weights of the lever arms

To test the hypothesis that the adaxial lever arm functions as a counterweight causing the connective's backswing into the neutral position, the weight of the adaxial lever arm and the abaxial lever arm was determined in 14 species.

The connectives were cut out of the flower and the abaxial and adaxial parts were separated at the joint. Then the parts were separately weighed on a scale (Sartorius *basic* BA210S, Sartorius, Göttingen, Germany). To avoid wilting, the freshly separated parts were stored in a humid chamber. Preceding tests confirmed this method to be most effective. The weight of the separated parts varied extremely, if stored in water or a dry chamber.

3.3.2 Experiments on lever mobility and stability

The angle of deflection of the lever necessary for a successful pollen-deposition was tested and the maximum angle of deflection which is possible in the flower was determined. In other words this test should figure out whether the lever is at its maximum point of deflection when it hits the pollinator.

To determine the angle for pollen positioning, the upper petals were partially removed, the flower was put in front of a protractor and, while moving the lever, the angle between neutral position and the position of pollen deposition was noted.

To determine the exact lever position of pollen deposition in melittophilous flowers, the body of an averagely big bumble bee was used. The bumble bee was inserted into the flower till the pollen sacs hit the bumble bee. To determine the exact lever position of pollen deposition in ornithophilous flowers a paper model of a hummingbird's head (*Nectarinia violacea*) was used.

To determine the maximum angle of deflection in the flower, the lever was moved until the pollen sacs hit the lower lip or the adaxial arm touched the corolla tube. In order to test which maximum angle of deflection the joint could stand, the stamina were cut out of the flower. The filament was locked into position and the lever was moved far over its point of pollination to the very point at which the joint was ripped off.

To test the stability and flexibility of the connective, the abaxial part of the lever arm was fixed while the adaxial part of the lever arm was bent backwards till the connective or the joint broke.

3.3.3 Histological investigations

In order to identify and to quantify changes of the cellular structure within the joint caused by the lever movement, histological sections of the joint were made in neutral position and after lever deflection. The condition of a deflected lever was achieved by preventing the levers backswing with needles which were pierced through the petals.

Joints of *S. patens, S. pratensis, S. glutinosa, S. forskahlii* and *S. canariensis* were investigated. The whole flowers were fixed in ethanol (70%) for at least 24h. From now on it was important that the joints get as less air contact as possible. The joints were cut out of the flowers while the flowers and joints were kept under ethanol (70%). The deflected joints remained in their deflected position and did not swing back into neutral position. The joints had not become stiff either. Now the joints were dehydrated in an ascending ethanol series (75%, 80%, 85%, 90%, 95%, 60min each) then moved to a 1:1 mix of 95%EtOH and Rotihistol (#6640.1, Roth, Karlsruhe, Germany) and after 2h moved to 100% Rotihistol for an other 2h. After this the joints were transferred into a 1:1 mix of Rotihistol and Paraplast tissue embedding media (by McCormick Scientific, St. Louis, USA) and stored over night at 61 °C in a heating cabinet. During the following three days the joints were moved to fresh liquid Paraplast twice a day while they remained in the heating cabinet at a temperature of 58-61°C. After this procedure the joints were embedded in Paraplast. The fresh Paraplast blocks were first cooled down to room temperature and then stored at 4°C for 48h until they were completely hardened.

Sections of 8µm, 10µm or 12µm thicknesses were made with a rotary microtome (Leitz Wetzlar, Germany). Longitudinal sections were made as well as transversal sections. The joint was cut longitudinally as well as transversally (see fig. 3.2). To expand the selection set, the slices were stained with three different methods according to respective protocol, toluidine-blue [SAKAI 1973, GERLACH 1977], astra-blue/safranine [BUKATSCH 1972, GERLACH 1977] or with Delafield's Haematoxyline-eosine [see GERLACH 1977].

The sections were finally dehydrated in an ascending alcohol series, bathed in Rotihistol for 2-3 minutes, mounted with coverslips (Eukitt, Fluka 402907) and dried overnight at room temperature.

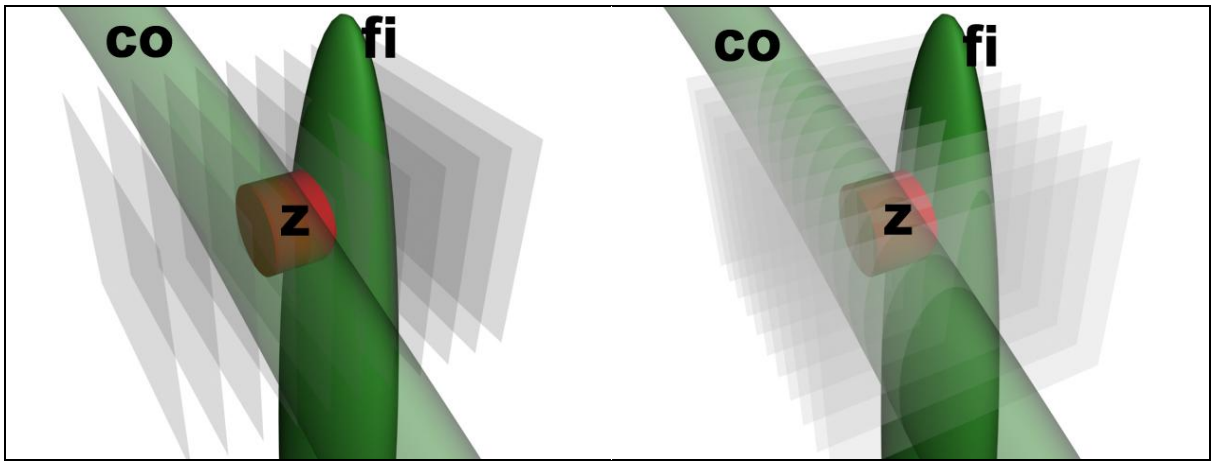


Fig. 3.2: Section plane through the joint area. Left: transversal sections of the ligament and longitudinal sections of filament and connective, right: longitudinal sections of the ligament and filament and transversal section of the connective. (co: connective, fi: filament, z: ligament) - original drawing by M. Gröteke.

3.4 Results

3.4.1 The weights of the lever arms

Tab 3.1: The adaxial and the abaxial lever arm were measured. In the majority of the cases the abaxial lever arm was heavier than the adaxial lever arm.

	average mass abaxial lever [mg]	dev	**average mass adaxial lever [mg]**	dev	**ratio of abaxial to adaxial lever**	**n**
S. aethiopis	1.16	0.12	0.07	0.12	1:17.4	8
S. africana-lutea	4.20	0.00	2.25	0.07	1:1.9	6
S. austriaca	0.67	0.14	0.05	0.12	1:13.3	7
S. canariensis	1.39	0.31	0.54	0.18	1:2.6	12
S. exserta	2.27	0.21	1.18	0.04	1:1.9	14
S. forskahlii	2.43	0.95	0.54	0.20	1:4.5	13
S. glutinosa	1.59	0.50	0.27	0.23	1:5.9	13
S. involucrata	5.41	0.43	9.74	0.43	1:0.6	14
S. mexicana	11.13	0.83	11.51	0.74	1:1	18
S. patens	1.80	0.18	0.49	0.05	1:3.7	14
S. pratensis	1.40	0.51	0.42	0.28	1:3.4	14
S. sclarea	3.97	0.46	1.50	0.23	1:2.6	10
S. thermarum	2.40	0.14	1.40	0.00	1:1.7	2
S. uliginosa	0.89	0.11	1.24	0.39	1:0.7	14
S. verticillata UL	no lever	no lever	no lever	no lever	no lever	-
S. verticillata tube	no lever	no lever	no lever	no lever	no lever	-

Except for three of the investigated species the abaxial (=fertile) lever arm always is heavier than the adaxial (=sterile) lever arm (tab. 3.1). In one of these three, in *S. mexicana*, the abaxial part weighs as much as the adaxial part, giving a ratio of 1:1. Only in *S. involucrata* and *S. uliginosa* the adaxial arm is heavier than the abaxial arm. *S. involucrata* the abaxial lever weighs 5.41 +/-0.43mg and the adaxial lever arm weighs 9.74 +/-0.43mg. So the adaxial arm is almost twice as heavy as the abaxial arm. In *S. uliginosa* the abaxial lever arm weighs 0.89 +/-0.11mg and the adaxial lever arm weighs 1.24+/-0.39mg. The ratio of abaxial to adaxial part is therefore 1:0.7. These two species remain the only exceptions among the investigated species. In all the other species the abaxial arm was heavier and longer than the adaxial arm.

3.4.2 Experiments on lever mobility and stability

The following results should give information about the mobility of the lever and its stability (tab. 3.2). The deflection necessary for pollen deposition was investigated, as well as the maximum degree of deflection within and without the flower and the maximum bend the connective can withstand. To deposit pollen, the levers had to be deflected by 40-50° in *S. canariensis, S. forskahlii, S. glutinosa, S. pratensis, S. sclarea* and *S. aethiopis. S. uliginosa* had the lowest pollination-angle of the investigated melittophilous species. The ornithophilous species like *S. africana-lutea, S. involucrata, S. mexicana, S. exserta* and *S. thermarum* had a pollination-angle of 10-20°. The only exception here was *S. patens* with an angle of 30-40°. But the angle necessary for pollination is not the maximum angle possible in an intact flower. All levers could be deflected for several degrees beyond this point of pollination till they hit the lower lip or could not move anymore because the adaxial arm was blocked by the corolla tube (tab. 3.2).

In all species, the joint could tolerate wider deflections than necessary for pollination, within the flower and also when cut out of the flower without petals obstructing the lever's movement (tab. 3.2).

Tab. 3.2: The lever can perform wider deflexions than necessary for pollination.

	measured angles in flower		**measured angles out of flower**			n
	normal angle necessary for deposition	maximum angle possible in the flower	maximum angles out of flower (plastic deformation)	rip off of joint	maximum angle connective can be bent	
S. aethiopis	30°	50°-60°	120°	180°	90°	7
S. africana-lutea	10-20	80°	120°	180°	90°	4
S. canariensis	40-50°	50-60°	120°	180°	90°	7
S. forskahlii	40-50°	70-80°	160-170°	180°	90°	7
S. glutinosa	50°	60-70°	180°	180°	90°	7
S. involucrata	20°	30-40°	110°	180°	90°	7
S. mexicana	10°	20°	130-150°	180°	90°	7
S. patens	30-40°	50°	150°	180°	90°	7
S. exserta	10°	20°	120°	180°	90°	5
S. pratensis	45-50°	70°	90-180°	180°	90°	7
S. sclarea	50°	60-70°	120°	120-130°	90°	7
S. thermarum	10°	20-30°	170°	180°	90°	3
S. uliginosa	30°	40°	90-100°	100°	90°	7

A plastic deformation occurred mostly between 100° and 180° of deflection. The small *S. uliginosa* had the most fragile joint. A plastic deformation was noticed at 90-100°. In some cases *S. glutinosa* and *S. pratensis* could stand angles up to 180° and still the levers swung back. But even in these two species no joint could stand an angle of more than 180°. Beyond this point plastic deformation or breakdown of the joint was unavoidable (tab. 3.2).

The tests of stability and flexibility of the connective showed that the connective can withstand deformations of up to nearly 90°. No connective could be bent for more than 90°. When bent backwards for an angle greater than 90° they all broke (tab. 3.2).

3.4.3 Histological investigations

Connective and filament comprise three different cell types: epidermis, parenchyma and a central vascular bundle (fig. 3.3). Lignin containing cell structures appear red due to staining by astra-blue/safranine. The isodiametic parenchyma cells have large lumina and no particularly thickened cell walls. Intercellulars occur in the parenchyma, especially nearby the ligament's branch-off (fig. 3.4). The epidermis is made of up to three rows of bigger cells.

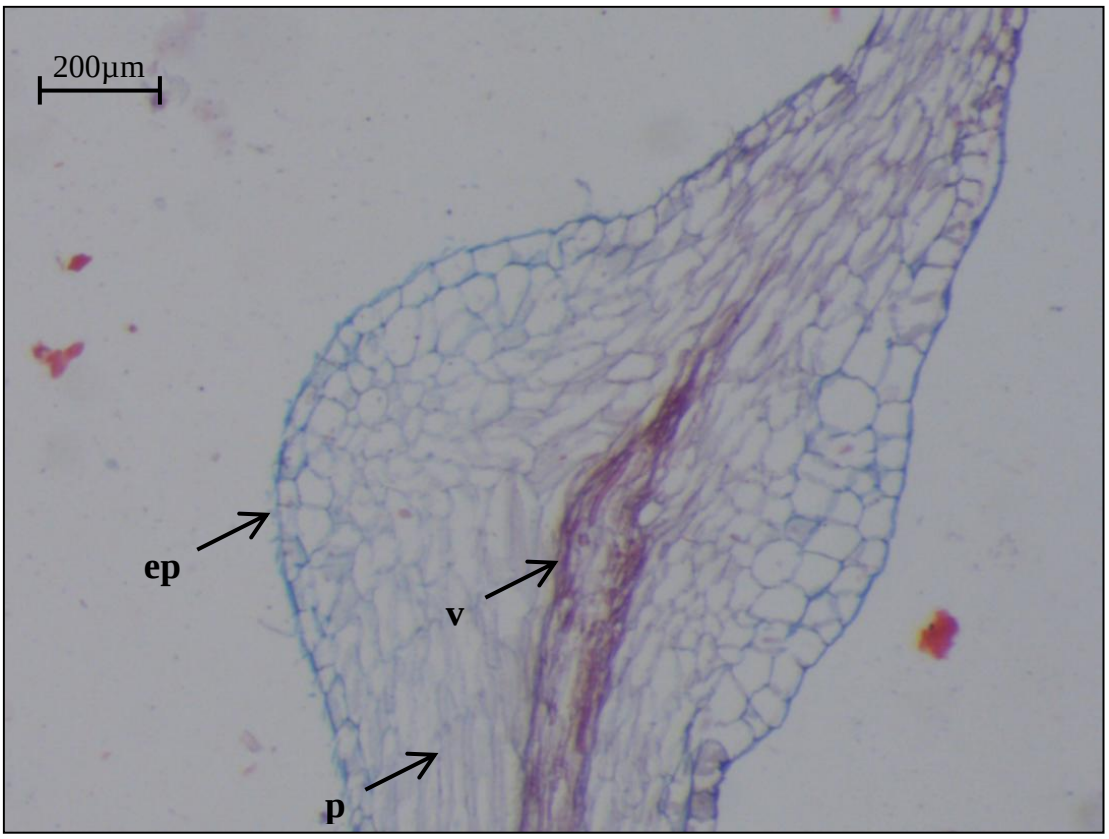


Fig. 3.3: Longitudinal section of the filament of *S. glutinosa*, astra-blue/safranine staining (ep: epidermis, v: vascular bundle, p: parenchyma).

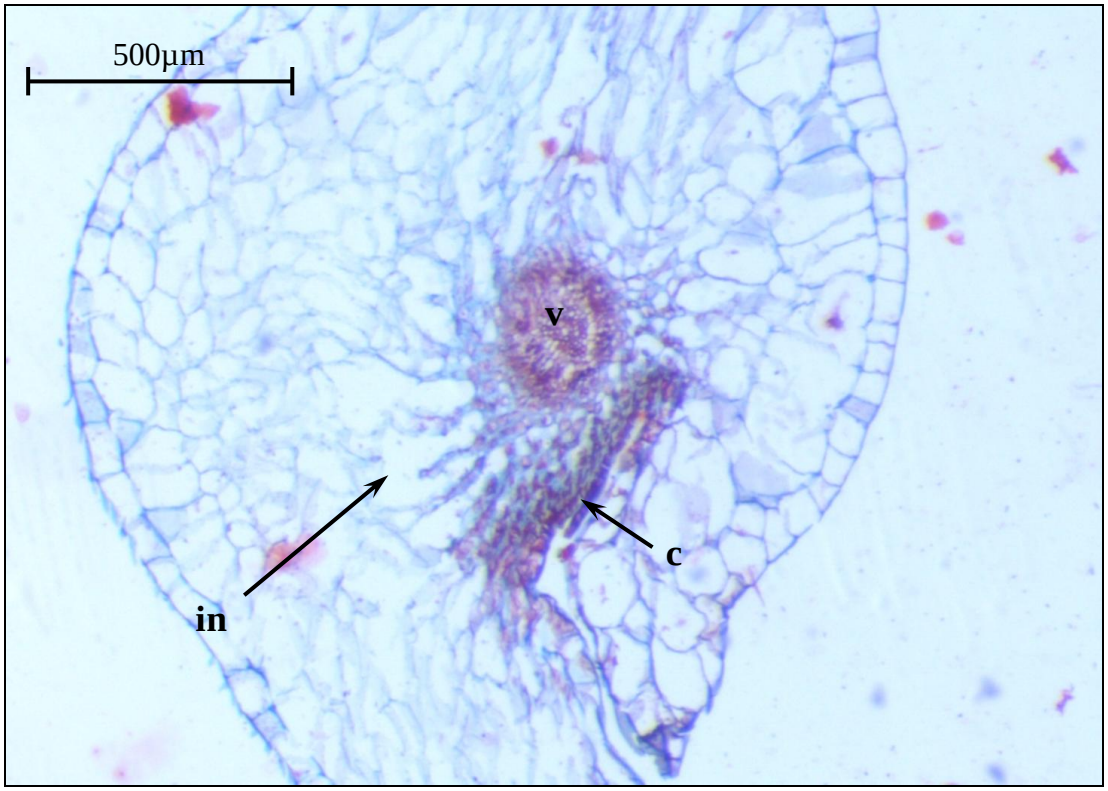


Fig. 3.4: Intercellulars occur near the branch-off of the ligament. Collenchyma cells also increase in quantity. *S. glutinosa*, astra-blue/safranine staining (c: collenchyma, in: intercellulars, v: central vascular bundle).

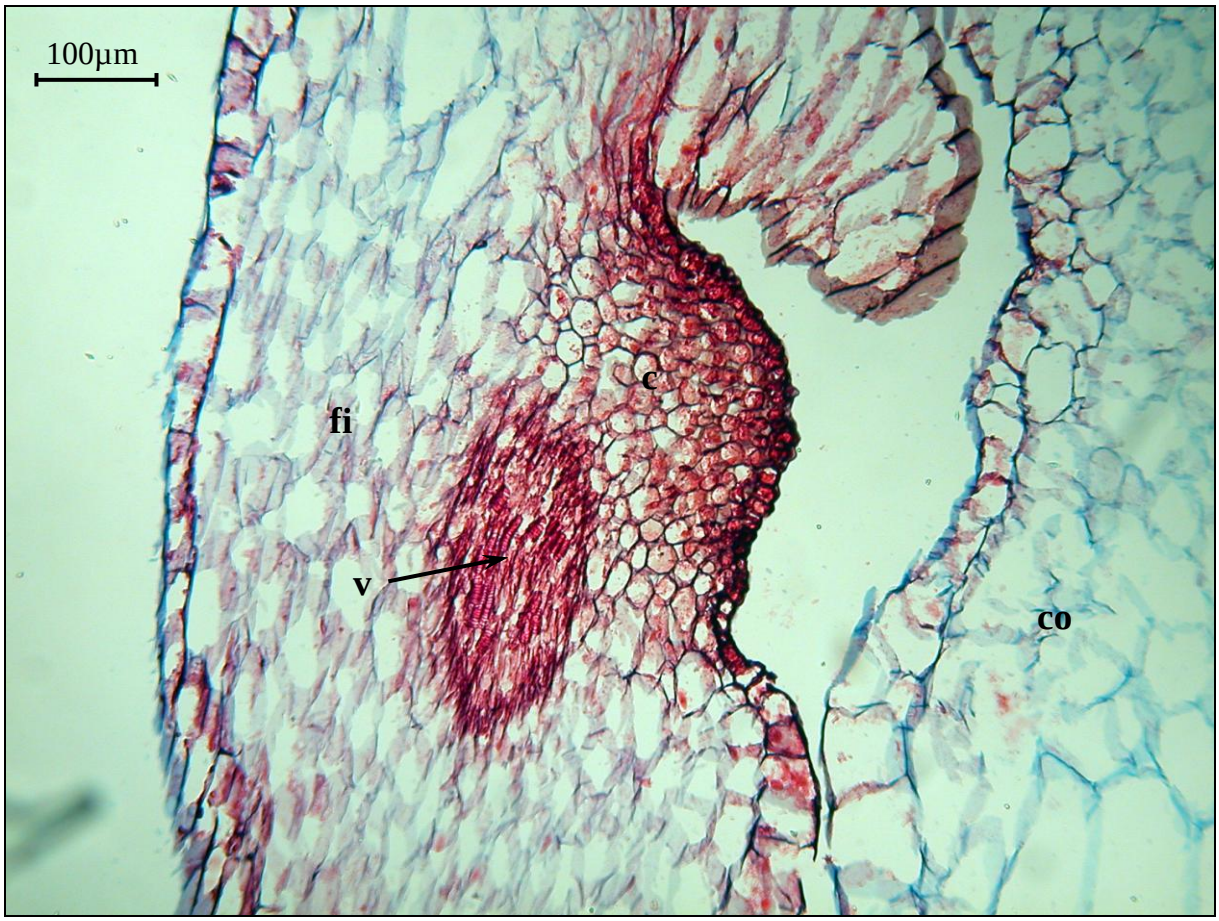


Fig. 3.5: Longitudinal section of the filament of *S. glutinosa*. Close to the point where the vascular bundle branches off into the ligament (c: collenchyma, co: connective, fi: filament, v: vascular bundle); photo by M. Gröteke.

At the point where the vascular bundle branches off into the ligament smaller and thick-walled collenchyma cells appear (fig. 3.4 & 3.5). These collenchyma cells flank the vascular bundle on its way into the ligament. The latter bears a central vascular bundle, which is surrounded by collenchyma, which again is bordered by big epidermal cells (fig. 3.7 – 3.11). Intercellulars can be found in the ligaments collenchyma tissue, too (fig. 3.7). In general, the ligament's transverse section is oval (figs. 3.6 & 3.7). But the intensity of this shape differs from species to species. The ligament of *S. glutinosa* is more orbital and is less oval shaped, while the most elliptic transverse section of a ligament can be found in *S. patens.* Here the axial ratio is close to 2:1 (fig. 3.7).

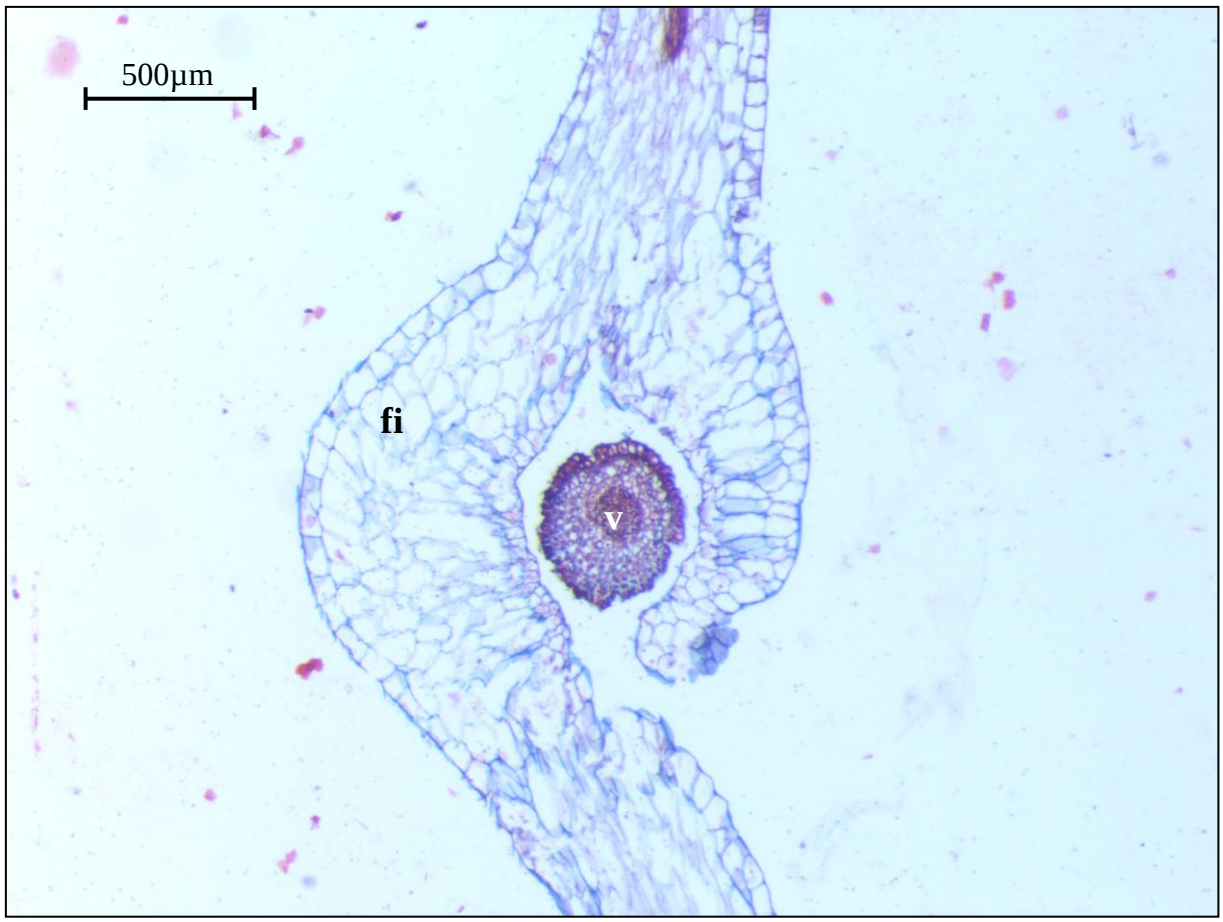


Fig. 3.6: Transversal section of the ligament of *S. glutinosa*, near the filament, astra-blue/safranine staining**.** The ligaments profile is very orbital, compared to the ligaments of other species. The bulges of the filament still enclose the ligament (fi: filament, v: ligament).

No twisting of "cell-fibres" or cell-rows was detected. The cell rows within the ligament of a deflected lever remained straight, parallel and no twisting was recognized (fig. 3.8 & 3.10). Cells of the deflected ligament showed no difference to the cells of the undeflected ligament (fig. 3.8-3.11). A changing of the cell-structure was observed in connective and ligament tissue nearby the transition to the ligament. At the very point where the ligament inserts to the connective and the filament respectively, the tissue appears to be deformed and twisted (fig. 3.12). Intercellulars appear to be deformed, too.

Longitudinal sections of the ligament showed that the deflected ligament appeared to be thinner in diameter compared to the undeflected ligament; just like it was stretched (figs. 3.8 & 3.10). In *S. glutinosa* the ligament of an undeflected lever was 400µm thin (fig. 3.9). The ligament of a deflected lever was 350µm thin (fig. 3.8). In *S. canariensis* the ligament of an undeflected lever was 250µm thin (fig. 3.11). The ligament of a deflected lever was 150µm thin (fig. 3.10).

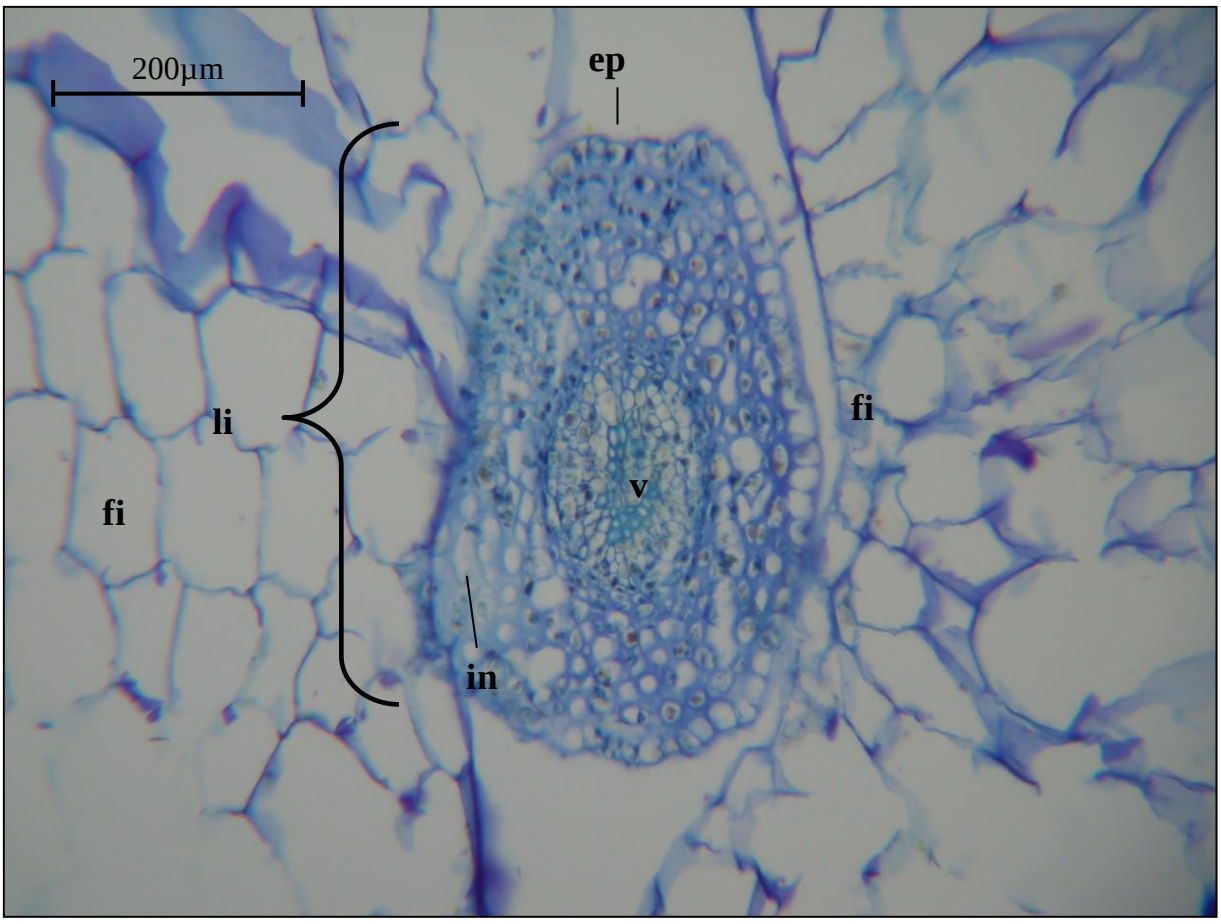


Fig. 3.7: Transversal section of the ligament of *S. patens* (toluidine staining) surrounded by the bulges of the filament. The central vascular bundle is surrounded by collenchyma tissue (ep: epidermis, fi: filament, in: intercellulars, li: ligament, v: vascular bundle).

3.4.4 Morphological observations

During the histological investigations a more or less intensive dent of the filament was discovered in which the connective fits in. Connective and filament form bulges which partially encloses the ligament. The bulge of the filament is smaller than the bulge of the connective. A longitudinal section of the ligament area reveals these bulges as enlarged epidermal cells of connective and filament respectively (fig. 3.13). In a longitudinal section of connective and filament the central ligament is surrounded by the bulges (fig. 3.6 & 3.7).

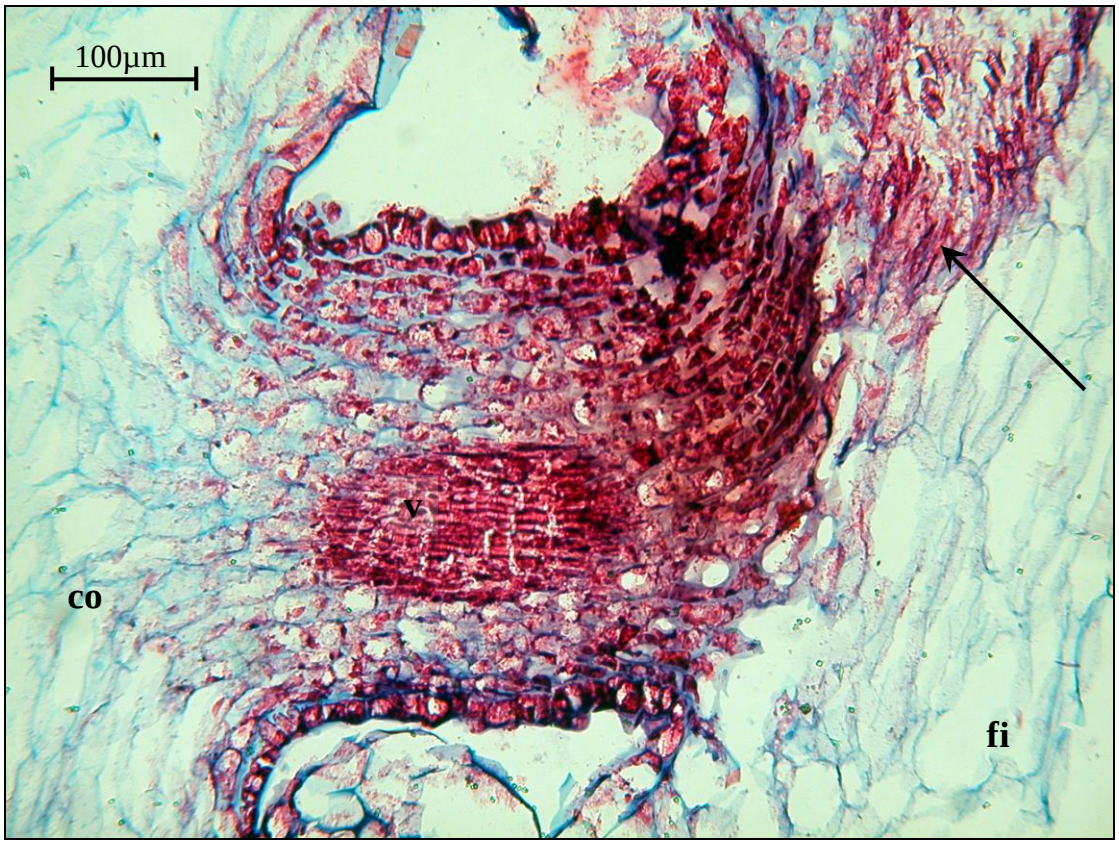


Fig. 3.8: Deflected joint of *S. glutinosa*, longitudinal section, astra-blue/safranine staining (co: connective, fi: filament, v: vascular bundle, arrow: deformed cells); photo by M. Gröteke.

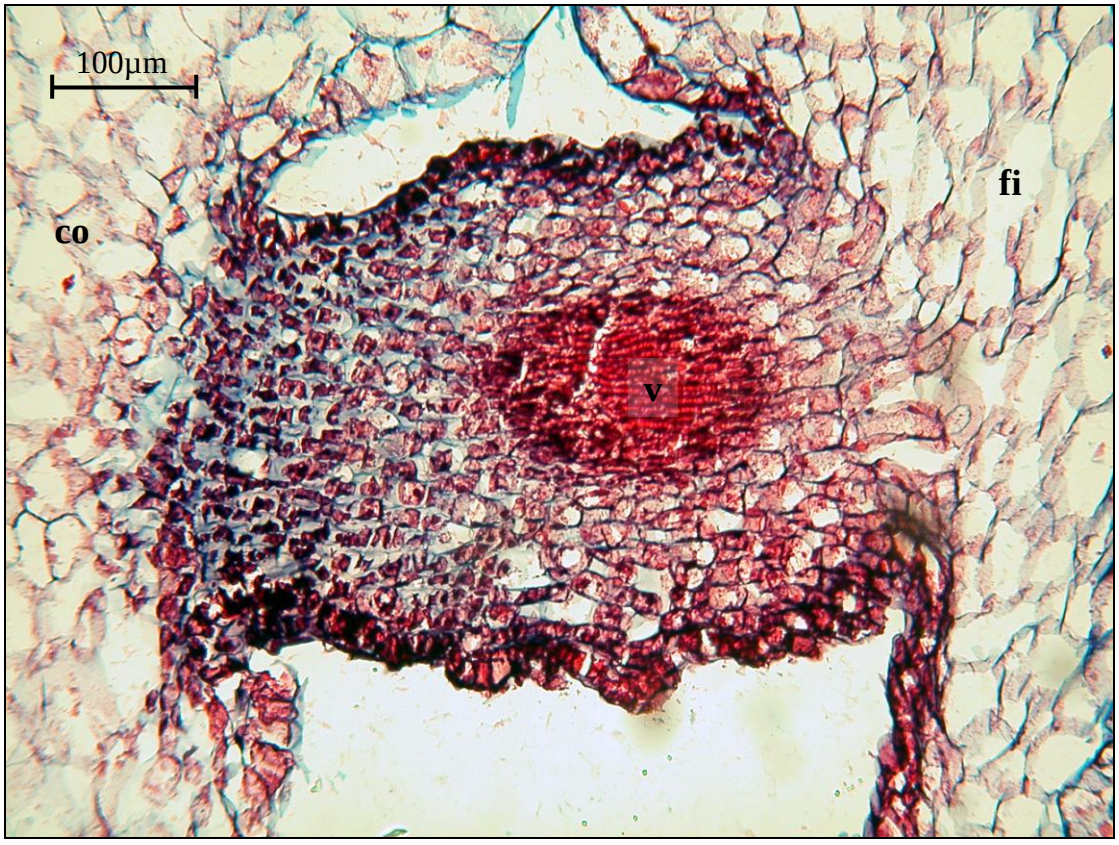


Fig. 3.9: Undeflected joint of *S. glutinosa* longitudinal section, astra-blue/safranine staining (co: connective, fi: filament, v: vascular bundle); photo by M. Gröteke

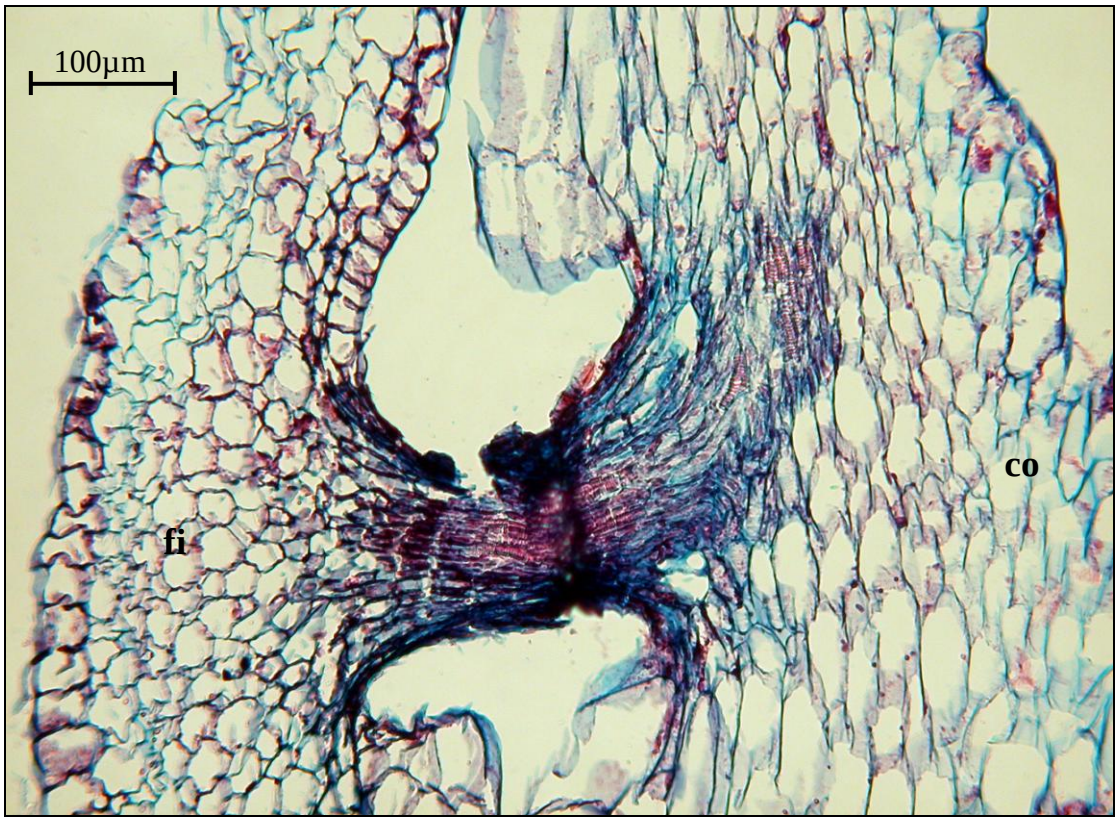


Fig. 3.10: deflected joint of *S. canariensis*, longitudinal section astra-blue/safranine staining**.** (co: connective, fi: filament), photo by M. Gröteke.

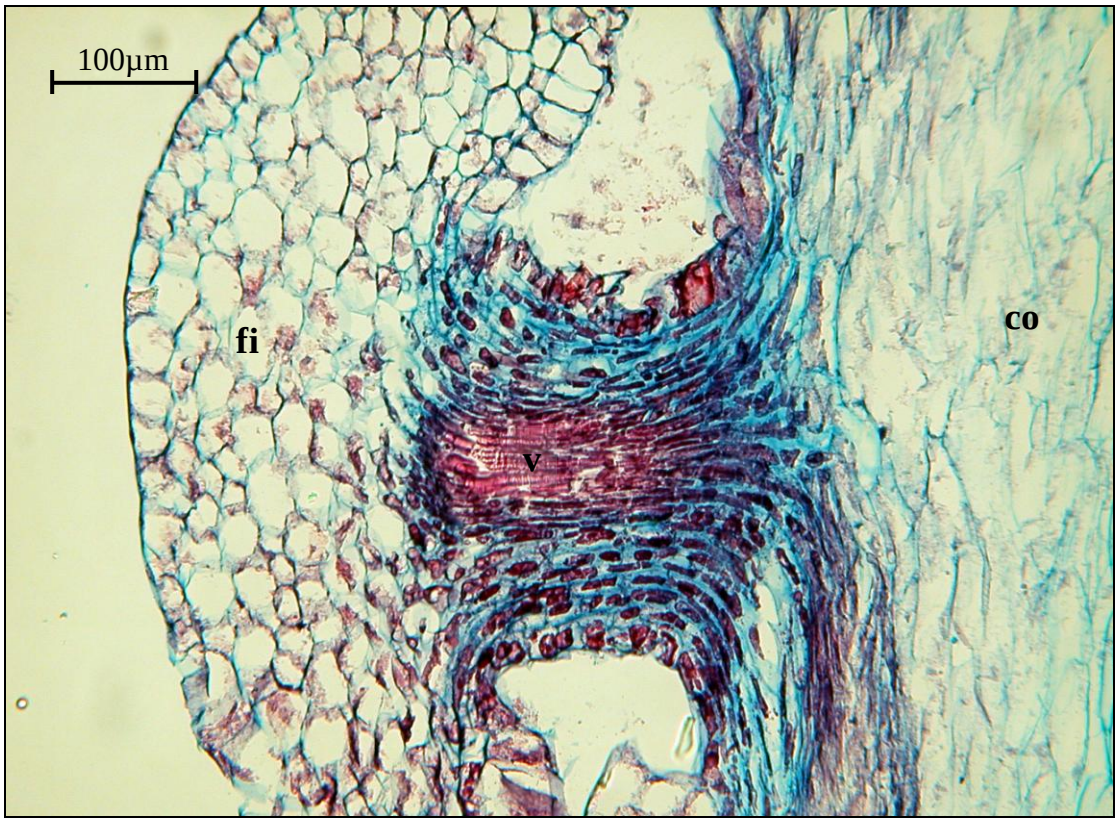


Fig. 3.11: Undeflected joint of *S. canariensis* longitudinal section, astra-blue/safranine staining**.** (co: connective, fi: filament, v: vascular bundle); photo by M. Gröteke.

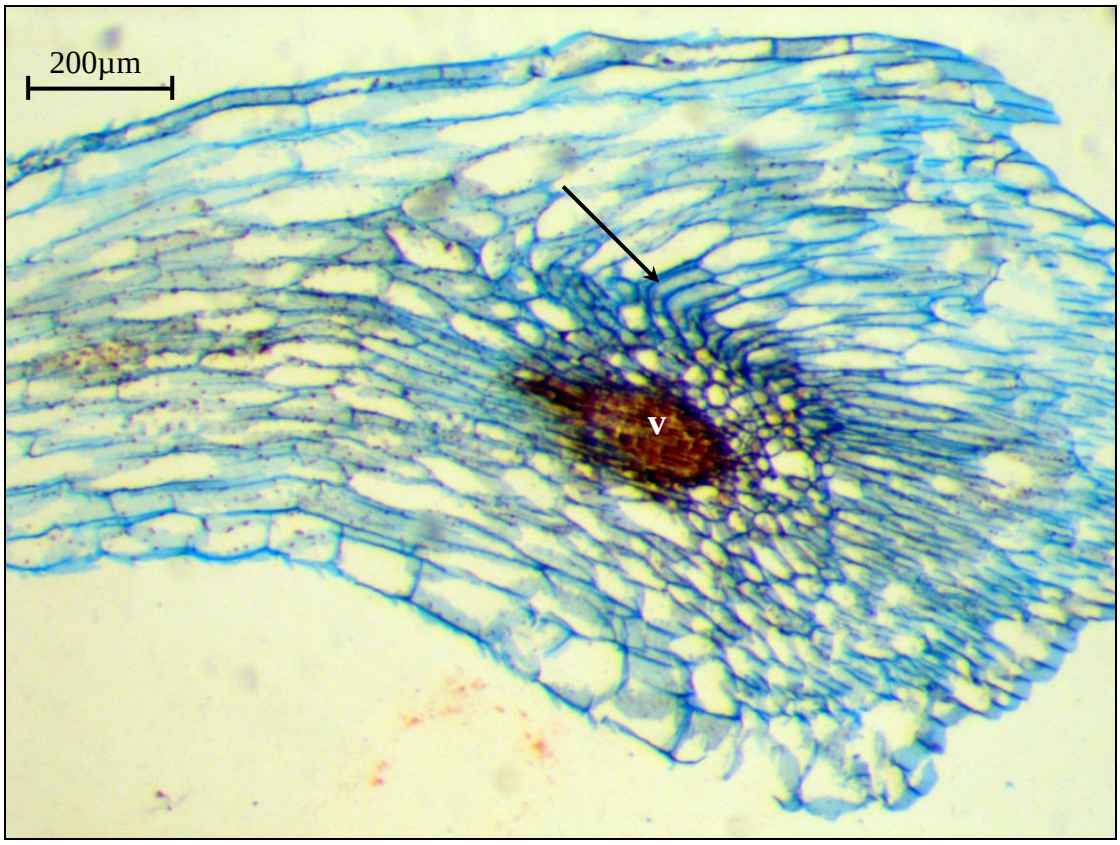


Fig. 3.12: Deformed parenchyma cells in the filament of *S. canariensis* are visible nearby the insertion point of the ligament (v: vascular bundle, arrow: deformed cells).

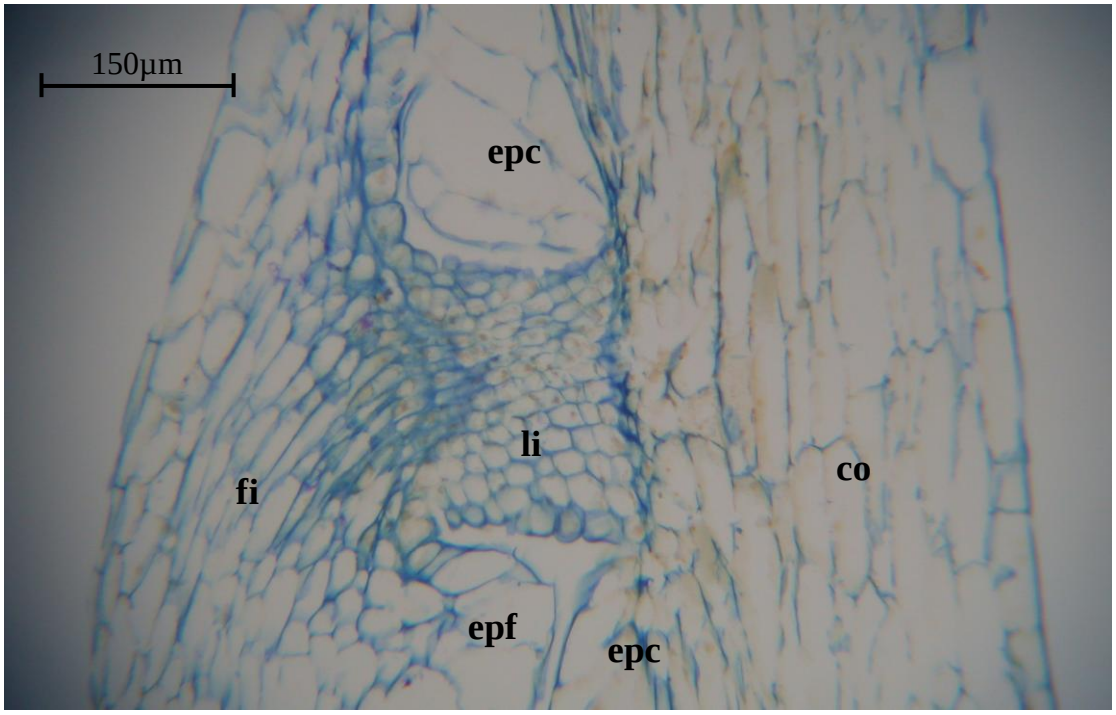


Fig. 3.13: *S. pratensis*, longitudinal section of the ligament area, toluidine-blue staining. Big epidermal cells of the connective form a bulge which encloses the ligament (co: connective, epc: epidermal cells of connective, epf: epidermal cells of filament, fi: filament; li: ligament).

3.5 Discussion

3.5.1 The weight of the lever arm

One assumption of how the lever is re-erected was that the adaxial lever arm could function as a counterweight heaving the abaxial arm back into its neutral position. But the weighing of the levers and its parts showed that only in *S. involucrata* and in *S. uliginosa* the adaxial lever arm is heavier than the abaxial arm and therefore able to pull its counterpart up again (tab. 1.4). In all other species the abaxial arm is heavier and/or longer than the adaxial arm. CORRENS (1891) also mentioned that the species he investigated have an abaxial lever arm, which is 2-4 times longer than the adaxial arm. He stated these findings for *S. pratensis, S. sclarea, S. horminum* and *S. glutinosa*. A further species, *S. hispanica*, however, had a relation of abaxial to adaxial arm of 1:1 [CORRENS 1891]. Now, considering the law of the lever it is impossible that the adaxial arm functions as a counterweight when it is lighter and shorter than the abaxial arm. Only in the exceptional cases where the adaxial arm is heavier than the abaxial arm, it could act as a counterweight and pull the abaxial lever arm to return it to its neutral position. In all other species this is not possible, due to the heavy abaxial lever arm. So the re-erection of the lever has to be managed in some other way.

3.5.2 Experiments on lever mobility and stability

In order to detect the limit of the joint, several angles of deflection of the lever were tested (tab. 3.2). It turned out that depending on the species, angles of 10-50° were necessary for successful pollination. While ornithophilous species tend to have smaller pollination-angles than melittophilous species due to narrower corolla tubes. But the minimum lever deflection of pollination was always feasible without the lever touching the petals. So the lever had more free moving space than necessary within the flower. Outside the flower the maximum angle the joint could stand was always 180°. Investigations on other species of the genus *Salvia* show similar results [CORRENS 1891]. CORRENS (1891) also made experiments regarding the angle of deflection of the lever. He found out that angles of 20-60° were necessary for pollination, which resembles the findings of this work. CORRENS (1891) also confirmed that wider angles were possible within the flower, e.g. 50-70° in *S. glutinosa*. Furthermore, he found out that the joint could stand much wider angles than necessary for pollination. He even managed a deflection of the lever of *S. pratensis* up to 210° till the joint

ripped off [CORRENS 1891]. This very result could not be reproduced, but apart from this exception, all results of this work referring to the lever mobility and the joint's stability confirm CORRENS' results or are confirmed by him.

As already assumed in previous publications [THIMM 2003, CLAßEN-BOCKHOFF et al. 2004b, KÖHLER unpubl., chapter one & two], current results show that the joint is a very flexible and mechanically resistant construction, designed to function repeatedly and with repeating precision.

3.5.3 Histological investigations

CORRENS (1891) conjectured the driving force for the lever's backswing in the ligament itself. Cell-fibres in the ligament should be twisted and elongated (s. fig. 3.1). When releasing the tension, the cell-fibres re-erect the lever. Longitudinal and transversal sections of the ligament could not reveal any twisted cell-fibres within the ligament. No deformation or disarrangement of cells within the ligament was visible.

Nevertheless, longitudinal sections of the ligament lead to the assumption that the ligament really is thinner when deflected (figs. 3.8-3.11). This again leads to the assumption that the ligament is stretched during deflexion and therefore gets thinner and longer, which would confirm CORRENS' thesis. But no hint could be found that it was elongated as well. If the ligament should function like a spring and therefore get twisted and stretched, it should get thinner and longer when deflected. Instead the ligament only appeared to be thinner, but not elongated or twisted. The reason why the ligament appears to be thinner when deflected could be its oval shaped diameter. When the ligament does not get stretched and twisted but remains stiff it has to rotate and follow the lever's movement when the lever is deflected, otherwise it would break. As a consequence of the rotation of the stiff ligament its former upper side has been rotated for several degrees and is now pointing to the front. As the ligament's diameter is larger in dorsi-ventral diameter than in equatorial diameter it now appears thinner when looked at from the front. A further fact that should provide an indication of the appropriateness of this assumption is that the described phenomenon was more obvious in species with a more oval shaped ligament (figs. 3.8-3.11).

Another assumption is referring to CORRENS' theory of twisting cell fibres. The ligament's cells should function as a pressure accumulator [KÖHLER unpubl.]. The cell walls of the ligament's cells are assumed to be highly elastic and watertight. Due to the deformation of the ligament during deflexion, former isodiametic cells would be deformed but due to the

incompressibility of water the cells' volumes' would not change. Flattened and elongated cells would be visible in a deflected ligament. These cells would be under considerable strain and in an effort to loose tension and get back to their original form the cells would re-erect the lever [KÖHLER unpubl.]. Such extremely deformed cells could not be detected in a deflected ligament. Furthermore, no proof could be found that the collenchyma cells of the ligament are watertight.

Several movements in the plant kingdom are the result of a change of turgor in cells. Even rapid and repeatable movements can be performed by this phenomenon. Extensor cells and flexor cells would interact like in *Mimosa* or *Samanea saman* [SATTER et al. 1990, BRAAM 2005]. But neither extensors or flexors nor any transfer of cell fluids was detected.

The histological sections reveal no cellular deformations or disarrangements in the ligament. So obviously and contradictory to earlier hypotheses [CORRENS 1891] the cells within the ligament do not change form or position, when the lever is deflected. In fact, they stay in place and their thickened cell walls seem to ensure a certain stiffness of the ligament.

So if there are no cellular changes within the ligament. And if the ligament is a stiff axis of rotation, inevitably some other tissue has to deal with the appearing forces and manage the backswing of the staminal lever. The recent histological investigations indicate that tissue of filament next to the joint is involved in this process. Comparable to a mainspring this tissue temporarily has to absorb the forces when the lever is deflected and has to release them in order to re-erect the lever. The observations that were made during these investigations are not beyond doubt, several sections of deflected levers were without noteworthiness referring to the tissue in filament and connective, but on the other hand several sections seemed to reveal deformed cells in the filament. Earlier investigations indicate that the deflection of the lever leads to deformed cells in the connective as well as in the filament [Gröteke unpubl.], but the recent investigations could not affirm these findings. No deformed cells were found in the connective.

Connective and filament each fit in a small recess [CORRENS 1891]. In the recent sections the bulges of ligament and connective are visible, too (e.g. fig. 3.13). When deflected the connective is heaved out of this recess and the gap/distance between filament and connective grows. This leads to tension, which additionally could support the backswing of the lever.

The movement of the lever can be repeated at least 20 times in a row without affecting its function [chapter 1 & 2]. During the repeated measurements, a drop of needed force was recognized. The repeating movement of the lever did not affect the reliability of the lever

mechanism at all [chapter two] but this drop of force can be interpret ted as some kind of fatigue [see also KÖHLER unpubl.]. Furthermore, the lever does not swing back in flowers which have been stored in alcohol. So, living tissue seems to be necessary for a functioning lever. The theory of elastic cell walls working against the incompressible water [KÖHLER unpubl.] becomes more interesting again. In the filament deformed parenchyma cells and deformed intercellulars were found. Both were near the ligament's point of insertion. These cells could be the very tension accumulator [see KÖHLER unpubl.] which is responsible for the lever's backswing. The intercellulars, which were already mentioned by Correns (1891) support such a deformation of the tissue and give space when the tissue is compressed.

General Conclusion

Force measurements on pollinators and on *Salvia*-flowers showed that the common pollinators are able to move the *Salvia*-lever. Field observations confirmed a wide range of pollinators for different *Salvia*-species [TWERASER 2000]. The lever mechanism is not harder to overcome than other floral structures like e.g. the hairy ring inside the flower tube [chapter one]. Furthermore, sometimes the forces to move the lever are outperformed by the forces to extricate the lever from the upper petals [chapter one]. It is most unlikely that the lever is meant to be a barrier to select pollinators by force. It is far too easy to overcome even by small insects and there are other floral structures that demand comparable or even higher forces. The lever is no device for pollinator-selection. Pollinators in all likelihood will be selected by flower-shape and quality of nectar rather than by the staminal lever.

The assumption that it could serve as a device for pollen positioning or pollen placement [FAEGRI & VAN DER PIJL 1971, Grant 1994a, CLAßEN-BOCKHOFF et al. 2003] seems more likely by far. It could be shown that the lever repeatedly can perform a precise movement for pollen placement. Due to the specific range of coverage each species has its own spot on a visitor's body to deposit the pollen. Due to its curved form, the lever can serve different pollinators as it compensates different body sizes. Furthermore, the curved form avoids smearing and ensures a precise pollen placement [chapter two]. The precise pollen placement allows pollinator sharing in co-occurring species. It minimizes the risk of hybridisation and waste of pollen and increases the male fitness. The possibility to use a wide range of pollinators increases the chance of pollination, as well.

The question of the backswing of the lever could not be answered satisfyingly. The lever's re-erection is likely not managed by the adaxial lever arm, functioning as a counterweight. In most of the investigated species the adaxial arm is too light to fulfil this

task [chapter three]. A twisting of cell fibres, as recommended by CORRENS (1891), was not detected. Therefore the possibility of a spring mechanism that is located inside the ligament seems most unlikely. Recent investigations give a hint that the mechanism that is responsible for the re-erection of the lever is located in the filament. Although the investigations are not unambiguous, deformed cells in the filament near the insertion of the ligament were detected. These cells could be part of the spring mechanism, as they are deformed when the tissue has to take the tension of the levers rotation. Further studies will be necessary to confirm the hypothesis that the spring mechanism is located in the tissue near the insertion of the ligament. However, by the actual standard of knowledge this is the most plausible theory.

Annex

Tab. A.1: An Analysis of Variance (Bonferroni) referring to the measured forces to move the lever points out equalities and differences between the species.

univariat

ONEWAY ANOVA

force

	summ of square	df	mean of squares	F	significance
between groups	68978.964	15	4598.598	166.92	.000
inside groups	132792.119	4820	27.550		
entire	201771.084	4835			

Post-Hoc-Tests:

multiple comparisons

dependent variable: force

Bonferroni

(I) species	(J) species	average difference (I-J)	standard error	significance	95%-confidence interval	
					lower limit	upper limit
S. aethiopis	*S. africana-lutea*	-1.39668	.8342	1.000	-4.3431	1.5498
	S. austriaca	-1.15630	.4879	1.000	-2.8796	.5670
	S. canariensis	.61559	.8036	1.000	-2.2228	3.4540
	S. exserta	3.76694(*)	.5337	.000	1.8820	5.6519
	S. forskahlii	2.06121(*)	.4564	.001	.4492	3.6732
	S. glutinosa	3.40708(*)	.4307	.000	1.8859	4.9283
	S. involucrata	-4.58955(*)	.5858	.000	-6.6586	-2.5205
	S. mexicana	-5.45571(*)	.7300	.000	-8.0342	-2.8772
	S. patens	-16.30014(*)	.5681	.000	-18.3066	-14.2937
	S. pratensis	2.06463(*)	.4039	.000	.6380	3.4913
	S. sclarea	-3.45211(*)	.5255	.000	-5.3082	-1.5960
	S. thermarum	4.12482(*)	.9131	.001	.9000	7.3497
	S. uliginosa	1.12473	.4407	1.000	-.4320	2.6815
	S. verticillata (upper lip)	4.00691(*)	.6089	.000	1.8562	6.1576
	S. verticillata (tube)	-.85074	.6104	1.000	-3.0068	1.3053
S. africana-lutea	*S. aethiopis*	1.39668	.8342	1.000	-1.5498	4.3431
	S. austriaca	.24039	.8025	1.000	-2.5940	3.0748
	S. canariensis	2.01227	1.0256	1.000	-1.6100	5.6346
	S. exserta	5.16362(*)	.831	.000	2.2281	8.0991
	S. forskahlii	3.45790(*)	.7838	.001	.6897	6.2261
	S. glutinosa	4.80376(*)	.7691	.000	2.0875	7.5201
	S. involucrata	-3.19287(*)	.8655	.027	-6.2498	-.1359
	S. mexicana	-4.05903(*)	.9690	.003	-7.4814	-.6366
	S. patens	-14.90345(*)	.8536	.000	-17.9184	-11.8885
	S. pratensis	3.46132(*)	.7544	.001	.7968	6.1258
	S. sclarea	-2.05543	.8259	1.000	-4.9725	.8616
	S. thermarum	5.52150(*)	1.1134	.000	1.5891	9.4539
	S. uliginosa	2.52142	.7747	.137	-.2149	5.2578

	S. verticillata (upper lip)	5.40359(*)	.8813	.000	2.2908	8.5164
	S. verticillata (tube)	.54594	.8824	1.000	-2.5706	3.6625
S. austriaca	*S. aethiopis*	1.15630	.4879	1.000	-.5670	2.8796
	S. africana-lutea	-.24039	.8025	1.000	-3.0748	2.5940
	S. canariensis	1.77189	.7707	1.000	-.9501	4.4938
	S. forskahlii	3.21751(*)	.3954	.000	1.8208	4.6143
	S. glutinosa	4.56338(*)	.3655	.000	3.2725	5.8542
	S. involucrata	-3.43326(*)	.5397	.000	-5.3394	-1.5272
	S. mexicana	-4.29941(*)	.6936	.000	-6.7491	-1.8497
	S. patens	-15.14384(*)	.5204	.000	-16.9818	-13.3059
	S. exserta	4.92323(*)	.4826	.000	3.2187	6.6277
	S. pratensis	3.22093(*)	.3335	.000	2.0430	4.3989
	S. sclarea	-2.29581(*)	.4735	.000	-3.9683	-.6233
	S. thermarum	5.28111(*)	.8842	.000	2.1583	8.4039
	S. uliginosa	2.28103(*)	.3773	.000	.9485	3.6136
	S. verticillata (upper lip)	5.16321(*)	.5647	.000	3.1688	7.1576
	S. verticillata (tube)	.30556	.5663	1.000	-1.6947	2.3058
S. canariensis	*S. aethiopis*	-.61559	.8036	1.000	-3.4540	2.2228
	S. africana-lutea	-2.01227	1.0256	1.000	-5.6346	1.6100
	S. austriaca	-1.77189	.7707	1.000	-4.4938	.9501
	S. exserta	3.15135(*)	.8004	.010	.3243	5.9784
	S. forskahlii	1.44562	.7511	1.000	-1.2073	4.0986
	S. glutinosa	2.79149(*)	.7358	.018	.1928	5.3902
	S. involucrata	-5.20514(*)	.8361	.000	-8.1582	-2.2521
	S. mexicana	-6.07130(*)	.9428	.000	-9.4012	-2.7414
	S. patens	-16.91572(*)	.82381	.000	-19.8252	-14.0062
	S. pratensis	1.44904	.7204	1.000	-1.0955	3.9936
	S. sclarea	-4.06770(*)	.7950	.000	-6.8756	-1.2598
	S. thermarum	3.50923	1.0907	.156	-.3429	7.3614
	S. uliginosa	.50914	.7417	1.000	-2.1106	3.1288
	S. verticillata (upper lip)	3.39132(*)	.8524	.008	.3805	6.4021
	S. verticillata (tube)	-1.46633	.8535	1.000	-4.4810	1.5483
S. forskahlii	*S. aethiopis*	-2.06121(*)	.4564	.001	-3.6732	-.4492
	S. africana-lutea	-3.45790(*)	.7838	.001	-6.2261	-.6897
	S. austriaca	-3.21751(*)	.3954	.000	-4.6143	-1.8208
	S. canariensis	-1.44562	.7511	1.000	-4.0986	1.2073
	S. exserta	1.70572(*)	.4507	.019	.1138	3.2977
	S. glutinosa	1.34587(*)	.3222	.004	.2077	2.4840
	S. involucrata	-6.65077(*)	.5114	.000	-8.4569	-4.8446
	S. mexicana	-7.51692(*)	.6718	.000	-9.8897	-5.1442
	S. patens	-18.36135(*)	.4910	.000	-20.0954	-16.6273
	S. pratensis	.00342	.2854	1.000	-1.0049	1.0117
	S. sclarea	-5.51333(*)	.4410	.000	-7.0710	-3.9556
	S. thermarum	2.06360	.8672	1.000	-.9992	5.1265
	S. uliginosa	-.93648	.3355	.634	-2.1217	.2487
	S. verticillata (upper lip)	1.94569(*)	.5377	.036	.0466	3.8448

	S. verticillata (tube)	-2.91195(*)	.5394	.000	-4.8172	-1.0067
S. glutinosa	*S. aethiopis*	-3.40708(*)	.4307	.000	-4.9283	-1.8859
	S. africana-lutea	-4.80376(*)	.7691	.000	-7.5201	-2.0875
	S. austriaca	-4.56338(*)	.3655	.000	-5.8542	-3.2725
	S. canariensis	-2.79149(*)	.7358	.018	-5.3902	-.1928
	S. exserta	.35985	.4246	1.000	-1.1401	1.8598
	S. forskahlii	-1.34587(*)	.3222	.004	-2.4840	-.2077
	S. involucrata	-7.99664(*)	.4885	.000	-9.7222	-6.2711
	S. mexicana	-8.86279(*)	.6546	.000	-11.1748	-6.5508
	S. patens	-19.70722(*)	.4671	.000	-21.3572	-18.0572
	S. pratensis	-1.34245(*)	.2422	.000	-2.1980	-.4869
	S. sclarea	-6.85919(*)	.4143	.000	-8.3227	-5.3957
	S. thermarum	.71774	.8539	1.000	-2.2983	3.7338
	S. uliginosa	-2.28235(*)	.2996	.000	-3.3407	-1.2240
	S. verticillata (upper lip)	.59983	.5160	1.000	-1.2228	2.4225
	S. verticillata (tube)	-4.25782(*)	.5178	.000	-6.0869	-2.4288
S. involucrata	*S. aethiopis*	4.58955(*)	.5858	.000	2.5205	6.6586
	S. africana-lutea	3.19287(*)	.8655	.027	.1359	6.2498
	S. austriaca	3.43326(*)	.5397	.000	1.5272	5.3394
	S. canariensis	5.20514(*)	.8361	.000	2.2521	8.1582
	S. exserta	8.35649(*)	.5814	.000	6.3030	10.4099
	S. forskahlii	6.65077(*)	.5114	.000	4.8446	8.4569
	S. glutinosa	7.99664(*)	.4885	.000	6.2711	9.7222
	S. mexicana	-.86616	.7656	1.000	-3.5703	1.8380
	S. patens	-11.71058(*)	.6131	.000	-13.8761	-9.5451
	S. pratensis	6.65419(*)	.4651	.000	5.0113	8.2970
	S. sclarea	1.13744	.5739	1.000	-.8895	3.1644
	S. thermarum	8.71437(*)	.9417	.000	5.3882	12.0405
	S. uliginosa	5.71429(*)	.4974	.000	3.9573	7.4713
	S. verticillata (upper lip)	8.59646(*)	.6511	.000	6.2967	10.8962
	S. verticillata (tube)	3.73881(*)	.6526	.000	1.4340	6.0437
S. mexicana	*S. aethiopis*	5.45571(*)	.7300	.000	2.8772	8.0342
	S. africana-lutea	4.05903(*)	.9690	.003	.6366	7.4814
	S. austriaca	4.29941(*)	.6936	.000	1.8497	6.7491
	S. canariensis	6.07130(*)	.9428	.000	2.7414	9.4012
	S. exserta	9.22265(*)	.7265	.000	6.6566	11.7887
	S. forskahlii	7.51692(*)	.6718	.000	5.1442	9.8897
	S. glutinosa	8.86279(*)	.6546	.000	6.5508	11.1748
	S. involucrata	.86616	.7656	1.000	-1.8380	3.5703
	S. patens	-10.84442(*)	.7521	.000	-13.5009	-8.1879
	S. pratensis	7.52034(*)	.6373	.000	5.2694	9.7713
	S. sclarea	2.00360	.7205	.654	-.5413	4.5485
	S. thermarum	9.58053(*)	1.0376	.000	5.9157	13.2454
	S. uliginosa	6.58044(*)	.6613	.000	4.2449	8.9160
	S. verticillata (upper lip)	9.46262(*)	.7834	.000	6.6955	12.2297
	S. verticillata (tube)	4.60497(*)	.7846	.000	1.8337	7.3763

S. patens	*S. aethiopis*	16.30014(*)	.5681	.000	14.2937	18.3066
	S. africana-lutea	14.90345(*)	.8536	.000	11.8885	17.9184
	S. austriaca	15.14384(*)	.5204	.000	13.3059	16.9818
	S. canariensis	16.91572(*)	.8238	.000	14.0062	19.8252
	S. exserta	20.06707(*)	.5635	.000	18.0767	22.0574
	S. forskahlii	18.36135(*)	.4910	.000	16.6273	20.0954
	S. glutinosa	19.70722(*)	.4671	.000	18.0572	21.3572
	S. involucrata	11.71058(*)	.6131	.000	9.5451	13.8761
	S. mexicana	10.84442(*)	.7521	.000	8.1879	13.5009
	S. pratensis	18.36477(*)	.4426	.000	16.8015	19.9280
	S. sclarea	12.84802(*)	.5558	.000	10.8850	14.8110
	S. thermarum	20.42495(*)	.9308	.000	17.1374	23.7125
	S. uliginosa	17.42487(*)	.4764	.000	15.7421	19.1077
	S. verticillata (upper lip)	20.30704(*)	.6352	.000	18.0634	22.5507
	S. verticillata (tube)	15.44939(*)	.6367	.000	13.2006	17.6982
S. exserta	*S. aethiopis*	-3.76694(*)	.5337	.000	-5.6519	-1.8820
	S. africana-lutea	-5.16362(*)	.8311	.000	-8.0991	-2.2281
	S. austriaca	-4.92323(*)	.4826	.000	-6.6277	-3.2187
	S. canariensis	-3.15135(*)	.8004	.010	-5.9784	-.3243
	S. forskahlii	-1.70572(*)	.4507	.019	-3.2977	-.1138
	S. glutinosa	-.35985	.4246	1.000	-1.8598	1.1401
	S. involucrata	-8.35649(*)	.5814	.000	-10.4099	-6.3030
	S. mexicana	-9.22265(*)	.7265	.000	-11.7887	-6.6566
	S. patens	-20.06707(*)	.5635	.000	-22.0574	-18.0767
	S. pratensis	-1.70230(*)	.3975	.002	-3.1062	-.2984
	S. sclarea	-7.21905(*)	.5206	.000	-9.0577	-5.3804
	S. thermarum	.35788	.9102	1.000	-2.8570	3.5728
	S. uliginosa	-2.64220(*)	.4349	.000	-4.1781	-1.1063
	S. verticillata (upper lip)	.23997	.6047	1.000	-1.8957	2.3757
	S. verticillata (tube)	-4.61768(*)	.6062	.000	-6.7588	-2.4766
S. pratensis	*S. aethiopis*	-2.06463(*)	.4039	.000	-3.4913	-.6380
	S. africana-lutea	-3.46132(*)	.7544	.001	-6.1258	-.7968
	S. austriaca	-3.22093(*)	.3335	.000	-4.3989	-2.0430
	S. canariensis	-1.44904	.7204	1.000	-3.9936	1.0955
	S. exserta	1.70230(*)	.3975	.002	.2984	3.1062
	S. forskahlii	-.00342	.2854	1.000	-1.0117	1.0049
	S. glutinosa	1.34245(*)	.2422	.000	.4869	2.1980
	S. involucrata	-6.65419(*)	.4651	.000	-8.2970	-5.0113
	S. mexicana	-7.52034(*)	.6373	.000	-9.7713	-5.2694
	S. patens	-18.36477(*)	.4426	.000	-19.9280	-16.8015
	S. sclarea	-5.51674(*)	.3864	.000	-6.8817	-4.1518
	S. thermarum	2.06018	.8407	1.000	-.9093	5.0297
	S. uliginosa	-.93990(*)	.2597	.036	-1.8572	-.0226
	S. verticillata (upper lip)	1.94227(*)	.4939	.010	.1977	3.6868
	S. verticillata (tube)	-2.91537(*)	.4958	.000	-4.6666	-1.1642
S. sclarea	*S. aethiopis*	3.45211(*)	.5255	.000	1.5960	5.3082
	S. africana-lutea	2.05543	.8259	1.000	-.8616	4.9725

	S. austriaca	2.29581(*)	.4735	.000	.6233	3.9683
	S. canariensis	4.06770(*)	.7950	.000	1.2598	6.8756
	S. exserta	7.21905(*)	.5206	.000	5.3804	9.0577
	S. forskahlii	5.51333(*)	.4410	.000	3.9556	7.0710
	S. glutinosa	6.85919(*)	.4143	.000	5.3957	8.3227
	S. involucrata	-1.13744	.5739	1.000	-3.1644	.8895
	S. mexicana	-2.00360	.7205	.654	-4.5485	.5413
	S. patens	-12.84802(*)	.5558	.000	-14.8110	-10.8850
	S. pratensis	5.51674(*)	.3864	.000	4.1518	6.8817
	S. thermarum	7.57693(*)	.9055	.000	4.3789	10.7750
	S. uliginosa	4.57684(*)	.4248	.000	3.0765	6.0772
	S. verticillata (upper lip)	7.45902(*)	.5975	.000	5.3488	9.5693
	S. verticillata (tube)	2.60137(*)	.5990	.002	.4856	4.7171
S. thermarum	*S. aethiopis*	-4.12482(*)	.9131	.001	-7.3497	-.9000
	S. africana-lutea	-5.52150(*)	1.1134	.000	-9.4539	-1.5891
	S. austriaca	-5.28111(*)	.8842	.000	-8.4039	-2.1583
	S. canariensis	-3.50923	1.0907	.156	-7.3614	.3429
	S. exserta	-.35788	.9102	1.000	-3.5728	2.8570
	S. forskahlii	-2.06360	.8672	1.000	-5.1265	.9992
	S. glutinosa	-.71774	.8539	1.000	-3.7338	2.2983
	S. involucrata	-8.71437(*)	.9417	.000	-12.0405	-5.3882
	S. mexicana	-9.58053(*)	1.0376	.000	-13.2454	-5.9157
	S. patens	-20.42495(*)	.9308	.000	-23.7125	-17.1374
	S. pratensis	-2.06018	.8407	1.000	-5.0297	.9093
	S. sclarea	-7.57693(*)	.9055	.000	-10.7750	-4.3789
	S. uliginosa	-3.00008	.8590	.058	-6.0342	.0340
	S. verticillata (upper lip)	-.11791	.9563	1.000	-3.4955	3.2596
	S. verticillata (tube)	-4.97556(*)	.9573	.000	-8.3566	-1.5946
S. uliginosa	*S. aethiopis*	-1.12473	.4407	1.000	-2.6815	.4320
	S. africana-lutea	-2.52142	.7747	.137	-5.2578	.2149
	S. austriaca	-2.28103(*)	.3773	.000	-3.6136	-.9485
	S. canariensis	-.50914	.7417	1.000	-3.1288	2.1106
	S. exserta	2.64220(*)	.4349	.000	1.1063	4.1781
	S. forskahlii	.93648	.3355	.634	-.2487	2.1217
	S. glutinosa	2.28235(*)	.2996	.000	1.2240	3.3407
	S. involucrata	-5.71429(*)	.4974	.000	-7.4713	-3.9573
	S. mexicana	-6.58044(*)	.6613	.000	-8.9160	-4.2449
	S. patens	-17.42487(*)	.4764	.000	-19.1077	-15.7421
	S. pratensis	.93990(*)	.2597	.036	.0226	1.8572
	S. sclarea	-4.57684(*)	.4248	.000	-6.0772	-3.0765
	S. thermarum	3.00008	.8590	.058	-.0340	6.0342
	S. verticillata (upper lip)	2.88218(*)	.5245	.000	1.0297	4.7346
	S. verticillata (tube)	-1.97547(*)	.5262	.021	-3.8342	-.1168
S. verticillata (upper lip)	*S. aethiopis*	-4.00691(*)	.6089	.000	-6.1576	-1.8562
	S. africana-lutea	-5.40359(*)	.8813	.000	-8.5164	-2.2908
	S. austriaca	-5.16321(*)	.5647	.000	-7.1576	-3.1688

	S. canariensis	-3.39132(*)	.8524	.008	-6.4021	-.3805
	S. exserta	-.23997	.6047	1.000	-2.3757	1.8957
	S. forskahlii	-1.94569(*)	.5377	.036	-3.8448	-.0466
	S. glutinosa	-.59983	.5160	1.000	-2.4225	1.2228
	S. involucrata	-8.59646(*)	.6511	.000	-10.8962	-6.2967
	S. mexicana	-9.46262(*)	.7834	.000	-12.2297	-6.6955
	S. patens	-20.30704(*)	.6352	.000	-22.5507	-18.0634
	S. pratensis	-1.94227(*)	.4939	.010	-3.6868	-.1977
	S. sclarea	-7.45902(*)	.5975	.000	-9.5693	-5.3488
	S. thermarum	.11791	.9563	1.000	-3.2596	3.4955
	S. uliginosa	-2.88218(*)	.5245	.000	-4.7346	-1.0297
	S. verticillata (tube)	-4.85765(*)	.6734	.000	-7.2360	-2.4793
S. verticillata (tube)	*S. aethiopis*	.85074	.6104	1.000	-1.3053	3.0068
	S. africana-lutea	-.54594	.8824	1.000	-3.6625	2.5706
	S. austriaca	-.30556	.5663	1.000	-2.3058	1.6947
	S. canariensis	1.46633	.8535	1.000	-1.5483	4.4810
	S. exserta	4.61768(*)	.6062	.000	2.4766	6.7588
	S. forskahlii	2.91195(*)	.5394	.000	1.0067	4.8172
	S. glutinosa	4.25782(*)	.5178	.000	2.4288	6.0869
	S. involucrata	-3.73881(*)	.6526	.000	-6.0437	-1.4340
	S. mexicana	-4.60497(*)	.7846	.000	-7.3763	-1.8337
	S. patens	-15.44939(*)	.6367	.000	-17.6982	-13.2006
	S. pratensis	2.91537(*)	.4958	.000	1.1642	4.6666
	S. sclarea	-2.60137(*)	.5990	.002	-4.7171	-.4856
	S. thermarum	4.97556(*)	.9573	.000	1.5946	8.3566
	S. uliginosa	1.97547(*)	.5262	.021	.1168	3.8342
	S. verticillata (upper lip)	4.85765(*)	.6734	.000	2.4793	7.2360

* The difference of the means is significant to the 0.05 confidence level.

Tab. A.2a: t-test comparing the lever forces of melittophilous and ornithophilous species – including *S. patens.*

t-test group statistics:

	pollinator	N	mean	deviation	standard error of mean
force	melittophilous	3974	3.2684	2.99076	.04744
	ornithophilous	862	9.0325	12.87387	.43849

test of independent samples:

		levene test for variational equality		t-test for mean equality						
		F	signifi-cance	T	df	sig. (2-sided)	mean difference	sandard error of difference	95%-confidence interval of difference	
									lower	upper
force	deviations are the same	1296.2	.000	-25.26	4834	.000	-5.764	.228	-6.21	-5.32
	deviations are not the same			-13.06	881.2	.000	-5.764	.441	-6.63	-4.89

Tab. A.2b: t-test comparing the lever forces of melittophilous and ornithophilous species – without *S. patens.*

t-test group statistics:

	pollinator	N	mean	deviation	standard error of mean
force	melittophilous	3974	3.2684	2.99076	.04744
	ornithophilous	497	5.4207	8.31127	.37281

test of independent samples:

		levene test for variational equality		t-test for mean equality						
		F	signifi-cance	T	df	sig. (2-sided)	mean difference	sandard error of difference	95%-confidence interval of difference	
									lower	upper
force	deviations are the same	431.72	.000	-9.83	4469	.000	-1.8853	.19169	.1916	-2.261
	deviations are not the same			-5.01	513.4	.000	-1.8853	.37605	.3760	-2.624

Tab. A.3a: t-test comparing the lever forces of Old World-species and New World-species – including *S. patens.*

t-test group statistics:

	origin	N	mean	deviation	standard error of mean
force	old world	89	4.010	3.368	.357
	new world	562	10.041	15.455	.651

test of independent samples:

		levene test for variational equality		t-test for mean equality						
		F	sig.	T	df	sig. (2-sided)	mean difference	standard error of difference	95% confidence interval of difference	
									lower	upper
force	deviations are the same	47.06	.000	-3.66	651	.000	-6.032	1.645	-9.262	-2.801
	deviations are not the same			-8.11	602.5	.000	-6.032	.743	-7.491	-4.572

Tab. A.3b: t-test comparing the lever forces of Old World-species and New World-species – without *S. patens.*

t-test group statistics:

	origin	N	mean	deviation	standard error of mean
force	old world	89	4.010	3.368	.357
	new world	407	5.7382	9.01207	.44671

test of independent samples:

		levene test for variational equality		t-test for mean equality						
		F	sig.	T	df	sig. (2-sided)	mean difference	standard error of difference	95% confidence interval of difference	
									lower	upper
force	deviations are the same	13.746	.000	1.81	494	.070	1.7531	.96588	-.1445	3.650
	deviations are not the same			3.04	373.29	.003	1.7531	.57626	.6200	2.886

Tab. A.4: comparism of forces measured in picked flowers and flowers, which were left on the plant during force measurement.

	on plant or not on plant	N	mean	deviation	standard error of mean
picked vs on plant	on plant	77	.00418	.00096763	.00011027
	picked	77	.00419	.00096367	.00010982

test of independent samples:

		levene test for variational equality		t-test for mean equality						
		F	sig.	T	df	sig. (2-sided)	mean of difference	standard error of difference	95% confidence interval of difference	
									upper	lower
force	deviations are the same	4.117	.044	-.050	152	.960	-.000	.0001	-.0003	.0002
	dev's are not the same			-.050	151.99	.960	-.000	.0001	-.0003	.0002

Tab. A.5: Force-differences between 1st and following measurements. A decline of force was detected with proceeding measurments.

	difference 1st and 20th measurement	difference 2nd and 20th measurement	difference 1st and 5th measurement	difference 2nd and 5th measurement	difference 1st and 10th measurement	difference 2nd and 10th measurement
average drop of force	52.78%	45.49%	28.94%	12.25%	39.11%	27.32%
deviation	19.17%	21.31%	14.86%	9.00%	16.43%	16.90%

Tab A.6a: Analyis of correlation to test the influence of inner and outer structures on the demanded forces – including *S. patens.*

analysis of correlation including *S. patens*						
	absolute length of abaxial lever arm (a)	distance covered by lever (b+c)	distance joint pollen sacs (b)	flower height (h)	flower length (l)	mass of lever
slope	0,47	0,44	0,57	0,40	0,19	0,24
intercept	-3,21	-1,43	-1,14	-0,64	0,74	4,81
r (correlation)	0,68	0,61	0,62	0,68	0,50	0,29
r^2	0,46	0,37	0,38	0,47	0,25	0,09
p	0,01**	0,03*	0,02*	0,00**	0,05*	0,33
* significant at the 95% level of confidence						
** significant at the 99% level of confidence						

Tab A.6b: Analyis of correlation to test the influence of inner and outer structures on the demanded forces – without *S. patens.*

analysis of correlation without *S. patens*						
	absolute length of abaxial leverarm (a)	distance covered by lever (b+c)	distance joint pollensacs (b)	flower heigth (h)	flower length (l)	mass of lever
slope	0,18	0,16	0,01	0,14	0,06	0,36
intercept	1,62	2,48	4,88	2,78	3,37	2,76
r (correlation)	0,36	0,33	0,01	0,23	0,21	0,76
r^2	0,13	0,11	0,00	0,05	0,04	0,57
p	0.23	0.3	0.97	0.45	0.49	0.00**
* significant at the 95% level of confidence						
** significant at the 99% level of confidence						

Tab. A.7: Analysis of Variance (Bonferroni) referring to the joint-pollen sac-distance reveals the differences and equalities between several species.

ONEWAY ANOVA

joint-pollen sac distance

	square summ	df	mean of squares	F	significance
between groups	5401.364	13	415.490	255.152	.000
inside groups	273.571	168	1.628		
entire	5674.934	181			

Post-Hoc-Tests

multiple comparisons

dependent variable: joint-pollen sac distance
Bonferroni

(I) species	(J) species	mean difference (I-J)	standard error	sig-nificance	95%-confidence interval	
					upper limit	lower limit
S. aethiopis	*S. africana-lutea*	-8.77157(*)	.52835	.000	-10.6330	-6.9101
	S. austriaca	-4.01107(*)	.50201	.000	-5.7797	-2.2424
	S. canariensis	-2.54857(*)	.52835	.000	-4.4100	-.6871
	S. forskhalii	-2.62524(*)	.50201	.000	-4.3939	-.8566
	S. glutinosa	-2.86690(*)	.50201	.000	-4.6355	-1.0983
	S. involucrata	3.40052(*)	.51415	.000	1.5891	5.2119
	S. mexicana	-7.32571(*)	.48232	.000	-9.0250	-5.6265
	S. patens	-19.35473(*)	.49150	.000	-21.0864	-17.6231
	S. exserta	-7.06739(*)	.46055	.000	-8.6900	-5.4448
	S. pratensis	-.94607	.42914	1.000	-2.4580	.5659
	S. sclarea	-6.58000(*)	.44029	.000	-8.1312	-5.0288
	S. thermarum	-5.71607(*)	.72347	.000	-8.2650	-3.1672
	S. uliginosa	4.45518(*)	.56556	.000	2.4626	6.4477
S. africana-lutea	*S. aethiopis*	8.77157(*)	.52835	.000	6.9101	10.6330
	S. austriaca	4.76050(*)	.54639	.000	2.8355	6.6855
	S. canariensis	6.22300(*)	.57068	.000	4.2124	8.2336
	S. exserta	1.70418	.50855	.090	-.0875	3.4959
	S. forskhalii	6.14633(*)	.54639	.000	4.2213	8.0713
	S. glutinosa	5.90467(*)	.54639	.000	3.9797	7.8297
	S. involucrata	12.17209(*)	.55756	.000	10.2077	14.1365
	S. mexicana	1.44586	.52835	.626	-.4156	3.3073
	S. patens	-10.58315(*)	.53675	.000	-12.4742	-8.6921
	S. pratensis	7.82550(*)	.48030	.000	6.1333	9.5177
	S. sclarea	2.19157(*)	.49029	.001	.4642	3.9189
	S. thermarum	3.05550(*)	.75494	.007	.3957	5.7153
	S. uliginosa	13.22675(*)	.60530	.000	11.0942	15.3593
S. austriaca	*S. aethiopis*	4.01107(*)	.50201	.000	2.2424	5.7797
	S. africana-lutea	-4.76050(*)	.54639	.000	-6.6855	-2.8355

	S. canariensis	1.46250	.54639	.744	-.4625	3.3875
	S. exserta	-3.05632(*)	.48113	.000	-4.7514	-1.3612
	S. forskhalii	1.38583	.52096	.780	-.4496	3.2212
	S. glutinosa	1.14417	.52096	1.000	-.6912	2.9796
	S. involucrata	7.41159(*)	.53267	.000	5.5349	9.2882
	S. mexicana	-3.31464(*)	.50201	.000	-5.0833	-1.5460
	S. patens	-15.34365(*)	.51084	.000	-17.1434	-13.5439
	S. pratensis	3.06500(*)	.45116	.000	1.4755	4.6545
	S. sclarea	-2.56893(*)	.46178	.000	-4.1958	-.9420
	S. thermarum	-1.70500	.73675	1.000	-4.3007	.8907
	S. uliginosa	8.46625(*)	.58245	.000	6.4142	10.5183
S. canariensis	*S. aethiopis*	2.54857(*)	.52835	.000	.6871	4.4100
	S. africana-lutea	-6.22300(*)	.57068	.000	-8.2336	-4.2124
	S. austriaca	-1.46250	.54639	.744	-3.3875	.4625
	S. exserta	-4.51882(*)	.50855	.000	-6.3105	-2.7271
	S. forskhalii	-.07667	.54639	1.000	-2.0017	1.8483
	S. glutinosa	-.31833	.54639	1.000	-2.2433	1.6067
	S. involucrata	5.94909(*)	.55756	.000	3.9847	7.9135
	S. mexicana	-4.77714(*)	.52835	.000	-6.6386	-2.9157
	S. patens	-16.80615(*)	.53675	.000	-18.6972	-14.9151
	S. pratensis	1.60250	.48030	.095	-.0897	3.2947
	S. sclarea	-4.03143(*)	.49029	.000	-5.7588	-2.3041
	S. thermarum	-3.16750(*)	.75494	.004	-5.8273	-.5077
	S. uliginosa	7.00375(*)	.60530	.000	4.8712	9.1363
S. forskhalii	*S. aethiopis*	2.62524(*)	.50201	.000	.8566	4.3939
	S. africana-lutea	-6.14633(*)	.54639	.000	-8.0713	-4.2213
	S. austriaca	-1.38583	.52096	.780	-3.2212	.4496
	S. canariensis	.07667	.54639	1.000	-1.8483	2.0017
	S. exserta	-4.44216(*)	.48113	.000	-6.1372	-2.7471
	S. glutinosa	-.24167	.52096	1.000	-2.0771	1.5937
	S. involucrata	6.02576(*)	.53267	.000	4.1491	7.9024
	S. mexicana	-4.70048(*)	.50201	.000	-6.4691	-2.9318
	S. patens	-16.72949(*)	.51084	.000	-18.5293	-14.9297
	S. pratensis	1.67917(*)	.45116	.025	.0897	3.2687
	S. sclarea	-3.95476(*)	.46178	.000	-5.5817	-2.3278
	S. thermarum	-3.09083(*)	.73675	.004	-5.6865	-.4952
	S. uliginosa	7.08042(*)	.58245	.000	5.0284	9.1325
S. glutinosa	*S. aethiopis*	2.86690(*)	.50201	.000	1.0983	4.6355
	S. africana-lutea	-5.90467(*)	.54639	.000	-7.8297	-3.9797
	S. austriaca	-1.14417	.52096	1.000	-2.9796	.6912
	S. canariensis	.31833	.54639	1.000	-1.6067	2.2433
	S. exserta	-4.20049(*)	.48113	.000	-5.8956	-2.5054
	S. forskhalii	.24167	.52096	1.000	-1.5937	2.0771
	S. involucrata	6.26742(*)	.53267	.000	4.3908	8.1441
	S. mexicana	-4.45881(*)	.50201	.000	-6.2275	-2.6902
	S. patens	-16.48782(*)	.51084	.000	-18.2876	-14.6881
	S. pratensis	1.92083(*)	.45116	.003	.3313	3.5103
	S. sclarea	-3.71310(*)	.46178	.000	-5.3400	-2.0862
	S. thermarum	-2.84917(*)	.73675	.014	-5.4448	-.2535
	S. uliginosa	7.32208(*)	.58245	.000	5.2700	9.3741

S. involucrata	*S. aethiopis*	-3.40052(*)	.51415	.000	-5.2119	-1.5891
	S. africana-lutea	-12.17209(*)	.55756	.000	-14.1365	-10.2077
	S. austriaca	-7.41159(*)	.53267	.000	-9.2882	-5.5349
	S. canariensis	-5.94909(*)	.55756	.000	-7.9135	-3.9847
	S. exserta	-10.46791(*)	.49379	.000	-12.2076	-8.7282
	S. forskhalii	-6.02576(*)	.53267	.000	-7.9024	-4.1491
	S. glutinosa	-6.26742(*)	.53267	.000	-8.1441	-4.3908
	S. mexicana	-10.72623(*)	.51415	.000	-12.5376	-8.9148
	S. patens	-22.75524(*)	.52278	.000	-24.5971	-20.9134
	S. pratensis	-4.34659(*)	.46464	.000	-5.9836	-2.7096
	S. sclarea	-9.98052(*)	.47495	.000	-11.6538	-8.3072
	S. thermarum	-9.11659(*)	.74507	.000	-11.7416	-6.4916
	S. uliginosa	1.05466	.59295	1.000	-1.0344	3.1437
S. mexicana	*S. aethiopis*	7.32571(*)	.48232	.000	5.6265	9.0250
	S. africana-lutea	-1.44586	.52835	.626	-3.3073	.4156
	S. austriaca	3.31464(*)	.50201	.000	1.5460	5.0833
	S. canariensis	4.77714(*)	.52835	.000	2.9157	6.6386
	S. exserta	.25832	.46055	1.000	-1.3642	1.8809
	S. forskhalii	4.70048(*)	.50201	.000	2.9318	6.4691
	S. glutinosa	4.45881(*)	.50201	.000	2.6902	6.2275
	S. involucrata	10.72623(*)	.51415	.000	8.9148	12.5376
	S. patens	-12.02901(*)	.49150	.000	-13.7606	-10.2974
	S. pratensis	6.37964(*)	.42914	.000	4.8677	7.8916
	S. sclarea	.74571	.44029	1.000	-.8055	2.2969
	S. thermarum	1.60964	.72347	1.000	-.9392	4.1585
	S. uliginosa	11.78089(*)	.56556	.000	9.7883	13.7734
S. patens	*S. aethiopis*	19.35473(*)	.49150	.000	17.6231	21.0864
	S. africana-lutea	10.58315(*)	.53675	.000	8.6921	12.4742
	S. austriaca	15.34365(*)	.51084	.000	13.5439	17.1434
	S. canariensis	16.80615(*)	.53675	.000	14.9151	18.6972
	S. exserta	12.28733(*)	.47016	.000	10.6309	13.9438
	S. forskhalii	16.72949(*)	.51084	.000	14.9297	18.5293
	S. glutinosa	16.48782(*)	.51084	.000	14.6881	18.2876
	S. involucrata	22.75524(*)	.52278	.000	20.9134	24.5971
	S. mexicana	12.02901(*)	.49150	.000	10.2974	13.7606
	S. pratensis	18.40865(*)	.43944	.000	16.8604	19.9569
	S. sclarea	12.77473(*)	.45034	.000	11.1881	14.3613
	S. thermarum	13.63865(*)	.72963	.000	11.0681	16.2092
	S. uliginosa	23.80990(*)	.57342	.000	21.7897	25.8301
S. exserta	*S. aethiopis*	7.06739(*)	.46055	.000	5.4448	8.6900
	S. africana-lutea	-1.70418	.50855	.090	-3.4959	.0875
	S. austriaca	3.05632(*)	.48113	.000	1.3612	4.7514
	S. canariensis	4.51882(*)	.50855	.000	2.7271	6.3105
	S. forskhalii	4.44216(*)	.48113	.000	2.7471	6.1372
	S. glutinosa	4.20049(*)	.48113	.000	2.5054	5.8956
	S. involucrata	10.46791(*)	.49379	.000	8.7282	12.2076
	S. mexicana	-.25832	.46055	1.000	-1.8809	1.3642
	S. patens	-12.28733(*)	.47016	.000	-13.9438	-10.6309
	S. pratensis	6.12132(*)	.40452	.000	4.6961	7.5465

	S. sclarea	.48739	.41633	1.000	-.9794	1.9542
	S. thermarum	1.35132	.70915	1.000	-1.1471	3.8497
	S. uliginosa	11.52257(*)	.54712	.000	9.5950	13.4501
S. pratensis	*S. aethiopis*	.94607	.42914	1.000	-.5659	2.4580
	S. africana-lutea	-7.82550(*)	.48030	.000	-9.5177	-6.1333
	S. austriaca	-3.06500(*)	.45116	.000	-4.6545	-1.4755
	S. canariensis	-1.60250	.48030	.095	-3.2947	.0897
	S. exserta	-6.12132(*)	.40452	.000	-7.5465	-4.6961
	S. forskhalii	-1.67917(*)	.45116	.025	-3.2687	-.0897
	S. glutinosa	-1.92083(*)	.45116	.003	-3.5103	-.3313
	S. involucrata	4.34659(*)	.46464	.000	2.7096	5.9836
	S. mexicana	-6.37964(*)	.42914	.000	-7.8916	-4.8677
	S. patens	-18.40865(*)	.43944	.000	-19.9569	-16.8604
	S. sclarea	-5.63393(*)	.38130	.000	-6.9773	-4.2905
	S. thermarum	-4.77000(*)	.68917	.000	-7.1980	-2.3420
	S. uliginosa	5.40125(*)	.52096	.000	3.5658	7.2367
S. sclarea	*S. aethiopis*	6.58000(*)	.44029	.000	5.0288	8.1312
	S. africana-lutea	-2.19157(*)	.49029	.001	-3.9189	-.4642
	S. austriaca	2.56893(*)	.46178	.000	.9420	4.1958
	S. canariensis	4.03143(*)	.49029	.000	2.3041	5.7588
	S. exserta	-.48739	.41633	1.000	-1.9542	.9794
	S. forskhalii	3.95476(*)	.46178	.000	2.3278	5.5817
	S. glutinosa	3.71310(*)	.46178	.000	2.0862	5.3400
	S. involucrata	9.98052(*)	.47495	.000	8.3072	11.6538
	S. mexicana	-.74571	.44029	1.000	-2.2969	.8055
	S. patens	-12.77473(*)	.45034	.000	-14.3613	-11.1881
	S. pratensis	5.63393(*)	.38130	.000	4.2905	6.9773
	S. thermarum	.86393	.69616	1.000	-1.5887	3.3166
	S. uliginosa	11.03518(*)	.53018	.000	9.1673	12.9031
S. thermarum	*S. aethiopis*	5.71607(*)	.72347	.000	3.1672	8.2650
	S. africana-lutea	-3.05550(*)	.75494	.007	-5.7153	-.3957
	S. austriaca	1.70500	.73675	1.000	-.8907	4.3007
	S. canariensis	3.16750(*)	.75494	.004	.5077	5.8273
	S. exserta	-1.35132	.70915	1.000	-3.8497	1.1471
	S. forskhalii	3.09083(*)	.73675	.004	.4952	5.6865
	S. glutinosa	2.84917(*)	.73675	.014	.2535	5.4448
	S. involucrata	9.11659(*)	.74507	.000	6.4916	11.7416
	S. mexicana	-1.60964	.72347	1.000	-4.1585	.9392
	S. patens	-13.63865(*)	.72963	.000	-16.2092	-11.0681
	S. pratensis	4.77000(*)	.68917	.000	2.3420	7.1980
	S. sclarea	-.86393	.69616	1.000	-3.3166	1.5887
	S. uliginosa	10.17125(*)	.78144	.000	7.4181	12.9244
S. uliginosa	*S. aethiopis*	-4.45518(*)	.56556	.000	-6.4477	-2.4626
	S. africana-lutea	-13.22675(*)	.60530	.000	-15.3593	-11.0942
	S. austriaca	-8.46625(*)	.58245	.000	-10.5183	-6.4142
	S. canariensis	-7.00375(*)	.60530	.000	-9.1363	-4.8712
	S. exserta	-11.52257(*)	.54712	.000	-13.4501	-9.5950
	S. forskhalii	-7.08042(*)	.58245	.000	-9.1325	-5.0284
	S. glutinosa	-7.32208(*)	.58245	.000	-9.3741	-5.2700

S. involucrata	-1.05466	.59295	1.000	-3.1437	1.0344
S. mexicana	-11.78089(*)	.56556	.000	-13.7734	-9.7883
S. patens	-23.80990(*)	.57342	.000	-25.8301	-21.7897
S. pratensis	-5.40125(*)	.52096	.000	-7.2367	-3.5658
S. sclarea	-11.03518(*)	.53018	.000	-12.9031	-9.1673
S. thermarum	-10.17125(*)	.78144	.000	-12.9244	-7.4181

* The mean difference is significant to the 0.05 confidence level.

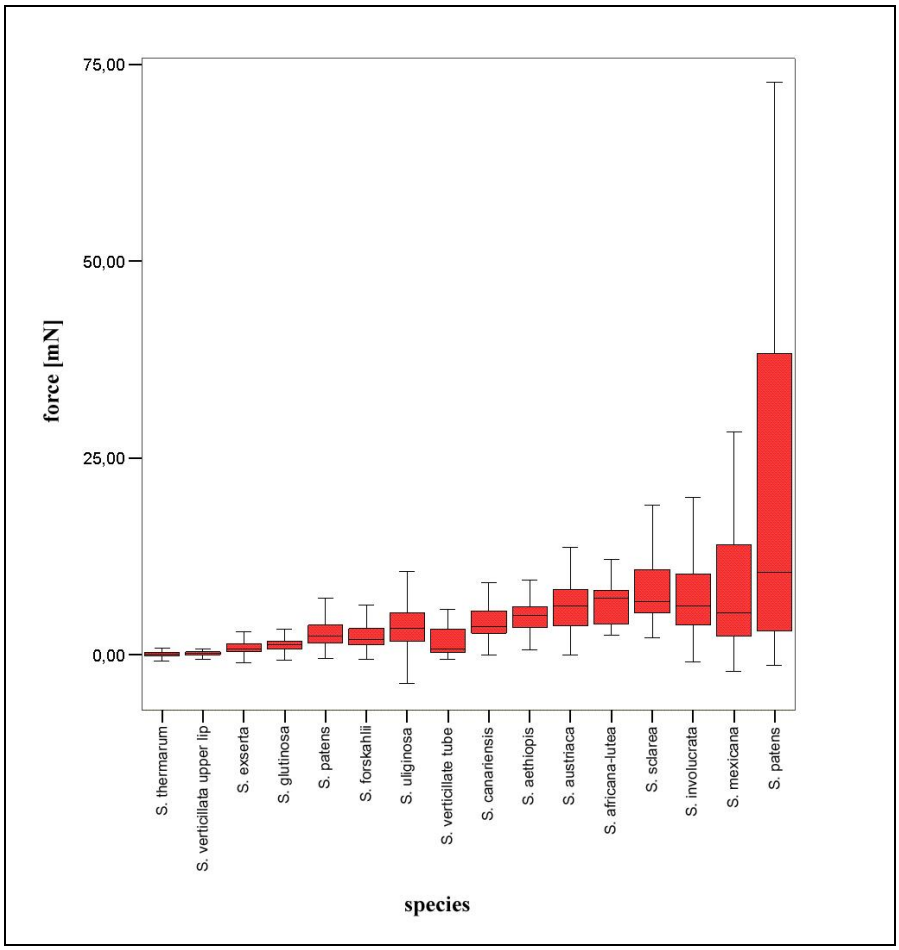


Fig. A.1 shows the Boxplots for all measured species. The forces differ extremely. All the species share a certain range of forces. The hairy ring of *S. verticillata* (tube) lies within the middlefield of the other species. Its upper lip however needs compareable low force to be moved. *S. patens* occupies the widest range of measured forces

Figs. A.2-A.7: Regression analysis of floral structures and the measured forces.

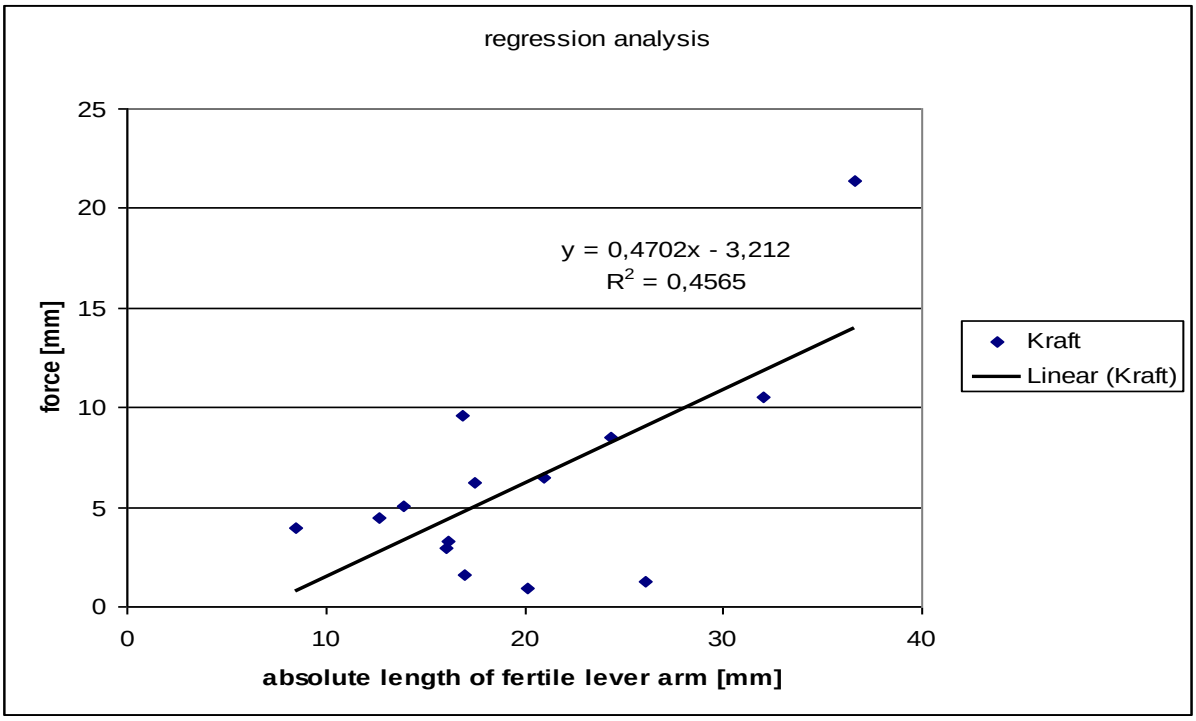


Fig. A.2: Regression analysis of absolute lever length and the measured forces.

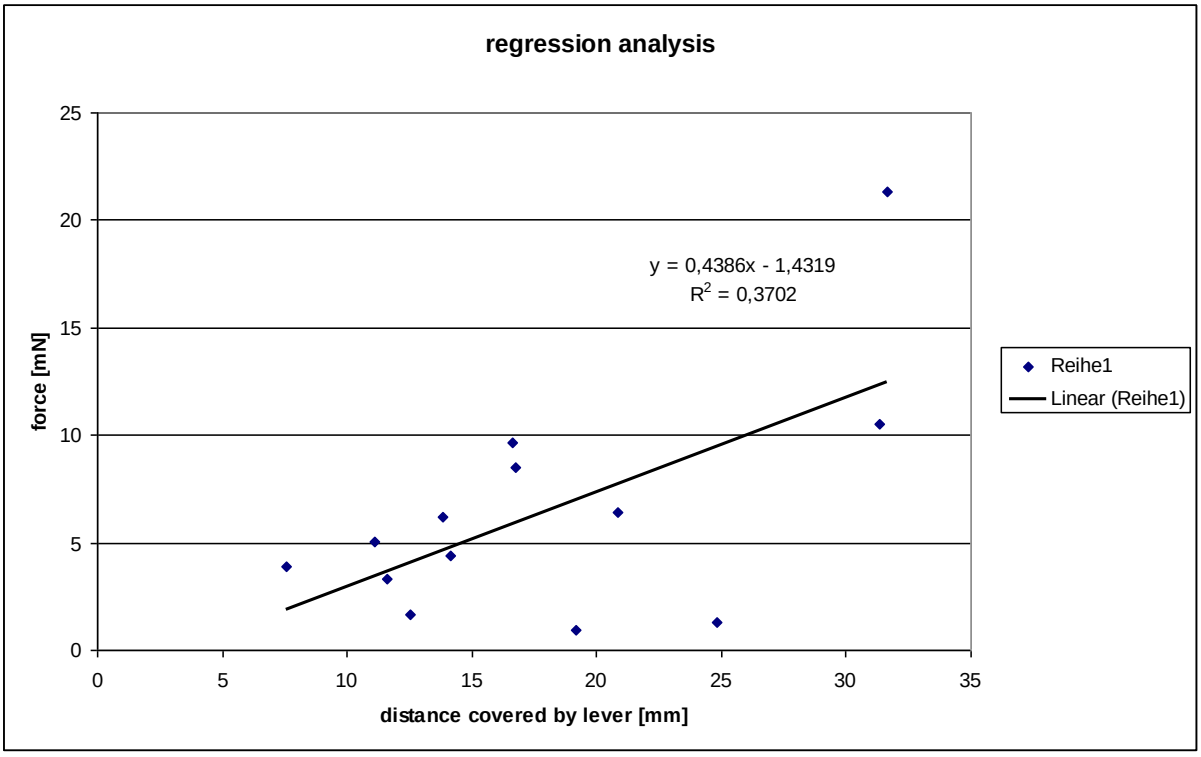


Fig. A.3: Regression analysis of distance covered by lever and the measured forces.

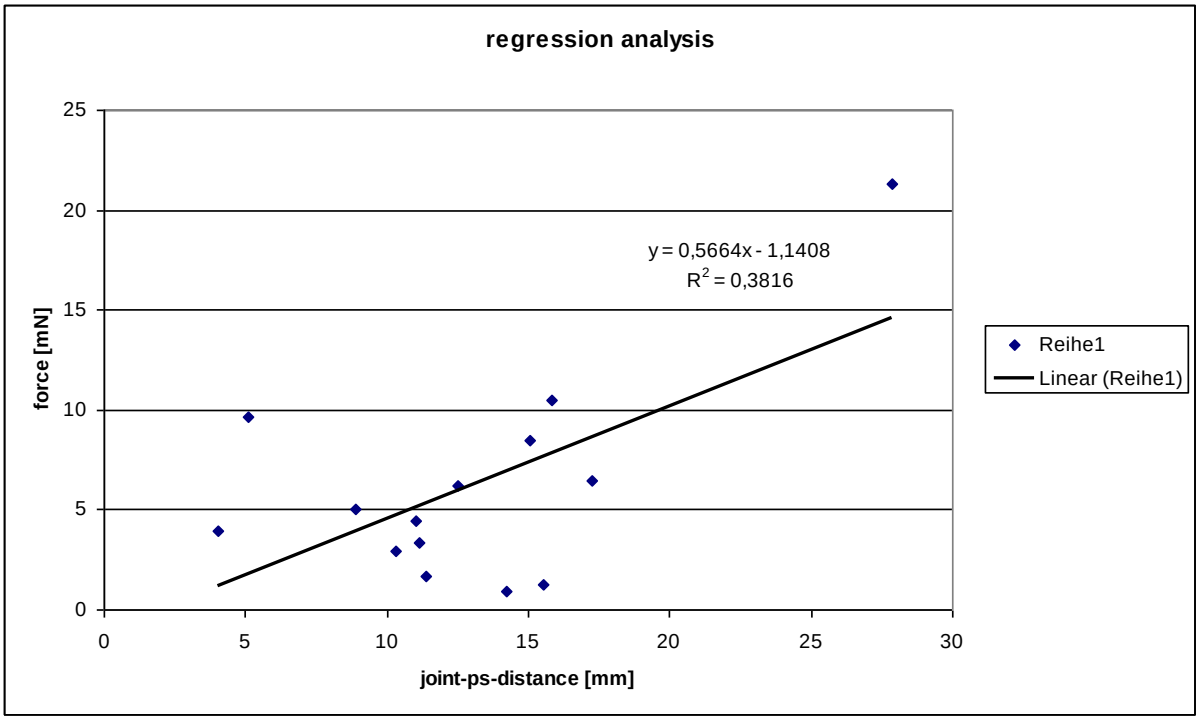


Fig. A.4: Regression analysis of joint-pollen sac distance and the measured forces.

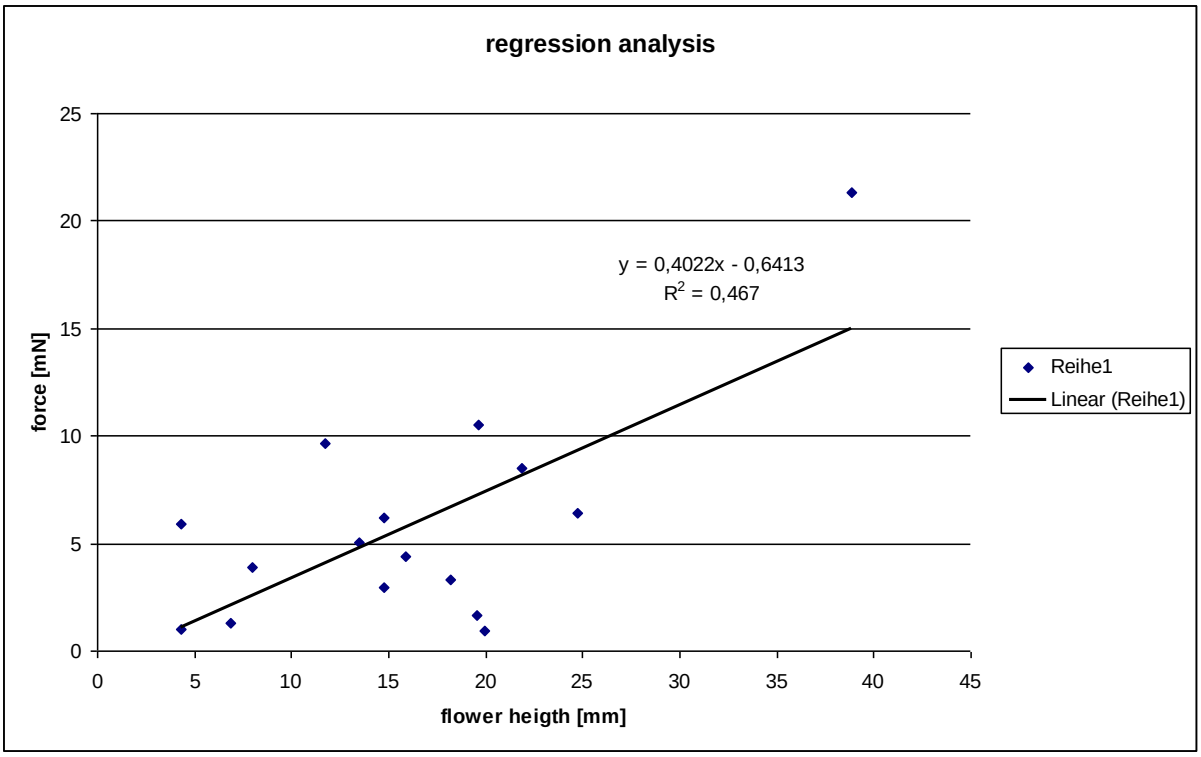
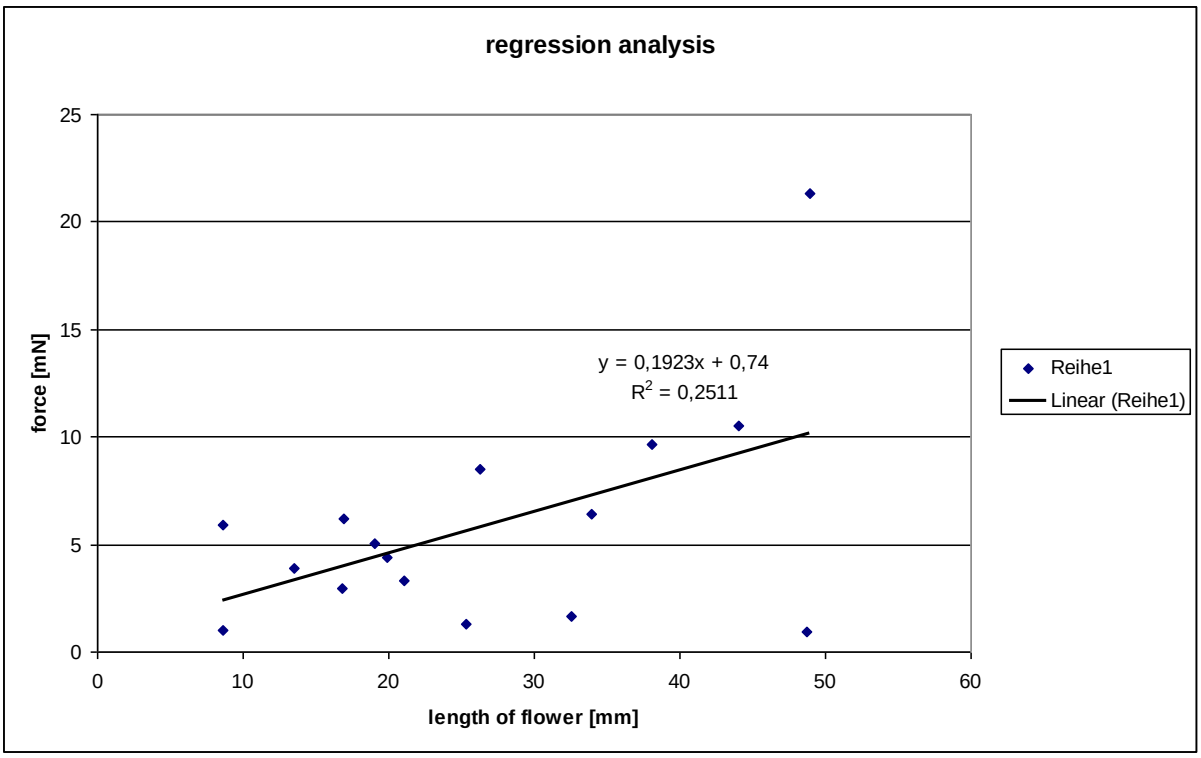


Fig. A.5: Regression analysis of flower height and the measured forces.



Fig, A.6: Regression analysis of flower lengtht and the measured forces.

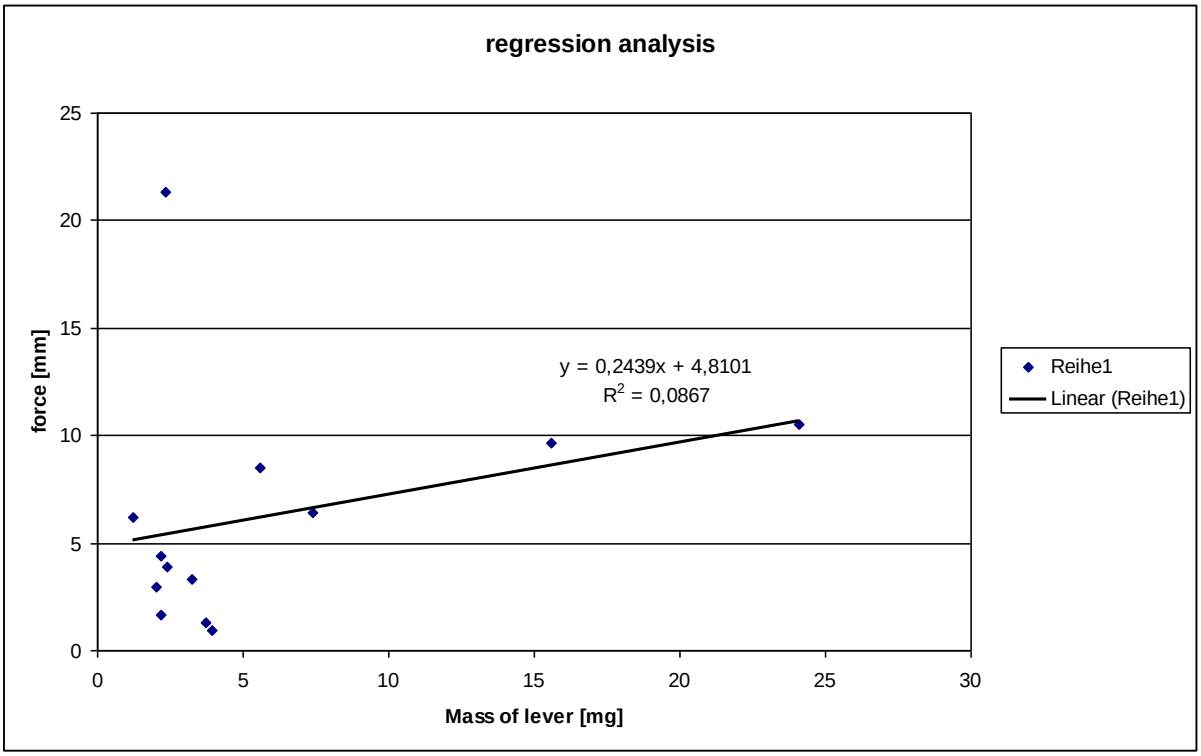


Fig. A.7: Regression analysis of the mass of the levers and the measured forces. r^2=0.09 indicates a correlation, but this result is not significant.

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Acknowledgements

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Curriculum vitae

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Erklärung:

Hiermit versichere ich, daß ich die vorliegende Arbeit selbständig verfaßt und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Mainz September, 2008 Sascha Thimm