Functional Integration of Floral Plant Traits: Shape and Symmetry, Optical Signal, Reward and Reproduction in the Angiosperm Flower

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I. GENERAL INTRODUCTION

I.1 FOREWORD

Flowering plants comprise probably about 450,000 extant species worldwide (Pimm and Joppa, 2015; Joppa et al., 2011). Round about a third of them is at risk of extinction, and they are going extinct at least 1,000 times the pre-human background extinction rate (Pimm and Joppa, 2015), comparable to estimations on vertebrate extinction: The accelerated, human-induced loss of biodiversity is already labelled as the 6th mass extinction (Ceballos et al., 2015). Mankind's role in this process and its responsibility to find a solution to it are understood and expressed univocally by the world´s nations since the first Earth summit 1992 in Rio de Janeiro. Cardinale et al. (2012) conclude on basis of two decades of scientific research that biodiversity loss has an alarming impact on ecosystem functions: Biodiversity loss decreases the stability of ecosystem functions and diminishes the spectrum of functional traits of organisms that constitute these functions.

A key ecosystem function is pollination, provided by the organisms involved, their functional traits and plant-pollinator as well as plant-environment interactions. The 2016 IPBES assessment on pollinators, pollination and food production states: Approximately 90 % of wild flowering plants depend at least to some extent on animal pollination. 75 % of food crops also depend on animal pollination, with an estimated annual market value of global crop production of $235 billion – $577 billion directly attributable to pollinators (2015, US $: IPBES, 2016; Lautenbach et al., 2012). Diversity of wild animal pollinators is critical to crop pollination, even when pollinators such as honeybees are managed by humans: The majority of pollinator species are wild. Where Red List assessments are available, they show high levels of threat for pollinators, i.e. often more than 40 % of wild bee species may be threatened. Plant-pollinator interactions contribute to many other economic and social values beside crop pollination, such as medicine, production of biofuels, fibres or construction materials, and also to cultural values and good quality of human life: They contribute to education and recreation, are a source of inspiration, i.e. for art, music, science and religion, are symbols of identity and constitute a globally significant heritage (cf. IPBES, 2016). The basis for this ecosystem service and the interface for plant-pollinator interactions is the angiosperm flower.

However: What is a flower?

Flowers fascinate mankind. We use them to celebrate important events, please our senses and express love (cf. Buchmann, 2015). From a biological perspective, flowers are, in essence, the sex organs of angiosperms – specialised structures that secure reproduction, predominantly via outcrossing (cf. Willmer, 2011; Buchmann, 2015). In order to secure reproduction, flowers rely on abiotic (wind, water) or biotic pollen vectors (animal taxa). Probably between 130,000 and 300,000 animal taxa are regular flower visitors, and at least 25,000 species of bees are obligate flower visitors (Willmer, 2011, citing Buchmann and Nabhan, 1996; Kearns et al., 1998). From an evolutionary perspective, flowers are a key innovation: Offering food to animal visitors in the form of pollen or nectar is a key mutualism that secures sexual reproduction of plants, and is hypothesised to have caused rapid speciation in early angiosperm evolution (Lunau, 2000; Pellmyr, 1992; Willmer, 2011, citing Dodd et al., 1999, and Kay et al., 2006). Diversification and establishment of major angiosperm lineages synchronously took place 135–

130 million years ago (Magallón et al., 2015). The common ancestor of all seed plants, angiosperms and gymnosperms, originated much earlier, likely 310-350 Ma ago (Sauquet et al., 2017, citing Doyle, 2012, and Magallón et al., 2013).

Main objective of this dissertation is to contribute to better understanding of the functional aspects of angiosperm flowers and their interplay, constituting the basis for successful plant-pollinator and plant-environment interactions and siring success:

In its core functions, flowers produce pollen grains, the male gametophytes, dispense them, organise the receipt of incoming pollen and guide their genetic material to the ovules, which contain the female gametophytes (cf. Willmer, 2011). Organisation of pollen receipt and guidance can be divided into aspects of mechanical fit between floral structures and pollen vectors (realised via floral morphology, architecture and symmetry), see section I 2.1 of this introduction, as well as plant presentation traits (Willmer, 2011, citing Thomson, 1983), see section I 2.2. Plant presentation traits are introduced with focus on pollinator attraction and optical flower signal (I 2.2.1), as well as reward (I 2.2.2). Basic parameters of reproduction in angiosperms, such as sexual systems, pollen and ovule production, the relevance of the pollen to ovule ratio and its correlation to breeding systems, are introduced in section I 3. In section I 4 the concept of pollination syndromes is presented, which offers an integrated view of floral structure, attraction, reward and parameters of reproduction: Pollination syndromes are groups of floral phenotypes that reflect specialisation towards pollen vectors. Afterwards specific hypotheses (I 5) and goals (I 6) of this work are presented, an overview of the dissertation (I 7) is given and the author's contributions to the chapters (I 8) are listed.

I.2 ORGANISATION OF POLLEN RECEIPT AND GUIDANCE

In the following, floral strategies of pollen receipt and guidance are described separately for aspects of mechanical fit between floral structures and pollen vectors (section I 2.1), as well as for plant presentation traits, flower signal and reward (section I 2.2).

I.2.1 FLORAL STRUCTURE – MORPHOLOGY, ARCHITECTURE, SYMMETRY AND SHAPES

Floral structure can be described by four **fundamental morphological components** that are present in most flowers, but can vary strongly between species. These components are arranged from outer to inner as sepals, petals, stamens and carpels (cf. Willmer, 2011):

Sepals and petals (tepals, if undifferentiated) form the perianth. Sepals as the outermost structure and protect the inner, sexual flower organs. Petals also have protective function, but often play an important role in pollinator attraction, i.e. due to distinctive colour. Sepals can also be of importance for pollinator attraction in some species.

The stamens are the male sexual flower organs, in sum called the androecium. Each stamen is formed of a filament that bears the anther as its apical end. The anther itself contains the male gametophytes or pollen grains.

Carpels are the female organs in the centre of a flower that contain the ovules, in sum called the gynoecium. One or several carpels constitute the pistil, the female reproductive structure. A flower can bear one or more pistils. In general, pistils can be described by three components: The stigma is the apical and pollen-receptive part, sometimes on tip of a style that connects to the ovary, which contains the ovules.

Male and female flower organs come in very different shapes and colours and can therefore serve additional roles, i.e. as visual or olfactory attractants.
Diversity of flower shapes in angiosperms is realised on two structural levels: While variation in floral morphology addresses changes in the arrangement and number of floral organs, variation of **floral architecture** addresses the modification of floral organs (Jeiter et al., 2017; Jeiter and Weigend, 2018) via synorganisation, fusion and differential growth rates (Endress, 1996).

Flower symmetry is a key trait in angiosperm flower structure (Endress, 1996, 1999). It is caused by repetitions of structural units, which are assessed in relation to the principal axis of the flower (Neal et al., 1998). Four types of flower symmetry are usually distinguished, namely "asymmetric" (without any symmetry plane), "monosymmetric" or "zygomorphic" (also called "bilateral", one symmetry plane), "disymmetric" (two symmetry planes) and "actinomorphic" (also called "polysymmetric" or "radially symmetric", with several symmetry planes). Actinomorphic and zygomorphic flower symmetry are the most common types.
Many hypotheses try to explain the role of flower symmetry for plant-pollinator interactions. Neal et al. (1998) present four major categories: (a) environmental conditions (i.e. protection from rain etc.), (b) perception by the pollinators, (c) information processing (i.e. learning abilities and innate preferences) by pollinators, and (d) activity patterns (i.e., behaviour and movement of pollinators) controlled by flower symmetry.
Plant-animal interactions related to symmetry patterns are commonly understood as a driving force in evolution and species diversification (Endress, 1999). Actinomorphy is considered to be ancestral in angiosperms (Endress, 2011; Reyes et al., 2016). Fossil records indicate a relatively late origin of zygomorphy in angiosperm evolution, dating back to the upper Cretaceous (Crepet, 1996; Endress, 1999). Amongst the basal magnoliids zygomorphic flowers are absent, and only seldom to find among basal eudicots. They are much more common and represented in larger groups among rosids and asterids, however (Endress, 1999; Reyes et al., 2016). The occurrence of zygomorpic flowers in the upper Cretaceous is often interpreted as a selective advantage due to pollinator preference and/or fostered pollen economy and precise pollen placement, coinciding with the appearance of bees round about 123 million years ago (Cardinal and Danforth, 2013; Reyes et al., 2016). Contrary to this, actinomorphic flowers offer approach and landing of potential pollinators as well as reward from every direction (cf. Endress, 1999; Willmer, 2011).
Reyes et al. (2016) showed in their reconstruction of floral symmetry evolution a minimum of 130 origins of zygomorphy in angiosperms, almost double the amount of previous estimations, and 69 reversals to actinomorphy. The authors conclude selective advantages of this symmetry type in some circumstances, but not in others, due to the absence of zygomorphy in basal angiosperms and its uneven distribution in clades in which it is present.

Beyond floral symmetry, specific **floral shapes** are described in botany in order to treat the arrangement of floral organs, floral morphology and floral architecture in a holistic way, in example (cf. Willmer, 2011):

Open disk or bowl flowers are the simplest and most common flower shape, allowing free access for most flower visitors. The petals display a flattened disk when the flower is fully opened, or form a bowl when remaining in a more erect orientation.
Tubular or funnel-shaped flowers are characterised by elongated perianths, which are at least fused at their base to form the tube. Nectar reward is concealed by this flower type, and can only be accessed with suiting mouthparts. Tubular flowers can show actinomorphic as well as zygomorphic flower symmetry. The later do control pollinators more strongly by their build-up, nototribic pollen position and usually also by consistent orientation of flowers on the plant.
Trumped-shaped and bell-shaped flowers are a variation of actinomorphic, funnel-shaped flowers: The tube is more open and allows pollinators to enter the flower with their whole body. Sexual flower organs are usually arranged at the centre.
Spherical or urceolate flowers have a narrow mouth and are also contracted at the base, while the corolla in between widens out.
Many other flower shapes are described in botany, and arrangement of floral structures does not stop at the individual flower level: In many species flowers are grouped in **inflorescences**. Some reproductive structures of plants are called flowers, but are actually inflorescences, i.e. composite flowers of Asteraceae, or brush blossoms (headed inflorescences with elongated stamens constituting the "brush"). Flower shapes and inflorescence structures are presented in more detail in the following chapters of this thesis, if necessary.

I.2.2 Plant presentation traits – floral attractants and reward

Animal-pollinated plants show plant presentation traits that secure successful interaction with animal taxa. Plant presentation traits can be divided into floral attractants and rewards (Willmer, 2011, citing Thomson, 1983).
Floral attractants comprise various stimuli for the sensory systems of animal taxa in order to draw them to the flower. Floral rewards go beyond attraction and satisfy substantial needs of the flower visitors, like nutritional needs, in order to ensure repeated visitation and therefore pollination (Dafni, 2005).

I.2.2.1 Floral attractants

Flowers address the sensory systems of potential pollen vectors in medium or even long distance via attractants. **Olfactory signals** are very successful to this end, but are not further analysed here. Advertising signals in focus of this thesis are visual flower cues.
Flower shape and size are important optical attractants. Floral display size is sometimes measured as the total expanse of blooming flowers on a plant individual. Here, display size is defined as the expanse of single flowers or functional units of reproductive structures (i.e. inflorescences) that can be optically perceived and measured from frontal and lateral view.
Further, **optical advertising via flower colour** is one of the most important floral attractants, either in the light spectrum that can be perceived by the human eye, or beyond. Many animal taxa are able to perceive the ultraviolet (UV) spectrum. Several methods exist to measure and standardise colour and its perception. In practice, these methods often produce similar results: Using the human eye to rate colours is therefore still widespread (Willmer, 2011, citing Kevan, 1978, and Chittka and Kevan, 2005),

and also the method of choice for the research presented here. UV light pattern are detected by spectral photography and application of specialised lens filters.

The following aspects of colour and UV signal in flowers were of special research interest in the last years, and their relevance for plant-pollinator interactions has been demonstrated:

Colour preferences of pollinator taxa: Flower colour signal shows a lot of common features across different plant taxa, and not all possible colour hues are realised to the same extent in angiosperm flowers, indicating a coevolution with pollinator cognition. There is reasonable evidence that pollinating animal taxa show a (learned or innate) preference for certain colours, as follows (cf. Willmer, 2011, citing Kevan, 1983, and Scogin, 1983):

Bees: Blue, purple, pink, white; yellow in less advanced and/or short-tongued types. Beetles: Cream, dull or light green; red or orange in few species. Butterflies and diurnal moths: Red, pink, purple; yellow in less advanced types. Moths: White or cream. Flies: White, cream, yellow/green. Carrion flies: Brown, deep red, purple. Wasps: Brown and green. Bats: White, cream, dull green, dull purple. Non-flying mammals: Red, brown, dull shades. Birds: Red, orange.

Reddish flower colour is classically assumed as a good indicator for bird-pollination. Moreover, it is hypothesised to be a double strategy to simultaneously attract birds and distract bees due to their bad perception of red hues (Chittka et al., 2001, citing Raven, 1972). However, bees do visit red flowers that are bird-pollinated, and can learn to distinguish red hues (Chittka and Waser, 1997). Chittka et al. (2001) point out that there is no need for exclusivity, and simultaneous attraction of birds and distraction of bees might both be of advantage, even if positive changes by natural selection are minimal.

Anther dummy signal: Also described as mimic stamens, fist described by Osche (Lunau, 2006, citing e.g. Osche, 1979). Pollen and anthers show a yellow colouration due to the UV light protective function of flavonoid pigments. This colouration represents a primary food signal that could be perceived by insects early in angiosperm evolution. Colour vision in insects evolved earlier than flower colour (Lunau, 2006, citing Chittka, 1996). Anther dummy signals copy the yellow colouration of pollen and anthers and present it as colour pattern on other parts of the flower. Sometimes anther dummies also copy the structure of anthers. Anther dummy signals cause the same innate pollinator responses like the original cues: Approach, targeting with antennae, landing, proboscis extension and intake were experimentally demonstrated for several Hymenoptera and Diptera species (Lunau, 1990, 2006; Lunau and Wacht, 1994), see also figure I.1. Anther dummy signals are no typical mimicry systems, because model and mimicry stamens of the same plant species often do not resemble each other (cf. Lunau, 2006).

Colour purity and optical contrast within a flower: A gradient of centripetally increasing spectral purity has been demonstrated for melittophilous flowers (Lunau, 1992): The UV pattern usually shows a "bulls-eye" effect (Silberglied, 1979), with an UV absorbing flower centre and UV reflecting peripheral parts, see figure I.2. Stronger UV absorption enhances spectral purity, without altering the dominant wavelength of the optical signal in a significant way. Wavelengths that can be perceived by bees (bee-yellow, bee-bluegreen, bee-purple) strongly increase in spectral purity by this effect. UV absorption is also coupled to bee-white flowers, because UV reflection would impair spectral purity of these flowers massively (Lunau, 1992, citing Daumer, 1958). Stronger UV reflection has been detected in fewer cases, leading to enhanced spectral purity of bee-ultraviolet or bee-violet (i.e. strong UV-reflection in poppy flowers; van der Kooi and Stavenga, 2019).

Nectar guides are a variation of colour contrast in melittophilous flowers, such as contrasting blotches, concentric colour rings or lines converging towards the flower centre, etc. (Willmer, 2011).

Centripetally increasing spectral purity and colour contrasts serve as cues for innate orientations of potential pollinators, such as approach, antennal contact, landing reaction, proboscis extension and intake (Lunau, 1988, 1990, 1991, 1992).

It is likely that the diversity of colours and their hues are necessary cues for discrimination learning, beyond innate orientations. Learning ability of one sort or another has been found in virtually all pollinating animal taxa tested (Weiss, 2001, citing Alloway, 1973).

Optical contrast to the background: Optical signal pattern of the environment are also of relevance for pollinator attraction. In many cases, entomophilous flowers contrast to their background due to higher spectral purity. Soil or leaves usually show a low spectral purity, i.e. by only weak peaks of green leaf colour in the bee-yellow range, reflection of incident light by waxy leaf-surfaces, light dispersion on uneven surfaces, etc. (Barthlott and Rosen, 1991; Lunau, 1992).

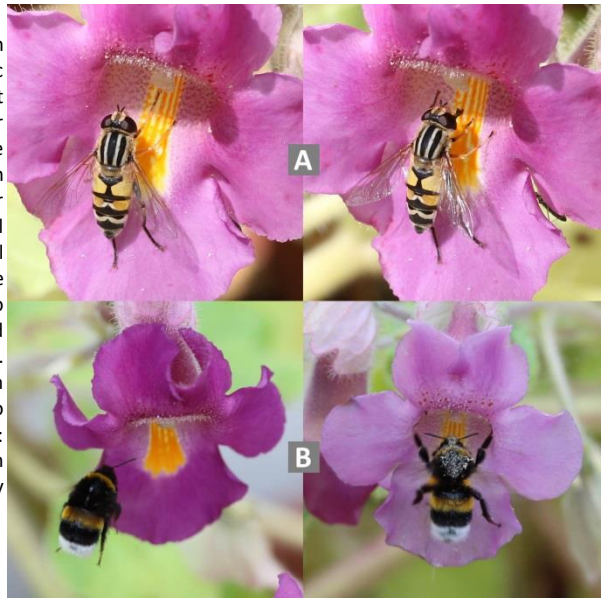

FIGURE I.1: FLOWER SIGNALS AND ANIMAL BEHAVIOUR
Flowers of *Proboscidea louisianica* (Mill.) Thell., a native plant of North America, and behaviour of flower visitors in Bonn University Botanic Gardens, Germany. Flowers show a pink colour and a dominant yellow stripe on ground of the sympetalous corolla, an anther dummy signal. Mind also the sharp contrast of flower colour to the background of greenish leaves. **A.** An innate proboscis extension can be observed when syrphid flies get in contact with the anther dummy signal. **B.** Optical flower signals trigger and control several other innate behavioural responses, such as approach and antennal orientation (B-left) or landing and body orientation (B-right), here observed for *Bombus terrestris* L. Innate reactions of insect taxa to optical colour cues were verified in experimental settings, isolated from other possible cues (odour, flower size and symmetry, etc.). Although optical flower signals trigger similar innate reactions in different insect taxa (here even shown for a plant non-native to Europe), successful pollination is influenced by many other factors: The bumblebee is a potential pollen vector due to successful pollen placement on the upper thorax, but the syrphid fly is not. Pictures by A. W. Mues.

FIGURE I.2: MAKING THE INVISIBLE VISIBLE – UV LIGHT PATTERN
Frontal flower displays photographed in full-spectrum light (left) and UV light (right). **Upper row:** Masked flower type of *Utricularia subulata* L. The lower lip is closing the flower tube, and the body weight of a potential pollinator is necessary to bow down the lip and get access to the nectar. The UV light spectrum shows a bulls-eye effect in the flower centre, which is not visible in daylight and can also be interpreted as a three-dimensional anther dummy signal due to shape and colouration. **Lower row:** Bulls eye effect of *Pulmonaria mollis* Wulfen ex Hornem. The flower shows a whitish centre in daylight coupled to UV light absorption, which is a sharp contrast to the otherwise pinkish and UV reflecting corolla. Scale bars indicating 1 cm. Pictures by A. W. Mues.

I.2.2.2 FLORAL REWARD

Floral rewards satisfy needs of animal visitors and come in many ways, such as oils or floral tissues as food reward, resins and gums as antibacterial and antifungal building material in brood sites, scents that are (likely) used for attraction and guidance of other individuals, or flowers that are directly functioning as a brood place, cf. Willmer (2011) for an overview. However, the most common floral rewards are pollen and nectar as food rewards.

Pollen as a reward is collected deliberately by some animal taxa, and it provides a balanced and nourishing diet when compared to other possible food sources offered by plants (Willmer, 2011). Round about 20,000 plant species exclusively offer pollen as reward for potential pollinators and produce it in high amounts, likely to compensate for the high pollen demand of pollen collecting bees (Willmer, 2011). Indeed, "pollen-only" flowers produce significantly more pollen than species that offer nectar reward (Cruden, 2000).

Nectar reward is offered by 70 to 80 % and therefore the majority of extant angiosperm species (Abrahamczyk et. al., 2017 a, citing Heywood et al., 2007). It is essentially a mixture of sugar and water, with concentrations typically ranging from 10 % to 75 % (Willmer, 2011). Nectar reward is produced by specialised structures called nectaries, which are derived from different floral tissues. Nectaries can come in very different shapes and sizes: Usually they are arranged inside and near the base of flower (i.e. formed from parts of or all of a sepal, petal, stamen or ovary), so that flower visitors have to insert their mouth parts or even their whole body to access the nectar – and in return become pollen vectors by getting in contact with the anthers (Willmer, 2011). Nectar spurs are also very common structures that are formed as more or less elongated tubes from the flower base. Nectar spurs protect nectar from environmental conditions, and only flower visitors with a sufficient proboscis length can access the nectar.

Beside water, **sugars** are the predominant components of nectar reward, usually glucose, fructose and sucrose. It has long been hypothesised about the relevance of the proportions of these sugars for adaption to pollinator guilds. Scientific evidence is contradictory at present (Willmer, 2011), and the proportion of sugar components might rather be a consequence of phylogenetic constraints (Abrahamczyk et. al., 2017 a, citing e.g. van Wyk et al., 1993; Nicolson and Thornburg, 2007). Sugar proportions might also be restricted by flower shapes in interplay with environmental conditions (Abrahamczyk et. al. 2017 a, citing e.g. Baker and Baker, 1983; Nicolson et al., 2007). However, Abrahamczyk et. al. 2017 a show a (weak) evolutionary adaption of nectar sucrose proportion in their extensive study of asterids, with high sucrose percentages shown for specialist-pollinated and low proportions shown for generalist-pollinated plants.

Amino acids in nectar rewards seem to be indicative for pollinator groups: Very high amino acid content is often found in flowers visited by carrion flies, elevated levels correlate to pollination by Lepidoptera, moderate levels are found in flowers visited by wasps, bees, flies and hawkmoths, and very low levels correlate to pollination by birds and bats (Willmer, 2011, citing Baker and Baker, 1973, 1986).

Other components of nectar reward (i.e. lipids, scents etc.) are insufficiently researched, although scattered evidence for their relevance in plant-pollinator interaction does exist: Johnson et al. (2006) e.g.

showed that dark nectar colour and bitter taste are relevant filters for flower visitors in a bird-pollinated plant.

The most important **nectar parameters** for the assessment of plant-pollinator interactions are nectar volume, nectar concentration and sugar content. Analysis of these parameters requires rigorous standardisation of the experimental assessment: The amount and composition is highly influenced by environmental conditions, such as water availability and heat, age of the flower, time of day etc. The following statements therefore refer to retrieved species profiles on average and general pattern of plant-pollinator interactions.

The **nectar volume** produced by a flower strongly influences the interaction with possible pollinators and is indicative for pollinator guilds. Importantly, nectar volume is not only of caloric relevance due to sugar intake, but also serves as a water resource, and nectar feeders have to manage their water balance in relation to nectar uptake. This is of special importance in arid environments, and for bees (except *Apis*), that do not collect free water (Willmer, 1986, 1988, 2011). Large avian and mammalian pollinators are rewarded with the highest nectar amounts, ranging between several hundred µl to some ml. Flowers that have adapted to Diptera show very low amounts, often less than 0.05 µl, and bee pollinated flowers are somewhere in between, nectar volume is usually ranging between .01 and 10 µl (Willmer, 2011).

The **amount of sugar** that is contained in nectar reward is often used a caloric measurement for energy uptake, and also an indicator for the bodily requirements of pollinator groups. Total sugar contend of nectar reward is a deduced variable, calculated from nectar volume and the measured concentration of the nectar.

Nectar concentration is the most important indicator for pollinator groups: The sucking or licking mouthparts of the different pollinating animal taxa (and therefore nectar uptake via capillary adhesion or suction) predefine the preferred nectar concentrations, due to the interdependence of nectar concentration with nectar viscosity (Kingsolver and Daniel, 1995; Krenn et al., 2005; Willmer, 2011). Table I.1 gives an overview of preferred nectar concentrations for important pollinator taxa, taken from Nicolson et al. (2007) and the references therein. The information presented can be reduced to the simple rule that long-tongued pollinators cannot drink strongly concentrated nectars due to viscosity effects: In general, long-tongued hummingbirds, bats and lepidopterans seek diluted sources between 15 and 30 %, while longer-tongued bees prefer 30 to 50 %, short-tongued bees can cope with 45 to 60 % and flies can even deal with concentrations between 65-70 % due to lapping nectar uptake (cf. Willmer, 2011).

TABLE I.1: PREFERRED NECTAR CONCENTRATIONS OF POLLINATOR TAXA
The table presents preferred ranges or arithmetic means of nectar concentration, according to pollinator taxa. Cited from Nicolson et al. (2007) and references therein.

pollinator taxa	reported nectar concentration	reference
Diptera: Tabanidae, Nemestrinidae (with proboscis, nectar-sucking)	25-30 %	Goldblatt and Manning (2000)
Lepidoptera	35-45 %	Kingsolver and Daniel (1995)
Hymenoptera (bees)	30-50 %	Waller (1972)
Hummingbirds / Sunbirds	Ø 25 % / Ø 21 %	Nicolson and Fleming (2003)

There is a relevant relationship between flower shapes and nectar concentration: longer, tubular flowers are correlated to lower nectar concentrations, while open flowers are correlated to more concentrated

nectar (cf. Willmer, 2011). Due to this correlation, a broader functional classification of flower types and their flower visitors is presented by Nicolson et al. (2007; citing Corbet, 2006), which is cutting across insect orders and is presenting **pollinator guilds**: In general, flower types with fully exposed or only moderately concealed nectar and higher concentration are matching **short-tongued** pollinators like Diptera, beetles or short-tongued Hymenoptera. Complementary, flowers with abundant, deeply concealed nectar are matching **long-tongued** Hymenopthera, Diptera or Lepidoptera.

I.3 REPRODUCTION: SEXUAL SYSTEMS, BREEDING SYSTEMS, GAMETE PRODUCTION AND P/O RATIO

In order to secure siring success, largely immobile and sessile plant organisms have to transmit pollen (respectively male gametophytes) effectively to ovules (and the female gametophytes they contain) via abiotic or biotic vectors. Compared to floral structure and floral advertising, this core aspect of pollination and floral ecology is largely "cryptic" to animal pollen vectors, but determines reproductive success in the long term (cf. Willmer, 2011).

Unfortunately, plant reproductive biology covers a variety of concepts and confusing terminology, Neal and Anderson (2005) take a stand on this issue. An important aspect of plant reproduction, sometimes covered by the label breeding system, are **sexual systems** – the arrangement of reproductive structures within flowers and their distribution between plant individuals of a species (cf. Bawa and Beach, 1981; Renner, 2014): Flowers are usually hermaphroditic, containing both male and female sexual flower organs, therefore also called bisexual or perfect flowers. Unisexual or imperfect flowers are called pistillate (female, lacking stamens) or staminate (only male, lacking pistils). Species with both sexes on the same plant are called monoecious. When sexes are separated between plant individuals, the species is called dioecious. Separate sexes occur in round about 6 % of extant species (Renner and Ricklefs, 1995). Between 10 and 15 % of angiosperms show mixed strategies, the majority of species is bisexual however, at least 80 % (Willmer, 2011).

In this thesis, the term **breeding system** is used when treating genetic relatedness and pairing of gametes between or within plant individuals, out of reasons of continuity to the bulk of literature cited (esp. research done by R. W. Cruden, see below). Breeding systems represent basic strategies of sexual plant reproduction that range from self-pollination, called autogamy, to outcrossing and pollen transfer between individuals, called xenogamy. These terms can be further defined by epithets that express the rigidity of the respective strategy, with "facultative" describing an optional but predominantly occurring pattern, and "obligate" describing the respective pattern as exclusive and fixed. Cleistogamy describes an extreme form of obligate autogamy, the self-pollination within non-opening flowers. It is important to note in this context that the presence or absence of a physiological or genetic **self-incompatibility** is a powerful strategy of plants to control the degree of outcrossing.

Siring success strongly relies on successful pollen dispersal and receipt: Only round about 1 % of all produced pollen grains successfully reach a stigma (Willmer, 2011, citing Harder, 2000). Minnaar et al. (2019) present 16 barriers that diminish the probability of pollen grains to reach a conspecific stigma along three main phases of pollen transfer: Before (e.g. pollen grains remaining in anthers), during (e.g. grooming behaviour of an animal pollen vector) and after the pollen transfer (e.g. pollen lost on self-stigmas that otherwise could have reached other plant individuals for outcrossing).

When viewing pollination from an economic point of view, pollen transfer should be as effective as possible to save resources: Pollen and ovules should be produced in balanced proportions and in regard to the respective breeding system. Cruden (1977) first proposed the pollen to ovule ratio per flower, in short **p/o ratio**, as a proxy for plant breeding systems. In general, autogamous species should have much lower p/o ratios than xenogamous species, since little or no pollen is lost in transfer between male and female flower organs. Table I.2 shows the thresholds for p/o ratios that Cruden presented in 1977.

TABLE I.2: POLLEN TO OVULE RATIOS AS INDICATOR OF BREEDING SYSTEMS
The table shows retrieved arithmetic means and standard errors for different breeding systems, as presented by Cruden (1997).

breeding system	p/o ratio $\bar{X}$ ± s.e.
cleistogamy	4.7 ± .7
obligate autogamy	27.7 ± 3.1
facultative autogamy	168.5 ± 22.1
facultative xenogamy	796.6 ± 87.7
obligate xenogamy	5859.2 ± 936.5

In the last decades a lot of research has been conducted on this topic (cf. Cruden and Jensen, 1976; Cruden and Miller-Ward, 1981; Cruden and Lyon, 1985; Small, 1988; Kirk, 1993; Lopez et al., 1999; Cruden, 2000; Michalski and Durka, 2009; Alarcón et al., 2011; Lozada-Gobilard et al., 2019). The initially proposed relationship between p/o ratio and breeding system still seems to hold true, especially within individual genera and families. However, between unrelated plant groups huge differences in p/o ratios of taxa with the same breeding system may be discovered. Moreover, p/o ratios are influenced by the sexual system (e.g. plants only bearing hermaphroditic flowers show lower p/o ratios), the pollen vector (e.g. wind-pollinated plants show substantially higher p/o ratios) and many other details of the pollination process, like behaviour, body size and pollen bearing area of an animal pollinator (cf. Cruden, 2000).

I.4 POLLINATION SYNDROMES, SUITES OF FLORAL PLANT TRAITS AND PLANT-ANIMAL INTERACTION

Pollination syndromes describe groups of floral phenotypes that represent specialised plant-animal interactions. The general idea that interactions between flowers and pollinators are promoted by floral features was already proposed in the 18th century by Kölreuter (1761) and Sprengel (1793), while the concept of pollination syndromes was founded in the 19th century by Delpino (1868–1875). It became a centre of scientific interest in the midst of the 20th century again, and the works of Vogel (1954), van der Pijl (1961) and Grant and Grant (1965) are usually cited as starting points, cf. Fenster et al. (2004) and Vogel (2006). The research of Grant (1949) on pollination systems acting as isolating mechanisms in angiosperms should be counted amongst them, and references therein indicate that respective ideas had its precursors. Today, pollination syndromes are understood as suites of floral plant traits that are the result of convergent evolution, due to common characteristics of abiotic or biotic pollen vectors. Pollination syndromes integrate the different organisational levels of floral structure, advertisement and parameters of reproduction that were described in the previous sections.

Pollination syndromes have been the subject of substantial critique (e.g. Herrera, 1996; Waser et al., 1996; Ollerton et al., 2009), essentially focusing on the degree of **specialisation vs. generalisation** in

plant-pollinator interactions (respectively allophilic, generalist flowers vs. euphilic, specialised flowers). Critics discard the pollination syndromes concept because it allegedly does not successfully describe the diversity of floral phenotypes, is unable to predict the pollinators of most plant species and narrows down the perspective of convergent floral adaption to the idea of a single most effective pollinator or functional pollinator guild (cf. Ollerton 2009). Selection of floral traits might be shaped simultaneously by many different pollinating animal taxa, and also by non-pollinator agents. Strauss and Whittall (2006) e.g. present an overview on biotic and abiotic factors like herbivory, spread of disease or climatic conditions that can exert influence on selection and evolutionary shaping of floral diversity. Complementary, generalist flowers that are not dependent on specific pollen vectors are often proposed as rather the rule than the exception (cf. Waser et al., 1996). Three levels of generalisation are distinguished by Ollerton et al. (2007): Ecological generalisation refers to the number of pollinating animal taxa involved in the plant-pollinator interaction, while functional generalisation refers to pollinator guilds that serve as pollen vectors (typically family level or above) and phenotypic generalisation refers to the adaption of floral traits itself.

In the last decades however, a strong body of scientific evidence has also emerged that supports the idea of pollination syndromes (e.g. Faergi and van der Pijl, 1979; Baker and Hurd, 1986; Culley et al., 2002; Friedman and Barrett, 2009; Johnson, 2010; Gómez et al., 2008; van der Niet and Johnson, 2012; Newman et al., 2014; Rosas-Guerrero et al., 2014; Abrahamczyk et al., 2017 b; Johnson and Wester, 2017; Dellinger et al., 2018; Ibañez et al., 2019). There are many good reasons to defend floral specialisation via pollination syndromes (cf. Willmer, 2011): A specialised form of plant-animal interaction improves foraging efficiency and reduces interspecific competition for the animal pollen vector. Moreover, specialisation is not necessarily riskier than generalisation. Many additional strategies can compensate for the restriction to fewer pollinators, such as longevity or self-fertilisation, to secure reproduction.

Pollination syndromes and their characteristic suites of floral traits are presented in table I.3, showing basic differences between abiotic and biotic pollination as well as descriptions of classical biotic pollination syndromes. Figure I.3 illustrates selected pollination syndromes and pollen vectors. As for **abiotic pollination syndromes**, wind pollination or anemophily is the most important form: It relies on wind dispersal of large amounts of pollen that by chance might arrive at a conspecific stigma, evolved repeatedly in several lineages and is present in round about 18 % of angiosperm families (Culley et al., 2002, citing Ackerman, 2000). Water pollination or hydrophily is only realised by round about 3 % of angiosperms (Willmer, 2011). It shows commonalities with wind pollination due to abiotic pollen transport, such as missing floral advertising and longer stamens and styles for enhanced pollen dispersal and reception.

However, pollination by animal taxa, zoophily, is by far the most common form, realised by approximately 90 % of extant angiosperms (Willmer, 2011). **Classical biotic pollination syndromes** describe floral phenotypes that are indicative for pollination by bees, butterflies, moths, hawkmoths, flies, carrion flies, beetles, birds and bats. Floral traits associated with pollination by beetles (cf. table I.3) are also associated with generalist flowers and pollination by an assemblage of less prominent pollinators, like wasps. Respective floral suites can therefore be interpreted as a **generalist pollination syndrome** (cf. Willmer, 2011). Vogel (2006) presents estimations for the quantitative distribution of biotic pollination syndromes amongst angiosperms: Generalist flowers form the majority. Of the 340

families listed in Engler´s traditional syllabus, 140 families are strongly specialised (41.3 %) and another 26 families at least specialised to some degree (6.7 %). The share of melittophilous pollination syndromes has not been quantified, but most likely outnumbers the other syndromes. Bird pollination occurs at least in 687 genera in 113 families, with the majority of such families (47 %) restricted to the New World. Bat pollination is recognised in 148 families, with 47 genera occurring in the paleotropics and 134 genera occurring in the neotropics.

Review of evidence indicates that the concept of pollination syndromes is a promising approach for better understanding of plant-pollinator interactions and the integration of floral functions. However, its restrictions have to be acknowledged, and one should be aware of the methodological issues that characterise the majority of research undertaken on this topic so far:

- The pollination syndromes concept suffers from **incomplete understanding of pollinator taxa**. As an example, the importance of bee-flies (Bombyliidae) is strongly undervalued, like many other Diptera (i.e. Syrphidae, Tabanidae, Nemestrinidae), cf. Kastinger and Weber (2001): Although a clearly distinguishable bee-fly syndrome does not exist, significant correlations between aspects of floral organisation, floral advertisement and pollination by bee-flies have been detected. Flowers pollinated by these Diptera show flower traits that are more similar to bee- than to fly pollination syndromes (e.g. blue or violet corolla colour, funnel- or bell-shaped, narrow tubes, concealed nectar).
- The research of pollination syndromes is very often shrouded by insufficient differentiation between **flower visitors and true pollinators** that contribute to outcrossing (cf. Willmer, 2011).
- Pollination syndromes should rather be understood as a specialisation towards **pollinator guilds** than towards single animal species. Extreme specialisation is rather seldom the case. However, animal taxa can often be aggregated to functional groups that behave in similar ways, exert similar selection pressures and in turn generate correlations among floral traits (cf. Fenster, 2004). Pollinator guilds should therefore rather be interpreted as a form of specialisation than a form of generalisation (see above, against Ollerton, 2007).
- Even when dealing with true pollinators and pollinator guilds, one should be aware of the fact that different pollinating animal taxa can be pollen vectors of different value. Stebbins (1970) highlighted that only **frequent and effective pollinators** are of relevance, and can be accounted for the moulding of floral traits in the evolutionary long-run (cf. Fenster, 2004).
- Due to this, pollination syndromes are rather statistical than absolute constructs (Willmer, 2011) that contain **a clear centre with bad boundaries** (Vogel, 2006, citing van der Pijl; no exact source given).
- **Data quality** is a crucial factor. A large part of research on pollination syndromes relies on categorical data, at best allowing for rudimentary resolution. Exactly quantified floral traits have much higher power to retrieve pollination syndromes (Abrahamczyk et al. 2017 b). Ollerton´s article of 2009 in example, often cited as evidence for the invalidity of the pollination syndromes concept, analysed 41 alternative manifestations of 13 major flower traits: All of them are coded dichotomous however, a strong reduction of the natural conditions (cf. Ollerton et al., 2009).
- **Incomplete representation of floral traits and their interaction** is a common phenomenon in this field of research, too (cf. Fenster et al., 2004; Abrahamcyk et. al., 2017 b). Focus is usually placed on aspects of floral structure, flower signal and nectar reward, while reproductive core functions like pollen and ovule production or breeding systems are often neglected.

The points listed above show that research of the pollination syndromes concept is an interdisciplinary task to which zoologists as well as botanists can contribute equally well. This thesis is a botanical work. Main purpose and motivation for this work is therefore to address some of the topics that can be elucidated directly by botanical science: Integrative assessment of the different levels of floral function and intensified use of quantitative data on floral plant traits, aside with categorical information.

TABLE I.3: OVERVIEW OF REPORTED POLLINATION SYNDROMES, AND FLORAL PLANT TRAITS ASSOCIATED WITH THEM

The table presents general differences between biotic and abiotic pollination syndromes in angiosperms, as well as characteristics of classical biotic pollination syndromes. General profiles of abiotic and biotic pollination are presented after Friedman and Barrett (2009) and Culley et al. (2002). Classical biotic pollination syndromes are predominantly described after Willmer (2011) and Vogel (2006), bird and bat pollination were complemented after Proctor et al. (1996) and references therein. The table accounts for the fact that pollination by moths and hawkmoths represent variations of butterfly pollination, and all taxa are belonging to a long-tongued pollinator guild. As for bee pollination, a distinction between micro- and macromelittophilous syndromes according to body size is common, and a distinction of different pollinator guilds due to long- and short-tongued taxa is recommendable. Bat flowers and floral suites associated with it represent a mammalian pollination syndrome, and non-flying mammalian pollinators like rodents join into this syndrome. Floral traits associated with pollination by beetles are also associated with generalist flowers, and can therefore be interpreted as a generalist pollination syndrome.

trait	abiotic / esp. wind pollination	biotic pollination
stigmas and styles	feathery	simple and solid
p/o ratio	high – many pollen grains, ovules few or one	low – fewer pollen grains, many ovules
pollen diameter	10 – 50 µm	highly variable (often > 60 µm)
stamen filaments	long	variable
nectaries	absent or reduced	present
scent	absent or very weak	present
perianth	absent or reduced; if present, inconspicuous hues (greenish or whitish)	present and showy; often bi- or multicoloured, contrasting hues
flower type	usually unisexual	usually hermaphroditic
inflorescence structure	pendulous, catkin-like, often condensed; many flowers	variable, sometimes simple and diffuse; fewer flowers
inflorescence position and habitat	held away from vegetation, in open habitats	variable position on plant, in open and closed habitats

classical, biotic pollination syndromes

trait	***Melittophily*** -bees-	***Psychophily*** -butterflies- **variations: *Phalaenophily*** -moths- ***Sphingophily*** -hawkmoths-	***Myophily*** -flies-	***Sapromyophily*** -carrion flies-	***Cantharophily*** -beetles- also: **Generalist pollination syndrome**	***Ornithophily*** -birds-	***Chiropterophily*** -bats- non-flying mammals join in this syndrome
floral symmetry	bilateral or radial	bilateral or radial usually radial	radial	radial or bilateral	radial	radial or bilateral	bilateral or radial
floral shape	open, or short to medium tube can be subdivided by size: *Micro-* and *Macromelittophily*	long tube, or medium corolla length plus nectar spur moderate tube (moths); long tube and/or spur (hawkmoths)	smaller, open accessible flowers, bowl shaped or flat; often grouped as inflorescences	larger in size, possibly with deep trap, petal surfaces with hairs	small or medium-sized, open accessible flowers; bowl shaped or flat; often grouped as inflorescences	often tubular, short/medium tube length; often with nectar spur (deeper/wider than in insect flowers); firm but elastic structures, so that birds can perch on them	open, bowl, bell or brush type; robust structures
flower colour	pink, purple, blue, white, yellow; usually with nectar guides	often intense and attractive; deep pink, blue, cream, yellow, orange, red; often with yellow center; without nectar guides or only simple pattering inconspicuous: cream, greenish; moths also yellow, hawkmoths also white	white, cream, yellowish, greenish	dull red, purple, brown, sometimes greenish, often with mottling	often dull; white, cream, yellowish, greenish	vivid, predominantly red or orange; no nectar guides; some contrast present (yellow, white, sometimes blue)	(dull) white, cream, beige, dull shades of green and purple; no nectar guides
anthesis	dawn, day	day dusk, night often flowering *en masse*, grouped in inflorescences	day	day or night, relatively short-lived	day or night	day, often dawn; often long-lasting	dusk, night
scent	moderate, usually sweet	mild, sweet strong, sweet	mild, sweet or musty	strongly unpleasant, mimicking dead or decaying flesh or excreta	mild to moderate, fruity or musty but not unattractive	usually absent	strong, often fruity, sour, musty or fermenting
pollen amount and placement	moderate; placed on head, dorsal or ventral body	low (no feeding); placed on face and tongue, sometimes legs	low to moderate; placed on legs, face, thorax	moderate to abundant; covering the visitors body, often completely	often large quantities; placed on face, legs or underside	low; placed on forehead, beak, throat	high; placed on face, head
nectar reward	exposed or concealed, moderate volume and concentration	concealed, low volume and concentration; often amino-acid-rich	exposed, low volume, mod. to high concentration	little or none; if present, amino-acid-rich	exposed nectar, low volume, high concentration	usually concealed; high volume, low concentration	usually exposed, high volume, low to moderate concentration

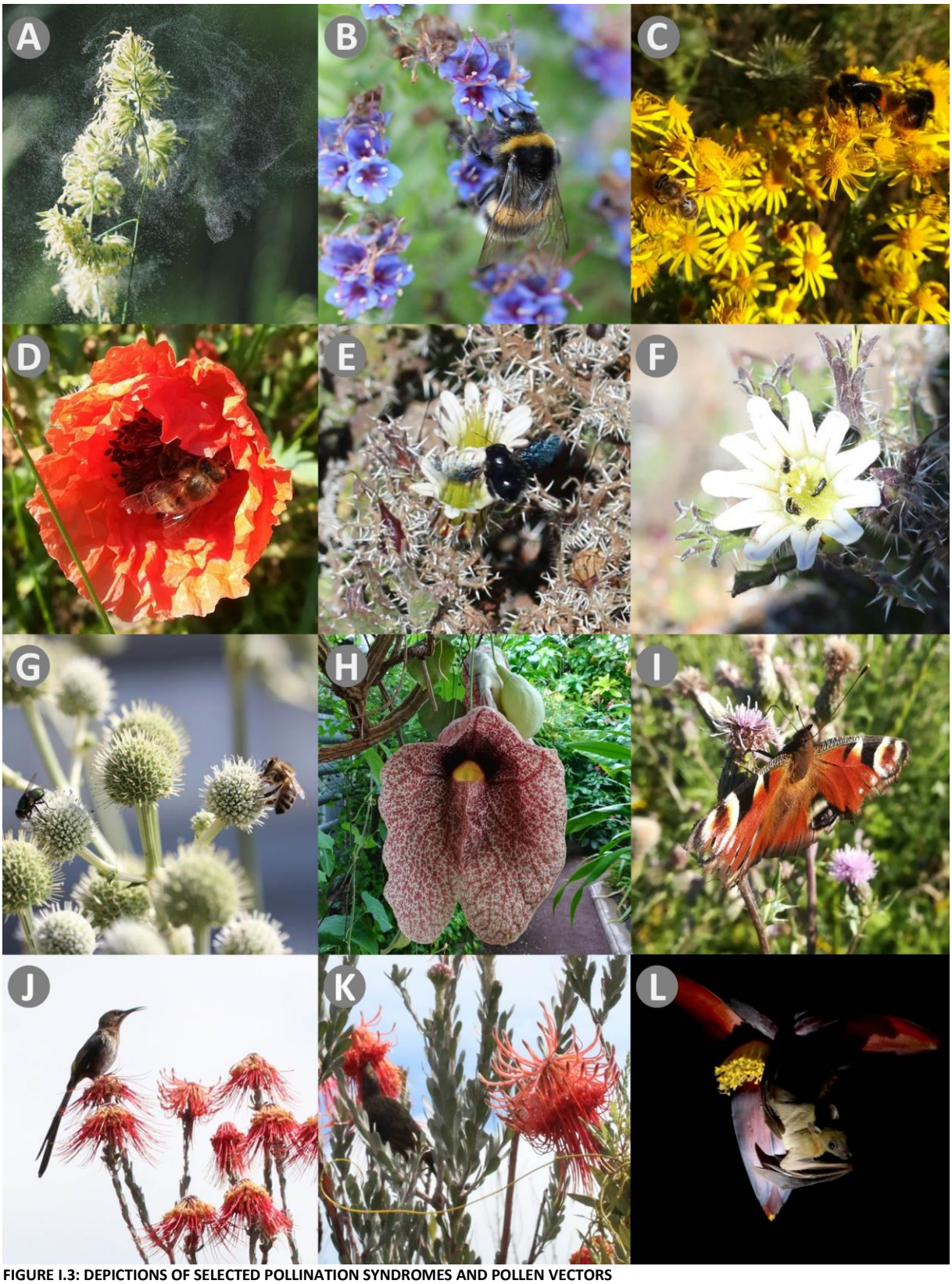

FIGURE I.3: DEPICTIONS OF SELECTED POLLINATION SYNDROMES AND POLLEN VECTORS
A. Pollen of grasses is distributed by the wind, and inflorescences show common features of anemophilous pollination syndromes, i.e. a reduced perianth, long and exserted anthers and extremely high pollen production; **B and C.** Brightly blue and yellow flower colour is common in melittophilous pollination syndromes: B. *Echium candicans* L.f. visited by a Bumblebee (*Bombus terrestris* L.) – Bonn University

Botanic Gardens, Germany; C. Bumblebees (*Bombus lapidarius* L.) and Honeybees (*Apis mellifera* L.) were observed as frequent visitors of *Jacobaea vulgaris* Gaertn. – Kirchhellen, Germany; **D.** Pollination syndromes are no clear-cut pattern: Although brightly red flower colour is often indicative for bird pollination, insect pollinated plants occasionally display this flower colour, too. Actinomorphic, red coloured poppy flowers (*Papaver rhoeas* L.) show a bulls-eye pattern due to darker anthers and UV reflecting petals (cf. section I 2.2.1). They offer a high amount of pollen as reward, which is easily accessed by honeybees – Weltacker, Berlin, Germany. **E.** A large carpenter bee, *Xylocopa* spec., visits *Codon royenii* L. The noisy arrival of this large pollinator, ending the silence of the Namaqualand semi-desert, is impressive: A pollen vector capable of long distance travel is of high importance for outcrossing between the scattered populations of this plant. *Codon royenii* displays features that are indicative for macromelittophily: The actinomorphic flowers are larger and occur on stouter spikes, able to bear the weight of *Xylocopa*. The pollination syndrome is further supported by day blooming, white to yellowish flowers with nectar guides on the petals of the gamopetalous corolla, and nectar in moderate amount and concentration – Springbok, South Africa; **F.** Beetles were also observed as visitors of *Codon royenii*, and may play a role as pollinators on short distance within *Codon* populations – Springbok, South Africa; **G:** *Eryngium paniculatum* Cav. & Dombey ex F.Delaroche displays a generalist pollination syndrome, here visited by a blow fly (*Lucilia* spec.) and a honeybee (*Apis mellifera* L.). Wasps, bumblebees and beetles were also frequently observed. Flowers are greenish and have a strong smell, experienced as unpleasant by humans. Nectar and pollen can be accessed by short-tongued pollinator guilds – Bonn University Botanic Gardens, Germany; **H.** *Aristolochia gigantea* Mart. shows a carrion-fly pollination syndrome, mimicking animal carcasses by reddish-brown and yellowish colour pattering and unpleasant scent. Attracted flies are trapped by the U-shaped corolla (see flower in lateral view, behind), and released again after some time to secure deposition and transfer of new pollen to the animal vectors – Bonn University Botanic Gardens, Germany; **I.** *Cirsium arvense* (L.) Scop. displays a psychophilous pollination syndrome: The mauve coloured and sweet smelling flowers are blooming during day, and nectar is concealed. Butterflies are common flower visitors. Here, *Aglais io* L. inserts its long lepidopteran proboscis into the heads of *Cirsium* to access nectar reward – Kirchhellen, Germany; **J and K.** The rocket pincushion (*Leucospermum reflexum* Buek ex Meissn.) shows characteristics of a bird pollination syndrome: Flower colour is reddish, and inflorescences are stout enough to bear the body weight of birds. Flowers are even directed straight downwards during anthesis, offering a landing platform. Cape Sugarbirds (*Promerops cafer* (L.)) were observed as flower visitors, feeding from nectar – Helderberg Nature Reserve, Somerset West, South Africa; **L:** *Musa* spec. visited by a fruit bat, showing a chiropterophilous pollination syndrome: The pendent inflorescence is stout enough to bear mammalian visitors, flowers are coloured cream and bracts are dull-purple. Picture credits: A by Alex Jones, L by U. Wilsan, both "Unsplash" internet source, free licence, electronically retrieved 22.09.2019. Pictures B to K by A. W. Mues.

I.5 HYPOTHESES

Pollination syndromes represent floral suites that have originated and diversified in interaction with biotic and abiotic pollen vectors. Plant trait pattern that constitute respective syndromes have been used extensively to predict pollen vectors. However, research in this field has seemingly suffered from poor data quality, especially overreliance on categorical data, and insufficient integration of important floral plant traits, especially related to the floral core function of reproduction and gamete production. If so, assessment of plant traits via quantitative data and better representation of different aspects of floral function (i) should allow for clearer description and retrieval of pollination syndromes, (ii) this integrative approach should allow for better prediction of pollen vectors, (iii) should allow for better understanding of the evolution and diversification of plant-pollinator interaction by combination with the latest phylogenies, and (iv) allow for better understanding of the inheritance of these plant traits by experimental botany (crossing experiments).

I.6 GOALS

Based on the hypotheses presented, the following research questions were selected. The objectives represent special cases of plant-animal interactions that could help to elucidate the validity of the pollination syndromes concept. The selected model organisms contain several taxonomic levels, ranging from species to order level.

I.6.1 RESEARCH QUESTIONS

1. Do floral plant traits and their diversification show functional integration in order Geraniales, backing up reported pollinator guilds?

2. Does flowering time at the fringe of the growth season affect floral plant traits of winter- and autumn-flowering members of Hamamelidaceae?
3. Do floral plant traits of carnivorous plants show special pattern, due to nutrition deprived habitats and a possible pollinator-prey conflict?
4. Does floral diversity of *Streptocarpus* subgenus *Streptocarpus* mirror pollination syndromes and functional integration of floral plant traits within groups of floral phenotypes, as well as differentiation between them?
5. Is hybridisation of closely related but florally diverse members of *Streptocarpus* subgenus *Streptocarpus* prevented by postzygotic pollination barriers, without necessity of isolating effects of pollination syndromes?
6. If hybridisation is successful between members of *Streptocarpus* subgenus *Streptocarpus*, do hybrids show functional floral plant traits, possibly by inheritance of pollination syndromes, or is attraction and guidance of biotic vectors disturbed and therefore isolating gene flow?

I.6.2 Specific Objectives

1. Compare diversification of floral plant traits and pollinator guilds within order Geraniales. Quantify gamete production (pollen and ovule production, p/o ratio) and nectar reward (amount, concentration, sugar content). Analyse their pattern within order Geraniales via hierarchical clustering and Non-metric Multidimensional Scaling (NMDS). Test for functional integration via vector fitting of remaining data on floral function, especially floral morphology, symmetry and described pollinator guilds. Analyse the evolutionary history of these patterns via mapping onto the latest phylogeny of the order.
2. Compare diversification of floral plant traits and flowering time for members of family Hamamelidaceae. Quantify gamete production, assess other levels of floral function (morphology, signal, reward) and analyse their pattern separately and combined within the family via hierarchical clustering and NMDS. Test for functional integration via reciprocal vector fitting. Test the explanatory value of flowering time for the retrieved patterns via Permutational Multivariate Analysis of Variance (PERMANOVA). Analyse the evolutionary history of the retrieved pattern via phylogenetic mapping.
3. Compare diversification of floral plant traits between carnivorous genera *Drosera*, *Dionaea* and *Pinguicula*. Assess floral plant traits (gamete production, nectar reward, floral morphology, optical flower signal) as well as true breeding systems via pollination experiments. Analyse correlations between variables of floral function. Test for differences of gamete production between retrieved breeding systems via group comparisons, in order to elucidate a possible pollinator-prey conflict and its effect on floral function.
4. Compare diversification of floral plant traits within *Streptocarpus* subgenus *Streptocarpus*. Assess floral plant traits (gamete production, nectar reward, floral structure, optical flower signal) and analyse their pattern via hierarchical clustering and NMDS. Test for functional integration via reciprocal vector fitting. Analyse the evolutionary history of the retrieved pattern via phylogenetic mapping.
5. Compare siring success and hybrid seed set between three closely related members of *Streptocarpus* subgenus *Streptocarpus*. Perform crossing procedures within the parental and F1

generation and count the seed set. Analyse possible factors of influence on seed set (maternal and paternal effects, ripening time, fruit length, generational level, pollination procedure) via Generalised Linear Modelling (Generalised Estimating Equations).

6. Compare functionality of floral plant traits of hybrid offspring between members of *Streptocarpus* subgenus *Streptocarpus*. Assess floral plant traits (nectar reward, floral architecture, optical flower signal) for parental species and hybrids, and analyse the retrieved pattern via hierarchical clustering and NMDS. Assess severe floral malfunction as well as the degree of trait instability via coefficients of variation for hybrids. Assess the heredity of the retrieved pattern.

I.7 Overview of the Dissertation

This work is a monograph. The subsequent chapters are intended to be published in peer reviewed scientific journals. Due to this, chapters II to VII are structured in the style of journal articles (abstract, introduction, materials and methods, results, discussion, literature), in order to allow for extraction, further development and subsequent submission. General conclusions are drawn in chapter VIII. A summary of the thesis in English is presented in chapter IX. Appendices to chapters II to VII are presented at the end of the thesis in chapter X, as well as the curriculum vitae of the author (XI).

In **chapter II** diversification of floral plant traits and pollinator guilds are presented for members of order Geraniales (objective 1). The order is small but florally diverse, and therefore particularly suitable for comparative studies. Core variables of reproduction (gamete production) and nectar reward are analysed in depth. Integration of floral plant traits and correlations to reported pollinator guilds are tested. Floral morphology and floral symmetry appear as unrelated to gamete production and nectar reward, arguing against the hypothesis of higher resource efficiency in zygomorphic flowers. Phylogenetic mapping reveals phylogenetic constrains of gamete production, nectar reward and pollination syndromes.

In **chapter III** diversification of floral plant traits and flowering time for Hamamelidaceae are studied (objective 2). Hamamelidaceae are a small family which is known for the peculiar flowering time of some of its members in late autumn or winter. Analysis of floral plant traits and their functional integration reveals clear clusters of wind- and animal pollinated species, as well as a mixed pollination mode. Phylogenetic analysis shows that animal pollination was evolutionary reinvented at least three times from an ancestral wind pollination mode. Pollination syndromes and flowering time appear to be recombined quite freely during evolution. Surprisingly, animal pollination appears to be common in winter flowering species.

In **chapter IV** floral plant traits of carnivorous plants are compared between Droseraceae and genus *Pinguicula* (objective 3). Carnivorous plants are animal pollinated, and the potential capture of legitimate pollinators has long been researched under the term pollinator-prey conflict, usually focusing on sorting between pollinators and prey by the trap types. Here, floral plant traits and their functional integration are analysed, and experimentally retrieved true breeding systems are presented. The detected p/o ratios are extremely low, thus indicating high resource efficiency and reliability of pollen transfer even in xenogamous species – clearly arguing against a pollinator-prey conflict. Optical flower signal ensures a sorting of pollinators from prey.

In **chapter V** floral plant traits and their functional integration are analysed for 18 members of *Streptocarpus* subgenus *Streptocarpus*, with focus on reproduction (gamete production, breeding system), nectar reward, optical flower signal and floral structure (objective 4). The subgenus contains a broad diversity of floral phenotypes that are indicative of different biotic pollination syndromes. Analysis of floral plant traits shows low integration of floral functions however, and almost free combination of floral trait clusters along the course of evolution. The data indicate two separate evolutionary driving forces: Floral architecture controlling for pollinator access, pollen placement and gamete production, and the interaction of floral signal and nectar reward as an adjustment to sensory capacities and nutritional needs of pollinator taxa.

In **chapter VI** postzygotic pollination barriers are analysed for three closely related but florally diverse members of *Streptocarpus* subgenus *Streptocarpus* via crossing experiments and hybridisation (objective 5). Seed set was vigorous: Even in experimental groups with lowest siring success, seed set has to be regarded high enough to allow for the establishment of hybrid swarms in nature. Praezygotic pollination barriers and pollinator guidance via floral plant traits appear to be of high importance for reproductive isolation, especially for sympatric species with overlapping flowering time.

Chapter VII presents the crossability of nine species of *Streptocarpus* subgenus *Streptocarpus*, as well as functionality and inheritance of floral plant traits for hybrid offspring (objective 6). The majority of hybrids appeared fully functional in terms of floral architecture, nectar reward and optical flower signal. Hybrids showed floral trait pattern for all three analysed levels of floral function that are already present in the paternal generation, representing a general compatibility to pollinator guilds. Only 5 out of 40 hybrids showed severe malfunctions of the (male) reproductive system. Due to this, establishment of functional hybrid swarms and hybrid speciation is likely in case of freak pollination events.

Chapter VIII represents general conclusions of this thesis. Main results are reviewed and discussed, and objectives for future research are given.

I.8 CONTRIBUTION TO CHAPTERS

Chapter II: Mues, A. W., Kelch, A., Ackermann, M. and Weigend, M. Diversification of floral function in Geraniales – why so dapper? Breeding system related variables not correlated to pollinator guilds and flower symmetry.

General concept and research question by A. W. Mues and M. Weigend.

Collection of processed raw data for gamete production predominantly done by: Alexandra Kelch (unpublished Diploma Thesis 2011, Freie Universität Berlin). Additional sampling: Alexa Brox collected data for *Francoa appendiculata* and *Melianthus dregeanus*; T. Joßberger collected data for *Greyia flanaganii*, *Greyia sutherlandii*, *Melianthus villosus* and *Viviania elegans*; A. W. Mues collected data for *Erodium manescavi*, *Francoa sonchifolia*, *Geranium sanguineum*, *G. reuteri, G. sylvaticum*, *G. yunannense* and *Hypseocharis bilobata*.

Collection of processed raw data for nectar production predominantly done by: Alexandra Kelch (unpublished Diploma Thesis 2011, Freie Universität Berlin). Additional sampling: M. Ackermann collected data for genus *Greyia* and *Melianthus comosus*, *M. pectinatus* and *M. villosus*. T. Joßberger collected data for genus *Viviania* and *Geranium reuteri*. M. Ackermann and T. Joßberger both collected

data for *Francoa appendiculata* and *Melianthus dregeanus*. A. W. Mues collected data for *Geranium sanguineum*, *G. sylvaticum, G. yunannense* and *Hypseocharis bilobata*.
Data collection and literature research for categorisation of other variables done by A. W. Mues.
Data processing, statistical analysis and interpretation done by A. W. Mues.
Chapter written by A. W. Mues. Supervision by M. Weigend.

Chapter III: Mues, A. W., Hoff, L., Luebert, F. and Weigend, M. Frost flowers – Pollination syndromes of Hamamelidaceae independent from flowering time.
General concept and research question by A. W. Mues and M. Weigend.
Collection of raw data for genera *Corylopsis* (*C. glabrescens*, *C. pauciflora*, *C. spicata*, *C. veitchiana*, *C. willmottiae*), *Fothergilla* (*F. gardenii*, *F. major*) and *Fortunearia* (*F. sinensis*) done by L. Hoff (unpublished project work, bachelor´s programme biology, university of Bonn).
Collection of raw data for genera *Corylopsis* (*C. sinensis*), *Disanthus* (*D. cercidifolius*), *Distyliopsis* (*D. tutcheri*), *Distylium* (*D. myricoides*, *D. racemosum*), *Hamamelis* (*H. japonica*, *H. mollis*, *H. vernalis*, *H. virginiana*), *Loropetalum* (*L. chinense*), *Parrotia* (*P. persica*), *Parrotiopsis* (*P. jacquemontiana*), *Sinowilsonia* (*S. henryi*) and *Sycopsis* (*S. sinensis*) done by A. W. Mues.
Literature research for categorisation of flowering time done by A. W. Mues.
Assessment concept, data processing, statistical analysis and interpretation done by A. W. Mues.
Analysis of DNA-sequencing, retrieval of phylogenetic trees and testing of phylogenetic signal performed by F. Luebert.
Chapter written by A. W. Mues, except phylogenetic methods written by F. Luebert.
Supervision by M. Weigend.

Chapter IV: Mues, A. W., Brauwers, S. and Weigend, M. No indication of a pollinator-prey conflict in floral functional traits of carnivorous plants of the active flypaper type – *Pinguicula* (Lentibulariaceae) and *Drosera* (Droseraceae).
General concept and research question by A. W. Mues and M. Weigend.
Sampling of raw data done by A. W. Mues and S. Brauwers (unpublished project work and bachelor thesis, university of Bonn). Gametic variables (count of pollen and ovules): 48.8 % by S. Brauwers, 51.2 % by A. W. Mues. Nectar measurements: 48.9 % by S. Brauwers, 51.1 % by A. W. Mues. Sampling of morphological data (display sizes, flower opening and spur length) done by A. W. Mues. Photographic documentation of flower colour and UV signal done by S. Brauwers (63.4 %) and A. W. Mues (36.6 %), documentation of colour and UV signal of trap leaves done by S. Brauwers. Testing of breeding systems done by A. W. Mues.
Data processing, statistical analysis and interpretation done by A. W. Mues.
Chapter written by A. W. Mues, supervision by M. Weigend.

Chapter V: Mues, A. W., Liu, T. and Weigend, W. Plasticity of flower traits in *Streptocarpus*: Floral architecture, optical signal, nectar reward and reproductive system largely disjunct and evolutionary recombined.
General concept and research question by A. W. Mues and M. Weigend.

Sampling of raw data done by A. W. Mues and T. Liu (internship and unpublished project work, Bonn University). Assessment of gametic variables (count of pollen and ovules): 61.1 % by T. Liu, 38.9 % by A. W. Mues. Assessment of nectar production: 56.7 % by T. Liu, 43.3 % by A. W. Mues. Photographic documentation and measurement of flower display sizes done by A. W. Mues. Measurement of floral architecture: 61.1 % by T. Liu, 38.9 % by A. W. Mues. Analysis of UV signal (flowers and leaves) done by A. W. Mues. Breeding systems tested by A. W. Mues.
Collection of DNA samples by A. W. Mues, sequencing by N. Schmandt, construction of phylogenetic tree by J. C. Pinilla and A. W. Mues.
Data processing, statistical analysis and interpretation of results done by A. W. Mues.
Chapter written by A. W. Mues, supervision by M. Weigend.

Chapter VI: Mues, A. W. and Weigend, M. Vigorous hybrid seed set between three closely related species of *Streptocarpus* subgenus *Streptocarpus* indicate postzygotic barriers of no relevance for reproductive isolation.
General concept and research question by A. W. Mues and M. Weigend.
Sampling of raw data, counting of seed material, data processing, statistical analysis and interpretation of results done by A. W. Mues.
Chapter written by A. W. Mues, supervision by M. Weigend.

Chapter VII: Mues, A. W., Nicolin, L., Hoff, L. and Weigend, M. Functional floral architecture, optical signal and nectar reward in *Streptocarpus* hybrids, allowing for homoploid hybrid speciation.
General concept and research question by A. W. Mues and M. Weigend.
Sampling of raw data done by L. Hoff (internship), T. Liu (internship and unpublished project work), L. Nicolin (unpublished bachelor thesis) and A. W. Mues. All: Nees Institute for biodiversity of plants, university of Bonn. Raw data of parental species is identical with raw data presented in chapter V. Crossing experiment performed by A. W. Mues. Photographic documentation and measurement of flower display sizes for parental species done by A. W. Mues. Photographic documentation of F1 hybrids predominantly done by L. Nicolin, and measurement of flower display sizes for F1 hybrids predominantly done by L. Hoff (8.5 % added by A. W. Mues). As for measurements of other variables of floral architecture, parental species: 77.8 % by T. Liu, 22.2 % by A. W. Mues; F1 hybrids: 82.3 % L. Hoff, 12.7 % L. Nicolin and 5.1 % A. W. Mues. Analysis of UV signal done by A. W. Mues for parental species, and predominantly done by L. Nicolin for F1 hybrids. Nectar measurements of parental species performed by T. Liu (77.8 %) and A. W. Mues (22.2 %). Assessment of hybrid nectar production performed by L. Nicolin.
Data processing, statistical analysis and interpretation of results done by A. W. Mues.
Chapter written by A. W. Mues, supervision by M. Weigend.

I.9 LITERATURE

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II. DIVERSIFICATION OF FLORAL FUNCTION IN GERANIALES – WHY SO DAPPER? BREEDING SYSTEM RELATED VARIABLES NOT CORRELATED TO POLLINATOR GUILDS AND FLOWER SYMMETRY

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II.1 ABSTRACT

Background and aims: Geraniales are a small but florally diverse order of five families, allowing for comparative studies on diversification of floral function. Here we present a detailed analysis on variables related to breeding system (pollen production, ovule numbers, p/o ratios) and variables that have been considered as instrumental for pollinator interaction: Nectar reward pattern (amount, concentration, sugar production per flower) and pollinator positioning (flower symmetry). We further analyse floral morphological aspects (anther number, secretory tissue) and growth habit as factors considered closely tied to reproductive strategies. Reported pollinator guilds are tested for explanatory value of the analysed variables. Pollinator guilds and retrieved plant trait pattern are mapped onto the latest phylogeny of the order and discussed from an evolutionary perspective.

Methods: Pollen and ovule counting, nectar measurements (amounts, sugar concentration), hierarchical clustering, Non-metric Multidimensional Scaling (NMDS), vector fitting.

Key results: Pollinator guilds are not correlated to breeding system related variables, but to nectar reward pattern. Pollinator positioning via flower symmetry is of no explanatory value for the analysed variables. Other floral morphological variables (anther number, secretory tissue) are also of no explanatory value, however growth habit is: Plants of stouter growth habit show higher resource investment into breeding system related variables and nectar reward. Phylogenetic constraints are present: Gamete production and investment in nectar reward is very high in phylogenetically neighbouring genera *Greyia* and *Melianthus*. Pollen production is highest in genus *Balbisia*. Geraniaceae s. str. show lower gamete production and nectar amount.

Conclusions: Breeding system and pollination mode appear as separate aspects of the floral ecology of Geraniales. The uncoupling of flower symmetry from pollen and nectar production discount *pollen position hypothesis* and *reward wastage hypothesis* as explanations for the flower symmetry in the order.

Key words: Geraniales, floral function, breeding system, pollinator guilds, symmetry, nectar, reward, pollen, ovules, p/o ratio.

II.2 INTRODUCTION

On the one hand, comparative studies on floral function are often focused to genera and species, mostly because species numbers at the family and order levels and the degree of divergence are too high for generating or analysing relevant datasets. On the other hand, the higher degree of expected divergence in functional traits makes a higher-level study particularly interesting. Here we investigate the order Geraniales, a group of five families and ca. 900 species in the Rosid II clade (Angiosperm Phylogeny Group, 2009; 2016; Berger et al., 2016). Their phyletic diversity is particularly high in the southern hemisphere: Melianthaceae are largely restricted to southern Africa while Vivianiaceae and Hypseocharitaceae are restricted to South America. Francoaceae is represented in southern South America by *Francoa* and *Tetilla*, while *Greyia* is restricted to southern Africa. The only wide-ranging family with representatives on the northern hemisphere are Geraniaceae. Only the genus *Geranium* is subcosmopolitan in temperate and Mediterranean climates, *Pelargonium* has a clear centre of diversity on southern Africa, *Monsonia* is restricted to Africa and southwest Asia and *Erodium* has its main distribution area in the Mediterranean region. Palazzesi et al. (2012) provided a phylogeny based on a broad sampling at the genus level. They propose the recognition of five families: Francoaceae (genera *Francoa*, *Greyia*, *Tetilla*), Geraniaceae (*Erodium*, *Geranium*, *Monsonia*, *Pelargonium*), Melianthaceae (*Bersama*, *Melianthus*), Hypseocharitaceae (*Hypseocharis*) and Vivianaceae (*Balbisia*, *Rhynchotheca*, *Viviania*). Sytsma et al. (2014) retrieves the same clades based on essentially the same dataset.

In their current circumscription, Geraniales include a range of genera previously assigned to other families because of their superficially very different vegetative and floral morphology, such as *Francoa* (formely Saxifragaceae) or *Hypseocharis* (formerly Oxalidaceae; Engler, 1898; Chronquist, 1981; Ehrendorfer, 1983; Takhtajan, 1997). Flower morphology is particularly diversified and flower structure and biology of some individual genera and species of Geraniales have been studied extensively in the recent past (e.g. Struck, 1997; Ronse de Craene and Smets, 1999; Aldasoro et al., 2001; Weigend, 2005; Linder et al., 2006; Fiz et al., 2008; Endress, 2010; Jeiter et al., 2017 a, b). With the well-resolved phylogenies available (Palazzesi et al., Sytsma et al.) a closer study of the evolution of floral function at the order level is of particular interest. The evolution of floral function and plant-pollinator interactions is a complex field, but an understanding of the phylogenetic history together with in depth information on functional traits, preferably linked to pollinator observations, permit an identification of evolutionary trends in individual lineages, including possible phylogenetic constraints on particular characters or suites of characters.

In this study we investigate a range of functional traits, with a special emphasis on variables related to the breeding system (pollen, ovules, p/o ratio) and nectar reward (amount, sugar concentration, absolute sugar production per flower). Breeding systems represent the basic strategies of sexual plant reproduction to ensure persistence of species in their environment, either by relying on selfing (autogamy) or pollen transfer between individuals (xenogamy). The pollen to ovule ratio per flower as a proxy for plant breeding systems was first proposed by Cruden (1977), and its general validity has repeatedly been demonstrated. The theory generally states that autogamous species should have much lower p/o ratios than xenogamous species, since little or no pollen is lost in transfer between male and female flower organs, and p/o ratios should also be influenced by pollination mode and details of the pollination process, i.e. body size of the pollinator, size of the pollen bearing area, pollinator behaviour

(e.g. grooming, pollen consumption). These overall correlations seem to hold true, especially when looking at species within individual genera and families, but between unrelated plant groups huge differences in the p/o ratios of taxa with the same breeding system may be observed (compare Cruden and Jensen, 1979; Cruden and Miller-Ward, 1981; Cruden and Lyon, 1985; Small, 1988; Kirk, 1993; López et al., 1999; Cruden, 2000; Michalski and Durka, 2009; Alarcón et al., 2011; Lozada-Gobilard et al., 2019). Beside breeding system and pollination mode, other factors like growth habit may also have influence on the p/o ratio, with higher p/o ratios found in woody perennials than in herbs (Michalski and Durka, 2009).

We further use flower symmetry as a key trait of flower morphology and architecture controlling plant-pollinator interaction (Neal et al., 1989; Endress, 1996, 1999). Symmetry determines the type of interaction between a potential pollinator and the flower and influences expenditure of nectar and pollen, which may also influence breeding system. According to the number of symmetry planes four types of floral symmetry are used in conventional terms: "asymmetric" (without any symmetry plane), "monosymmetric" or "zygomorphic" (one symmetry plane), "disymmetric" (two symmetry planes) and “polysymmetric”, "radially symmetric" or "actinomorphic" (with several symmetry planes).

Neal et al. (1989) present an overview on flower symmetry and the vast field of hypotheses that try to explain its role in plant-pollinator interactions. In general, Neal et al. (p. 355) sort hypotheses in four major categories, based on the phase of the pollination process in which selection acts on flower symmetry: (a) environmental conditions (e.g. protection from rain, etc.), (b) perception by the pollinators, (c) information processing (i.e. learning abilities and innate preferences) by pollinators, and (d) activity patterns (i.e. behaviour and movement) of the pollinators on the flower. Here we focus on category (d), especially on the *pollen position hypothesis* and the *reward wastage hypothesis*. The pollen position hypothesis suggests that zygomorphic flowers are restricting pollinators in their possibilities to approach flowers, and therefore leading to precise pollen placement and stigma contact of the pollinator during the pollination process (Armbruster et al., 1994; Laverty, 1980; Macior, 1974), while actinomorphic flowers do not constrain the pollinator (Faegri and van der Pijl, 1979; Harper, 1979; Leppik, 1972). The *reward wastage hypothesis* is similar, stating that zygomorphic flowers only allow specialised pollinators access to reward, which is often hidden and only accessible by advanced behavioural patterns and/or fitting mouthparts. They thus discourage non-pollinating flower visitors, presumably increasing pollination efficiency.

Fortunately, reports on flower visitors and potential pollen vectors are considerable for the order (although not complete, and there is no comprehensive view of floral functional traits in most of the smaller, southern hemisphere groups). The data here presented is therefore also discussed with respect to pollination syndromes: There has been frequent criticism about the usefulness of the pollination syndromes approach, especially because strict specialisation of a plant species to single pollinator guilds is rather uncommon (e.g. Waser et al., 1996; Ollerton et al., 2009), and this theory cannot cover all the complex facets of pollination in nature. We agree with Willmer (2011), that pollination syndromes should be understood as a statistical rather than an absolute constructs, meaning that particular floral parameters are more likely in plant species visited more frequent by a particular type of pollinator. The validity of pollination syndromes has found many advocates in the course of scientific endeavour (e.g. Delpino, 1868–1875; Vogel, 1954; van der Pijl, 1961; Baker and Hurd, 1986; Faergi and van der Pijl, 1979; Johnson, 2010; Gómez et al., 2008; van der Niet and Johnson, 2012; Newman et al., 2014; Rosas-

Guerrero, et al. 2014; Abrahamczyk et al., 2017; Johnson and Wester, 2017; Dellinger et al., 2018; Ibañez et al., 2019). In Geraniales, a wide range of different pollination syndromes have been reported, including rare types such as bird-pollination in *Melianthus* and pollination by mega-nosed flies in *Pelargonium* (e.g. Daniels, 1987; Johnson and Steiner, 1997; Struck, 1997; Kozuharova, 2002; Henning, 2003; Linder et al., 2006).

We complement the broad and already existing and/or accessible knowledge on floral morphology and architecture, pollination syndromes and phylogenetic history of the order with quantitative data on breeding system related variables and nectar award. With this paper we are addressing the following questions about the interplay of these levels:

Are pattern of breeding system related variables (pollen and ovule production, p/o ratio) and nectar reward (amount, concentration, total sugar production) related to pollinator guilds?

Are these pattern mirrored by basic aspects of floral architecture like flower symmetry, a floral key trait for pollinator positioning?

Are observed patterns of breeding system related variables, nectar reward and pollination syndromes phylogenetically constrained?

II.3 Materials and Methods

All plants were cultivated either outdoors or in the greenhouse (at Botanischer Garten und Botanisches Museum, Freie Universität Berlin (Botanic Garden Berlin-Dahlem), resp. Botanische Gärten der Universität Bonn (Bonn Botanical Gardens)). The bulk of the samples were obtained April to July 2010. Pollen and ovule data from some South American taxa were taken from liquid-preserved material collected in the wild. Species numbers assessed for the genera of Geraniales are as follows: *Balbisia* (3 species), *Viviania* (2), *Francoa* (2), *Tetilla* (1), *Greyia* (3), *Melianthus* (4), *Erodium* (4), *Geranium* (5), *Monsonia* (2), *Pelargonium* (9) and *Hypseocharis* (1).

II.3.1 Assessment of gamete production and nectar reward

As for **nectar analysis**, flowers or inflorescences were covered with gauze ca. 24 h before sampling to prevent flower visits and nectar or pollen removal. All nectar was removed from the flowers with microcapillaries, either inserted between petals onto the receptacle of a flower or placed against the sectioned base of the hypanthium in spurred species. Sugar concentration was measured in degrees Brix with a hand-held refractometer (neoLab, type 'universal'). For flowers with very low nectar production nectar from several flowers had to be pooled for the measurement and nectar/sugar amounts were afterwards calculated for individual flowers.

Nectar sugar production per flower was derived from nectar volume and nectar concentration after Galetto and Bernadello (2005), with *x* being the measured concentration:

$$\frac{mg}{\mu l} = 0.00226 + (0.00937x) + (0.0000585x^2)$$

In order to estimate **pollen production**, closed anthers of individual flowers were cut off and dried in tubes for at least three days. Afterwards glycerol and distilled water (1:1) were added, the amount depending on the size of the pollen sample (anther size and number – 25 to 1500 µl). Samples were

mixed 2 to 5 minutes in a laboratory mill, then placed into an ultrasonic bath for 15 minutes to disperse the pollen grains in the solvent. Samples were then vortexed to ensure equal suspension of pollen grains before counting. The number of pollen grains was counted in a hemocytometer (Fuchs-Rosenthal ruling pattern). Per sample, pollen grains on five squares (each 1x1 mm) of the hemocytometer chamber were counted. Total pollen amount of the sample was calculated with the following formula (Neuendorf, 2013):

$$\text{pollen per sample} = \frac{\text{counted pollen grains}}{\text{counted surface (mm}^2) \cdot \text{ chamber depth (mm)} \cdot \text{ dilution (1} \div \text{amount of diluent)}}$$

Pollen production per anther was derived by division of pollen number per sample by the number of anthers per sample. Pollen production per flower was subsequently calculated by multiplying anther pollen production with the anther number per flower.

The number of viable **ovules** per flower is fixed and single-digit for many species of Geraniales. For species with varying ovule number, ovaries were opened carefully and ovules were counted under a stereomicroscope. Afterwards p/o ratios were calculated by dividing pollen production per flower by the ovule number per flower.

Due to unequal sample sizes for gametic and nectar variables, data points were excluded randomly with RAND function in Microsoft Excel until the lowest common denominator per species and variable was found. A sample size of five data points was chosen as lowest acceptable threshold. As for nectar data, the remaining sample size is usually ten, but 25 for Francoaceae and *Melianthus*. Nectar information is scanty for *Viviana* and *Hypseocharis* and was not used for further analysis, while information on nectar production of *Tetilla* is missing. As for gametic variables, remaining sample sizes usually range between seven and twelve data points, but only five for *Melianthus*. Twelve data points were obtained for ovule number of *Tetilla*, but only one data point was available for pollen production, and therefore also p/o ratio. Due to non-normality of the dataset, data points selected for analysis were used for data exploration and description of data bandwidth, while arithmetic means on species level were used for further analysis.

II.3.2 Level of measurement and Statistical Analysis

Variables analysed are either ratio scaled or nominal. Ratio scaled variables are all variables of gamete production and nectar reward: Pollen production per anther and flower, number of viable ovules per flower, p/o ratio, produced nectar amount in µl, nectar sugar concentration in percent and total nectar sugar production per flower in mg. Further, anther number per flower (ranging from 4 to 15 for the species analysed) as well as number of secretory organs (ranging from 1 to 10 in rewarding species, otherwise absent) are ratio scaled.

Nominal variables are flower symmetry, growth habit and pollinator guild, as well as additional information on secretory organs. R function "factor" was used to introduce respective variables to calculations, and levels per variable were introduced as unordered to denote nominal level of measurement. **Secretory tissue** for nectar rewarding species was classified after Jeiter et al. (2017 b), and information was extrapolated for the genus level, with two nominal variables assessed: Position of secretory organs (coded 1 for extrastaminal, 2 for both extra- and interstaminal and 3 for interstaminal),

and possible linkage between the vasculature of secretory organs and the main vasculature of the plant (coded 1 for not linked and 2 for linked).

Flower symmetry was coded in three categories: Actinomorphic flowers with several symmetry planes allow pollinators to approach sexual flower organs and possible reward freely, and were coded with 1. Disymmetric *V. marifolia* was included in this category. Non-functionally zygomorphic flowers with a single symmetry plane for the corolla (due to shape, colouration etc.), but a radially symmetric androecium and gynoecium, were coded with 2. Functionally zygomorphic flowers with a single symmetry plane, and therefore coercing potential pollinators to approach nectar respectively touch anthers and stigmatic surfaces in a distinctive way, were coded with 3.

Growth habit was classified in three categories, based on observation and literature, and coded 1 for herbs (i.e. annual or perennial plants without a persistent woody stem, including geophytes), 2 for subshrubs (i.e. woody plants with size below 1.5 m) and 3 for shrubs (i.e. woody plants with size above 1.5 m, including small trees).

Pollinator guilds were derived from flower visitors reported in literature, as well as from features generally accepted as indicators of pollination syndromes, namely flower colour, flower shape and visibility of nectar site. Three superordinate functional pollinator guilds are distinguished here, generally reflecting commonalities between pollinators in regard to suitable nectar reward (amount, viscosity), bodily requirements and "mechanical fit" for successful interactions with the flowers: Pollinators with short proboscis (short-tongued flies and bees, including beetles, coded with 1), pollinators with long proboscis (long-tongued flies and bees, butterflies, moths and hawkmoths, coded with 2) and avian pollinators (coded with 3).

Detailed information on continuous and nominal variables on species level are presented in the appendix (tables A X.1.1 to A X.1.4).

Cluster analysis and multivariate ordination were performed with R package vegan, version 2.5-1 (compare Oksanen, 2013): Hierarchical clustering was performed separately with arithmetic means of gametic data (all species used) and nectar data (only nectar-bearing species with complete assessment of nectar variables). Cluster analyses were conducted with average linkage, and dissimilarity matrices were produced via Bray Curtis index. Linkage method was selected by means of R function cophenetic, and average linkage performed best. Optimal number of clusters was selected after visual inspection of cluster dendrograms, Elbow-method and R package NBclust (see appendix, figure A X.1.1).

Ordination was performed by means of Non-Metric Multidimensional Scaling (NMDS), with same dissimilarity matrices used as for hierarchical clustering. Function metaMDS was used for iterative testing and selection of the solution with smallest stress, with stress between .05 and .1 interpreted as good, and stress below .05 as very good (McCune et al., 2002). Data were standardised by square root transformation and Wisconsin double standardisation. In order to interpret the obtained ordinations, nominal variables were introduced via factor function and fitted onto the ordination by means of function "envfit" (999 permutations). As for the ordination of gametic variables, vector fitting was performed with flower symmetry, pollinator guild, growth habit, and anther number. As for the ordination of nectar reward, variables regarding the morphology of the secretory tissue replaced anther number for vector fitting.

II.4 RESULTS

II.4.1 POLLINATOR GUILDS

For detailed information on reported flower visitors and derived pollinator guilds please see appendix, table A X.1.4.

As for Vivianiaceae, long- and short-tongued hymenopteran flower visitors are reported for genus *Balbisia* in literature. Freely accessible, saucer-shaped flowers in combination with missing nectar reward make no requirements on proboscis length, and successful plant-pollinator interactions are possible with short-proboscid pollinators. In genus *Viviania*, hypocrateriform flower shape and hidden nectar reward require long-proboscid pollinators, and Hymenoptera, Lepidoptera and long-proboscid flies are reported as flower visitors.

In Francoaceae, hypocrateriform flower shape of genus *Francoa* in combination with exposed nectar reward and observations of hymenopteran flower visitors indicate functional interactions with short-tongued pollinators. Same has to be assumed for *Tetilla* with its bilabiate flower shape and exposed nectar reward, although observations of flower visitors are missing. As for genus *Greyia*, bowl-shaped or urceolate flower shape, exposed nectar reward, brightly red flower colour and observations of avian flower visitors point out a bird pollination syndrome.

Genus *Melianthus* shows tubular-bilabiate flower shape, exposed nectar with dark colouration and reddish flower colour as optical attractants, indicating a bird pollination syndrome. This is verified by reports on avian flower visitors.

As for Geraniaceae, most of the analysed species of genera *Erodium*, *Geranium* and *Monsonia* indicate short-proboscid pollinators: Flowers are openly accessible, bowl or saucer-shaped, nectar is exposed and reports on flower visitors include long- and short-probiscid taxa. Amongst observed flower visitors, short-tongued Hymenoptera and Diptera as well as Coleoptera are very likely the most important pollinators. Only *Geranium reuteri* indicates specialisation for long-proboscid pollinators due to hypocrateriform flower shape and five separate nectar compartments in which reward is hidden, known as revolver flower structure in *Geranium* section *Robertium* (Jeiter et al., 2017 a; Endress, 2010). However, genus *Pelargonium* predominantly shows funnel-shaped "flag blossom" flowers with nectar reward hidden in a hypanthial nectar tube, indicating specialisation for long-tongued pollinators. In line with this, long-tongued Hymenoptera, Diptera and Lepidoptera are described as flower visitors. Only exception is brightly red flowering *P. fulgidum*, which is visited by birds.

In Hypsocharitaceae, *H. bilobata*, flowers are bowl shaped and flies were observed as flower visitors in nature (M. Weigend, personal observation).

II.4.2 FLOWER SYMMETRY

Actinomorphic flower type is predominant in Vivianiaceae, except for some disymmetric species of genus *Viviania* (here *V. marifolia*). Francoaceae show actinomorphic flowers in genus *Francoa* and weak, non-functional zygomorphy in genus *Greyia*, while flowers of *Tetilla* are functional zygomorphic. Flowers of Melianthaceae are characterised by zygomorphy, which is functional and strong in *Melianthus*, but only weak in *Bersama* (not assessed here). In Geraniaceae s. str. the actinomorphic flower type is the most common. Exception is genus *Pelargonium* with functional and strong flower zygomorphy, and

some species of *Erodium* with non-functional zygomorphic flowers. *Hypseocharis* is actinomorphic. See figure II.1 for depictions of Geraniales flowers.

II.4.3 Growth habit

In Vivianiaceae, analysed *Balbisia* species are all subshrubs, while *Viviania elegans* is herbaceous and *V. marifolia* a subshrub, too. For Francoaceae, both analysed *Francoa* species are herbs, while *Greyia* consists of shrubs or small trees. *Melianthus* consists of shrubs only. In Geraniaceae s. str. a broad spectrum of possible life forms is realised, with *Erodium* and *Geranium* usually representing herbs (applicable for all species analysed here), and herbs and subshrubs in *Monsonia*. In genus *Pelargonium* herbs, subshrubs and shrubs are present. *Hypseocharis* is herbaceous.

II.4.4 Gamete production

Gamete production was analysed for 34 Geraniales species, nectar production for 32 species. Please see figure II.2 for data bandwidth of ovule number, pollen production per flower, p/o ratio, nectar amount, nectar sugar concentration and total sugar production per flower on genus level. Compare tables A X.1.2 and A X.1.3 in the appendix for additional information on species level.

Ovule number per flower ranges from a fixed number of 5 in Geraniaceae s. str. to 472 ovules on average in *Greyia radlkoferi*. In Geraniaceae s. str., originally ten ovules are produced, but five are abortive. In *Melianthus*, ovule number is fixed to a number of eight; exception is *Melianthus villosus* with 16 ovules per flower. In *Hypseocharis bilobata* ovule number is variable, and 48 ovules are produced on average. In Vivianiaceae fixed as well as variable ovule numbers are present: While members of genus *Viviania* produce six ovules per flower, ovule number is variable in *Balbisia*, on average 117 (*B. meyeniania* – 92, *B. verticillata* – 98, *B. peduncularis* – 161). Ovule production is highest in members of Francoaceae, and ovule numbers are variable, too: On average, *Tetilla hydrocotylaefolia* produces 252 ovules per flower, *Francoa* produces 273 (*F. sonchifolia* – 147, *F. appendiculata* – 400) and *Greyia* has 317 (*G. sutherlandii* – 231, *G. flanaganii* – 247, *G. radlkoferi* – 472).

Pollen production per flower ranges from 1 547 pollen grains on average in *Pelargonium myrrhifolium* to 927 775 in *Balbisia verticillata*. Within the order, pollen production per flower is lowest in members of Geraniaceae s. str.: *Pelargonium* has an average of 3 442 pollen grains (lowest in *P. myrrhifolium* - 1 547, highest in *P. odoratissimum* – 5 068). Seven anthers are common, but five or six are also present in the genus. *Geranium* has an average pollen grain number of 3 786 (lowest in *G. versicolor* – 2 369, highest in *G. yunnanense* – 5 880), and 10 anthers per flower. *Erodium* has an average of 7 545 pollen grains (*E. pelargoniflorum* – 6 510, *E. manescavi* – 8 580), and five anthers per flower. *Monsonia* has an average of 11 559 pollen grains (*M. emarginata* – 6 499, *M. marlothii* – 16 619), and 15 anthers. Geraniaceae are followed by *Tetilla* with 14 160 pollen grains (only one data point), and anther number is eight. In genus *Viviania* 22 113 pollen grains are produced on average (*V. marifolia* – 20 827, *V. elegans* – 23 400), and anther number is 10.

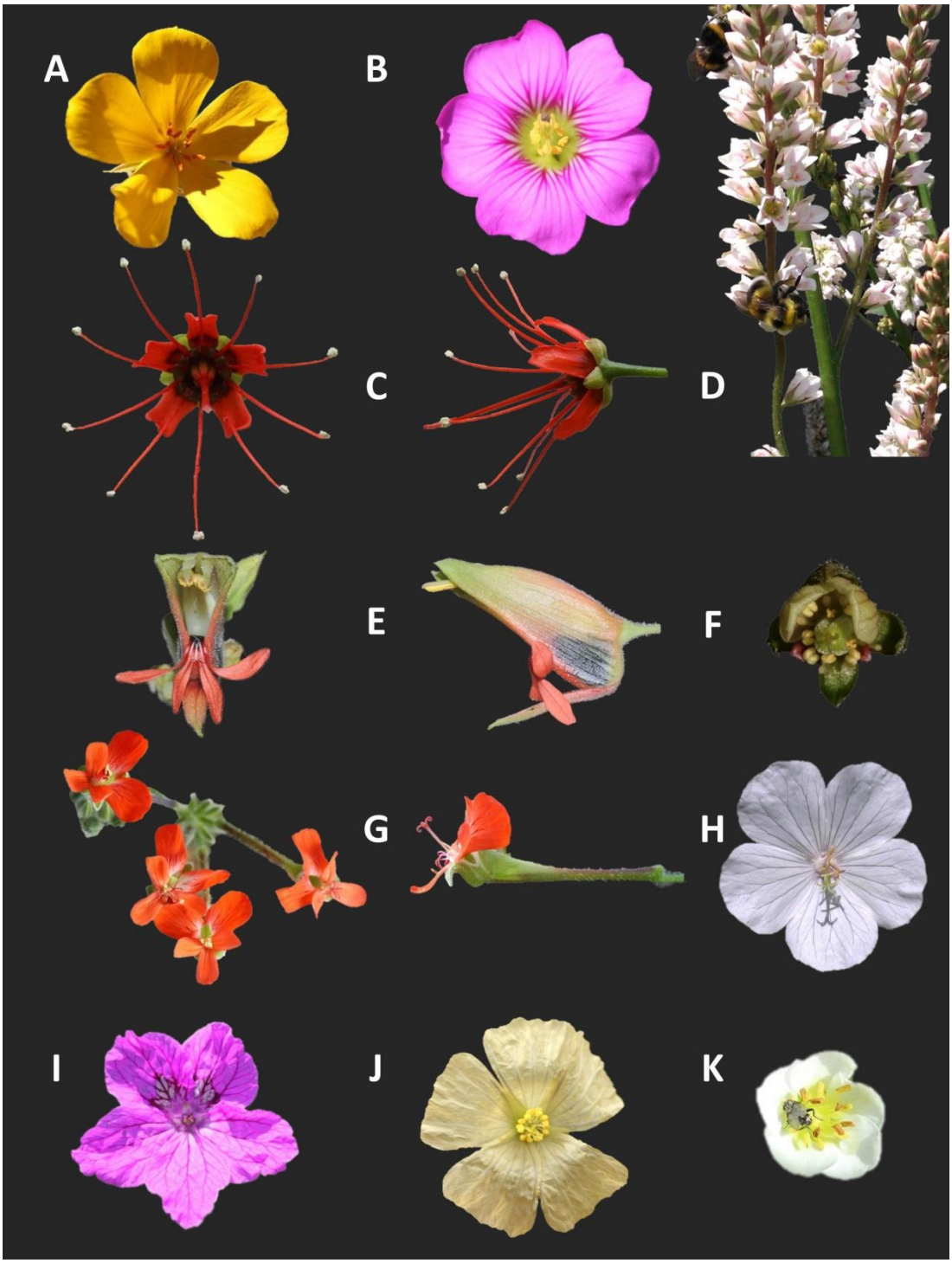

FIGURE II.1: SHAPE, SYMMETRY AND COLOUR SIGNAL OF GERANIALES FLOWERS

(A) *Balbisia meyeniana*, actinomorphic, frontal view; (B) *Viviania elegans*, actinomorphic, frontal view; (C) *Greyia sutherlandii*, non-functional zygomorphic, frontal and lateral view; (D) *Francoa appendiculata*, actinomorphic, inflorescences visited by bumblebees; (E) *Melianthus comosus*, functional zygomorphic, frontal and lateral view; (F) *Tetilla hydrocotylaefolia,* functional zygomorphic, frontal view (G) *Pelargonium fulgidum*, functional zygomorphic, frontal view onto inflorescence and lateral view onto a single flower; (H) *Geranium yunnanense*, actinomorphic, frontal view; (I) *Erodium manescavi*, non-functional zygomorphic, frontal view; (J) *Monsonia emarginata*, actinomorphic, frontal view; (K) *Hypseocharis bilobata*, actinomorphic, frontal view, flower visited by a fly. *Melianthus*, *Greyia* and *P. fulgidum* show a bird pollination syndrome, other species are entomophilous. Pictures not to scale. Picture credits: A and J by M. Weigend, F by H. H. Hilger, others by A. W. Mues.

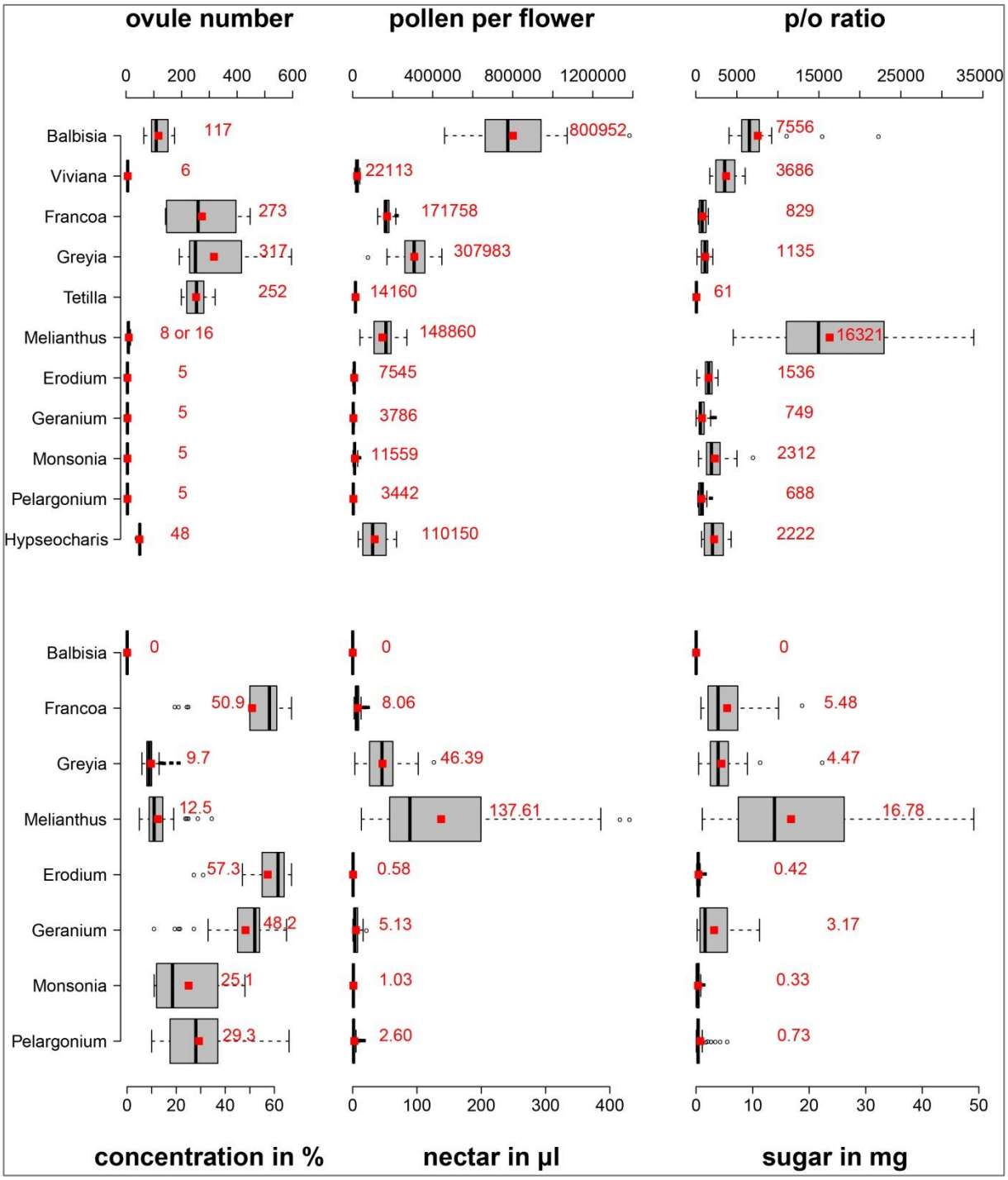

FIGURE II.2: DATA BANDWIDTH OF GAMETE PRODUCTION AND NECTAR REWARD IN GERANIALES

Data bandwidth (boxplots and arithmetic means, in red) of variables related to breeding system and nectar reward in genera of Geraniales. Presented are pollen production, number of viable ovules and p/o ratio (upper row) as well as nectar sugar concentration in percent, nectar amount in µl and sugar content in mg (lower row) per flower. Nectar is also produced in *Hypseocharis bilobata* (average: 0.55 µl) and *Viviana* (*V. elegans*: 0.73 µl; traces for *V. marifolia*), but nectar concentration and sugar production could not be assessed. No nectar information available for *Tetilla*, but secretory tissue present.

Total pollen production per flower is distinctly higher for the following taxa: *Hypseocharis bilobata* has 110 150 pollen grains on average, and 15 anthers per flower. In genus *Melianthus* 148 860 pollen grains are produced on average (lowest in *M. comosus* – 90 520, highest in *M. dregeanus* – 191 520), and anther number per flower is four. *Francoa* has 8 anthers per flower and an average of 171 758 pollen grains (*F. appendiculata* – 159 183, *F. sonchifolia* – 184 333). As for *Greyia*, anther number is 10, and arithmetic mean of pollen grain number is 307 983 (lowest in *G. radlkoferi* – 246 850, highest in *G. flanaganii* – 343 500). Pollen production is highest in genus *Balbisia*, anther number is 10 and 800 952 pollen grains are produced on average (lowest in *B. meyeniana* – 610 800, highest in *B. verticillata* – 927 775).

Pollen production per anther showed a similar pattern like pollen production per flower, compare table A X.1.2 in the appendix. Pollen production per anther is lowest in members of Geraniaceae s. str.: *Geranium* produces 379 pollen grains per anther on average, *Pelargonium* has 512, *Monsonia* 771 and *Erodium* 1 509. *Tetilla* follows with 1 770 (only one data point). Arithmetic mean for *Viviania* is 2 211 pollen grains per anther. Distinctly higher numbers are produced by the other genera: Arithmetic mean for *Hypseocharis bilobata* is 6 908, and *Francoa* produces 21 879. *Greyia* has 30 798 pollen grains per anther on average, and *Melianthus* produces 37 215 pollen grains per anther. The highest pollen production was found in *Balbisia* again, with 80 095 pollen grains per anther on average.

Pollen to ovule ratio ranges from 61 in *Tetilla hydrocotylaefolia* (only one data point, however) to 23 940 in *Melianthus dregeanus*. Besides *Tetilla*, lower but already three-digit p/o ratios are present in two genera of Geraniaceae s. str.: *Pelargonium* has an average p/o ratio of 688 (lowest in *P. myrrhifolium* – 309, highest in *P. odoratissimum* – 1 014), and *Geranium* has 749 (lowest in *G. reuteri* and *G. versicolor* – both 474, highest in *G. yunnanense* – 1 176). This is followed by the remaining members of Francoaceae: As for *Francoa*, average p/o ratio is 829 (*F. appendiculata* – 400, *F. sonchifolia* – 1 258). *Greyia* has an average p/o ratio of 1 135 (lowest in *G. radlkoferi* – 530, highest in *G. sutherlandii* – 1 449) Next is *Erodium* with a p/o ratio of 1 536 (*E. pelargoniflorum* – 1 302, *E. manescavi* – 1 770). *Hypseocharis bilobata* has a p/o ratio of 2 222, and *Monsonia* has 2 312 (*M. emarginata* – 1 300, *M. marlothii* – 3 324). This is followed by Viviianiaceae, *Viviania* has a p/o ratio of 3 686 (*V. marifolia* – 3 471, *V. elegans* – 3 900), and *Balbisia* produces a ratio of 7 556 on average (lowest in *B. peduncularis* – 5 426, highest in *B. verticillata* – 10 584). Genus *Melianthus* is exceeding the other genera by far, average p/o ratio is 16 321 (lowest in *M. villosus* – 9 148, highest in *M. dregeanus* – 23 940).

II.4.5 NECTAR PRODUCTION

Regarding **nectar production and secretory tissue** (see Jeiter et al., 2017 a, b; table A X.1.1 in the appendix), *Balbisia* shows no secretory organs and no signs of nectar production. *Melianthus* is characterised by one large nectar gland and *Pelargonium* shows a single small gland in a spur-like cavity. Other Geraniaceae as well as *Hypseocharis* and *Viviania* show five glands. Francoaceae show 8 glands for *Francoa* and *Tetilla* and a ring nectary with ten appendices for *Greyia*. The position of the secretory structures is extrastaminal for Geraniaceae s. str., *Hypseocharis* and *Melianthus*, and interstaminal for *Francoa* and *Tetilla*. For *Greyia* and *Viviania* secretory tissue is extra- as well as intrastaminally placed.

Main pedicel vasculature is linked to the vasculature of the secretory tissues in Geraniaceae, *Hypseocharis* and *Melianthus*, while not linked in Francoaceae and *Viviania*.

Nectar production for rewarding species ranges from 0.19 µl per flower for *Pelargonium myrrhifolium* up to 265.11 µl per flower for *Melianthus dregeanus*. In Vivianiaceae, *Balbisia* produces no nectar, while minute amounts are produced by *Viviania elegans* (0.73 µl), and only traces are present in *V. marifolia*. Low amounts of nectar are also produced by members of Geraniaceae: *Erodium* produces an average of 0.58 µl nectar (*E. pelargoniflorum* – 0.43 µl, *E. manescavi* – 0.74 µl). Arithmetic mean for *Monsonia* is 1.03 µl (*M. emarginata* – 0.87 µl, *M. marlothii* – 1.20 µl). *Pelargonium* produces 2.60 µl on average (lowest in *P. myrrhifolium* – 0.19 µl, highest in *P. tetragonum* – 9.86 µl). Arithmetic mean for *Geranium* is 5.13 µl (lowest in *G. sanguineum* – 1.73 µl, highest in *G. reuteri* – 9.77 µl). *Hypseocharis bilobata* produces 0.55 µl nectar on average. As for Francoaceae, *F. appendiculata* produces 8.06 µl (no data for *F. sonchifolia*), while *Greyia* has an average of 46.39 µl (*G. radlkoferi* – 33.18 µl, *G. flanaganii* – 40.78 µl, *G. sutherlandii* – 65.20 µl). Genus *Melianthus* has highest nectar production, 137.61 µl on average (*M. pectinatus* – 50.50 µl, *M. comosus* – 68.52 µl, *M. villosus* – 166,32 µl, *M. dregeanus* – 265.11 µl).

Nectar sugar concentration ranges from 8.2 % for *Greyia sutherlandii* to 60.9 % for *Erodium pelargoniflorum*. The two genera with highest nectar production are characterised by distinctly lower nectar sugar concentration: In *Greyia*, average concentration is 9.7 % (*G. sutherlandii* – 8.2 %, *G. radlkoferi* – 10.1 %, *G. flanaganii* – 10.9 %). *Melianthus* produces 12.5 % on average (lowest in *M. dregeanus* – 8.6 %, highest in *M. comosus* – 16.0 %, others in between). Nectar sugar concentration is markedly higher in *Francoa appendiculata* (50.9 % on average), as well as in members of Geraniaceae s. str.: Genus *Monsonia* produces 25.1 % on average (*M. emarginata* – 13.7 %, *M. marlothii* – 36.4 %). Arithmetic mean for *Pelargonium* is 29.3 % (lowest in *P. crispum* – 16.2 %, highest in *P. odoratissimum* – 58.6 %). *Geranium* produces 48.2 % on average (lowest in *G. versicolor* – 28.3 %, highest in *G. reuteri* – 58.2 %). Arithmetic mean for *Erodium* is 57.3 % (*E. manescavi* – 53.7 %, *E. pelargoniflorum* – 60.9 %).
For both *Viviania* species and *Hypseocharis* nectar could not be quantified further in terms of nectar concentration and sugar production: Higher nectar sugar concentrations have to be assumed due to fast crystallisation of nectar after extraction, however.

Sugar production per flower ranges from 0.07 mg for *Pelargonium myrrhifolium* to 25.47 mg for *Melianthus villosus.* For members of *Geraniaceae* s. str. sugar production per flower is low: *Monsonia* species have 0.33 mg on average (*M. emarginata* – 0.12 mg, *M. marlothii* – 0.55 mg). *Erodium* produces 0.42 mg (*E. pelargoniflorum* – 0.34 mg, *E. manescavi* – 0.51 mg). Arithmetic mean for *Pelargonium* is 0.73 mg on average (lowest in *P. myrrhifolium* – 0.07 mg, highest in *P. tetragonum* – 3.72 mg). *Geranium* produces 3.17 mg on average (lowest in *G. versicolor* – 0.81 mg, highest in *G. reuteri* – 7.19 mg). As for Francoaceae, *Greyia* has an average sugar production of 4.47 mg (lowest in *G. radlkoferi* – 3.29 mg, highest in *G. sutherlandii* – 5.40 mg), and *Francoa appendiculata* produces 5.48 mg. *Melianthus* shows highest sugar production on genus level, 16.78 mg on average (*M. pectinatus* – 5.89 mg, *M. comosus* – 11,54 mg, *M. dregeanus* – 24,20 mg, *M. villosus* – 25.47 mg).

II.4.6 NMDS ORDINATIONS AND VECTOR FITTING

Results of hierarchical clustering and separate NMDS ordinations for gametic variables and nectar reward as well as vector fitting of explanatory variables are presented in figure II.3.

Clustering of gametic variables (pollen production per anther and flower, ovule numbers, p/o ratio) and NMDS ordination show the emergence of four clusters: In general, cluster 1 represents Geraniaceae s. str., with viable ovules fixed to 5, pollen production per flower < 10 000, but usually much lower, and a p/o ratio seldom above 1 000. Cluster 2 is represented by *Tetilla*, *Viviania* and *Monsonia marlothii*, with ovule numbers fixed or variable, floral pollen production of ca. 10 000 to 20 000 and a p/o ratio of approximately 3 000 to 4 000, except for *Tetilla*. Cluster 3 is represented by genera *Greyia*, *Francoa*, *Melianthus* and *Hypseocharis*, with ovule numbers fixed or variable, floral pollen production higher, ranging between 90 000 and 340 000, and a p/o ratio that is quite variable, but extremely high for *Melianthus* (ca. 9 000 to 24 000). Cluster 4 is represented by *Balbisia*, with very high ovule and pollen number (ca. 600 000 to 930 000 pollen grains per flower), and a p/o ratio ranging from ca. 5 000 to 11 000.

For gametic ordination, only growth habit (p = .029, r^2 = .213) is of explanatory value, showing that analysed species of higher gamete production are long-lived woody plants (subshrubs, shrubs, small trees). Flower symmetry (p = .556, r^2 = .039), anther number (p = .656, r^2 = .030) as well as pollinator guilds (p = .463, r^2 = .049) are non-significant and of no explanatory value for the gametic variables assessed, and vectors therefore are not shown.

Clustering and NMDS ordination of nectar variables (produced nectar amount, nectar sugar concentration and sugar production per flower) also retrieves four clusters: Clusters 1 and 2 are represented by Geraniaceae s. str. plus *Francoa appendiculata*. Both clusters show much lower nectar amount when compared to other members of the order. Nectar concentration in cluster 1 (ranging from 13.7 % in *M. emarginata* to 28.3 % in *G. versicolor*) is markedly lower than in cluster 2 (ranging from 32.1 % in *P. myrrhifolium* to 60.9 % in *E. pelargoniflorum*). Cluster 3 represents genus *Greyia*, *Melianthus comosus* and *M. pectinatus*: It shows considerably higher nectar and sugar production than clusters 1 and 2, and nectar sugar concentration is low, between 8 and 16 %. Cluster 4, *Melianthus dregeanus* and *M. villosus*, shows extremely high nectar and sugar production per flower, but concentration is similar to cluster 3.

For nectar ordination, growth habit (p = .001, r^2 = .579) is of explanatory value again, showing that analysed species of higher nectar amount and sugar production are long-lived woody plants. Pollinator guilds are also of explanatory value (p = .001, r^2 = .651), pointing to specific nectar reward pattern for the classified pollinator guilds. Non-significant are flower symmetry (p = .117, r^2 = .164) and all morphological variables of secretory tissue (number of secretory organs: p = .616, r^2 = .040; position of secretory organs: p = .278; r^2 = .107; secretory and main vasculature linked: p = .071, r^2 = .213), and vectors therefore are not shown.

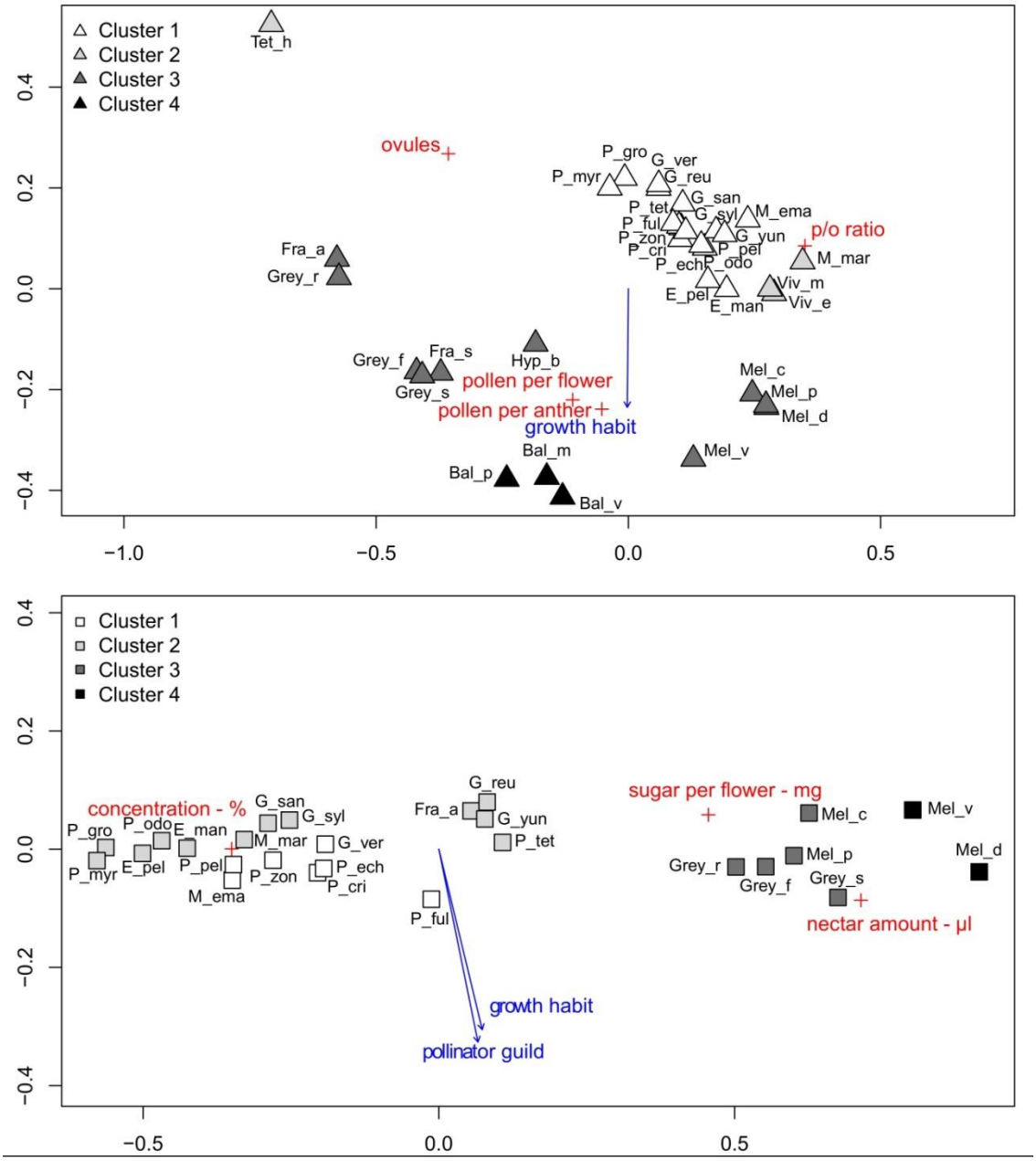

FIGURE II.3: NMDS ORDINATIONS FOR GAMETE PRODUCTION AND NECTAR REWARD

Presented are separated NMDS ordinations for gametic (top) and nectar variables (bottom). For *Viviania*, *Tetilla* and *Hypseocharis* data on nectar production is insufficient or missing. Retrieved hierarchical clusters are indicated by colouration: For each dataset, four clusters were retrieved. **Gametic clusters:** △ = cluster 1, in general Geraniaceae s. str., viable ovules fixed to 5, pollen production per flower < 10 000, usually much lower, p/o ratio seldom > 1 000; ▲ = cluster 2, *Tetilla*, *Viviania* and *Monsonia marlothii*, ovule number fixed or variable, floral pollen production ca. 10 000 to 20 000, p/o ratio ca. 3 000 to 4 000, except for *Tetilla*; ▲ = cluster 3, genera *Greyia*, *Francoa*, *Melianthus*, *Hypseocharis*, ovules fixed or variable, floral pollen production higher, ranging between ca. 90 000 and 340 000, p/o ratio variable, extremely high for *Melianthus* (ca. 9 000 to 24 000); ▲ = cluster 4, genus *Balbisia*, ovule number variable and high, pollen production extremely high, ca. 600 000 to 930 000, p/o ratio ranging between ca. 5 000 and 11 000. For gametic ordination, only growth habit is of explanatory value. Two convergent solutions obtained after 20 trials, model fit is very good (stress = 0.029). **Nectar clusters:** □ = cluster 1, low nectar and sugar production, concentration between 13.7 and 28.3 %; ■ = cluster 2, low nectar amount and sugar production, nectar sugar concentration > 32.1 %; ■ = cluster 3, considerably higher nectar and sugar production than clusters 1 and 2, nectar sugar concentration low (between 8 and 16 %); ■ = cluster 4, extremely high nectar and sugar production, nectar sugar concentration similar to cluster 3. Nectar clusters 1 and 2 represented by Geraniaceae s. str. and *Francoa appendiculata*, clusters 3 and 4 by *Greyia* and *Melianthus*. For nectar ordination, pollinator guild and growth habit are of explanatory value. Two convergent solutions obtained after 20 trials, model fit is very good (stress = 0.003).

II.4.7 PHYLOGENETIC MAPPING OF PLANT TRAITS AND POLLINATOR GUILDS

Figure II.4 shows the latest phylogeny of Geraniales (after Palazzesi et al., 2012, and Sytsma et al., 2014), with retrieved gametic and nectar clusters, flower symmetry and growth habit mapped onto it, as well as retrieved pollinator guilds. As can be seen, resource investment in gamete and nectar production is high in phylogenetically neighbouring genera *Greyia* and *Melianthus*, with extreme observations made for genus *Melianthus*. Phylogenetically closely related genera within Geraniaceae s. str. are strongly conserved in their gamete production, showing fixed ovule numbers as well as low pollen production and p/o ratios, when compared to other families of the order. Production of nectar reward is similarly low in Geraniaceae, and only somewhat higher nectar concentration and sugar production rates within the family can be reported for genera *Erodium* and *Geranium*. *Balbisia* shows highest pollen production of the order, which is coupled to missing nectar reward – a rare exception in Geraniales. Other genera are in between the described patterns: *Francoa* is similar to *Greyia* in pollen and ovule production, p/o ratio and sugar production per flower, however nectar amount is lower and nectar concentration is higher. While we are not able to provide solid measurements on nectar amount or concentration for *Tetilla*, *Viviania* and *Hypseocharis*, nectar is definitely present in low amounts in these genera. Moreover, *Viviania* differs strongly from closely related *Balbisia* in regard to gamete production, with fixed and low instead of high and variable ovule numbers, and only moderate pollen production. For *Hypseocharis* a trend towards higher pollen production has to be stated, compared to closely related Geraniaceae s. str.

Regarding flower symmetry, fully functional zygomorphic flowers have evolved three times in Geraniales (*Tetilla*, *Melianthus*, *Pelargonium*), non-functionally zygomorphic flowers two times (*Greyia*, *Erodium*), other genera are actinomorphic.

Regarding phylogenetic pattern of growth habit, the woody habit of *Balbisia* (subshrubs), *Greyia* and *Melianthus* (shrubs, small trees) have to be highlighted as distinctive patterns on the phylogenetic tree, coupled to high investment in gamete and/or nectar production.

Pollinator guilds show predominance of short-probiscid pollinators in Geraniaceae s. str.; only exception is genus *Pelargonium* with specialisation on long-probiscid pollinators and one bird-pollinated species, *P. fulgidum*. Phylogenetically neighbouring *Hypseocharis* shares short-proboscid pollinators (flies) with Geraniaceae s. str. Birds are reported as flower visitors of *Melianthus* and *Greyia*, therefore two genera of Geraniales are completely specialised on bird pollination, without any exception on species level. As for the remaining genera, short-probiscid pollinators are predominant again (*Balbisia*, *Francoa*, *Tetilla*), only *Viviania* shows specialisation on long-probiscid pollinators.

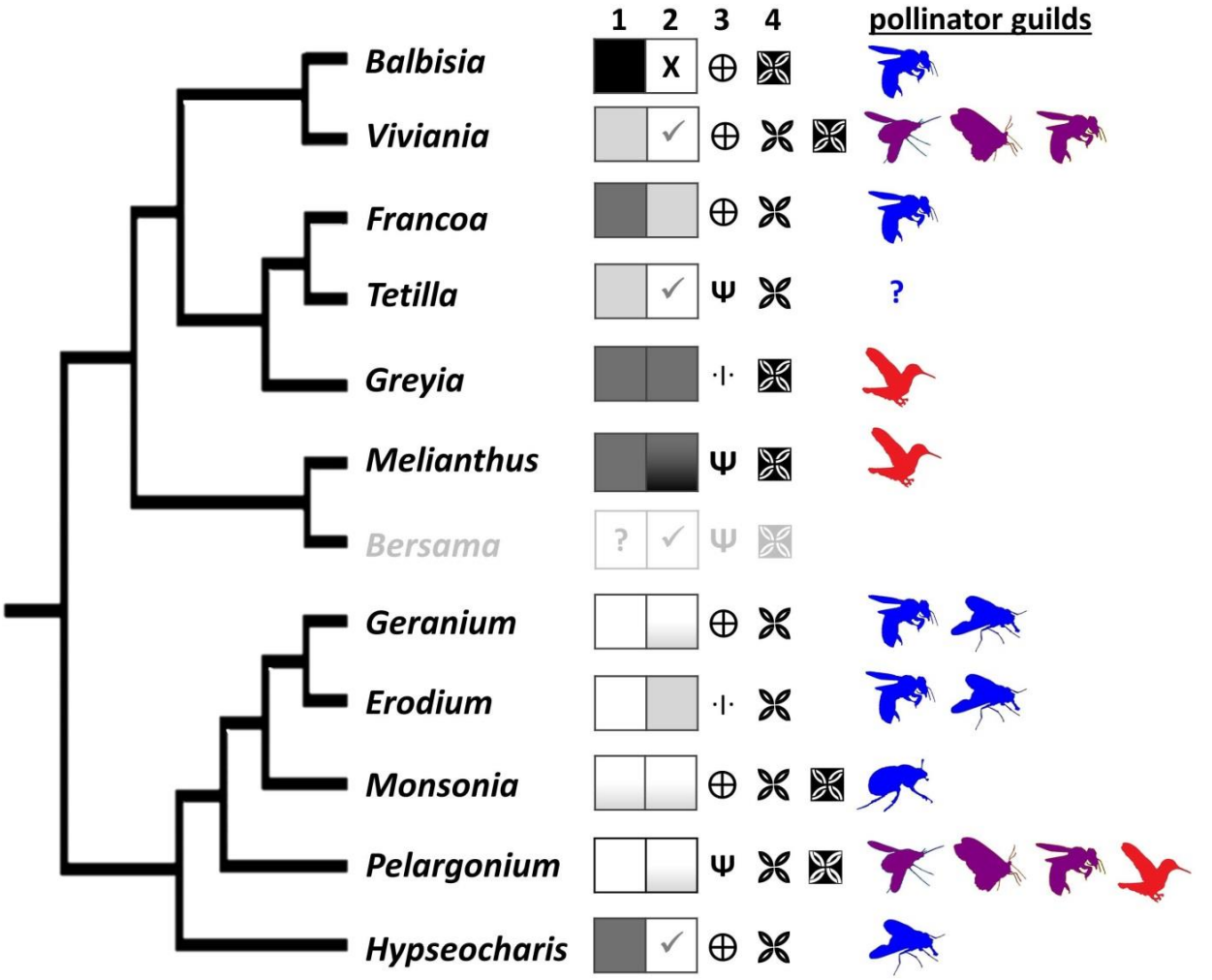

FIGURE II.4: PHYLOGENETIC MAPPING OF PLANT TRAITS AND POLLINATOR GUILDS OF GERANIALES
1 = gametic clusters as described before, darker colours indicating higher gamete production; 2 = nectar clusters as described before, darker colours indicating higher nectar production; (for 1 and 2: ✓ = nectar present but data insufficient for calculation, x = no nectar production, ? = missing information); 3 = floral symmetry (actinomorphic ⊕, including cross symmetry, non-functional ·|· or functional zygomorphic flowers Ψ); 4 = growth habit, ⋇ = herbs, ⋇ = subshrubs, shrubs and small trees (woody). Icons for reported flower visitors represent following taxa: Flies, long-probiscid flies, beetles, bees/bumblebees, butterflies/hawkmoths and nectarivorous birds (see appendix, table A X.1.4). Pollinator guilds are indicated by colouration: ■ = short-proboscid pollinators (short-tongued Diptera and Hymenoptera, including beetles); ■ = long proboscid pollinators (long-tongued Diptera and Hymenoptera, Lepidoptera); ■ = avian pollinators.

II.5 DISCUSSION

II.5.1 MISSING RESOURCE EFFICACY DESPITE POLLINATOR POSITIONING VIA FLOWER SYMMETRY

Geraniales are a counterevidence to the generally assumed correlation of floral symmetry and resource management: Low p/o ratios and low sugar production can be observed in actinomorphic as well as zygomorphic flowers, and functional zygomorphy is *not* linked to resource efficiency. Therefore, neither the pollen position hypothesis nor the reward wastage hypothesis can account for flower symmetry in Geraniales.

Additionally, core morphological variables like anther number, number and position of nectar glands and linkage of secretory tissue to main plant vasculature are also of no explanatory value for gamete production and nectar reward.

Bird pollinated genera *Greyia* and *Melianthus* deserve special attention in this context: Bird pollination is realised by different floral shape and symmetry in phylogenetically closely related taxa. In using birds as pollen vectors, plants have to produce enough pollen to cover relevant areas of the birds bodies (pollen-bearing areas; Cruden, 1981, 2000) in order to ensure contact with the stigma of another flower and thus pollination. In *Greyia*, pollen and nectar is presented by only weakly zygomorphic flowers not controlling for placement of pollen onto the pollinator, whereas *Melianthus* shows highly specialised, zygomorphic and bilabiate blossoms. Further, pollen production per flower in *Melianthus* is lower than in *Greyia*, but pollen production per anther is very similar in both genera: Equivalent pollen production per anther in combination with reduced anther number hints at a more effective placement and/or a smaller pollen bearing area, beside zygomorphy. *Melianthus* flowers therefore should be more effective – however they are not: Extreme resource investment into gamete production and nectar reward is reflected by highest p/o ratios and highest total sugar production per flower found in the whole order. What could be the reasons?

For *Melianthus*, Linder et al. (2006) are suggesting from field observation and literature (Marloth, 1908, 1925; Skead, 1967; Maclean, 1993) that the pollination system is a generalist one, demanding for more resource investment – most bird species are able to access the shallow and open flowers. Zygomorphy of *Melianthus* therefore rather might reflect the need for protection of nectar against desiccation in a dry environment, and a general need to attract avian pollinators. Our controlled nectar data support the hypothesis of Linder et al. (2006) that interspecific variation of *Melianthus* in nectar volume is related to the aridity of the habitats, showing that species from dry habitats (*M. comosus* and *M. pectinatus*) do produce significantly less nectar than species from wet habitats (*M. dregeanus* and *M. villosus*).

An additional cause of missing resource efficacy might be pollen and nectar theft by bees (cf. Huryn and Moller, 1995). The bilabiate flower shape of *Melianthus* is insufficient to prevent nectar- and pollen collection from bees (see Westerkamp and Claßen-Bockhoff, 2007), and the species might have to compensate for the loss of pollen material.

II.5.2 Pollinator guilds correlate with nectar, but not with variables of the breeding system

As another core result, pollinator guilds in Geraniales are mirrored by the nectar reward pattern we found for the order. In general, a high amount of nectar production in correlation with low nectar sugar concentration (~20 %) is understood as indicator for bird pollination (Nicolson and Fleming, 2003; Nicolson, 2007). Low nectar amount and intermediate concentration (~25 – 30 %) is seen as suitable reward for Diptera and Lepidoptera (Kingsolver and Daniel, 1995; Goldblatt and Manning, 2000; Nicolson, 2007) and low nectar production in combination with higher concentration (~30–50 %, Waller, 1972; Roubik and Buchmann, 1984) is assumed to be most suitable for Hymenoptera. However, as Willmer (2011) clearly points out, optimum nectar concentration depends on mouthpart morphology due to correlation between viscosity and concentration: while long-tongued pollinators need lower concentration (~15 – 30 %), short-tongued pollinators can cope with much higher concentrations (45 –

60 % or even higher, becoming crystalline), and there is an often reported relationship between longer, tubular flowers and lower concentration and long-tongued insect visitors, and higher concentration in openly accessible flowers and short-tongued insect pollinators (Willmer, 2011).
Our data confirm these general statements: High nectar production coupled with lower concentration is found in bird pollinated genera *Greyia* and *Melianthus*, and this pattern is also reflected within the phylogenetic constrains of Geraniaceae s. str. (see below) by bird pollinated *Pelargonium fulgidum*. Other nectar rewarding species of Geraniales are entomophilous, and a general coupling of short-tongued pollinators to species with higher nectar concentrations (*Geranium*, *Erodium*, *Francoa*) as well as a coupling of long-tongued pollinators to lower concentrations (*Pelargonium*) is evident.
This interplay between nectar reward pattern and pollinator guilds (already categorised on basis of flower visitors, flower colour as visual attractant, general flower shape and visibility of nectar site) strongly supports the pollination syndromes concept.
Interestingly, pollinator guilds are uncoupled from breeding system related variables. Although hard facts on true breeding systems of the order are scarce to almost non-existent in literature (many published data for Geraniales are only inferred on basis of p/o ratios and Cruden´s theory), the generally high p/o ratios point to predominantly xenogamous breeding systems - often approximating a ratio of 1 000 in Geraniaceae, and way above in most other families. When considering xenogamy as true breeding system for most of the species analysed, the extreme bandwidth of gamete production we have demonstrated here clearly points to additional factors influencing the p/o ratio.
Growth habit is one of these factors: Woody species (subshrubs, shrubs or small trees) surpass herbal species in their gamete production and p/o ratios, possibly as a counterbalance for higher inbreeding depression in larger and long-lived than smaller and shot-lived plants (Michalski and Durka, 2009; Husband and Schemske, 1996).
Additionally, pollen as predominant reward has to be accounted as another relevant factor: Highest pollen production coupled to missing nectar reward is constrained to genus *Balbisia*, and higher pollen production coupled to traced of nectar reward is present in *Hypseocharis bilobata*: The pattern of both taxa are indicative for pollen flowers. Pollen production is also higher in *Francoa*, making pollen a likely reward for Hymenoptera in the field, as observed for plants in Bonn Botanical Gardens.

II.5.3 POLLINATOR GUILDS AND PLANT TRAITS ARE PHYLOGENETICALLY CONSTRAINT

Seldom exceptions (like bird-pollinated *P. fulgidum* in Geraniaceae) underline the general observation that pollination syndromes are phylogenetically constrained in Geraniales. Switch to bird pollination occurred three times: two times on genus level for *Greyia* and *Melianthus*, and additionally on species level within *Pelargonium* for *P. fulgidum*. *Pelargonium* is otherwise constrained to long-tongued insect pollinators, similar to genus *Viviania*. As for other genera, short-probiscid insect pollinators are predominant.
Similarly, detected patterns of gamete production and nectar reward are phylogenetically constrained, with highest resource investment in genera *Balbisia*, *Greyia* and *Melianthus*, and lowest investment in Geraniaceae s. str. (other genera in between).
Although phylogenetic constrains are obvious for pollinator guilds, gamete production and nectar reward, it is remarkable that these constrains are largely uncoupled. The described correlation between pollinator guilds and nectar reward is realised within phylogenetic constrains of the respective genera,

and the phylogenetic constrains of gamete production are completely uncoupled from the retrieved pollinator guilds: Bird pollination, in example, is realised in genera *Greyia* and *Melianthus*, together with extremely high gamete production. However, bird pollination is also realised in *Pelargonium fulgidum*, showing only round about 1 % percent of the total pollen production per flower of *Greyia sutherlandii*, although both are even sharing the same pollinator: *Nectarinia chalybea* (see table A X.1.4, appendix). Entomophilous syndromes show a similar pattern, with both long- and short-tongued pollinators being the relevant pollen vectors for Geraniales species with lower *or* higher gamete production, covering the whole bandwidth of gamete production of the order.

This uncoupling might be best interpreted in terms of pollinators acting as drivers of speciation (see van der Niet and Johnson, 2012): Modifications in floral architecture, reward and attraction pattern *within* the general framework of a phylogenetic trajectory have led to radiation of species and stabilisation of the interplay of pollinator guilds and genera of Geraniales. While nectar reward is a necessary interface for successful plant-pollinator interaction, the observed pattern of gametic variables are primarily shaped, stabilised and constrained by other evolutionary forces than pollinator interaction, and therefore combined almost freely with pollinator guilds in Geraniales.

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II.7 Literature

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III. FROST FLOWERS – POLLINATION SYNDROMES OF HAMAMELIDACEAE INDEPENDENT FROM FLOWERING TIME

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III.1 ABSTRACT

Background and Aims: Hamamelidaceae are a family of 82 species, comprising several extant taxa flowering "out of season" in late autumn and winter. Flower function and pollination syndromes are poorly documented in this family. We collected data from 22 species and 12 genera on floral functional plant traits: Pollen and ovule production, p/o ratio, optical flower signal, nectar reward as well as flower and inflorescence morphology. Floral plant trait clusters are retrieved in order to test the validity of the floral syndromes concept. Moreover, interplay between retrieved floral functional pattern and flowering time is tested, and data are presented in a phylogenetic context.

Methods: Pollen and ovule counting, nectar measurements (amounts, sugar concentration), UV light photography, morphometry, Cryo-Scanning Electron Microscopy (CSEM), hierarchical clustering, Non-metric Multidimensional Scaling (NMDS), vector fitting, phylogenetic analysis.

Key results: Three major floral functional trait clusters are retrieved via hierarchical clustering an NMDS (complete dataset, stress: 0.092, good fit; two convergent solutions after 20 tries). Flowering time appears as disjunct from the retrieved clusters (PERMANOVA: non. sig., p = .124).

Conclusions: Clear floral functional clusters indicative for wind resp. animal pollination are retrieved, but also a separate group with ambophilous, mixed pollination mode (corresponding to the genera *Fothergilla* and *Parrotiopsis*). Surprisingly, animal pollination appears to be common in winter flowering species, and pollination syndromes and flowering time appear to be recombined quite freely during evolution. Zoophily was evolutionary reinvented five to six times from an ancestral anemophilous condition in Hamamelidaceae. Ambophily is apparently stabilised in two genera, at least dating back to the Eocene, and possibly even the Upper Cretaceous, arguing against this representing an ephemeral transitional stage.

Key words: Hamamelidaceae, flowering time, floral function, plant traits, pollination syndromes, anemophily, zoophily, ambophily, hierarchical clustering, NMDS, phylogeny.

III.2 INTRODUCTION

Hamamelidaceae (Saxifragales), a subcosmopolitan tropical to temperate family, show a broad variety of flower and inflorescence morphology. Like most other core eudicots (except Gunnerales), floral diversity is based on a structural plan (CEFG – Core Eudicot Floral Groundplan) characterised by whorled phyllotaxy, alternation of organs in adjacent whorls, a perianth consisting of a distinct calyx and corolla, and a meristic pattern with a pentamerous (or tetramerous) perianth and androecium and a dimerous (or trimerous) gynoecium (Magallón et al., 2007). In Hamamelidaceae there are representatives with petalous and apetalous flowers and the family thus consists of taxa displaying the full CEFG or only some of its components, therefore being a favourable model for the evolution of floral function.

Another interesting feature of Hamamelidaceae is the fact that some temperate representatives notoriously flower "out of season", i.e. in autumn or winter (see figure III.1). This could be expected to influence floral function in these taxa, since species flowering at the extreme margins of the growth season have to cope with several constraints, abiotic (i.e. extreme temperatures, water scarcity) and biotic (i.e. pollinator scarcity).

FIGURE III.1: WINTER FLOWERING HAMAMELIDACEAE IN BONN BOTANICAL GARDEN
Top row: Habitus of *Hamamelis mollis* (left) and close-up of inflorescences (right). Bottom row: *Sycopsis sinensis* (left) and *Parrotia persica* (right). Pictures by A. W. Mues, taken on January 31, 2019.

Endress (1989 a) offered the first elaborate classification of Hamamelidaceae R. Br., describing four subfamilies, three of them not subdivided: Rhodoleioideae Harms (genus *Rhodoleia* Champ. ex Hook),

Exbucklandioideae Reinsch (genera: *Disanthus* Maxim. (later separated as Disanthoideae Harms (Takhtajan, 1997)), *Mytilaria* Lecomte, *Chunia* Chan, *Exbucklandia* R. W. Br.), and Altingioideae Reinsch (genera: *Liquidambar* L., *Altingia* Nor., *Semiliquidambar* Chang). Altingioideae are today classified as the independent family Altingiaceae Horan. (Takhtajan, 1997; Angiosperm Phylogeny Group, 2003). The fourth, larger subfamily, Hamamelidoideae Reinsch, is subdivided in four tribes: Eustigmateae Harms (three genera: *Eustigma* Gardn. & Champ., *Fortunearia* Rehd. & Wils., *Sinowilsonia* Hemsl.), Corylopsideae Harms (one genus, *Corylopsis* Sieb. & Zucc.), Fothergilleae A. DC. (eight genera: *Molinadendron* Endress, *Fothergilla* Murray in L., *Parrotiopsis* Schneider, *Parrotia* C. A. Mey., *Sycopsis* Oliv., *Distyliopsis* Endress, *Distylium* Sieb. & Zucc., *Matudaea* Lundell). The fourth tribe Hamamelideae A. DC., is further divided in three subtribes: Hamamelidinae Endress subtrib. nov. (genus: *Hamamelis* L.), Loropetalinae Endress subtrib. nov. (four genera: *Loropetalum* R. Br. ex Reichb., *Tetrahyrium* Benth., *Maingaya* Oliv., *Embolanthera* Merr.) and Dicoryphinae Endress subtrib. nov. (five genera: *Dicoryphe* Du Petit-Thouars, *Trichocladus* Pers., *Ostrearia* Baill., *Neostrearia* L. S. Smith, *Noahdendron* Endress, Hyland & Tracey).

Regarding floral morphology, the full CEFG is present in Rhodoleioideae, Disanthoideae, genus *Mytilaria* (Exbucklandioideae) as well as in three of four tribes of subfamily Hamamelidoideae, namely Corylopsideae, Hamamelideae and Eustigmateae (however only very small petals in *Eustigma*, and petals rudimentary in *Fortunearia* and *Sinowilsonia*). The perianth is missing or highly reduced in tribe Fothergilleae, subfamily Exbucklandioideae, genera *Chunia* and *Exbucklandia*, and in Altingiaceae. In addition to variations of the CEFG on flower level, presentation of flowers in inflorescences also varies widely between taxa (Bogle, 1970; Morley and Chao, 1977; Endress, 1989 b; Mione and Bogle, 1990; Endress, 1993; Magallón, 2007).

Regarding the high floral diversity present in Hamamelidaceae, Endress (1977, p. 324 ff.) already stated the general idea that *"Here, obviously, an adaptive radiation has led to mainly bee, fly, bird, and wind pollinated flowers …"*, emphasising the importance of plant-animal interactions for the understanding of floral diversity in this family. There has been frequent criticism about the usefulness of the pollination syndromes approach (e.g. Waser et al., 1996; Ollerton et al., 2009). We agree with Willmer (2011) that it is unfortunate that the syndrome concept has been criticised for something it was never intended for: A pollination syndrome should be understood as a statistical rather than an absolute construct, meaning that a particular set of floral characters is more likely for specific biotic or abiotic vectors in order to secure outcrossing. There is a broad body of evidence for a predictive value of floral characters on both zoophilous and abiotic pollination systems (e.g. Delpino, 1868–1875; Vogel, 1954; van der Pijl, 1961; Baker and Hurd, 1986; Faergi and van der Pijl, 1979; Johnson, 2010; Gómez et al., 2008; van der Niet and Johnson, 2012; Newman et al., 2014; Rosas-Guerrero et al., 2014; Abrahamczyk et al., 2017; Johnson and Wester, 2017; Dellinger et al., 2018; Ibañez et al., 2019; abiotic/wind: Michalski and Durka, 2010; Molina et al.,1996; Culley et al., 2002; Friedman and Barrett, 2009).

The present article aligns with this research. Flower function is poorly documented in Hamamelidaceae, and we therefore collected a broad sample of floral plant traits. The retrieved dataset was subsequently analysed with a multivariate, statistical approach in order to discuss the validity of pollination syndromes in Hamamelidaceae. To this end, 22 species from 12 genera are investigated with regard to flower morphology, optical signal, nectar reward, pollen and ovule production as well as p/o ratios – a common indicator for pollination efficiency. Hierarchical clustering and Non-metric Multidimensional Scaling (NMDS) are used to retrieve clusters of floral functional traits, and interplay of these characters

is tested via vector fitting and Permutational Multivariate Analysis of Variance (PERMANOVA). Results are further compared in a phylogenetic context.
With this paper we want to address the following research questions:
Are clusters of floral traits indicative for pollination syndromes in Hamamelidaceae?
Is there a relationship between floral traits and flowering time?
Are differences in floral traits and flowering time correlated with phylogenetic relatedness?

III.3 Materials and Methods

Samples for analysis of floral function were taken from the living collection of Bonn University Botanic Gardens, with primary focus on subfamily Hamamelidoideae, representing the bulk of floral diversity found in the family: *Corylopsis* (*C. glabrescens*, *C. pauciflora*, *C. sinensis*, *C. spicata*, *C. veitchiana*, *C. willmottiae*), *Distyliopsis* (*D. tutcheri*), *Distylium* (*D. myricoides*, *D. racemosum*), *Fothergilla* (*F. gardenii*, *F. major*), *Fortunearia* (*F. sinensis*), *Hamamelis* (*H. japonica*, *H. mollis*, *H. vernalis*, *H. virginiana*), *Loropetalum* (*L. chinense*), *Parrotia* (*P. persica*), *Parrotiopsis* (*P. jacquemontiana*), *Sinowilsonia* (*S. henryi*) and *Sycopsis* (*S. sinensis*). Additionally *Disanthus cercidifolius* (Disanthoideae) was analysed. Sampling of flowers took place between September 2015 (*Hamamelis virginiana*) to October 2016 (*Disanthus cercidifolius*).
Additionally, material from all species was collected and stored in silica gel for sequencing and phylogenetic analysis, together with sequences downloaded from GenBank (outgroups, see below).

III.3.1 Assessment of inflorescence and flower morphology

Individual flowers of the species investigated are often strongly reduced and synorganised into inflorescences as functional units. We therefore summarised functional parameters at inflorescence level, additional to the flower level. Twelve inflorescences per species were analysed under a stereo microscope: Flowers were either classified as perfect, staminate or pistillate. The total number of flowers per flower type was recorded, as well as the total number of stamens and ovules or every flower and inflorescence. For all species studied, the number of carpels is fixed to two.
Ten mid-anthetic flowers per species were measured to obtain data on anther length, filament length, style length, distance between anther and stigma, petal length and petal width, if applicable. Averages and standard deviations were calculated. In three cases, namely *Fortunearia sinensis*, *Parrotia persica* and *Sycopsis sinensis*, a distinctive enlargement of styles was observed during the pistillate phase. Two values for style length (pre and post style elongation) were therefore recorded. Morley and Chao (1977) described protogyny of flowers for *Corylopsis*. Filament elongation brings anthers with or above level of the stigmas when petals are fully expanded, and all flowers are functionally hermaphroditic. Due to this, morphometry of *Corylopsis* was done with randomly selected, fully opened flowers.
Terminal inflorescences (T) are found to be more vigorous than lateral inflorescences (L) in three species: *Distyliopsis tutcheri*, *Parrotia persica* and *Sycopsis sinensis*. In order to cater for this difference, T:L ratios were approximated by a complete count of inflorescences from a randomly selected branch. The obtained T:L ratios are 1:1 for *Distyliopsis tutcheri* (n = 25 inflorescences), 1:3 for *Parrotia persica* (n = 40) and 1:2 for *Sycopsis sinensis* (n = 51). T:L ratios were applied for subsequent sampling.

III.3.2 Assessment of pollen production, ovule numbers and p/o ratios

Twelve inflorescences per species were analysed for pollen and ovule production as well as p/o ratio. Ten anthers per inflorescence were randomly selected for the pollen count. Where different flower types (perfect vs. staminate) were present, samples representing each flower type were taken separately. If a certain flower type was represented with less than ten anthers in an inflorescence (for example staminate flowers in an inflorescence of predominantly perfect ones), the maximum number of available anthers was used.

Only fully developed and completely closed anthers were used for pollen counts. Anthers were removed with tweezers and dried in Eppendorf tubes for at least three days. During drying the pollen material usually was released from the anthers. In some cases anthers failed to open. These were carefully smashed open with a small pestle, which was subsequently rinsed with the solvent to recover adhering pollen material. A mixture of glycerol and distilled water (1:1) was used for dispersal of pollen grains during the counting procedure. Glycerol was applied to increase viscosity and slow down the sedimentation of pollen grains. Depending on anther size and pollen number, the amount of added solvent was adjusted, ranging from 100 µl (*Corylopsis, Disanthus*) to 800 µl (*Distylium*, *Parrotia*, *Parrotiopsis*, *Sinowilsonia*, *Sycopsis*). Samples were mixed 5 minutes in a laboratory mill (Retsch® MM200), and afterwards placed 10 minutes into an ultrasonic bath (Bandelin Sonorex RK52H). Immediately before counting, samples were vortexed for 30 sec. (neoLab® 7-2020). 20 µl of the suspension were pipetted on a Fuchs-Rosenthal hemocytometer, and pollen grains were counted in five 1 mm² squares under a binocular microscope. Total pollen amount of the sample was calculated with the following formula (Neuendorf, 2013):

$$\text{pollen per sample} = \frac{\text{counted pollen grains}}{\text{counted surface (mm}^2\text{)} \cdot \text{ chamber depth (mm)} \cdot \text{ dilution (1} \div \text{amount of diluent)}}$$

Pollen production per anther was afterwards calculated by division of pollen number per sample by the number of anthers per sample. In case of perfect and staminate flowers present in an inflorescence, the integrated average pollen production per anther was calculated, too:

$$\overline{x}\ \text{pollen per anther}(\,\varnothing\,,\,\male) = \frac{(\text{pollen per anther}^{\male} \cdot \text{number of anthers}^{\male}) + (\text{pollen per anther}^{\varnothing} \cdot \text{number of anthers}^{\varnothing})}{\sum \text{anthers}^{\male,\varnothing}\ \text{per inflorescence}}$$

Average pollen production per flower was calculated by multiplication of anther-specific pollen production with the average number of anthers per flower and flower type, for each inflorescence assessed. In case of perfect and staminate flowers present in an inflorescence, the integrated average pollen production per flower was calculated as follows:

$$\overline{x}\ \text{pollen per flower}(\,\varnothing\,,\,\male) = \frac{(\text{pollen per flower}^{\male} \cdot \text{number of flowers}^{\male}) + (\text{pollen per flower}^{\varnothing} \cdot \text{number of flowers}^{\varnothing})}{\sum \text{flowers}^{\male,\varnothing}\ \text{per inflorescence}}$$

Total pollen production per inflorescence and flower type was calculated by multiplication of anther-specific pollen number by the total number of anthers of each inflorescence and flower type. For inflorescences bearing perfect as well as staminate flowers, total pollen production of the respective inflorescences was calculated by adding up the values.

Flowers of all species analysed have two carpels, each of them usually containing one ovule. In genus *Corylopsis* three ovules per carpel are produced, with only one reaching maturity (Magallón, 2007).

Ovule number of most species is therefore fixed to two per flower, the only exception is *Disanthus* with six, rarely seven ovules per carpel.

Pollen to ovule ratios (p/o) were obtained by dividing the total pollen production per inflorescence by the number of ovules per inflorescence. First proposed by Cruden (1977), p/o ratios are commonly used as indicators for plant breeding systems and pollination syndromes: Pollination efficiency varies between different biotic or abiotic pollen vectors (compare Cruden and Jensen, 1979; Cruden and Miller-Ward, 1981; Cruden and Lyon, 1985; Small, 1988; Kirk, 1993; López et al., 1999; Cruden, 2000; Michalski and Durka, 2009; Alarcón et al., 2011; Lozada-Gobilard et al., 2019).

III.3.3 Assessment of inflorescence size

Ten inflorescences per species were pictured frontal and lateral, together with graph paper as a reference. Frontal inflorescence size is defined as the surface of the functional reproductive unit oriented orthogonal to the peduncle (representing the main axis), connecting the functional unit to the branch (top view). Lateral inflorescence size is defined as the surface of the functional unit oriented parallel to the main axis (side view). Inflorescences were photographed with single-lens reflex camera Canon EOS 600D. Display size was then measured and calculated by means of Adobe® Photoshop® CS6 imaging software and the Magnetic Lasso Tool, with 1 cm^2 of the graph paper scale set as reference. Except for coloured bracts of genus *Corylopsis* and *Parrotiopsis jacquemontiana*, bracts were excluded from measurement.

III.3.4 Assessment of UV light patterns

For each species, inflorescences were illuminated with UV light, and observed pattern were depicted from frontal and lateral view. Pictures were taken with a single-lens reflex camera (Nikon R D300s), with an infrared neutralizer (OPTIK MAKARIO IR NG 52D) in combination with a UV light filter (OPTIK MAKARIO SP 400 UV 52D). A photo series was created with a fixed aperture, but different shutter speeds (1/5, 1/4, 1/3, 1/2, 1/1.6, 1, 1.6, 2, 3, 4, 5 sec.).

III.3.5 Assessment of nectar reward

Flowers were protected from pollinators with a covering of gauze ca. 24 h before nectar sampling. Glass microcapillaries (0.5 and 1 µl minicaps®; Hirschmann Laborgeräte, Germany) were used for extracting the nectar, and nectar concentration was subsequently measured in degrees Brix with a hand-held refractometer (neoLab, type 'universal'). In some flowers nectar amount was too low to be successfully extracted, in others nectar had to be pooled from several flowers in order to be measured on the refractometer.

Nectar sugar production in mg per flower was afterwards calculated with the following formula, with *x* being the measured concentration (cf. Galetto and Bernardello, 2005):

$$\frac{mg}{\mu l} = 0.00226 + (0.00937x) + (0.0000585x^2)$$

The number of nectar measurements was standardised to *n* = 10 per species, surplus data was excluded randomly. Only five nectar measurements could be obtained for *Corylopsis glabrescens*.

III.3.6 Cryo-Scanning Electron Microscopy (CSEM)

For some species nectar could not be extracted with microcapillaries. In order to validate absence of nectar, flowers were studied under the CSEM. Flowers and inflorescences were fixed to a conducting carrier (SEM stub) and attached with conductive coal to prevent electric charging. Samples were cooled down with liquid nitrogen and examined for nectar droplets and nectar stomata. If necessary, the perianth was partly removed. Scanning was performed with a Cambridge Stereoscan 200 (Cambridge Instruments Ltd., Cambridge, UK).

III.3.7 Phylogenetic Analysis

Sequence data of two nuclear (ETS and ITS) and four plastid markers (*trnL-F*, *rps16*, *atpB-rbcL* and *psbA-trnH*) was generated for all species analysed in our study and complemented with additional sequences of Hamamlidaceae, downloaded from GenBank (provided by Xie et al., 2010). We used four species of the family Altingiaceae as outgroup. Altingiaceae has been retrieved as sister to the Hamamelidaceae in several phylogenetic studies (e.g. Soltis et al., 2013; Xiang et al., 2019).

DNA was extracted from silica-gel using a modified CTAB method (Doyle and Dickson, 1987) and amplified following Xie et al. (2010). PCR products were purified with the GeneJET PCR Purification Kit (Thermo Fisher Scientific Biosciences, St. Leon-Rot, Germany) following manufacturer's instructions. Cycle sequencing was performed using BigDye Terminator v.3.1 (Applied Biosystems, Foster City, California, U.S.A.). The resulting electropherograms were assembled using Geneious v.5.6.5 (Biomatters, Auckland, New Zealand) and the consensus sequences aligned with MAFFT v.6.850b (Katoh et al., 2002), followed by manual adjustments in PhyDE v.0.9971 (available at http:// www.phyde.de). Phylogenetic analyses were conducted via Maximum Likelihood (ML) method (Felsenstein, 1981) as implemented in RaxML v. 8.2.10 (Stamatakis, 2014) on the CIPRES Science Gateway (Miller et al., 2010). Since incongruence between nuclear and plastid datasets has been reported in previous phylogenetic studies of the Hamamelidaceae (Xie et al., 2010), ML analyses were conducted separately for concatenated, marker-partitioned matrices of nuclear and plastid markers. Analyses were conducted with unlinked partitions and branch support was calculated with 1,000 bootstrap replicates. Trees were rooted with the Altingiaceae.

III.3.8 Statistical Analysis

Data exploration and basic statistics were carried out with the IBM software package SPSS, version 24. Statistical procedures beyond data exploration were performed in R version 3.3.3 with arithmetic means on species level. Continuous, ratio scaled variables are pollen production per anther, anther number per flower, pollen production per flower, flowers per inflorescence, anthers per inflorescence, pollen per inflorescence, ovules per inflorescence, p/o ratio, anther length, filament length, style length, petal length, petal width, frontal inflorescence size and lateral inflorescence size. Categorical variables were assigned via R factor function, and factor levels were labelled as unordered (nominal) or ordered (ordinal). Categorical variables are the presence or absence of staminate flowers in inflorescences (ordered factor – coded 0 for absence, 1 for occasional occurrence and 2 for large proportions of staminate flowers), sexual functionality (unordered – coded 0 for joint male and female function within an inflorescence, 1 for functional unisexuality via temporal separation and 2 for full unisexuality via

separation of male and female inflorescences), presence or absence of showy colour signal of inflorescences (perianth organs, bracts or filaments, ordered, coded 0 or 1), colour signal of anthers (unordered – coded 1 for yellow anthers, 2 for red and 3 for dark red anthers), nectar reward (ordered – coded 0 for absence, 1 for nectar traces and 2 for substantial amounts of nectar measurable with our refractometer technique) and flowering time (unordered): Winter flowering species are coded 1, spring flowering species 2 and autumn flowering species 3. In spite of some minor differences in flowering time in cultivated conditions at Bonn Botanical Gardens, observed flowering times are usually in line with reported phenology for the species' native ranges (appendix, table A X.2.5). Flowering time was labelled as "winter flowering", describing species flowering in late winter or early spring, blooming noticeably earlier than "spring flowering" species, and "autumn flowering" was chosen for the other extreme of the spectrum, species flowering at the end of the season. Winter and autumn flowering species are therefore marking the marginal extremes of the growth period.

UV light signal of inflorescences was found to be strongly conserved and is therefore not included in the statistical analysis (see appendix, table A X.2.3 and figure A X.2.2).

Bivariate correlations are presented in the appendix, table A X.2.6: Due to non-normality of data, correlations between continuous, ratio scaled variables as well as between ratio scaled and ordinal variables were analysed with non-parametric Kendall tau rank correlation coefficient (τ_b). Correlations from .1 to .3 are rated as low, from .3 to .5 as moderate, from .5 and above as strong (cf. Cohen, 1988).

Cluster analysis and multivariate ordinations were performed in order to structure, analyse and visualise available information on floral plant traits. Both methods were performed with the arithmetic means of continuous variables and nominal coding of discrete variables with R package vegan, version 2.5-1 (Oksanen et al., 2013). Where both staminate and perfect flowers were present within inflorescences, flower type specific information for gametic variables had to be aggregated via arithmetic means to have species-specific profiles. To account for this, presence or absence of staminate flowers was introduced as a nominal variable. As for *Sinowilsonia henryi*, male and female inflorescences were analysed separately. Most variables are unambiguous, except number of flowers per inflorescence as well as frontal and lateral inflorescence size. Respective variables were averaged across male and female inflorescences. Only data of fully anthetic flowers were used.

Hierarchical clustering was performed in R with gametic data, with non-gametic data and with all variables at once. Ovule production per carpel and flower are highly conserved within the family and were therefore excluded from these procedures, but female gamete production is represented via ovule number per inflorescence. Clustering of all variables also included flowering time. Cluster analyses were conducted with complete linkage and Gower dissimilarity matrices, except for gamete production analysed via Bray Curtis index (only metric variables); cf. appendix, figures A X.2.4 and A X.2.5.

Ordination was performed in vegan by means of Non-metric Multidimensional Scaling (NMDS) in order to handle different measuring scales and non-normality of data. We used the same dissimilarity matrices as for hierarchical clustering. Function metaMDS was used for iterative testing and selection of the solution with smallest stress. Data were standardised by square root transformation and Wisconsin double standardisation. For NMDS with gametic variables, non-gametic variables were fitted onto the ordination as vectors (R function envfit, 999 permutations). For the second model the procedure was reversed, NMDS ordination was done with non-gametic variables and gametic variables were then fitted onto the ordination. Model stresses between .05 and .1 were interpreted as good (McCune et al., 2002).

For the final NMDS model all floral plant traits plus flowering time were used, and influence of flowering time as a source of variation in the Gower dissimilarity matrix was checked with Permutational Multivariate Analysis of Variance (PERMANOVA, 200 permutations applied). Phylogenetic signal was evaluated for all single variables as well as for NMDS-axes using Pagel's λ (Pagel, 1999) as implemented in the R-package Geiger v.2.0.6.2 (Harmon et al., 2008) for discrete and continuous data, depending on the variable. The ARD (all rates different) model was employed for all analyses of discrete variables. We used the trees based on both nuclear and plastid data separately, pruning from them the species for which no measurements were available. Pagel's λ approaches 1 when the association between morphological covariance and phylogenetic relatedness is maximum.

III.4 RESULTS

For the taxa analysed in depth here, the following inflorescence structures are realised, please compare depictions in figures III.2 and III.3: *Corylopsis* (thyrses, racemes), *Disanthus* (2-flowered), *Distyliopsis* (condensed panicles or botroids), *Distylium* (compound inflorescences: a lateral axis stemming from a branch is bearing small axillary inflorescences – spikes, racemes or panicles), *Fothergilla* (spikes), *Fortunearia* (racemes), *Hamamelis* (heads), *Loropetalum* (tightly clustered raceme or panicle), *Parrotia* (bracteate heads), *Parrotiopsis* (subcapitate spikes), *Sinowilsonia* (catkins; male: racemes, female: spikes) and *Sycopsis* (panicles, spikes or heads). In the following, data bandwidth of analysed variables is presented in line with the results of hierarchical clustering, please see figure III.4 for NMDS-ordination of clusters.

III.4.1 CLUSTERING OF GAMETIC VARIABLES RETRIEVES TWO CLUSTERS

Hierarchical clustering exclusively performed on gametic variables retrieves two clusters (cf. table A X.2.1 and figures A X.2.4 and A X.2.5 in the appendix): **Cluster A is generally built up of species characterised by high to very high gamete production when compared to the rest of the family, namely: *Sycopsis, Parrotia*, *Fortunearia*, *Sinowilsonia*, both species of *Fothergilla* and *Parrotiopsis*. Cluster B is built up of all other species analysed.**

Pollen production per inflorescence is remarkably higher in cluster A than in cluster B, approximating 1 million pollen grains per inflorescence or even way above this value. *Sycopsis* is marking the lower bound of cluster A, with 724 993.3 pollen grains on average. Monoecious *Sinowilsonia henryi* with its unisexual staminate inflorescences is marking the upper bound with 3 430 566.7 pollen grains. For species in cluster B, pollen production per inflorescence is in the tens to hundreds of thousands: The lowest production was found for *Disanthus* with 42 333.3 pollen grains on average. *C. willmottiae* shows maximum production in cluster B (643 650.0 pollen grains).

Pollen production per anther is similar in both clusters, ranging in the thousands to the tens of thousands, and transitions are smooth (lowest in *F. gardenii*, showing 1 749.0 pollen grains per anther, and highest for *Corylopsis pauciflora* (21 312.5, cluster B)).

Pollen production per flower is also similar in both clusters, ranging in the tens of thousands, in some cases surpassing the one hundred thousand (lower bound: *Hamamelis japonica* (Cluster B), 13 916.7 pollen grains per flower; upper bound: *Parrotia* (cluster A), 147 504.4 pollen grains per flower).

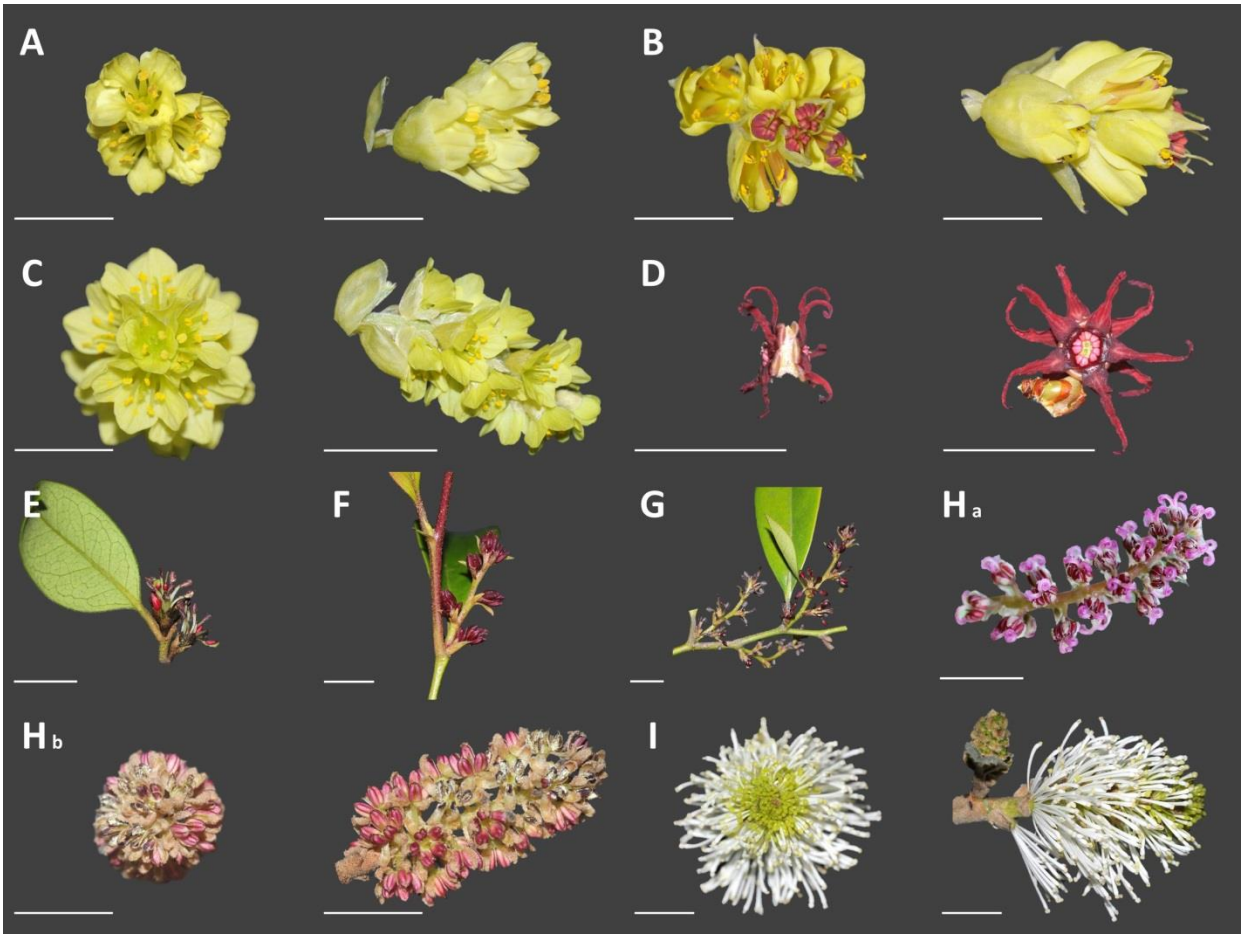

FIGURE III.2: HAMAMELIDACEAE, DEPICTIONS OF INFLORESCENCES – I
A. *Corylopsis pauciflora* (frontal and lateral view); B. *Corylopsis spicata* (frontal and lateral view); C. *Corylopsis veitchiana* (frontal and lateral view); D. *Disanthus cercidifolius* (frontal and lateral view); E. *Distyliopsis tutcheri* (anthetic, lateral view); F. *Distylium myricoides* (pre-anthetic, lateral view); G. *Distylium racemosum* (anthetic, lateral view); H a. *Fortunearia sinensis*, dichogamous and protandrous (pistillate phase, lateral view); H b. *Fortunearia sinensis* (male phase, frontal and lateral view); I. *Fothergilla gardenii* (frontal and lateral view). Scale bar = 1 cm. Pictures A, B, C, H, I taken by L. Hoff, pictures D, E, F, G taken by A. W. Mues.

Ovule production per inflorescence is usually in the higher dozens in cluster A, starting with 29.3 ovules in *Parrotiopsis* and going up to 193.5 ovules on average for *Sinowilsonia* and its pistillate inflorescences. Ovule numbers decline in dichogamous and protandrous *Fortunearia sinensis*: For preanthetic inflorescences 61.2 ovules were found on average, but only 23.08 % of the inflorescences (36 of 156 inflorescences analysed) reached female anthesis (cf. figure III.2). Due to this, average ovule number dropped to 14.1. Female anthesis and style elongation was also observed for dichogamous and protandrous *Parrotia persica* and *Sycopsis sinensis* for terminal inflorescences, while lateral

inflorescences showed no female phase (cf. figure III.3). This shifts average ovule number of preanthetic inflorescences towards a much lower level during anthesis, from 10.2 to 3.3 ovules for *Parrotia* and from 14.0 to 5.3 ovules for *Sycopsis*. Reduction of ovule number was therefore detected in three species of cluster A.

Ovule number per inflorescence is generally lower in cluster B, starting with *Distyliopsis* (2.5 ovules), and going up to 26.7 for *Corylopsis veitchiana*.

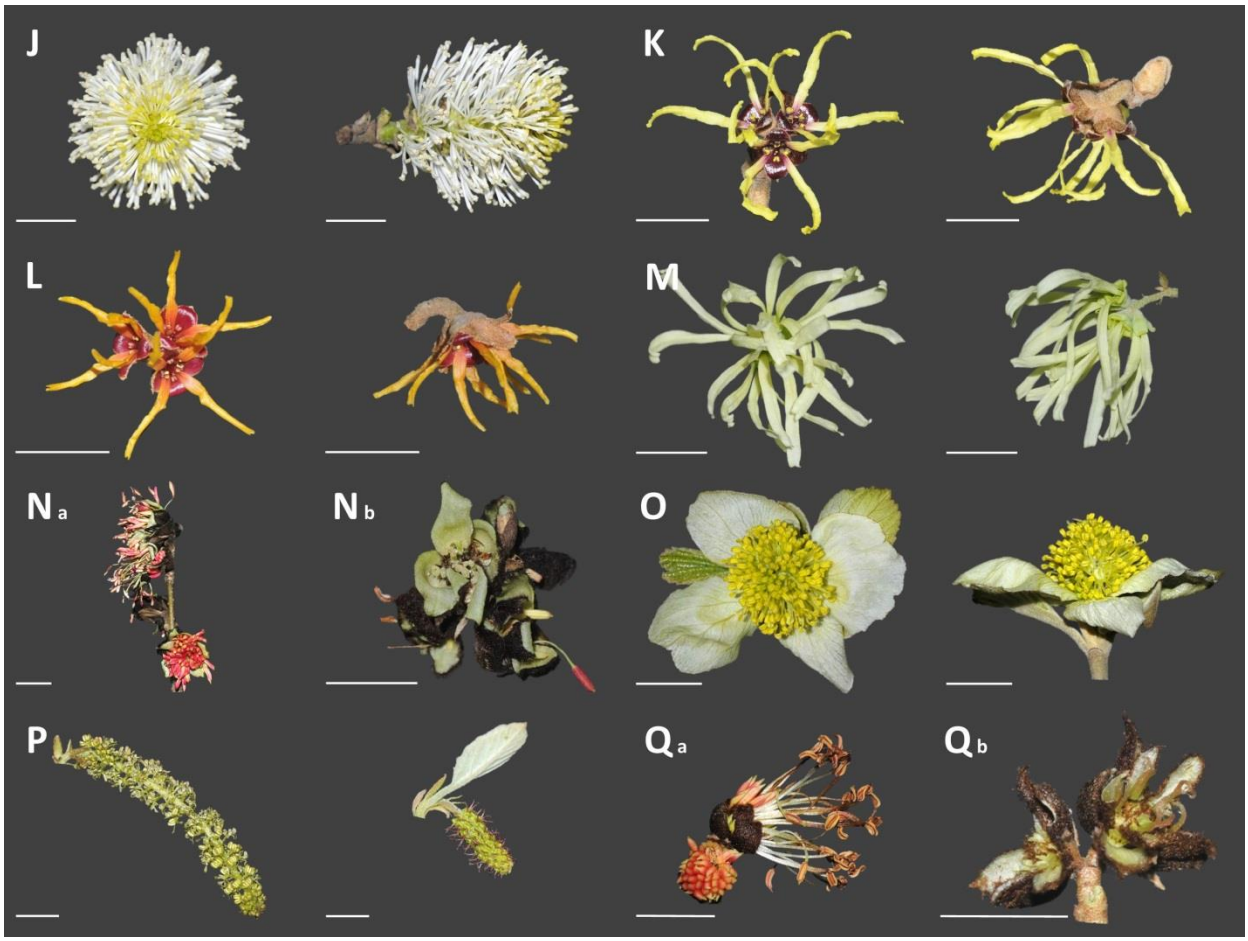

FIGURE III.3: HAMAMELIDACEAE, DEPICTIONS OF INFLORESCENCES – II

J. *Fothergilla major* (frontal and lateral view); K. *Hamamelis mollis* (frontal and lateral view); L. *Hamamelis vernalis* (frontal and lateral view); M. *Loropetalum chinense* (frontal and lateral view); N a. *Parrotia persica*, top of twig: protandrous inflorescences, bottom: pre-anthetic; N b. *Parrotia persica*, enlargement of styles during female phase (stamens have already fallen of), only observed for terminal inflorescences; O. *Parrotiopsis jacquemontiana* (frontal and lateral view); P. *Sinowilsonia henryi* (monoecious, with separate unisexual staminate and carpellate inflorescences, left: male, right: female); Q a. *Sycopsis sinensis*, top of twig: protandrous inflorescence, bottom: pre-anthetic; Q b. *Sycopsis sinensis,* terminal inflorescence on the right side (stamens removed): enlargement of styles during female phase; lateral inflorescence on left (stamens removed): no female phase observed. Scale bar = 1 cm. Pictures for J taken by L. Hoff, other pictures by A. W. Mues.

P/o ratio in cluster A is very high for species with reduced ovule numbers (p/o for *Fortunearia*: 164 247.5 (before anthesis: 37 908.3); *Sycopsis*: 1 725 748.5 (before anthesis: 51 084.7); *Parrotia*: 2 675 817.1 (before anthesis: 73 752.2)). For other species of cluster A, p/o ratios are scoring in the tens of thousands: *Sinowilsonia* is marking the lower bound (17 683.3), followed by both Fothergillas (*F. gardenii*: 19 590.4, *F. major*: 33 940.6) and *Parrotiopsis* (61 557.2). For cluster B, p/o ratios are

starting with *Disanthus*, showing a very low ratio of 1 691.6, while *Distylium racemosum* shows the highest (88 906.4).

III.4.2 Clustering of non-gametic variables retrieves three clusters

In a second step hierarchical clustering was performed with non-gametic data, precisely variables related to morphology, flower signal and nectar reward. As a result three major clusters were retrieved (cf. appendix, tables A X.2.2 to A X.2.4, as well as figures A X.2.4 and A X.2.5): **In general, cluster 1 contains species with optical signal, but lacking nectar (*Parrotiopsis*, both species of *Fothergilla*). Cluster 2 contains species showing optical flower signal as well as nectar reward, namely all species of genera *Corylopsis* and *Hamamelis*, as well as *Disanthus* and *Loropetalum* (the latter without nectar, however). Cluster 3 consists of species neither showing a clear optical flower signal nor nectar, namely *Sinowilsonia*, *Parrotia*, *Sycopsis*, *Fortunearia*, *Distyliopsis*, and *Distylium***.

Regarding **sexual systems**, all species of cluster 1 and 2 have fully functional hermaphroditic inflorescences. Cluster 3 shows more variation: Monoecious *Sinowilsonia henryi* shows completely separated unisexual staminate and carpellate inflorescences on the same plant. Although bisexual from their morphology, for the latter three cases protandrous, dichogamous inflorescences were observed, and therefore functional unisexuality: For *Fortunearia* only 23.08 percent of all inflorescences showed female anthesis (see ovule numbers), while for *Parrotia persica* and *Sycopsis sinensis* only terminal inflorescences reached the female phase after male anthesis.

Production of **staminate flowers** is absent in cluster 1 but considerable for some species in cluster 3, namely *Distyliopsis*, both species of *Distylium* and *Sinowilsonia* with its male inflorescences. For *Sycopsis* and *Parrotiopsis* (cluster 2) staminate flowers were observed only in rare cases.

The **number of flowers per inflorescence** is highly variable and taxon-specific and ranges from ca. 2 to nearly 100. Cluster 1 is belonging to the upper end of the family´s spectrum, with 14.8 flowers per inflorescence on average for *Parrotiopsis*, 24.2 flowers for *Fothergilla major* and 35.2 flowers for *Fothergilla gardenii*. In cluster 2 flower number is at the lower end of the spectrum, lowest in *Disanthus* (only two flowers) and going up to 13.3 flowers in *Corylopsis veitchiana*. Cluster 3 is quite similar to cluster 2 for the most part, starting with in average 2.1 flowers for *Distyliopsis*. However, two species with much higher flower numbers are present in cluster 3, too: *Fortunearia* has 30.6 flowers on average, and *Sinowilsonia* has 66.2 flowers for male and 96.8 flowers for female inflorescences.

Anther number per flower shows a similar pattern like flower number for the three clusters. Overall, observed anther number per flower ranges from 4 to 23 on average. Species in cluster 1 show the highest anther number, 18.6 anthers for *Parrotiopsis*, 22.0 for *Fothergilla major* and 22.7 for *Fothergilla gardenii*. In cluster 2 anther number is often fixed and low, to the number of four in genus *Hamamelis* and usually to the number of five in genus *Corylopsis*. Anther number is fixed to 5 for *Disanthus*. *Loropetalum* has 4.3 anthers per flower on average. Cluster 3 is in between, starting with 4.3 anthers for *Distylium myricoides* and going up to 11.5 for *Parrotia*.

Anther number per inflorescence shows the already described pattern: Cluster 1 represents the upper spectrum again, with 273.9 anthers per inflorescence on average for *Parrotiopsis*, 529.8 anthers for *Fothergilla major* and 800.3 anthers for *Fothergilla gardenii*. Cluster 2 shows much lower anther number per inflorescence, starting with only 10 anthers fixed for the two flowers of *Disanthus*, 11.3 to 12.7 anthers in genus *Hamamelis,* 27.7 anthers for *Loropetalum* and 12.8 to 66.8 anthers in genus *Corylopsis.*

Cluster 3 is in between cluster 1 and 2 again, showing dozens to hundreds of anthers per inflorescence. For *Distyliopsis and Disytlium* anther number is increased by vigorous production of staminate flowers, starting with 18.3 anthers on average for *Distyliopsis tutcheri*, followed by 36.8 anthers for *Distylium racemosum* and 42.3 anthers for *D. myricoides*. In cluster 3 highest anther numbers per inflorescence were obtained for species with fixed anther number per flower and higher flower number, namely *Fortunearia* with 152.9 anthers and *Sinowilsonia* with 327.9 anthers.

Regarding morphology of the reproductive organs, **anther length** is fixed to 0.5 mm in *Fothergilla* and to 1 mm in *Parrotiopsis* in cluster 1. In cluster 2 anther length is similar, and fixed to 1 mm in *Disanthus*, *Loropetalum* and genus *Hamamelis* (with only few deviations for *H. virginiana*). For genus *Corylopsis* anther length is ranging from 0.5 mm in *C. glabrescens* to 1.8 mm on average for *C. pauciflora*. Cluster 3 is characterised by longer anthers, starting with 1.2 mm on average for *Sinowilsonia*, followed by 2 mm length fixed for *Distyliopsis* and *Fortunearia*, 2.1 mm on average for *Distylium racemosum* and 2.9 mm for *D. myricoides*, 2.2 mm on average for *Sycopsis* and 3.2 mm for *Parrotia*.

Filament length is diverse within and between the three clusters. In cluster 1, filaments of *Parrotiopsis* are 5.0 mm on average, while both *Fothergilla* species show very long filaments, with 12.1 mm for *F. gardenii* and 13.4 mm for *F. major* on average. In cluster 2 filament length is starting with .1 mm fixed for *Loropetalum*, and is only somewhat longer in genera *Disanthus* and *Hamamelis* (.5 mm to 1.5 mm). Longer filaments were measured in genus *Corylopsis*, ranging from 3.4 mm (*C. glabrescens* and *C. willmottiae*) to 7.8 mm (*C. sinensis*). A similar diverse pattern is evident for cluster 3, starting with missing filaments for *Fortunearia* and going up to 8.9 mm for *Parrotia* and 14.8 mm for *Sycopsis*.

Style length shows high variation, too. In cluster 1, *Parrotiopsis* with 4.5 mm is rather in the middle range of the family´s spectrum, while both *Fothergilla* species are at the upper bound (*F. gardenii* – 9.3 mm; *F. major* – 9.9 mm). In cluster 2 style length is starting with .1 mm fixed for *Loropetalum*, going up to 2.3 mm for *H. japonica*, but is distinctly longer in genus *Corylopsis*, ranging from 5.0 mm in *C willmottiae* up to 11.0 mm on average for *C. sinensis*. In cluster 3 style length is starting with 1 mm fixed for *Distyliopsis tutcheri*, going up to 6.9 mm in *Distylium racemosum* and *Fortunearia*.

Largest **frontal inflorescence sizes** were observed for cluster 1, with 4.8 cm^2 for *Parrotiopsis* and 6.7 cm^2 on average for both species of *Fothergilla*. Next in line is *Loropetalum* with 4.2 cm^2, a member of cluster 2, such as the following genera: In genus *Hamamelis* average frontal size is ranging from .8 cm^2 for *H. virginiana* to 2.1 cm^2 for *H. mollis*. In genus *Corylopsis* frontal inflorescence size is ranging from 1.2 cm^2 for *C. pauciflora* to 3.3 cm^2 in *C. sinensis*. In cluster 3 frontal inflorescence size always stays below 2 cm^2, but is usually much lower. Smallest frontal inflorescence sizes were observed for *Distyliopsis* (.3 cm^2) and *Disanthus* (.2 cm^2, cluster 2).

Regarding **lateral inflorescence size**, cluster 1 is at the upper bound of the spectrum again (*Parrotiopsis* – 3.0 cm^2; *F. major* – 7.1 cm^2; *F. gardenii* – 7.6 cm^2), but larger lateral inflorescences were also observed for the two other clusters, namely for *Sinowilsonia* (cluster 3) with 2.5 cm^2 for female and 4.4 cm^2 for male inflorescences, *Fortunearia* (2.7 cm^2, cluster 3), *Loropetalum* (3.3 cm^2, cluster 2) and *Corylopsis sinensis* (6.3 cm^2, cluster 2). Other species of *Corylopsis* are ranging between 1.1 cm^2 (*C. glabrescens*) and 4.6 cm^2 (*C. veitchiana*). Lateral inflorescence sizes between one and two cm^2 were further obtained in cluster 3 for *Distylium*, *Sycopsis* and *Parrotia*. In genus *Hamamelis* (cluster 2) lateral inflorescence size is ranging between .5 cm^2 (*H. virginiana*) and 1.7 cm^2 (*H. mollis*). As with frontal inflorescence size, smallest lateral size was observed for *Disanthus* (.3 cm^2, cluster 2) and *Distyliopsis* (.4 cm^2, cluster 3).

Optical flower signal is present in cluster 1 and 2, but missing in cluster 3. In cluster 1 perianth organs are reduced or missing, but optical signal is realised by showy and white filaments in *Fothergilla* and white bracts in *Parrotiopsis*. In cluster 2 optical flower signal is realised by intense colour of perianth organs: Petals of *H. virginiana* and *H. mollis* are coloured yellow, and yellow-orange for *H. japonica* and *H. vernalis*. *Loropetalum* has white petal colour, but plants with reddish flowers do exist in the field, too. *Disanthus* has red flowers, and genus *Corylopsis* is flowering in yellow. In cluster 3 species are either apetalous or have only very reduced perianth organs.

As for **anther colour,** *Fothergilla* species in cluster 1 have a yellow colour when young, later turning white. Anthers and filaments of *Parrotiopsis* have a strong yellow colour. In cluster 2 stamens are yellow for *Hamamelis virginiana* and red for *H. mollis*, while *H. japonica* and *H. vernalis* have yellow thecas and otherwise reddish stamens. Stamens of *Loropetalum* have a greenish to yellow colour. In genus *Corylopsis* anthers are yellow for most of the species, except *C. sinensis* and *C. spicata* showing intense red anthers. Same anther colour is present in *Disanthus*, too. Most of the species of cluster 3 have dark red anthers, namely *Distyliopsis*, *Fortunearia*, both species of *Distylium* and *Parrotia*. The only exceptions are *Sinowilsonia* with greenish to yellow stamens and *Sycopsis* with yellow to orange anthers and white filaments.

Nectar reward is completely absent in cluster 1 and 3. In cluster 2 nectar reward is ranging from traces to substantial amounts: *Corylopsis* species, apart from *C. veitchiana*, have considerable nectar production (cf. table A X.2.4, appendix). Average nectar production per flower ranges from .03 µl (*C. glabrescens*) to .19 µl (*C. willmottiae*). Nectar concentration ranges from 22.6 percent (*C. spicata*) to 40.8 percent (*C. willmottiae*). Average sugar production per flower ranges from .01 mg (*C. glabrescens*) to .076 mg (*C. spicata*). *Hamamelis* and *Disanthus* have only traces of nectar production, documented by CSEM (cf. figure A X.2.3, appendix). Amounts were insufficient for measurements, but fast crystallisation was observed, indicating higher sugar concentrations. Absence of nectar production could be verified for *Loropetalum, Fothergilla* and *Parrotiopsis* via CSEM. Similarly, species in cluster 3 did not show any nectar production.

III.4.3 Reciprocal NMDS ordinations and vector fitting reveal high integration

Figure III.4 shows reciprocal NMDS ordinations and vector fitting of gametic and non-gametic variables. For NMDS ordination of gametic variables, model stress of 0.057 indicates a good fit, with two convergent solutions found after 20 tries. The ordination retrieves the split into two major gametic clusters A and B. Results of vector fitting of non-gametic variables onto this ordination are also presented in table III.1. Highly significant vectors with most explanatory value for gamete production are anther number per inflorescence (r^2 = .71, p = .002) and flower number per inflorescence (r^2 = .66, p = .001), followed by lateral inflorescence size (r^2 = .58, p = .002), anther length (r^2 = .55, p = .002), anther number per flower (r^2 = .44, p = .005) and showy colour signal (r^2 = .37, p = .009). Significant vectors are frontal inflorescence size (r^2 = .43, p = .011) filament length (r^2 = .34, p = .013), presence of (functional) unisexual inflorescences (r^2 = .32, p = .026) and nectar reward (r^2 = .31, p = .026). Most of the significant vectors are oriented towards cluster A, variables "nectar reward" and "showy colour signal" are oriented towards cluster B, however.

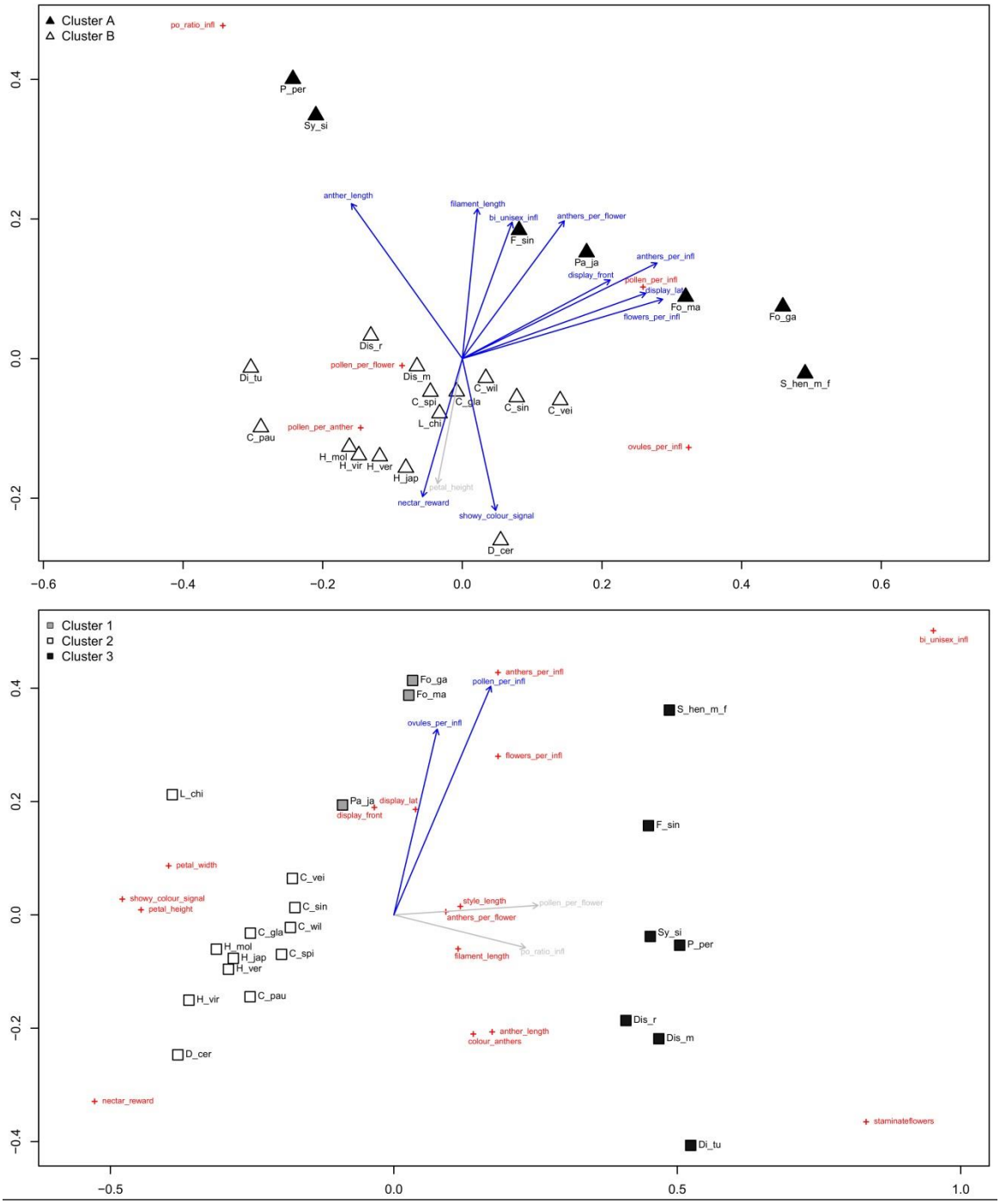

FIGURE III.4: RECIPROCAL NMDS AND VECTOR FITTING OF GAMETIC AND NON-GAMETIC DATASETS OF HAMAMELIDACEAE

Top: Result of NMDS for gametic variables (red), with retrieved species clusters A and B marked and non-gametic variables (morphology, signal, nectar reward) fitted as vectors onto the ordination. An NMDS stress value of 0.057 indicates good fit (two convergent solutions after 20 tries). Bottom: Result of NMDS for non-gametic variables (red), with retrieved species clusters 1, 2 and 3 indicated and gametic variables fitted onto the ordination. NMDS stress: 0.093, good fit (two convergent solutions after 20 tries). Vectors showing direction of most rapid change in the variable within the dataset; strength of the gradient is indicated by vector length, representing the correlation between the ordination and the vector. Significant vectors are coloured blue, vectors only appearing as a trend are coloured grey. Species names abbreviated.

TABLE III.1: VECTOR FITTING OF NON-GAMETIC VARIABLES ONTO GAMETIC ORDINATION OF GERANIALES
The first two columns show the direction cosines of the vectors, r^2 gives the squared correlation coefficient, *p* values are based on 999 random permutations of the data (significance codes: 0.001 = *** / 0.01 = ** / 0.05 = * / 0.1 = .).

vector	*NMDS1*	*NMDS2*	r^2	*p*	
anther length	-0.58211	0.81311	0.5505	0.002	**
filament length	0.10072	0.99491	0.3432	0.013	*
style length	0.57588	0.81753	0.1740	0.154	
anthers per flower	0.59472	0.80393	0.4450	0.005	**
flowers per inflorescence	0.95877	0.28417	0.6619	0.001	***
anthers per inflorescence	0.89763	0.44075	0.7132	0.002	**
presence/absence of staminate flowers	0.26759	0.96353	0.0457	0.652	
bi- vs. (funct.) unisexual inflorescences	0.34296	0.93935	0.3197	0.026	*
petal length	-0.19422	-0.98096	0.2460	0.069	.
petal width	0.83190	-0.55493	0.0193	0.800	
showy colour signal	0.21421	-0.97679	0.3661	0.009	**
anther colour	-0.72915	0.68435	0.1908	0.134	
nectar reward	-0.27680	-0.96093	0.3128	0.026	*
inflorescence size frontal	0.88293	0.46951	0.4250	0.011	*
inflorescence size lateral	0.94194	0.33577	0.5753	0.002	**

NMDS ordination of non-gametic variables (morphology, optical signal, nectar reward) also shows good fit (stress: 0.093, two convergent solutions found after 20 tries).
The ordination retrieves the functional clusters detected by hierarchical clustering. Vector fitting of gametic variables onto this ordination is presented in table III.2. Highly significant and strong vectors with most explanatory value for flower and inflorescence function are pollen production per inflorescence (r^2 = .76, p = .001) and ovule production per inflorescence (r^2 = .45, p = .002). Pollen production per flower and p/o ratio only appear as a trend, pollen production per anther is non-significant.

TABLE III.2: VECTOR FITTING OF GAMETIC VARIABLES ONTO NON-GAMETIC NMDS ORDINATION OF GERANIALES
The first two columns show the direction cosines of the vectors, r^2 gives the squared correlation coefficient, *p* values are based on 999 random permutations of the data (significance codes: 0.001 = *** / 0.01 = ** / 0.05 = * / 0.1 = .).

vector	*NMDS1*	*NMDS2*	r^2	*p*	
pollen per anther	0.68682	-0.72683	0.2143	0.110	
pollen per flower	0.99785	0.06553	0.2571	0.061	.
pollen per inflorescence	0.39057	0.92057	0.7600	0.001	***
ovules per inflorescence	0.22756	0.97376	0.4480	0.002	**
p/o ratio	0.97036	-0.24168	0.2269	0.071	.

III.4.4 FLORAL FUNCTION AND FLOWERING TIME ARE SEPARATED

Regarding flowering time, taxa identified as spring flowering are *Fothergilla*, *Parrotiopsis*, *Sinowilsonia*, *Corylopsis*, *Loropetalum*, *Fortunearia* and *Distylium*. *Disanthus* and *Hamamelis virginiana* flower in autumn, other *Hamamelis*, *Sycopsis*, *Parrotia* and *Distyliopsis* in winter (cf. appendix, table A X.2.5).
For the final model, hierarchical clustering was performed with gametic and non-gametic variables at once. In general, emerging plant trait clusters I, II and III are identical with non-gametic clusters 1, 2 and 3. However, *Sinowilsionia* appears as a separate cluster next to *Fothergilla* and *Parrotiopsis* (labelled cluster I a and I b). When flowering time is added to the calculations, the same clusters are retrieved in essence, but *Sinowilsonia* aligns with cluster 3 (cf. appendix, figures A X.2.4 and A X.2.5).

NMDS performed on gametic and functional variables plus flowering time is shown in figure III.5. An NMDS stress value of 0.092 indicates good fit of the model, with two convergent solutions found after 20 tries (ordination without flowering time similar; model stress: 0.093, good fit, two convergent solutions after 20 tries). Spring and winter flowering taxa are equally represented in clusters II and III. Permutational Multivariate Analysis of Variance shows that flowering time is separated from plant trait clusters (PERMANOVA: non. sig., p = .124).

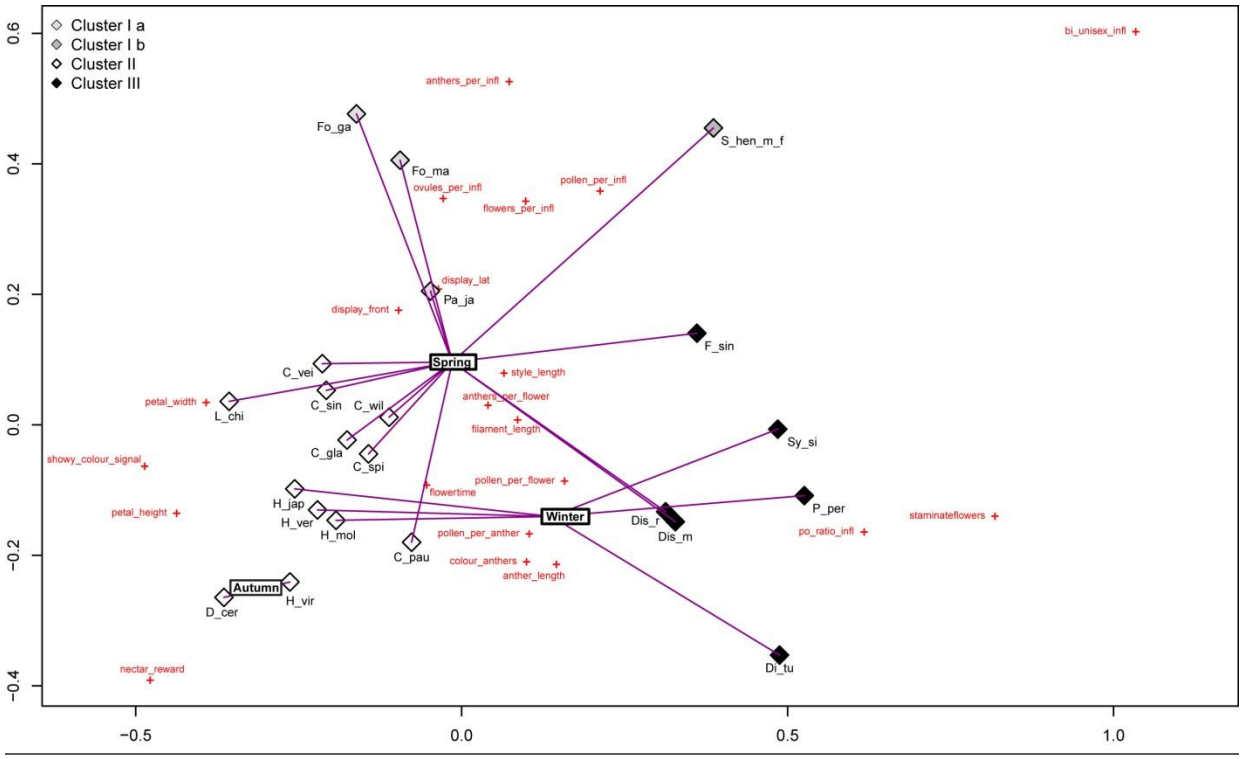

FIGURE III.5: NMDS OF GAMETIC AND NON-GAMETIC DATASETS COMBINED, AND VISUALISATION OF FLOWERING TIME
NMDS ordination performed on gametic and non-gametic variables (morphology, optical signal and nectar reward) at once, including flowering time. NMDS stress value: 0.092, good fit (two convergent solutions after 20 tries). Plant trait clusters retrieved from hierarchical clustering are indicated by colouration of rhombs. Flowering time visualised by centroids, showing flowering time disjunct from plant trait clusters (PERMANOVA: non. sig., *p* = .124). Species names abbreviated.

III.4.5 RETRIEVED CLUSTERS EVOLVED MORE THAN ONCE AND SHOW A STRONG PHYLOGENETIC SIGNAL

Results of phylogenetic analysis for ETS and ITS markers are presented in figure III.6. Retrieved floral trait clusters and flowering time are mapped onto the tree. In general, branch support is high. *Exbucklandia* (Hamamelidaceae) is sister clade to analysed members of the family, and Hamamelidaceae appear monophyletic. Within this clade *Disanthus* is sister to all other species, these forming two well supported clades. Genus *Hamamelis* is nested within the fothergillid clade. Representatives of tribe Eustigmateae, *Fortunearia* and *Sinowilsonia*, form a well-supported clade sister. *Corylopsis* together with *Loropetalum* form another clade. The described pattern is supported by phylogenetic analysis of chloroplast DNA, and trees are highly congruent (cf. cpDNA tree, figure A X.2.6 in the appendix).

Plant trait cluster I_a, represented by *Parrotiopsis* and both species of *Fothergilla*, is polyphyletic: While *Parrotiopsis* is sister to *Distylium*, *Distyliopsis*, *Sycopsis* and *Parrotia* (all cluster III), both *Fothergilla* species form a sister clade to the aforementioned species plus *Hamamelis*. *Sinowilsonia* (cluster I_b) and *Fortunearia* (cluster III), appear as sister clade to them. Cluster II is represented in three phylogenetic clades: Genus *Hamamelis*, genus *Corylopsis* plus *Loropetalum* as well as *Disanthus*, forming an isolated phylogenetic branch.

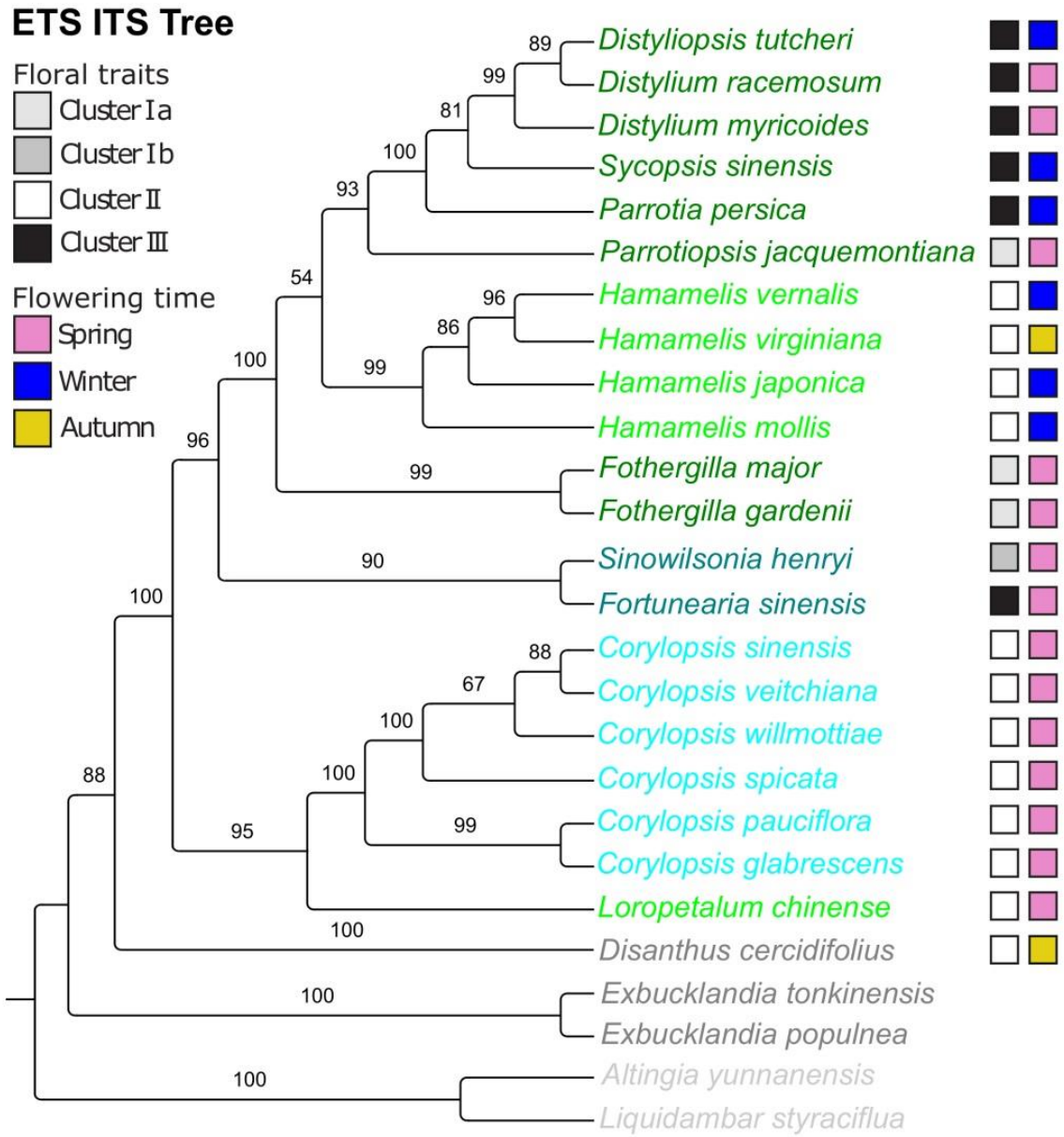

FIGURE III.6: ETS-ITS MAXIMUM LIKELIHOOD TREE FOR HAMAMELIDACEAE
Tree rooted with the Altingiaceae. Values corresponding to bootstrap support are presented above branches. Flowering time and retrieved clusters of floral plant traits indicated. Species names coloured according to taxonomic classification: Altingiaceae in light grey; Hamamelidaceae, subfamily Exbucklandioideae (including Disanthoideae) in dark grey. Hamamelidaceae, subfamily Hamamelidoideae: Tribe Hamamelideae in light green, tribe Fothergilleae in dark green, tribe Eustigmateae in bluish green, tribe Corylopsideae in aqua. Compare also cpDNA Tree in appendix (figure A X.2.6).

Phylogenetic signal could be traced better via nuclear- than chloroplast-based trees (see appendix, table A X.2.8 for exact test results). In general, phylogenetic signal is strong for all levels of analysis. Regarding aggregated information, such as NMDS species coordinates and retrieved clusters (gametic, floral function, combined plant traits), phylogenetic signal is strong without exceptions (pagel´s lambda ~ 1). Same is true for most isolated tests of floral plant traits, except for pollen production per anther and per flower, p/o ratio, filament length, presence of hermaphroditic vs. (functionally) unisexual inflorescences as well as showy colour signal of flowers, bracts or filaments (pagel´s lambda equaling 0 for all).

Although flowering time is not coupled to floral trait clusters, phylogenetic signal is present nevertheless: Spring flowering is present in the *Corylopsis* + *Loropetalum* clade, in analysed members of tribe Eustigmateae and genus *Fothergilla*, while occasionally occurring in other members of Fothergilleae. Genus *Hamamelis* tends to flower in autumn and winter.

III.5 DISCUSSION

III.5.1 RETRIEVED CLUSTERS OF FLORAL FUNCTION ARE HIGHLY INDICATIVE FOR POLLINATION SYNDROMES

Anemophily is present in members of clusters III and I_b: Species in these clusters are lacking optical flower signal and nectar reward, and pollen production is extremely high in order to secure pollination via transmission of male gametes through the air. Moreover, p/o ratio is dramatically increased in three species by reduction of ovules during anthesis. Phylogenetic results indicate that anemophily is represented by two clades, namely in the *Distylium*, *Distyliopsis*, *Sycopsis*, *Parrotia* clade, and the Eustigmateae, *Fortunearia* and *Sinowilsonia* clade.

Zoophily is present in members of cluster II: All species have a showy perianth and are able to reward possible pollinators by production of nectar, only exception is *Loropetalum* with missing nectar. Pollen production, ovule numbers and p/o ratios are much lower than in wind pollinated species of the family, also a common indicator for zoophily (cf. Cruden, 2000; Willmer, 2011). Phylogenetically, zoophily is represented by three clades analysed here, but for these clades only two evolutionary events are likely, due to the fact that evolution of zoophily in *Disanthus* is assumably plesiomorphic to the clade of *Corylopsis* and *Loropetalum*. Genus *Hamamelis* represents a separated evolution of zoophily in Hamamelidaceae then. Although not analysed here, additional evolutionary events fostering zoophily have to be assumed for Dicoryphinae (members such as *Trichocladus* and *Dicoryphe* do possess a showy perianth), as well as for *Rhodoleia*: Passerine pollination is verified for *Rhodoleia championii* (Gu et al., 2010).

Mixed pollination modes have to be assumed for *Parrotiopsis* and both species of *Fothergilla*, forming cluster I_a. While production of pollen and ovules rather resembles or even surpasses the production rates of anemophilous Hamamelidaceae in order to secure abiotic pollination, p/o ratios are lowered to some degree, which could be indicative for a more effective way of transmission of male gametes via animal vectors. A strong optical signal is here provided by bracts resp. filaments rather than the perianth, also arguing for biotic pollination. Nectar reward is missing, which might be suitable from a resource perspective when already investing in higher gamete production in order to secure abiotic pollination and/or pollinator reward (pollen as nutrition).

Flower visitors have been observed in Bonn Botanical Garden on species of clusters I_a and II (cf. figure A X.2.1, appendix), lending further support to the likelihood of animal vectors in the field. Moreover, colourful periants and showy bracts and filaments go hand in hand with UV light absorption (cf. figure A X.2.2, appendix). While not used for statistical analysis due to the strong blending with a showy visual signal, presence of UV light absorption together with showy colour is of relevance for pollinator attraction in purely zoophilous as well as ambophilous members of the family: UV light absorption plays a strong role for the purification of colour signal in order to attract pollinators (cf. Lunau, 1992, 2006), therefore also indicating zoophily. Although of no explanatory statistical value in comparison to other variables, the observed colouration of anthers in clusters I_a and II might play the same role. For the ambophilous species assessed here, pollinator attraction via UV absorption and colour is transferred to bracts (*Parrotiopsis*) and stamen filaments (*Fothergilla*). Contrary to this, the combination of dark red coloration and UV light absorption of anthers in purely anemophilous species of cluster III can be interpreted as a protective mechanism against pollen theft, due to missing or weak sensory capabilities of most insect taxa regarding red colour perception (Willmer, 2011; Chittka and Thomson, 2001).

For the dataset here discussed, zoophilous pollination modes join into a single cluster that separates from ambophily and anemophily. The zoophilous cluster is not divided further into distinctive **zoophilous pollination syndromes**. However, floral architecture and optical flower signal in *Hamamelis*, *Loropetalum* and *Disanthus*, as well as low amounts of fast crystallising (assumedly sugar rich) nectar traces in *Hamamelis* and *Disanthus* indicate short-probiscid pollinator guilds. Flies were observed as flower visitors fo *Hamamelis virginiana* in Bonn Botanical Garden. As for *Corylopsis*, bee pollination seems likely, due to optical flower signal, more concealed nectar reward, higher nectar amounts, nectar concentrations between approx. 30 to 40 % and anecdotal observations of bees as flower visitors in Bonn (see figure A X.2.1 in the appendix). Field observations are necessary to research the degree of pollinator specialisation or generalisation in zoophilous and ambophilous species of Hamamelidaceae. Moreover, enhanced quantification of variables that were only introduced as categorical to the calculations might further contribute to the differentiation of biotic pollination syndromes. Low nectar amounts with assumedly higher nectar concentration in *Hamamelis* and *Disanthus* e.g. could not be quantified with our method, but are very likely a crucial factor for the characterisation of a short-proboscid (fly) pollination syndrome.

Sinowilsonia (cluster I_b) represents a special case. It might be best interpreted as forming a wind pollination clade together with *Fortunearia* (cluster III) because of its high pollen production and the lack of nectar reward and optical signal. However, p/o ratio is much lower than in other species of gametic cluster A (*Parrotia*, *Sycopsis*, *Fortunearia*). It remains speculative, but ***Sinowilsonia*** **might alternatively also be interpreted as a species showing mixed pollination mode**, being highly similar in p/o ratios to both *Fothergillas* and *Parrotiopsis*, possibly because of improved effectiveness of pollen transmission by animal vectors beside possible wind pollination: Instead of showing an optical signal like cluster I_a, key attractant for animal vectors might be odour (see appendix, table A X.2.3), but more in depth research and field studies are needed on this topic.

III.5.2 Five to six independent origins of zoophily in Hamamelidaceae

Our phylogenetic analysis using the nuclear markers ITS and ETS and four plastid markers essentially retrieved the topology shown in Xie et al. (2010): Nuclear and plastid marker trees are highly congruent.

Subfamily Hamamelidoideae is retrieved as monophyletic, with *Disanthus* as basally branching taxon (Qiu et al., 1998; Soltis et al., 2000; Magallón et al., 2007). Latest phylogenetic analysis of Xiang et al. (2019), presenting the first time calibrated and complete genus-level sampling of Hamamelidaceae, is generally in line with our results. Only exception is genus *Hamamelis*: While nested within the fothergillid clade in our analysis, Xiang et al. (2019) retrieved the genus as separate clade, which diverged before tribe Fothergilleae.

Zoophily is scattered across three clades: Genus *Hamamelis*, genus *Corylopsis* plus *Loropetalum* as well as *Disanthus*. Although *Loropetalum* forms a clade with *Corylopsis*, floral morphology and inflorescence setup rather resembles *Hamamelis* and could be indicative for another additional origin of zoophily in Loropetalinae. Moreover, two additional clades not assessed here (Dicoryphinae and *Rhodoleia*) have to be counted amongst family members with zoophilous pollination mode, too. These five to six origins of zoophily might have to be considered as apomorphic. The phylogenetic distribution of zoophily is therefore of explanatory value for the high floral diversity in Hamamelidaceae, all zoophilous clades are characterised by extremly different and indepentently evolved floral morphology.

Latest Supermatrix approach for analysis of phylogenetic relationships and character evolution of Saxifragales by Soltis et al. (2013) shows phylogenentically neighbouring families to be anemophilous, missing a floral display (Altingiaceae, Cercidiphyllaceae, Daphniphyllaceae (monotypic, *Daphniphyllum*)). In flowering plants, anemophily is a derived condition, and has arisen independently in many families (Friedman and Barrett, 2009). Due to this, zoophilous pollination syndromes in Hamamelidaceae originated several times indepentendly from a putatively derived, anemophilous condition.

III.5.3 AMBOPHILY EVOLVED EARLY – ARGUING AGAINST AN EPHEMERAL TRANSITIONAL STAGE

It is an open debate in botany whether ambophily is a stable strategy or a transitional stage to either anemophily or zoophily (Culley et al., 2002). Our data, combined with time calibrated phylogenies of Hamamelidaceae reported in literature, might shed some light onto this question: An Eocene origin of mixed pollination modes of *Fothergilla* and *Parrotiopsis* has to be assumed at least (see Xie et al., 2010; Jaramillo et al., 2010). Moreover, Radke et al. (2005) report *Fothergilla* leaf fossils from the lower Eocene (49–50 million years ago) of northeastern Washington State, whereas the genus is today restricted to the eastern US. Furthermore, the latest time calibrated phylogeny with complete genus-level sampling and consideration of 22 fossils of Xiang et al. (2019) presents an estimated stem group age of 108.4 Ma years for Hamamelidaceae in the Lower Cretaceaous, much older than previously estimated (e.g. Magallón et al., 2015). Genera *Fothergilla* and *Parrotiopsis* appear to have a common ancestor, and estimated divergence time is 74.9 Ma year ago in the Upper Cretaceous.

Extant ambophilous taxa *Fothergilla* and *Parrotiopsis* have therefore to be considered old, and ambophily cannot represent an ephemeral transitional stage. It rather represents a functional and effective pollination strategy in Hamamelidaceae, that even withstood the family's evolutionary bottleneck of the Pleistocene Ice Age (Walther, 1980; Maslova, 2003; Mai, 2001; Shatilova and Mchedlishvili, 2011; Huang et al., 2017).

III.5.4 Flowering at the fringe of the growth season evolved disjunct from pollination syndromes, and at least three times in Hamamelidaceae

Different pollination syndromes have evolved at least three times at the extreme margins of the growth season: Wind pollinated genera *Distyliopsis*, *Parrotia* and *Sycopsis* form a clade and are winter flowering. All members of genus *Hamamelis* are flowering at the fringe of the season (autumn and winter) and show a zoophilous pollination syndrome. Phylogenetically isolated *Disanthus* is also zoophilous and autumn flowering. As for the zoophilous species, one might suggest that this investment in optical flower signal and nectar reward does not pay out due to the pollinator scarcity in autumn and winter, however it does, as the phylogenetic data shows: Biotic and abiotic pollination out of the growth season have developed several times, showing that the observed pattern are no dead ends, but were favoured several times during the course of the evolution. Flowering time and floral function appear as disjunct variables, that were recombined quite freely in Hamamelidaceae. However, some phylogenetic trends are obvious: Mixed-pollination syndromes seem to go hand in hand with spring flowering, and autumn flowering is coupled with zoophily for the species analysed.

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III.7 Literature

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IV. NO INDICATION OF A POLLINATOR-PREY CONFLICT IN FLORAL FUNCTIONAL TRAITS OF CARNIVOROUS PLANTS OF THE ACTIVE FLYPAPER TYPE – *PINGUICULA* (LENTIBULARIACEAE) AND *DROSERA* (DROSERACEAE)

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IV.1 ABSTRACT

Background and aims: The bulk of carnivorous (essentially insectivorous) plants are entomophilous. This has led to the hypothesis that these plants might suffer a "pollinator-prey conflict", compromising pollination services by trapping pollinators. The present study aims at investigating floral function in two prominent carnivorous genera based on a broad set of floral functional parameters. Breeding systems, pollen and ovule numbers and characters regarding optical flower signal (display size, colour, UV light pattern) and nectar reward are investigated for 19 species of *Pinguicula* (Lentibulariaceae) and 17 species of *Drosera*. Closely related *Dionaea* is added for comparison of floral functional traits.

Methods: Pollen and ovule counting procedures, pollination experiments, refractometer nectar analysis, UV light photography, Scanning Electron Microscopy (SEM), bivariate correlations, nonparametric group comparisons.

Key results: *Pinguicula* and Droseraceae differ strongly in flower symmetry and pollinator positioning. Patterns of optical signal in flowers (colouration, UV light reflection/absorption) are generally strongly contrasting to trap leaves. Nectar as a reward could be verified for *Pinguicula* and *Dionaea*, but not for *Drosera*. Autogamy as well as (facultative or obligate) xenogamy are found in Droseraceae, while *Pinguicula* is found to be (facultatively or obligately) xenogamous. Pollen is dispensed in tetrads in Droseraceae, but as single pollen grains in *Pinguicula*. The p/o ratios found in both genera are extremely low compared to other xenogamous, entomophilous species. They do not differ significantly between *Pinguicula* and xenogamous *Drosera* species.

Conclusions: Highly conserved optical flower signal, strongly contrasting to trap leaves, indicates an important role for segregation of pollinators and prey. The overall low p/o ratios in the carnivorous plants here investigated – independent of breeding system – clearly argue that there is no pollinator-prey conflict. The zygomorphic and highly sophisticated flower structure in *Pinguicula* and the synorganisation of pollen material into tetrads in Droseraceae likely represent alternative ways of optimising pollen transfer in xenogamous breeding systems.

Key words: Carnivory, pollinator-prey-conflict, *Drosera*, *Dionaea*, *Pinguicula*, p/o ratio, pollen dispersal unit, breeding system, optical flower signal, attraction.

IV.2 INTRODUCTION

It is remarkable that some of the most dangerous plants in the lifeworlds of insects also are having very conspicuous flowers – carnivorous plants. Seemingly a paradox comes with it, a pollinator-prey conflict inherently based on two competing needs: On the one hand, animals are trapped and used as energy source to compensate for the nutrition-deprived habitats of carnivorous plants, and on the other hand are needed for pollination to secure outcrossing and survival of the species in the long-run (figure IV.1). This raises the question how successful pollination is secured by carnivorous plants, because pollen loss by pollinators becoming prey could be considered as major problem to deal with.

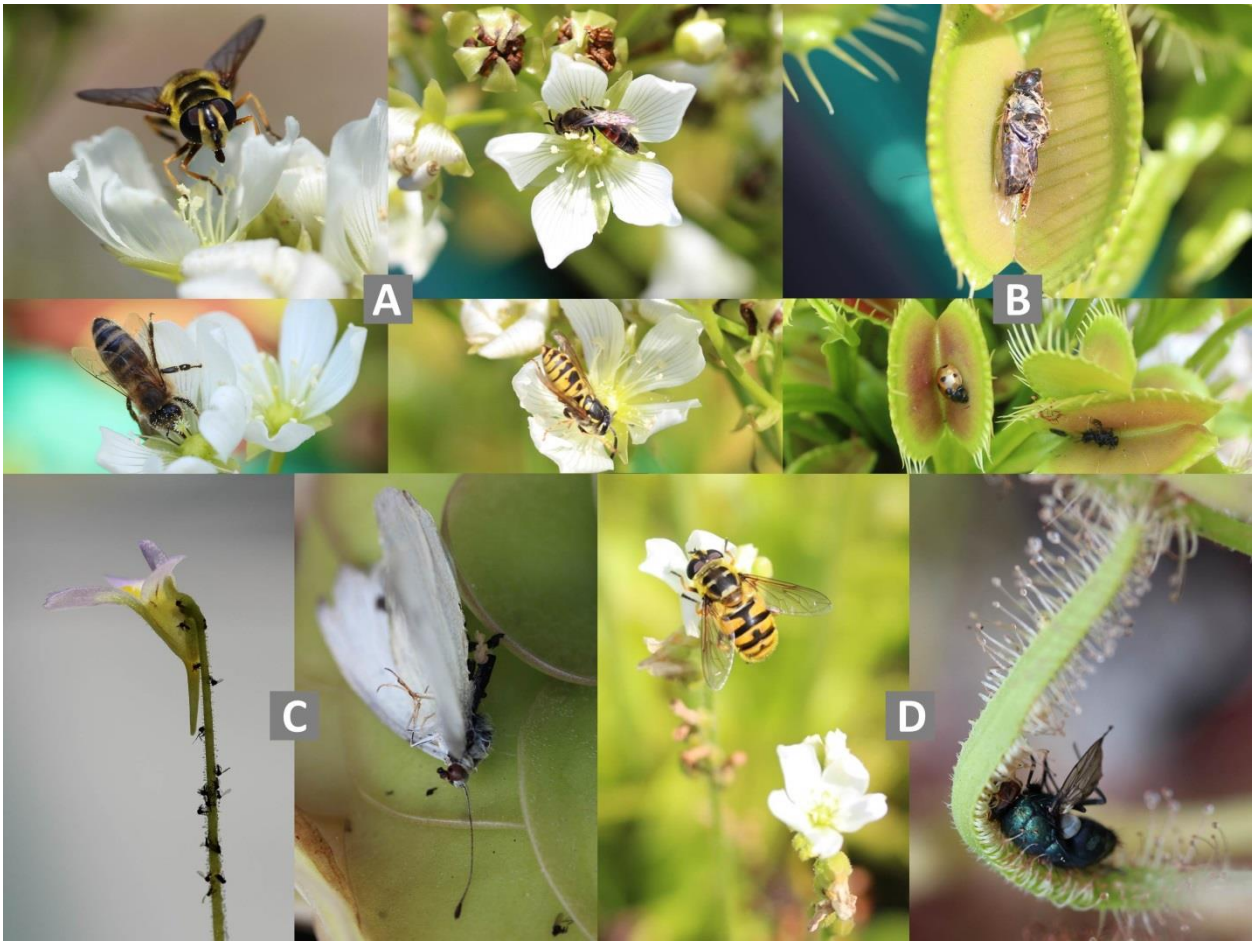

FIGURE IV.1: DEPICTIONS OF PLANT-ANIMAL INTERACTIONS IN DROSERACEAE AND *PINGUICULA*
Carnivorous plant families and genera evolved in zoophilous orders, and have maintained animal pollen vectors. Droseraceae and *Pinguicula* species are all insect-pollinated: For *Dionaea muscipula* various Diptera and Hymenoptera were observed as pollinators in Bonn University Botanic Gardens (A: Syrphid fly, upper left; honeybee, lower left; wild bee, upper right; wasp, lower right), which were also captured as prey, beside other insect taxa usually described as prey spectrum (B: Honeybee exoskeleton, top; exoskeletons of a ladybug and a fly, bottom). For genus *Pinguicula*, Bombyliidae spec. were observed visiting the flowers, and a pronounced nectar spur developed by most of the species makes pollination by long-tongued insect taxa likely (C, left: Fungus gnats caught on the sticky flower stalk of *P. hirtiflora var. hirtiflora*; C, right: Cabbage white butterfly caught on leaves of *P. hemiepiphytica*). For *Drosera*, observed insect taxa visiting the flowers were Hymenoptera and Diptera, which were also caught as prey (D, left: Syrphid fly visiting a flower of *D. capensis f. alba*; D, right: Fly caught by the sticky leaves of *D. capensis f. alba*). Pictures C-right and D-right by S. Brauwers, others by A. W. Mues.

The presence or absence of such a conflict in carnivorous plants is open to debate in botanical science. In their review article, Ellison and Gotelli (2009) point out that carnivorous plants have to deal with such a conflict: Although different types of carnivorous genera do show a specialisation to different prey taxa, the captured prey does not differ from the relative abundance of potential prey species in the environment. The degree of prey specialisation does not differ from simple passive traps, which capture rates can be largely understood on basic geometric variables like size, shape and orientation. However, this general conclusion might be too simplistic. In another review article, Jürgens et al. (2012) criticise that pollinator-prey conflict often has been assumed only on overlap of possible pollen vectors and prey taxa on higher taxonomic levels, disregarding the fact that flower visitors not necessarily are valid pollinators, and even a true pollinator-prey overlap must not necessarily lead to a pollinator-prey conflict, i.e. when pollen or pollinators are plenty, or only ineffective pollinators are caught. Although literature on carnivorous plants as a charismatic plant group is vast, pollinator-prey overlap has been quantified explicitly in only few published studies (Jürgens et al., 2012, citing: Moran, 1996; Zamora, 1999; Murza et al., 2006; Anderson, 2010; see also Jürgens et al., 2015; El-Sayed et al., 2016; Youngsteadt et al., 2018). The authors further point out that a valid pollinator-prey conflict only can occur under the following three preconditions: 1. dependence on animal pollen vectors for reproduction (i.e. no selfing mechanism), 2. pollen vectors and prey do overlap, and 3. pollination success is limited (in regard to quantity and/or quality of transferred pollen). To alleviate a possible pollinator-prey conflict, two possible mechanisms can occur in carnivorous plants (compare Jürgens et al., 2012, in the following): 1. reproductive assurance mechanisms, and 2. specialisation towards pollinator and prey, further distinguished into strategies of temporal and spatial separation, as well as specialised attraction and guidance of animal taxa.

Reproductive assurance mechanisms are vegetative reproduction as well as obligate or facultative autogamous breeding systems, allowing for propagation in absence of animal pollen vectors – which are indeed reported in literature (Wilson, 1995; Chen et al., 1997; Zamora, 1999; Murza and Davis, 2003; Murza and Davis, 2005; Murza et al., 2006; Sciligo, 2009). No general trend towards breeding systems seems to be present; the full spectrum from obligate autogamous to obligate xenogamous breeding systems is reported for carnivorous plants (Jürgens et al., 2012). However, the full picture is far from complete, and breeding systems are unknown for most carnivorous plant species.

As for specialisation towards pollinators and prey, temporal and spatial separation of traps and flowers can completely prevent pollinator-prey overlap in its extreme forms, i.e. when trap leaves and flowers are not present or active at the same time, or perfectly spatially separated by submerged aquatic traps in *Utricularia*, *Genlisea* and *Aldrovanda* (cf. Jürgens et al., 2012; Anderson and Midgley, 2001). In intermediate forms (i.e. flowers and traps active at the same time, but separated by the length of the flower stalk), temporal and spatial separation can alleviate pollinator-prey conflict, i.e. by partitioning insects of the same taxa or sorting of different taxa as prey or pollination agents, due to their diurnal, seasonal and spatial activity pattern (cf. Jürgens et al., 2012).

Attraction and guidance of pollinators and prey extends this sorting of animal taxa to the level of specialised optical and/or olfactory signal of traps and flowers, as well as reward. Minnaar et al. (2019) give an excellent overview of plant traits relevant for siring success of flowering plants in general, like floral display size (e.g. Harder and Barrett, 1995; Karron et al., 2012); corolla tube shape (Kulbaba and Worley, 2012, 2013); scent (Kessler et al., 2008; Larue et al., 2015), colour (e.g. overall colour (Stanton

et al., 1986), colour pattern (de Jager et al., 2016; Kemp et al., 2019), colour brightness and contrast (Sletvold et al., 2016)) as well as shape and symmetry (e.g. Møller, 1995; Gómez et al., 2006). In extension to flower symmetry and "mechanical fit in plant–pollinator interactions", securing the plant´s control over pollinator interactions, Minnaar et al. (2019) further highlight the importance of pollen presentation traits and pollen aggregation.

As for carnivorous plants, Jürgens et al. (2012) point out three possible attraction and guidance strategies to alleviate pollinator-prey conflict: 1. attraction of pollinators to flowers, 2. distraction of pollinators from traps and 3. attraction of prey taxa to traps. Astonishingly, studies explicitly quantifying the specific influence of plant traits on pollinator and prey attraction or distraction from flowers or traps are rare: Jürgens et al. (2015) experimentally tested the effect of leaf colour and flower-trap distance for *Drosera arcturi* and *D. spatulata*. Flower-trap distance had no significant effect on trapping of pollinators, but higher model flowers received more pollinator visitations. However, potential pollinators were trapped significantly less by traps with red leaves compared to green ones. Pollinator distraction was therefore induced by trap colour. El-Sayed et al. (2016) demonstrate utilisation of colour signal and spatial separation of traps and flowers in *Drosera spatulata* and *D. arcturi*, as well as additional chemical signals in *D. auriculata* to separate pollinators from prey taxa. Reports that only *indicate* the possible role of plant traits for luring of prey taxa to traps are more frequent, e.g.: Reward, like extra-floral nectar, offered by traps (Jürgens et al., 2012, citing Juniper et al., 1989; Dress et al., 1997; Deppe et al., 2000; Merbach et al., 2001), UV light reflection and/or absorption pattern in traps, especially close to the capture spots (Jürgens et al. 2012, citing Glossner, 1992; Moran, 1996; Moran et al., 1999; see also Joel et al. (1985) on UV pattern and glistening of mucilage, and Kurup et al. (2016) for distinct blue fluorescence emission at trap entrances), as well as odour emitted from traps (Jürgens et al., 2012, citing e.g. Miles et al. 1975; Moran, 1996; Moran et al., 1999; note Jürgens et al., 2009). However, scientific analysis of attraction or distraction of pollinator and prey taxa mainly focuses on attraction and guidance mechanisms of traps. Floral traits of carnivorous plants that could play a distinctive role are often neglected.

In the present paper we take an in-depth approach on floral plant traits for a broader sample of carnivorous plants. We focus our analysis on floral ecology of active flypaper traps *Drosera* (17 species analysed) and *Pinguicula* (19 species) in order to control for trap-dependent influences (i.e. trapping mechanism, resource efficiency in nutrition-deprived habitats). Monotypic *Dionaea muscipula* is added due to phylogenetic proximity to *Drosera*.

The selected model organisms are similar in regard to temporal and spatial separation of flowers and traps: Temporal separation is missing in *Drosera*, *Dionaea* and *Pinguicula*, with flowers and trap leaves being present and active at the same time in all of them. Spatial separation is realised to an intermediate degree, the model organisms are separating flowers from trap leaves more or less strongly by flower stalks. Focus of our analysis is therefore laid on plant traits related to attraction, guidance and control of pollinator and prey taxa, allowing for correlational analysis and exploration of interplay of these traits, like optical signal (floral display size, colour, UV light reflection pattern) and floral nectar reward. Actionmorphic flowers of Droseraceae and zygomorpic flowers of *Pinguicula* further allow for comparison of pollinator control via flower symmetry on efficiency of pollen transmission. Results will also be presented in regard to pollen presentation strategies: Pollen material is dispersed in tetrads in *Drosera* and *Dionaea*, but as single pollen grains in *Pinguicula* (Rodoni et al. 2010, Halbritter et al. 2012).

We present a broad sampling of breeding system related variables: Pollen and ovule production, pollen to ovule ratio (p/o) as well as experimentally retrieved true breeding systems in order to control for reproductive assurance mechanisms. We use the average p/o ratio per flower as indicator for effectivity of pollen transmission. Cruden (1977) proposed p/o ratios as indicators of plant breeding systems first: Autogamous species usually have much lower p/o ratios than xenogamous species, since pollen loss during transfer from male to female flower organs is minimal. However, siring success is influenced by many factors likely also influencing the p/o ratio (e.g. size of the pollen bearing area of the pollinator, or behavioural pattern like grooming, pollen consumption, etc.). While overall correlations between p/o ratios and true breeding systems seem to hold true in general (especially for species within genera and families), huge differences between taxa with the same breeding system may be observed (compare Cruden and Jensen, 1979; Cruden and Miller-Ward, 1981; Cruden and Lyon, 1985; Small, 1988; Kirk, 1993; López et al., 1999; Cruden, 2000; Michalski and Durka, 2009; Alarcón et al., 2011; Lozada-Gobilard et al., 2019). P/o ratios should be of explanatory value for possible pollinator-prey conflicts of carnivorous plants. Inefficient pollen transmission due to trapping of pollinators should demand for higher p/o ratios, like any other cause of pollen loss. Main research questions are therefore:

Are floral plant traits and characteristics of trap leaves indicative for attraction and guidance of pollinator and prey taxa, in order to alleviate a possible-pollinator-prey conflict?

Are pollen production, ovule numbers and p/o ratios indicative for breeding systems and possible pollinator-prey conflict?

Are attraction, guidance and control mechanisms, variables of gamete production and true breeding systems interrelated, showing an interplay of characters?

Do the differences in regard to mechanical control of pollinators and pollen dispersal between Droseraceae and *Pinguicula* exert an influence on breeding systems and gamete production?

IV.3 Materials and Methods

Living collections of Droseraceae and *Pinguicula* were cultivated either outdoors or in the greenhouse at Bonn University Botanic Gardens. Samples for pollen and ovule data, nectar production, morphology and UV signal were obtained from 2014 to 2016. Experimental testing of breeding systems was performed in 2015 and 2016.

For *Drosera*, 17 species were analysed: *D. adelae* F.Muell., *D. aliciae* Raym.-Hamet, *D. anglica* Huds, *D. binata* Labill., *D. callistos* N.G.Marchant & Lowrie, *D. capensis* L., *D. dichrosepala* Turcz., *D. filiformis* Raf., *D. intermedia Hayne*, *D. leioblastus* N.G.Marchant & Lowrie, *D. menziesii ssp. menziesii* R.Br. ex DC., *D. paleacea subsp. roseana* (N.G.Marchant & Lowrie) Schlauer, *D. paradoxa* Lowrie, *D. platystigma* Lehm., *D. regia* Stephens, *D. rotundifolia* L., *D. spatulata* Labill. Closely related *Dionaea muscipula* J.Ellis was added to the analysis.

Genus *Pinguicula* is represented by the following 19 species: *P. agnata* Casper, *P. cyclosecta* Casper, *P. ehlersiae* Speta & F. Fuchs, *P. emarginata* Zamudio & Rzed., *P. esseriana* B. Kirchn., *P. gigantea* Luhrs, *P. gracilis* Zamudio, *P. gypsicola* Brandegee, *P. hemiepiphytica* Zamudio & Rzed., *P. hirtiflora var. hirtiflora* Ten., *P. ibarrae* Zamudio, *P. jaumavensis* Debbert, *P. laueana* Speta & F. Fuchs, *P. medusina* Zamudio & Studnička, *P. mirandae* Zamudio & A. Salinas, *P. moctezumae* Zamudio & R.Z. Ortega, *P. moranensis* Kunth, *P. potosiensis* Speta & F. Fuchs and *P. rectifolia* Speta & F. Fuchs.

Only recognised species were used for calculations. However, the following four variations were also analysed and are described in the appendix: *D. capensis f. alba* L., *D. capensis f. rubra* L., *D. spatulata var. lovellae* Labill. and *P. moranensis f. alba* Kunth.

IV.3.1 ASSESSMENT OF FLORAL PLANT TRAITS

As for analysis of optical flower signal, colour variables are categorical and nominal. Flower colour was dichotomised for statistical analysis, testing predominantly white corolla colour (purely white flowers and white flowers with pink nectar guides) vs. reddish flower colour (predominantly violet to pink colouration, seldom orange or brightly red). Spur colour in *Pinguicula* is dichotomous (greenish vs. reddish).

UV signal of flowers was analysed as follows: Flowers were illuminated with UV light and pictures were taken for frontal and lateral flower displays with a single-lens reflex camera (Nikon R D300s), and an infrared neutralizer (OPTIK MAKARIO IR NG 52D) in combination with a UV light filter (OPTIK MAKARIO SP 400 UV 52D). Photos were created with a fixed aperture, but different shutter speeds. Degree of UV absorbance was coded according to strength of absorbance after 1 sec. UV light exposure. Analysed UV variables are categorical and ordinal, namely UV signal of the frontal and lateral display, UV signal of the nectar spur (dichotomous, reflection vs. absorbance – only for *Pinguicula*) and of anthers (dichotomous, reflection vs. absorbance – only for Droseraceae). The UV signal of the frontal and lateral flower display was coded as follows: Strong UV reflection was coded 1, UV reflection in combination with an absorbing centre was coded 2 (only some frontal flower displays), diffusely absorbing displays were coded 3 and strongly absorbing displays were coded 4. For spur and anthers, UV signal was coded 1 for reflection and 2 for absorption.

Presence of an anther dummy signal (stamens mimicked by floral guides, cf. Lunau, 2006), UV pattern of the anther dummy, anther colour, leaf colour and UV signal of leaf structures were also analysed, but are to homogenous for statistical analysis (see appendix, table A X.3.2).

Variables of nectar reward, gamete production and flower morphology are metric and ratio scaled.

Three variables were analysed for nectar reward: Amount of nectar production in µl, nectar sugar concentration in percent and amount of produced sugar in mg. For analysis of nectar production, flowers were covered with gauze ca. 24 h before sampling to prevent flower visits. Microcapillaries (0.5 and 1 µl minicaps® – Hirschmann Laborgeräte, Germany) were either inserted between petals onto the receptacle of a flower or placed against the sectioned base of the hypanthium in spurred species to check for presence of nectar and to collect it. Sugar concentration was measured in degrees Brix with a hand-held refractometer (neoLab, type 'universal'). For flowers with very low nectar production, nectar from several flowers was pooled and nectar/sugar amounts were afterwards calculated for the single flower level.

Nectar sugar production per flower was derived from nectar volume and nectar concentration after Galetto and Bernadello (2005), with *x* being the measured concentration:

$$\frac{mg}{\mu l} = 0.00226 + (0.00937x) + (0.0000585x^2)$$

Sample size for nectar measurements is ranging between five and 34 flowers per species. In total, nectar of 321 flowers was measured.

Regarding gamete production, ovule numbers, production of pollen dispersal units (afterwards referred as PDU) per flower and PDU/ovule ratios were analysed: A PDU is the smallest unit pollen grains are transported from flower to flower, either as single pollen grains or in more complex structures. In order to analyse the male reproduction system, PDUs were counted by means of a hemocytometer. Closed anthers of individual flowers were cut off and dried in tubes for at least three days for each sample. Afterwards glycerol and distilled water were added in proportion 1:1 (in total 50, 100 or 200 µl solvent added, depending on pollen production per species). Samples were mixed 2 to 5 minutes in a laboratory mill, and then placed into an ultrasonic bath for 15 minutes to loosen the PDUs from the anthers. Probes were then vortexed to ensure equal suspension before counting. Per sample, PDUs on five squares (each 1x1 mm) of the hemocytometer chamber were counted. Total PDU amount of the sample was calculated with the following formula (Neuendorf, 2013):

$$\text{PDUs per sample} = \frac{\text{counted PDU number}}{\text{counted surface (mm}^2\text{)} \cdot \text{ chamber depth (mm)} \cdot \text{ dilution (1} \div \text{amount of diluent)}}$$

Ovule numbers were counted under a stereomicroscope. PDU to ovule ratio was calculated by division, and pollen to ovule ratio was derived by multiplication of number of pollen grains per PDU. Correlations are not influenced by higher-order assemblage of pollen grains to PDUs, and correlations are therefore only shown for pollen production and p/o ratios. However, differences between PDU and pollen production per flower and between PDU/ovule and p/o ratio were tested statistically.

For variables of gamete production, sample size is usually 12. In few cases only six or three (*P. laueana*) data points were available. In total, PDU and ovule numbers of 459 flowers were counted.

Analysed variables of floral morphology are frontal and lateral display size in cm^2, as well as frontal to lateral display size ratio. For *Pinguicula*, length of nectar spur in cm and size of flower opening in cm^2 were also measured. Morphological data were retrieved from flower photographs by means of Adobe® Photoshop® CS6 imaging software and Magnetic Lasso Tool function. Sample size per morphological variable and species is usually 5 or 10. For five species only a single flower picture was available. In total, 306 flowers were measured for floral morphology.

For closer inspection of reproductive organs and PDUs, plant material of *Dionaea*, *Drosera capensis*, *Pinguicula cyclosecta* and *P. moranensis* were analysed via SEM (Scanning Electron Microscopy). Material was prepared via critical point drying, fixed to a conducting carrier (SEM stub) and attached with conductive coal to prevent electric charging. Scanning was performed with Cambridge Stereoscan 200 (Cambridge Instruments Ltd., Cambridge, UK).

Breeding systems were tested experimentally: Flowers were bagged and observed for occurrence of spontaneous autogamy in a first step. If absent, flowers were bagged and hand-pollinated in order to check for self-compatibility. Breeding system was coded as categorical and ordinal variable, according to proneness towards outcrossing: Spontaneous autogamy was coded 1, facultative xenogamy was coded 2 and obligate xenogamy was coded 3. For statistical testing, species were only categorised as autogamous or xenogamous, because obligate outcrossing was detected seldom.

IV.3.2 Statistical Analysis

Except description of data bandwidth, arithmetic means were used as species-specific profiles for analysis, due to unequal sample sizes and non-normality of data. Only nectariferous flowers were used

for calculation of arithmetic means of nectar variables, but ratio of dry to nectariferous flowers is reported in the appendix, table A X.3.3. Due to non-normality of data, correlations between ratio scaled variables as well correlations between ratio scaled and ordinal variables were calculated with Kendalls´s τ_b rank correlation coefficient. Correlations between dichotomous (nominal or ordinal) variables and ratio scaled variables were calculated with point-biserial correlation coefficient r_{pbi}, and correlations between dichotomous and ordinal variables were calculated with rank-biserial correlation coefficient r_{rb}. Group differences were analysed by Kruskal-Wallis test and subsequent group comparisons with Bonferroni correction via IBM SPSS, version 24. Boxplots were designed with base R, version 3.3.3, and arithmetic means were visualised with function 'beeswarm'.

IV.4 RESULTS

In the following section the observed data bandwidth is presented: For detailed, species specific information compare tables A X.3.1 to A X.3.5 in the appendix. For exemplary depictions of flowers and traps, colouration and UV light signal please compare figure IV.2.

IV.4.1 OPTICAL SIGNAL

Average frontal display size is 1.52 ± 1.81 cm^2 in Droseraceae (min.: *D. leioblastus*, 0.06 cm^2; max.: *D. binata*, 6.34 cm^2; *Dionaea*: 2.29 cm^2), and 3.49 ± 2.56 cm^2 in *Pinguicula* (min.: *P. emarginata*, 0.48 cm^2; max.: *P. moctezumae*, 9.55 cm^2). Average lateral display size is .54 ± .64 cm^2 in Droseraceae (min.: *D. leioblastus*, 0.02 cm^2; max.: *D. binata*, 2.29 cm^2; *Dionaea*: .83 cm^2), and 1.26 ± .81 cm^2 in *Pinguicula* (min.: *P. emarginata*, .22 cm^2; max.: *P. moctezumae*, 2.64 cm^2). The average frontal to lateral display size ratio is 3.03 ± .96 cm^2 in Droseraceae (min.: *D. aliciae* and *D. dichrosepala*, 1.7 cm^2; max.: *D. platystigma*, 4.9 cm^2; *Dionaea*: 2.9 cm^2), and 2.74 ± .67 cm^2 in *Pinguicula* (min.: *P. medusina*, *P. potosiensis* and *P. rectifolia*, 1.9 cm^2; max.: *P. ehlersiae*, 4.0 cm^2).

Regarding flower colour, white flowers are present in Droseraceae (*Dionaea* and eight species of *Drosera*) and *Pinguicula* (*P. gracilis* and *P. ibarrae*). Two species of *Pinguicula*, *P. agnata* and *P. emarginata*, show white flowers with pinkish nectar guides, while most of the other species analysed show a reddish, violet to pink colouration. In few cases flower colour is shifted more strongly to the red spectrum, with reddish-orange colours present in *D. callistos* and *D. platystigma*, and red flower colour in *D. adelae* and *P. laueana*.

Spur colour in *Pinguicula* is usually green, seven species are showing violet to pink spur colour however: *P. cyclosecta*, *P. gypsicola*, *P. hemiepiphytica*, *P. laueana*, *P. moctezumae*, *P. moranensis* and *P. potosiensis*.

UV light pattern of the frontal and lateral flower display shows full absorption for *Dionaea*. For *Drosera callistos*, *D. capensis*, *D. menziesii* and *D. platystigma* the frontal display shows reflection with a darker, absorbing flower centre, while the lateral display is UV reflecting. Other *Drosera* predominantly show a fully absorbing frontal display with a diffuse lateral UV signal. Only *D. spatulata* shows a reflecting lateral display beside frontal absorption, and *D. adelae* shows a diffuse UV signal from lateral as well as frontal view.

For *Pinguicula*, UV absorption is present in frontal and lateral flower displays of *P. emarginata*, *P. gracilis* and *P. hirtiflora*. An absorbing frontal display in combination with a UV reflecting lateral

display is present in *P. agnata*, *P. cyclosecta*, *P. gigantea*, *P. gypsicola*, *P. ibarrae*, *P. laueana*, *P. moctezumae* and *P. rectifolia*. A diffuse frontal absorption in combination with lateral reflection is present in *P. mirandae*, *P. moranensis* and *P. potosiensis*. For *P. ehlersiae*, *P. esseriana*, *P. hemiepiphytica* and *P. jaumavensis* reflecting frontal flower displays with a darker, absorbing centre and a reflecting lateral display were observed. Spurs usually show UV reflection, only *P. agnata*, *P. gigantea*, *P. gracilis*, *P. hirtiflora* and *P. potosiensis* have absorbing spurs.

Yellow anther colour in Droseraceae and presence of an anther dummy in *Pinguicula* are predominant pattern: Only *P. laueana* is missing respective anther dummy. The colour of the anther dummy is ranging from greenish-yellow to yellow or whitish in other *Pinguicula* species. As for anther colour, only *D. leioblastus* and *D. rotundifolia* are showing whitish anthers, others are yellow. Due to these very homogeneous patterns, both variables were excluded from further analysis. While UV signal of anthers in *Drosera* shows variation (six species reflecting, others showing absorption, same for *Dionaea*) and information is used for correlation analysis, the anther dummy signal in *Pinguicula* is consistently UV absorbing.

Similarly, trap leave colour is consistently green in *Pinguicula* and green to reddish in Droseraceae, due to reddish secreting hairs in *Drosera*. In some cases, leaves itself are showing a mixed green to red pattern (e.g. *Dionaea*). Additionally, leaves of all analysed genera show a homogenous, mixed pattern of UV absorption and reflection, in most cases because of reflecting mucilage above otherwise absorbing leave surfaces. Leaves of *Dionaea* also show a mixed UV pattern, however UV light reflexes between the trap teeth and a UV absorbing zone at the outer rim of the two trap lobes create a more complex setup (cf. figure IV.2).

IV.4.2 NECTAR REWARD

Nectar reward is not present in genus *Drosera*. *Dionaea* showed .34 µl nectar per flower on average, 28.2 % percent nectar sugar concentration and .1 mg sugar production. As for genus *Pinguicula*, nectar reward could not be detected for *P. agnata* and *P. emarginata* with the method applied. For the other species the average nectar production is 1.43 ± 2.38 µl per flower (min.: *P. hirtiflora*, .003 µl; max.: *P. moctezumae*, 9.66 µl). Due to limited nectar production, nectar sugar concentration and nectar sugar production could not be measured for species with shorter nectar spur: *P. gracilis* (.5 cm spur length), *P. hirtiflora* (.6 cm), *P. ibarrae* (.6 cm), *P. medusina* (.3 cm) and *P. mirandae* (.4 cm). For the remaining 12 species, average nectar concentration is 12.0 ± 8.7 % (min.: *P. potosiensis*, 5.1 %; max.: *P. gigantea*, 38.2 % – exceptionally high concentration, closest neighbour *P. ehlersiae* with 15.5 %). Average nectar sugar production is .20 ± .26 mg (min.: *P. gypsicola*, .02 mg; max.: *P. moctezumae*, .97 mg).

Nectar spurs of *Pinguicula* are measuring 1.79 ± 1.28 cm on average (min.: *P. medusina*, .3 cm; max.: *P. laueana*, 4.5 cm).

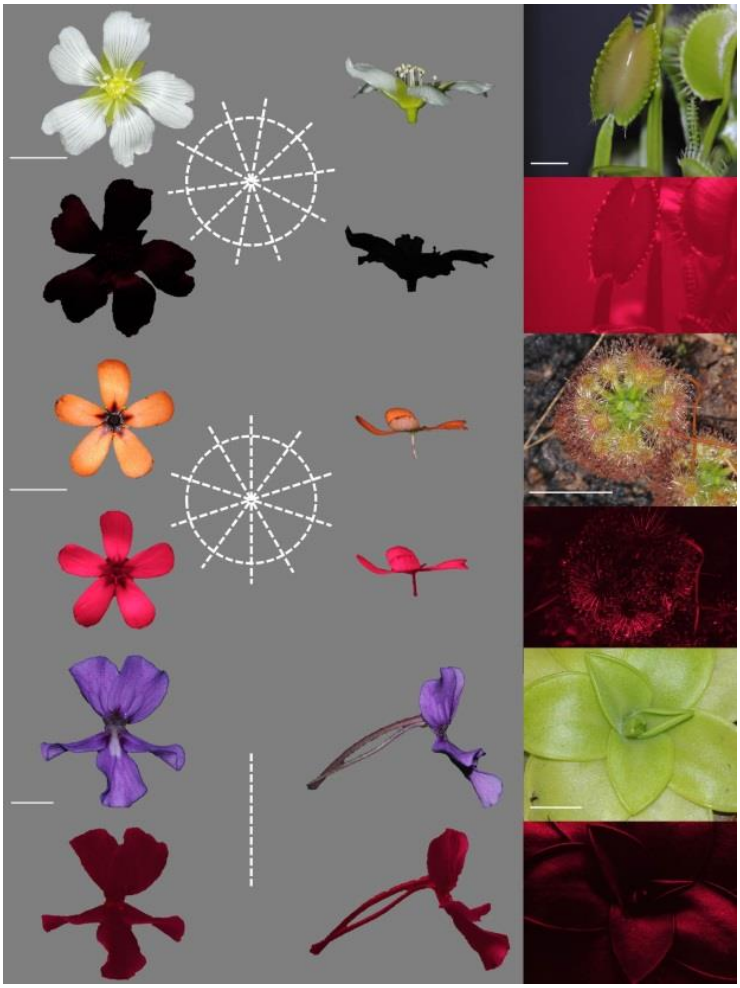

FIGURE IV.2: FLORAL SYMMETRY AND OPTICAL SIGNAL IN DAYLIGHT SPECTRUM AND UV LIGHT FOR FLOWERS AND TRAP LEAVES

The figure shows traps and flowers of monotypic *Dionaea* and selected species of *Drosera* and *Pinguicula*, exemplifying the observed bandwidth of colour and UV signal. As for Droseraceae, five abstract mirror planes are present and flowers are actinomorphic. Flowers of *Pinguicula* only show one mirror plane and are zygomorphic. Observed flower colours are ranging from white (present in all genera, here represented by *Dionaea*, top: frontal and lateral view) to pink/violett (in *Drosera* and *Pinguicula*, here: *P. potosiensis*, bottom) or seldom orange or red (here: *D. callistos*, centre). Regarding UV signal (in 2nd rows), flowers showed absorption (here: *Dionaea*), reflection (here: *D. callistos*, with darker center), or sometimes a diffuse signal (here: *P. potosiensis*). Leaf traps of all genera showed a mixed UV signal (right margin). Scale bar representing 1 cm. Pictures by A. W. Mues, except leaves of *Dionaea* by S. Brauwers.

IV.4.3 FLOWER MORPHOLOGY, SYMMETRY, POLLEN PRESENTATION AND AGGREGATION

Regarding flower morphology, *Dionaea* and *Drosera* share an actinomorphic flower structure. *Pinguicula* flowers are zygomorphic. Bowl-shaped flowers of Droseraceae are accessible from every direction, while flowers of *Pinguicula* control pollinator positioning and restrict access to pollen and nectar reward by a narrow flower opening, measuring .05 ± .03 cm^2 on average (min: *P. emarginata* and *P. potosiensis*, .01 cm^2; max.: *P. ibarrae*, .13 cm^2).

For depictions of reproductive organs and PDUs in Droseraceae and *Pinguicula* see figure IV.3. Anther number is usually fixed to five in *Drosera* and to two in *Pinguicula*: Only minimal, negligible fluctuations were observed. *Dionaea* shows 12.9 ± 2 anthers in average.

Regarding the male reproductive system, pollen is dispersed in packages of four in all analysed Droseraceae and PDUs are therefore pollen tetrads, while individual pollen grains are PDUs in all analysed species of *Pinguicula*. Anthers and stigma are in close proximity and accessible from every direction in *Drosera* and *Dionaea*. Pollen is released from two thecas per anther by longitudinal dehiscense. *Pinguicula* flowers are characterised by a more complex setup: Anthers are covered by a stigma lobe and stamens only show a single anther theca with transverse dehiscense. Due to this, pollen is released in a very controlled way into the space below the stigmatic lobe, and contained by glandular hairs on filaments and ovary.

IV.4.4 Gamete production and PDU/ovule ratios

Average amount of produced PDUs per flower is 1 179.2 ± 1 572.0 pollen tetrads in Droseraceae (min.: *D. leioblastus*, 177.1; max.: *D. menziesii*, 5 170.8; *Dionaea*: 1 333.3), corresponding to 4 716.9 ± 6 288.0 pollen grains when multiplied by a factor of four (min.: *D. leioblastus*, 708.3; max.: *D. menziesii*, 20 683.3; *Dionaea*: 5 333.3). In *Pinguicula*, average amount of produced pollen grains per flower is 13 278.1 ± 6 288.6 (min.: *P. emarginata*, 2 933.3; max.: *P. laueana*, 26 600.0).

Average ovule number of Droseraceae is 105.1 ± 97.0 ovules per flower (min.: *D. paleacea subsp. roseana*, 6.3; max.: *D. binata*, 327.9; *Dionaea*: 29.3). In *Pinguicula*, average ovule number is 276.6 ± 123.8 (min.: *P. hirtiflora*, 66.2, followed closely by *P. emarginata*, 66.3; max.: *P. ibarrae*, 485.0).

The PDU to ovule ratio is 17.0 ± 17.5 tetrads to ovules in Droseraceae (min.: *D. aliciae*, 1.0; max.: *Dionaea*, 74.7; max. for *Drosera*: *D. menziesii*, 33.4), corresponding to a p/o ratio of 68.1 ± 70.2 (min.: *D. aliciae*, 4.1; max.: *Dionaea*, 298.6; max. for *Drosera*: *D. menziesii*, 133.4). For *Pinguicula*, average p/o ratio is 55.0 ± 24.1 (min.: *P. ibarrae*, 21.4; max.: *P. mirandae*, 133.9).

IV.4.5 Breeding systems

Eight of the analysed species of Droseraceae are autogamous: *D. aliciae, D. anglica, D. capensis, D. filiformis, D. intermedia, D. rotundifolia, D. spatulata* as well as *Dionaea*. Most of the other *Drosera* species showed no seed set after hand pollination and are rated as obligate xenogamous. Only *D. regia* showed seed set after hand-pollination and is therefore rated as facultatively xenogamous. Spontaneous autogamy was not found in *Pinguicula*, and breeding systems are facultatively xenogamous for most species. Only five species showed self-incompatibility after hand pollination, and are therefore rated obligate xenogamous: *P. cyclosecta*, *P. ehlersiae*, *P. esseriana*, *P. gracilis* and *P. moctezumae*.

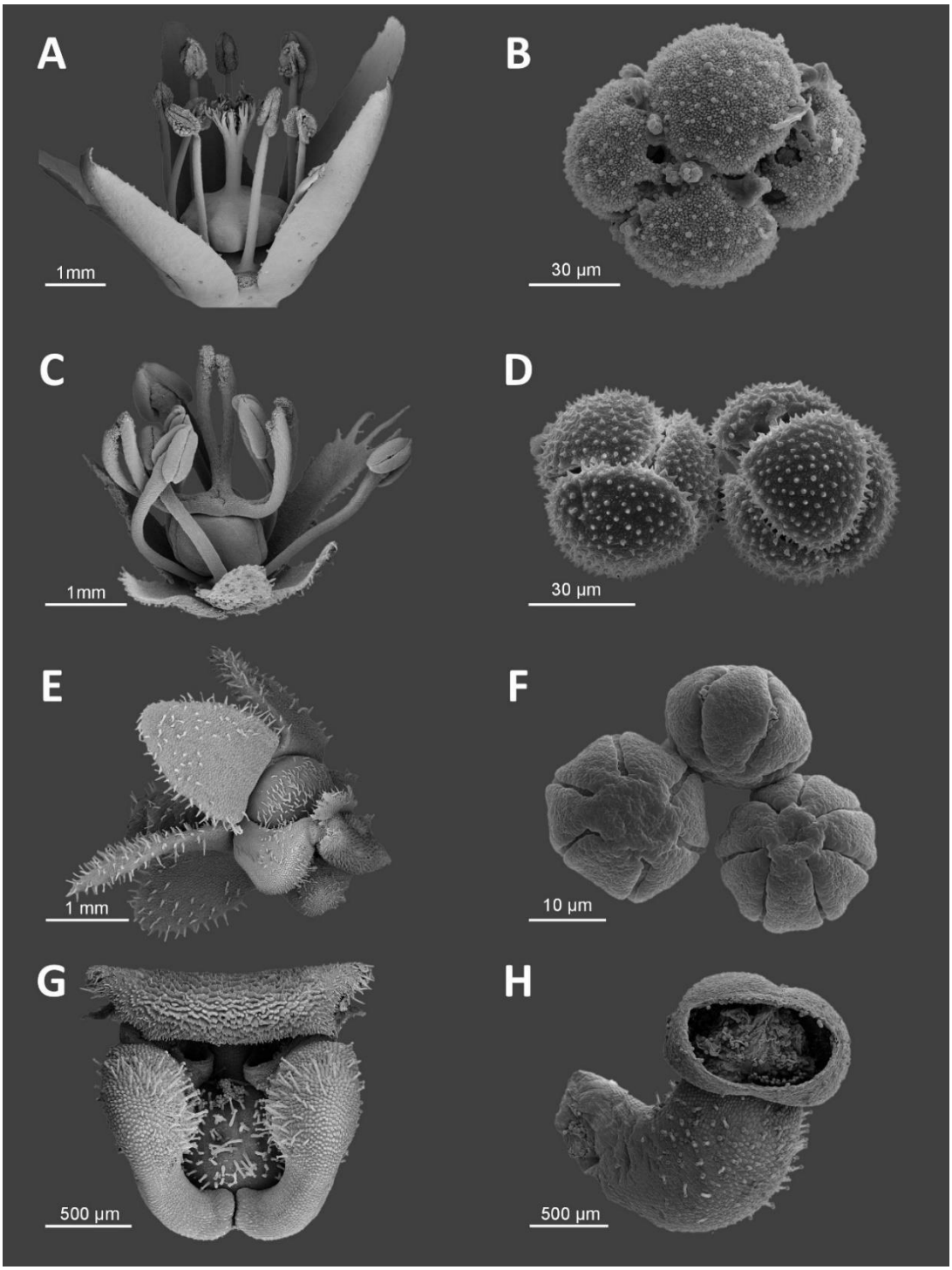

FIGURE IV.3: SEM PICTURES OF REPRODUCTIVE FLOWER ORGANS AND POLLEN MATERIAL

A: Flower of *Dionaea muscipula* (petals removed), anthers and stigma in close proximity; B: Pollen tetrad of *Dionaea muscipula*; C: Flower of *Drosera capensis* (petals removed), anthers and stigma in close proximity; D: Two pollen tetrads of *Drosera capensis*; E: Flower of *Pinguicula cyclosecta* (corolla removed), lateral view, receptive stigma lip bending over anthers to prevent selfing; F: Three pollen grains of *P. cyclosecta*; G: *P. cyclosecta*, frontal view onto filaments and anthers, covered by stigma lip; pollen released onto ovary and contained by glandular hairs; H: Stamen of *P. cyclosecta*, with single, transverse anther dehiscence. Scaling indicated, pictures by Andreas W. Mues and H.-J. Ensikat.

IV.4.6 INTERPLAY OF FLORAL FUNCTIONAL TRAITS

In Droseraceae (table IV.1), frontal and lateral flower display size are correlated strongly and highly significant (τ_b = .830, p = .000). Larger frontal and lateral display sizes are correlated with higher pollen number (frontal display: τ_b = .616, p = .000; lateral display: τ_b = .603, p = .000) and ovule production (frontal display: τ_b = .425, p = .014; lateral display: τ_b = .490, p = .004), but not p/o ratio. The ratio of frontal to lateral display size is correlated moderately and significantly to the flower colour (r_{pbi} = .487, p = .040). Pronounced frontal displays are therefore related to reddish flower colour. Display size ratio is further correlated negatively and significantly to the frontal UV signal (τ_b = -.457, p = .022), showing less UV absorption (respectively higher UV reflection) in pronounced frontal displays, and more absorption in smaller flowers. Display size ratio is also correlated to the breeding system (τ_b = .454, p = .023), showing a tendency for outcrossing when frontal displays are more pronounced.

TABLE IV.1: CORRELATIONS BETWEEN ANALYSED VARIABLES OF DROSERACEAE

For ratio scaled and ordinal variables, Kendalls´s τ_b is presented. For correlations between ratio scaled dichotomous variables (flower colour, UV signal of anthers), point-biserial correlation coefficient r_{pbi} is presented. For correlations between dichotomous and ordinal variables (UV pattern of frontal and lateral display), rank-biserial correlation coefficient r_{rb} is presented. No correlations shown for two dichotomous variables due to measurement level. The number of asterisks shows the significance of the observed correlation, with * = significant at the .05 level, ** = significant at the .01 level. Correlations from .1 to .3 are rated low, from .3 to .5 moderate, from .5 and above strong (Cohen, 1988). Correlations do not differ for pollen and PDUs, respectively p/o ratio and PDU/o ratio, due to simple scale conversion.

τ_b, r_{pbi}, r_{rb}	displ., lat.	display, front:lat	flower colour	UV, frontal	UV, lateral	UV, anthers	pollen p. flower	ovules	p/o ratio	breeding system
display, frontal	**.830****	.150	-.014	-.302	-.378	-.265	**.616****	**.425***	.150	-.009
display, lateral	.	-.020	-.048	-.165	-.298	-.237	**.603****	**.490****	.111	-.111
display, front:lat		.	**.487***	**-.457***	-.298	-.071	.184	-.190	.268	**.454***
flower colour			.	**-.615****	**-.711****		-.027	.128	-.270	.057
UV, frontal				.	**.659****	.116	-.293	-.029	-.165	-.319
UV, lateral					.	.111	-.323	-.330	.249	.074
UV, anthers						.	-.314	-.271	.049	-.202
pollen per flower							.	**.433***	.223	-.026
ovules								.	**-.346***	-.351
p/o ratio									.	**.523****

Flower colour is correlated highly significant and strongly negative to absorption of UV light (frontal UV signal: r_{rb} = -.615, p = .007 / lateral UV signal: r_{rb} = -.711, p = .001). Coloured flowers are therefore more often UV light reflecting, while white flowers are rather absorbing it. Frontal and lateral UV signal are strongly correlated (τ_b = .659, p = .003).

Optical flower signal via colouration or UV light pattern is not correlated to pollen and ovule production, p/o ratios and breeding system.

Pollen and ovule production are correlated moderately, positive and significantly with each other (τ_b = .433, p = .012). Ovule number is correlated negatively, significantly and moderately to the p/o ratio (τ_b = -.346, p = .045). Lower ovule numbers are therefore correlated to higher p/o ratios, while pollen production shows no correlation to p/o ratio. The p/o ratio is correlated strongly, highly significant and positively to the observed breeding system (τ_b = .523, p = .009): Outcrossers therefore do show higher p/o ratios when compared to autogamous species.

As for *Pinguicula* (table IV.2), frontal and lateral flower display size are also correlated strongly and highly significant with each other (τ_b = .825, p = .000), as well as with pollen number (frontal display: τ_b = .497, p = .003; lateral display: τ_b = .509, p = .002) and ovule production (frontal display: τ_b = .556, p = .001; lateral display: τ_b = .637, p = .000), but not p/o ratio. Moreover, display sizes are correlated moderately and highly significant to the nectar spur length (frontal display: τ_b = .439, p = .009; lateral display: τ_b = .427, p = .011), strongly to spur colour (frontal display: r_{pbi} = .540, p = .017; lateral display: r_{pbi} = .514, p = .024), as well as moderately to nectar amount (frontal display: τ_b = .493, p = .003; lateral display: τ_b = .457, p = .006) and produced sugar (frontal display: τ_b = .486, p = .016; lateral display: τ_b = .420, p = .037). Display size ratio is exclusively correlated to the breeding system (r_{pbi} = .603, p = .006). Moreover, no other variable is correlated to the breeding system. Species with more pronounced frontal displays are therefore rather obligate than facultative outcrossers in *Pinguicula*.

The size of the flower opening is not correlated to any other variable, but spur length is: Regarding flower signal, longer spurs are correlated significantly and moderately with reddish flowers (r_{pbi} = .497, p = .030), and highly significant and strongly with reddish spur colour (r_{pbi} = .824, p = .000). Moreover, spur length is correlated significantly, moderately but negative with the UV light signal of the lateral display (τ_b = -.395, p = .011), showing that longer spurs are correlated with less lateral UV absorption. Spur length is also correlated strongly and highly significant with the amount of nectar production (τ_b = .762, p = .000) and sugar content (τ_b = .575, p = .004), but not with nectar concentration (p = .511): Mind again that nectar concentration was not measurable for species with very short spurs. Spur length is also correlated highly significant and strongly to pollen production (τ_b = .637, p = .000), and moderately to ovule number (τ_b = .439, p = .009).

Further, reddish flower colour is correlated with higher pollen production (r_{pbi} = .517, p = .023). Spur colour shows three additional positive correlations: Higher nectar amount (r_{pbi} = .594, p = .007), higher pollen production (r_{pbi} = .754, p = .000) and higher ovule number (r_{pbi} = .458, p = .049) in species with reddish spurs. Regarding UV signal, the detected patterns for the frontal flower display and nectar spur show no relevant correlations at all. Only the UV signal of the lateral display shows additional negative and moderate to strong correlations beside the already mentioned ones (display size and spur length), namely with nectar amount (τ_b = -.464, p = .017), pollen (τ_b = -.511, p = .008) and ovules (τ_b = -.511, p = .008). The four species with higher lateral absorption (*P. emarginata*, *P. gracilis*, *P. hirtiflora* and *P. medusina*, see appendix, table A X.3.2) are therefore showing lower gamete production and nectar amount.

The amount of nectar reward is further correlated strongly and highly significant with the produced sugar content, where detectable (τ_b = .733, p = .000), as well as with gamete production (pollen: τ_b = .669, p = .000; ovules: τ_b = .516, p = .002). Similarly, sugar production is also correlated to gamete production (pollen: τ_b = .420, p = .037; ovules: τ_b = .530, p = .008). Nectar concentration is correlated to no other variable, however.

Pollen production and ovule number are correlated strongly and highly significant (τ_b = .591, p = .000). The p/o ratio is correlated to no other variable in *Pinguicula*.

TABLE IV.2: CORRELATIONS BETWEEN ANALYSED VARIABLES OF *PINGUICULA*

For ratio scaled and ordinal variables, Kendalls´s τ_b is presented. For correlations between ratio scaled and dichotomous variables (flower colour, spur colour, UV signal of spur, breeding system), point-biserial correlations r_{pbi} are presented. For correlations between dichotomous and ordinal variables (UV pattern of frontal and lateral display), rank-biserial correlation coefficient r_{rb} is presented. No correlations shown between two dichotomous variables due to measurement level. Mind that correlations to nectar concentration and sugar production do not include species with very short nectar spurs (*P. gracilis*, *P. hirtiflora*, *P. ibarrae*, *P. medusina*, *P. mirandae*), because nectar concentration and sugar could no be measured. The number of asterisks shows the significance of the observed correlation, with * = significant at the .05 level, ** = significant at the .01 level. Correlations from .1 to .3 are rated low, from .3 to .5 moderate, from .5 and above strong (Cohen, 1988).

τ_b, r_{pbi}, r_{rb}	display, lateral	display, front:lat	opening	spur length	flower colour	UV, frontal	UV, lateral	spur colour	UV, spur	nectar amount	conc.	sugar	pollen p. flower	ovules	p/o ratio	breeding system
display, frontal	**.825****	.287	.123	**.439****	.146	-.047	**-.588****	**.540***	.078	**.493****	.000	**.486***	**.497****	**.556****	-.018	.063
display, lateral	.	.111	.088	**.427****	.145	.000	**-.607****	**.514***	.141	**.457****	-.110	**.420***	**.509****	**.637****	.018	-,129
display, front:lat		.	.135	.240	-.028	-.109	-.145	.254	.058	.246	.243	.376	.135	.099	-.076	**.603****
opening			.	-.205	-.432	.125	-.010	-.238	.177	-.223	.110	.155	-.099	.029	-.076	-.233
spur length				.	**.497***	-.156	**-.395***	**.824****	-.306	**.762****	-.133	**.575****	**.637****	**.439****	.193	.132
flower colour					.	-.384	-.398			.286	.478	.294	**.517***	.213	.365	
UV, frontal						.	.360	-.035	.254	-.219	-.282	.000	-.187	-.078	-.016	-.114
UV, lateral							.	-.392	.307	**-.464***	-.351	-.351	**-.511****	**-.511****	-.029	.000
spur colour								.		**.594****	-.206	.437	**.754****	**.458***	.124	
UV, spur									.	-.211	.248	-.190	-.264	-.183	-.110	
nectar amount										.	-.022	**.733****	**.669****	**.516****	.106	.349
concen-tration											.	.244	-.110	.044	-.243	.108
sugar												.	**.420***	**.530****	.066	.448
pollen per flower													.	**.591****	.251	-.101
ovules														.	-.158	.030
p/o ratio															.	-.296

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IV.4.7 GROUP COMPARISONS FOR POLLEN PRESENTATION STRATEGIES AND BREEDING SYSTEMS

Gamete production was further analysed in regard to the experimentally retrieved breeding systems. The effect of pollen presentation as tetrads or single pollen grains was analysed via Kruskal-Wallis test and subsequent Bonferroni corrected group comparisons, compare figure IV.4. Average ovule number for autogamous Droseraceae including *Dionaea* is 135 ovules per flower, while xenogamous Droseraceae show 81 and generally xenogamous *Pinguicula* 277 ovules. Ovule production of xenogamous Droseraceae and *Pinguicula* differ significantly from each other (p = .002), while ovule number of autogamous Droseraceae does not (comparison with xenogamous Droseraceae: p = 1.000; with *Pinguicula*: p = .380).

As for the PDU production per flower, *Pinguicula* with in average 13 278 pollen grains differs significantly from the PDU number of Droseraceae when compared on tetrad-level (comparison with autogamous Droseraceae, in average 691 tetrads: p = .000; with xenogamous Droseraceae, in average 1 570 tetrads: p = .000). When synorganisation of pollen into tetrads is neglected and total pollen numbers are compared, both breeding groups of Droseraceae do not differ statistically from *Pinguicula* anymore (comparison with autogamous Droseraceae, in average 2 762 pollen grains: p = .071; with xenogamous Droseraceae, in average 6 280 pollen grains: p = .157). Breeding groups of Droseraceae do not differ significantly from each other in regard to PDU production, neither when compared on tetrad level, nor in total pollen numbers (p values ranging from .228 to 1.000).

As for PDU to ovule ratios, xenogamous Droseraceae do not differ from *Pinguicula* when interpreted as p/o ratios (p = 1.000). Similarly, the average p/o ratio of autogamous Droseraceae also does not differ from *Pinguicula* (p = .130) as long as *Dionaea* is included: when excluded, p/o ratios of autogamous Droseraceae do differ from *Pinguicula* (p = .015) – this is the only case that exclusion of *Dionaea* changes the significance of test results, pointing to a special relevance of higher higher p/o ratios for this species, like in xenogamous Droseraceae.

Synorganisation of pollen to tetrads does influence the ratio of male to female gametes in a significant way: On tetrad-level, autogamous Droseraceae do differ significantly from p/o ratios of *Pinguicula* (p = .001) and p/o ratios of xenogamous Droseraceae (p = .000). Similarly, the tetrad to ovule ratio of xenogamous Droseraceae does also differ significantly from p/o ratios of *Pinguicula* (p = .018) and xenogamous Droseraceae itself when interpreted as p/o ratio (p = .002).

In general, autogamous and xenogamous Droseraceae do differ when compared in regard to p/o ratios (p = .015; without *Dionaea*: p = .002), but not in regard to tetrad/ovule ratios (p = 1.000, without *Dionaea*: p = 1.000).

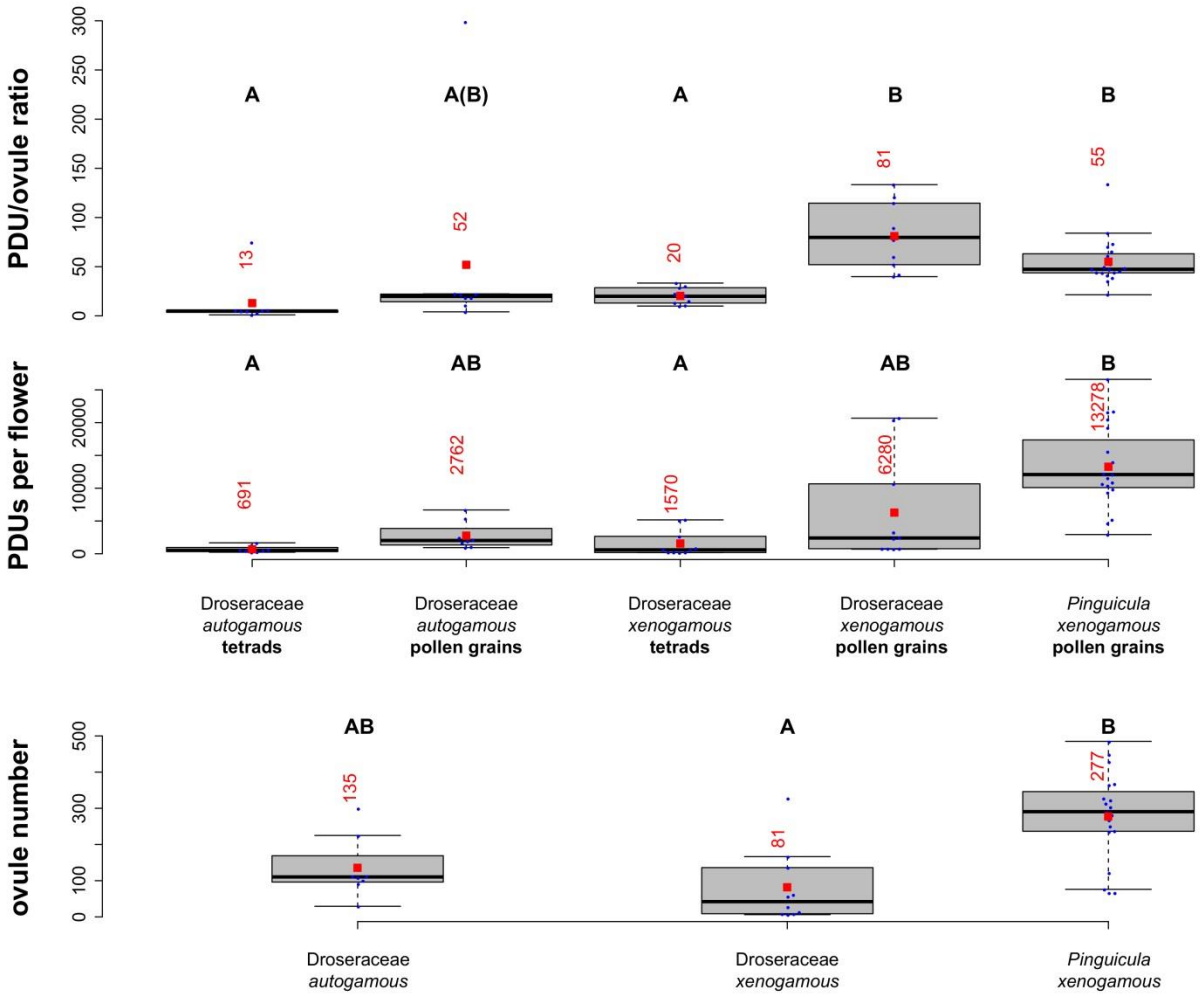

FIGURE IV.4: GROUP COMPARISONS FOR POLLEN PRESENTATION STRATEGIES AND BREEDING SYSTEMS

The figure presents the data bandwidth of variables related to breeding system (boxplots, arithmetic means in red and exact data points in blue). Presented are number of ovules (bottom), pollen production (centre) and pdu/o ratios (top) for autogamous (n = 8) and xenogamous Droseraceae (n = 10) as well as xenogamous *Pinguicula* (n = 19). Regarding pollen dispersal units, data for Droseraceae are presented as pollen tetrads as well as under perspective of pollen grains (tetrad number times four). Outliers for ovule number are *Drosera capensis* (autogamous) and *D. binata* (xenogamous) as well as *P. emarginata* and *P. hirtiflora*. Outliers for PDU/ovule ratio are *Dionaea* (extreme outlier when interpreted as p/o ratio) and *P. mirandae*. Letters above boxplots indicate groups that do not differ statistically by Kruskal-Wallis test and subsequent group comparisons, adjusted by Bonferroni correction. Regarding ovule number, xenogamous *Drosera* species differ from *Pinguicula*, but autogamous Droseraceae do not differ from both other groups. Produced pollen dispersal units per flower in Droseraceae do differ from pollen production in *Pinguicula* when interpreted as tetrads, but not when interpreted as pollen grain number. For PDU/o ratios, xenogamous *Drosera* species do not differ from *Pinguicula* when interpreted as p/o ratios. When interpreted as tetrad/ovule ratios, autogamous and xenogamous Droseraceae do differ from p/o ratios of *Pinguicula* and xenogamous Droseraceae. For autogamous Droseraceae, p/o ratios only match p/o ratios of *Pinguicula* and xenogamous Droseraceae when *Dionaea* as outlier is included (therefore group B put in bracets). Exclusion of *Dionaea* from Droseraceae does not affect other test results.

IV.5 Discussion

IV.5.1 Optical flower signal is crucial for sorting of pollinator and prey taxa

Optical flower signal in visible and UV light, contrasting the rather homogenous colouration and UV signal of the trap leaves, appears to be the crucial factor in sorting of pollinator and prey taxa for the species analysed, at least influencing the duration and/or frequency of pollinator interactions with flowers. The detected patterns of optical signal are generally highly conserved and invariant, to the point that some pattern of optical signal are to homogenous for statistical analysis, namely presence and UV light absorbance of anther dummy signal in *Pinguicula* flowers, anther colour in Droseraceae, as well as greenish to reddish colouration of trap leaves in combination with their mixed UV signal (reflecting mucilage above UV absorbing leaf surface). These very basic patterns of optical signal can be subsumed under a general need for contrast and its importance for attraction and guidance: The frontal flower display is contrasting strongly to the trap leaves via UV signal and flower colour for all genera analysed. Especially the UV light signal of the frontal flower display has to be highlighted. It is either showing reflection, sometimes in combination with a darker centre, or strong absorption. Moreover, colouration and UV signal of anthers and anther dummy signal have to be highlighted in regard to pollinator attraction and guidance. While openly visible anthers are only present in Droseraceae, anther dummy signal in *Pinguicula* is compensating for hidden anthers in a more complex flower structure. Anthers as well as anther dummies are contrasting very well to corolla colouration in visible light spectrum, and predominantly show UV light absorption in order to enhance clarity of the colour signal (Lunau, 2006).

IV.5.2 Functional integration of flower traits and reported pollinators

Beside these highly conserved patterns, the analysed interplay of optical flower signal with other plant traits is indicative for specialised attraction of pollinator guilds: The actinomorphic and saucer to bowl shaped flowers of Droseraceae, in combination with predominantly white or pinkish to violet flower colour and easily accessible anthers offering pollen as reward (beside nectar in *Dionaea*) are highly indicative for short-proboscid pollinator guilds (Diptera, Hymenoptera). Detected correlations between flower size and pollen production indicate an adaption to the body size of pollinators. More pronounced frontal flower displays (display size ratio) are further correlated to colour, UV signal and true breeding system, indicating higher resource investment in a pronounced and showy interface for plant-pollinator interactions in outcrossing species. Otherwise, flower size, flower signal and gamete production are largely uncorrelated. The p/o ratio is a valid indicator for breeding system in Droseraceae, showing higher values in xenogamous species. Field observations of flower visitors report a broad spectrum of short-probiscid pollinators: Scarabaeidae, Rutelinae/Hopliini (Goldblatt et al., 1998); Syrphidae, Calliphoridae, Dolichopodidae (Murza and Davis, 2005); syrphid flies and tachinid flies (Sciligo, 2009); bees, syrphid flies, and meloid beetles in *D. tracyi* (Wilson, 1995) – compare Jürgens et al. (2012). As for *Dionaea*, Hymenoptera (mainly bees) and Coleoptera are the most abundant pollen vectors (Youngsteadt et al., 2018).

In *Pinguicula*, zygomorphic flower structure and nectar reward hidden in spurs (Lustofin et al., 2019) are indicative for sucking nectar uptake of insect pollinators with a proboscis (Diptera, Lepidoptera, Hymenoptera), or even birds. Mind again that missing correlations to nectar concentration in the

dataset at hand are very likely caused by missing measurements for species with shorter nectar spurs: Fast crystallisation of minute nectar amounts in short spured species indicate higher nectar sugar concentrations as an adaption to pollinator guilds with shorter proboscis length (possibly Hymenoptera). In support of this hypothesis, *P. gigantea*, the only short spured species where nectar concentration could be measured, showed the highest concentration (38.2 %). As for long-spured species, nectar concentrations could be assessed and are generally low, representing an adaption to long-tongued pollinators. Similary like Droseraceae, stouter flowers (display sizes) correlate to higher investment in pollen and ovule production. Additionally, larger flowers are also correlated to higher nectar amount and sugar production, pointing to a general link to resource investment, and possibly body size and nutritional needs of pollinators. Reddish spur and display colour as well as longer spurs are also indicative for higher resource investment in nectar and pollen production in this dataset, assumedly due to pollinator specialisation. An example in this context is *P. laueana*: Bird pollination is likely and often stated in literature, indicated by red flower colour, missing anther dummy signal and suitable nectar reward. However, verification in the field is still missing (cf. Lustofin et al., 2019). As for breeding systems, only a pronounced frontal display size (display size ratio) is indicative for stronger outcrossing. Other variables are uncorrelated and p/o ratio is of no explanatory value, showing the breeding system generally disjunct from other floral plant traits.

Reported field observations of flower visitors are rare for *Pinguicula*. The following flower visitors were observed for *P. vulgaris*: Bees (identified: *Osmia caementaria* Gerst.), an unidentified Lepidopterid and two beetles (Heslop-Harrison, 2004, citing Muller (sic: Müller) 1881, 1886 (sic: 1883)). For *P. vallisneriifolia* following taxa were observed: beeflies (*Bombylius sp.*) and bees (*Andrena sp*., *Anthophora sp*., *Bombus terrestris*, *Halictus sp.*, *Lasioglossum sp.*, *Osmia cornuta*), hawkmoths, butterflies, calliphorid flies, small beetles and thrips (Zamora, 1999). For *P. moranensis* 14 species of Lepidoptera were observed, beside two Hymenopteran species and one Diptera (Villegas and Alcalá, 2018).

All in all, the concept of pollination syndromes (e.g. Delpino, 1868–1875; Vogel, 1954; van der Pijl, 1961; Baker and Hurd, 1986; Faergi and van der Pijl, 1979; Rosas-Guerrero et al., 2014; Johnson and Wester, 2017) can be a fruitful perspective in order to explain sorting of pollinators and prey in carnivorous plants via attraction and guiding. However, more field studies and observations of pollinator and prey taxa are urgently needed.

IV.5.3 Equally efficient: Zygomorphy in *Pinguicula* and pollen tetrads in Droseraceae

Droseraceae and *Pinguicula* are very different in regard to flower morphology: A zygomorphic flower structure, a small flower opening and a very sophisticated arrangement of reproductive organs are controlling pollinator interactions in *Pinguicula* (Villegas and Alcalá, 2018, give a detailed description for *P. moranensis*). This is opposed by actinomorphic flower structures in Droseraceae, allowing pollinators for uncontrolled access to the reproductive organs. Synorganisation of pollen material into tetrads heightens reproductive success in Droseraceae: Per pollen tetrad successfully dispersed as a single unit, four pollen grains are transmitted. Tetrad dispersal emerges as a statistically significant factor, influencing the interpretation of PDU/ovule ratios. Interestingly, almost identical pollen to ovule ratios have evolved for xenogamous breeding systems in phylogenetically unrelated lineages of carnivorous

plants. The pollination process is therefore equally efficient, realised by alternative strategies to improve effectiveness of pollen transmission.

IV.5.4 Autogamy as reproductive assurance mechanism in Droseraceae

While not realised in *Pinguicula*, spontaneous autogamy secures reproductive success in some Droseraceae. Autogamy is another strategy to enhance effectiveness of pollen transmission for the price of genetic recombination, correlated to lower pollen to ovule ratios. While autogamous Droseraceae do not differ from *Pinguicula* in regard to ovule production, ovule numbers are lowered significantly in xenogamous *Drosera* to enhance reproductive success. It should be noted that all analysed *Drosera* species showing xenogamous breeding systems are of Australian origin, except *D. regia*. Our findings complement the results of Chen, James and Stace (1997) who reported self-compatibilty in three out of 20 analysed Western Australian *Drosera* species. We can validate self-incompatibility for *Drosera menziesii ssp. menziesii*, and can newly report obligate xenogamy for the following Australian species: *D. adelae*, *D. binata*, *D. callistos*, *D. dichrosepala*, *D. leioblastus*, *D. paleacea subsp. roseana*, *D. paradoxa* and *D. platystigma*. While a phylogenetic signal underlying this pattern cannot be excluded, it has to be regarded of subordinate relevance for the ecological question of effective pollen transmission and functional breeding strategies in the context of a possible pollinator-prey conflict (see next section).
A special case is with autogamous *Dionaea*, and two explanations are possible for its higher p/o ratios: On the one hand, *Dionaea* is characterised by a very pronounced mellitophilous pollination syndrome. From observations in Bonn Botanical Garden, *Dionaea* is by far outdoing other Droseraceae (comparable in regard to flower shape, colouration and UV signal) in terms of hymenopteran flower visitations. The presence of nectar reward in *Dionaea* as pollinator attractant is not to be neglected in this context. On the other hand, two out of four plant individuals tested showed spontaneous autogamy, the others not. Both, the high frequency of hymenoptera visitations and therewith associated pollen loss, as well as presence of different breeding strategies in the genus, need to be researched further.

IV.5.5 Generally low p/o ratios indicate missing pollinator-prey conflict

When controlling for breeding strategy and taking a closer look at the mere production of pollen grains in relation to ovule numbers, the differences between Droseraceae and *Pinguicula* diminish: Xenogamous *Drosera* as well as *Pinguicula* species show a high resemblance in p/o ratios. When compared to p/o ratios reported in literature, the p/o ratios present here for xenogamous flypaper trap carnivory have to be rated as extremely low: The respective p/o ratios are rather resembling autogamous than xenogamous breeding systems. However, p/o ratios as indicators of breeding systems are no clear-cut thresholds, and research showed that the relationship between p/o ratios and breeding systems does vary between taxonomic units, usually holding on family level (Cruden, 2000), and is further influenced by biotic and abiotic environmental variables (Wilmer, 2011). In this sense, very low p/o ratios in xenogamous breeding systems of flypaper trap carnivory can be interpreted as a coping strategy in order to deal with the general resource limitation of the nutrition-deprived habitats. More importantly however, the extremely low p/o ratios in clearly xenogamous breeding systems of active

flypaper plants are a strong indicator that pollen transmission via animal vectors is highly effective, and pollinator-prey conflict of no relevance for outcrossing efficiency.

IV.6 ACKNOWLEDGEMENTS

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IV.7 LITERATURE

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V. PLASTICITY OF FLOWER TRAITS IN *STREPTOCARPUS*: FLORAL ARCHITECTURE, OPTICAL SIGNAL, NECTAR REWARD AND REPRODUCTIVE SYSTEM LARGELY DISJUNCT AND EVOLUTIONARY RECOMBINED

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V.1 ABSTRACT

Background and aims: Floral types of *Streptocarpus* are described as indicators of pollination syndromes in literature. We present quantitative measurements of key reproductive characters (pollen, ovules, p/o ratios), nectar reward (nectar amount, concentration, sugar content) and floral architecture, combined with categorical assessment of optical flower signal (daylight and UV light spectrum) and breeding system for 18 species of *Streptocarpus* subgenus *Streptocarpus*. The selected species cover the floral diversity of the genus to a large extent. We investigate the validity of the pollination syndromes concept in the genus and the functional integration of floral plant traits. Results are further presented and discussed in a phylogenetic context.

Methods: Pollen and ovule counting, hand pollination experiments, nectar measurements (amounts, sugar concentration), UV light photography, hierarchical clustering, Non-metric Multidimensional Scaling (NMDS), vector fitting, phylogenetic analysis.

Key results: Commonly described floral types of *Streptocarpus* are only explanatory for floral architecture. Gamete production and p/o ratios are of no explanatory value for the experimentally determined breeding systems. Flower size is correlated to pollen and ovule production. Flower length is correlated to nectar reward. Higher nectar amount and sugar production are correlated to higher pollen production. Higher nectar concentration is correlated to reddish flowers with stronger UV reflection, pointing to a coupling between the sensory capacities of pollinators and their nutritional needs. Otherwise, flower traits are combined freely amongst each other and along the retrieved phylogeny.

Conclusions: The analysed subsets of floral functional traits are largely uncorrelated and show extreme plasticity, not supporting the idea of clear-cut pollination syndrome pattern – only floral architecture is supported by floral types. Only few traits seem to be of importance for attraction and interaction with potential animal pollen vectors. Floral architecture and the link between optical attraction and reward appear as two different evolutionary driving forces. Both levels appear to be recombined freely. No clear phylogenetic pattern is evident for floral types, clusters of floral functional traits and pollination syndromes, indicating almost free recombination along the course of evolution.

Key words: *Streptocarpus*, pollination, floral architecture, nectar, reward, gametes, pollen, ovules, p/o ratio, flower signal, UV signal, floral function, clusters, ordination, NMDS, phylogeny.

V.2 INTRODUCTION

Streptocarpus Lindl., commonly known as Cape Primroses, have caught the attention of botanists since their discovery in the early 19th century, and are of considerable interest to horticulturists and plant breeders. Extensive breeding efforts during the last centuries have created a wide array of cultivars of high economic importance (Buta et al., 2010; Nishii et al., 2015). Not only is *Streptocarpus* characterised by a wide spectrum of very conspicuous flower shapes and colours. Species of *Streptocarpus* subgenus *Streptocarpus* also show highly unusual vegetative morphology and are missing a conventional shoot apical meristem: One of the cotyledons is growing out into the only leaf of the adult plant ("phyllomorph"), with inflorescences sprouting from the basal portion of the midrib (Hilliard and Burtt, 1971; Möller and Cronk, 2001).

Today, approximately 179 species of *Streptocarpus* are known, and the genus was recently expanded to include all African and Malagasy Gesneriaceae genera of subtribe Streptocarpinae, subfamily Didymocarpoideae tribe Trichosporeae (Möller et al., 2019, citing Darbyshire and Massingue, 2014; Nishii et al., 2015; Randrianasolo et al., 2018). Many species evolved very recently round about 1.5 million years ago, via radiation in the eastern South Africa (Möller et al., 2019; Möller and Cronk, 2001).

Floral diversity of *Streptocarpus* has been of long scientific interest. Hilliard and Burtt (1971), wrote: While "*the bare technical description 'corolla gametopetalous, five-lobed, zygomorphic*'" is able to describe almost the complete range of floral diversity in Gesneriaceae in a morphological way, "*one of the focal points of interest about Streptocarpus is that so great a proportion of the possible diversity is actually realized within this one genus*" when considering the "*wide variation in the shape of the corolla*" (Hilliard and Burtt, 1971: 34–35). Therefore, floral diversity in *Streptocarpus* is diversity of floral architecture, not floral morphology: floral architecture describes the flower as the outcome of synorganisation and fusion, differential growth rates (Endress, 1996), and the modification of floral organs (Jeiter et al., 2017; Jeiter and Weigend, 2018). Latest illustration of floral diversity was presented by Möller et al. (2019), based on the general framework of open tube, keyhole, personate, small-pouch, flat-faced *Saintpaulia* and bird-pollination flower types of Harrison et al. (1999), Hughes et al. (2006) and modifications by Nishii et al. (2015), further revised by knowledge of pollination mechanisms and observations and recently discovered species. All in all, Möller et al. (2019) identified seven main flower types and six subtypes for the open-tube type, of which five major flower types and three subtypes are analysed in this paper, see also figure V.1:

Flowers of the **small-pouch type** are predominantly small with short limbs and tubes, allowing pollinators to fully enter the corolla. The **keyhole type** is characterised by a laterally strongly compressed corolla opening, usually showing only a 2-3 mm narrow vertical slit, and an S-shaped cylindrical corolla tube. Pollinators cannot enter the corolla with their full body: Only the proboscis can be inserted to access nectar, and pollen material is also deposited on the proboscis of potential vectors. Flowers of the **personate type** are characterised by a lower corolla lip that is folded upward at a right angle, closing the gap between the lips. Pollinators are able to access the flowers by folding down the lower lip with their body weight. The **bird-pollination type** is characterised by tubular flowers with reddish colour signal to attract avian pollinators, only *S. dunnii* and *S. myoporoides* belong to this group. As for the **open tube flower type**, three subtypes have to be highlighted here:

A. The **open cylindrical tube** type with predominantly straight tubes; B. The **open tube type with pollinator chamber**, with corolla tubes showing an undilated proximal part (alignment channel; Westerkamp and Clasen-Bockhoff, 2007) and a dilated distal part (pollination chamber); C. The **Acanth flower type**, only realised in *S. liliputana*. The alignment channel is extremely long, the pollination chamber is strongly developed and the corolla shape reminds of Acanthaceae species growing in the habitat of the respective *Streptocarpus* species (Möller et al., 2019).

FIGURE V.1: DEPICITIONS OF FLOWER TYPES AND GROWTH HABITS OF SELECTED *STREPTOCARPUS* SPECIES
A. *S. burundianus* Hilliard & B.L.Burtt, small-pouch flower type (plurifoliate growth habit); B. *S. modestus* L.L.Britten, open cylindrical tube flower type (rosulate growth habit); C. *S. wilmsii* Engl., open cylindrical tube flower type (unifoliate growth habit); D. *S. bindseili* Eb.Fisch., open tube flower type with pollinator chamber (unifoliate growth habit); E. Same type as D, *S. longiflorus* (Hilliard & B.L.Burtt) T.J.Edwards (rosulate growth habit); F. *S. johannis* L.L.Britten, keyhole flower type (rosulate growth habit); G. *S. pole-evansii* I.Verd., personate flower type (plurifoliate growth habit); H. *S. dunnii* Mast., bird pollination type (unifoliate growth habit). Flower types assigned after Möller et al. (2019). Pictures by A. W. Mues.

The remarkable floral diversity in *Streptocarpus* is indicative for pollinator-driven speciation. There is strong scientific evidence that pollinator and floral shifts are correlated during the course of angiosperm evolution and diversification (compare Grant and Grant, 1965; Faegri and Van der Pijl, 1966; Stebbins, 1970; van der Niet and Johnson, 2012). With that comes the concept of pollination syndromes: Flowers that are characterised by a common set of floral characters (e.g. shape, colouration, pattern of nectar reward) are assumed to be pollinated by the same pollinator guild. Scientific research repeatedly supported the validity of the pollination syndromes concept (e.g. Delpino, 1868–1875; Vogel, 1954; van

der Pijl, 1961; Baker and Hurd, 1986; Faergi and van der Pijl, 1979; Gegear and Burns, 2007; Johnson, 2010; Gómez et al., 2008; van der Niet and Johnson, 2012; Newman et al., 2014; Rosas-Guerrero et al., 2014; Abrahamczyk et al., 2017; Johnson and Wester, 2017; Dellinger et al., 2018; Ibañez et al., 2019). As for *Streptocarpus*, Harrison et al. (1999) pointed out that the described floral types are indicative of at least four different pollination syndromes, namely: small fly pollination (pouch type), bee pollination (open tube and personate type), moth or butterfly pollination (keyhole type), and bird pollination (*S. dunnii*). However, pollination biology of *Streptocarpus* is still poorly known and pollinator information is almost as scarce as it was 50 years ago, when Hilliard and Burtt (1971) emphasised that only a functional understanding could explain the diversification. Möller et al. (2019) only report tabanid and/or nemistrinid long-proboscid flies for *S. primulifolius* (only two pollinator visits observed after about a week of observation; Bellstedt, Hughes, Möller, pers. obs.), and Potgieter and Edwards (2006) report *S. formosus* to be part of the pollination guild of the nemistrinid long-proboscid fly *Stenobasipteron wiedemanni*, along with 18 other plant species of six families (Acanthaceae, Balsaminaceae, Gesneriaceae, Iridaceae, Lamiaceae, and Orchidaceae). The involvement of long-tongued flies in the pollination of open tube flower types of *Streptocarpus* was unexpected (Möller et al., 2019) due to suggested bee pollination in earlier work (Harrison et. al., 1999). Long assumed bird-pollination of *S. dunnii* has been confirmed only a few years ago (one visit by the malachite sunbird, *Nectarinia famosa* L., observed by Bellstedt, Hughes, Möller in 2004; published in Möller et al., 2019). Thus, direct observational data on pollination in *Streptocarpus* is available only for a total of 3 species, representing 2 of the flower types outline above. More pollinator observations are urgently needed: For a comprehensive understanding of floral function the actual process of pollination needs to be studied in the field and needs to be correlated to floral functional traits.

However, in the absence of comprehensive pollinator data, floral function and pollination syndromes can be approximated by directly investigating floral functional traits (e.g. Gómez et al., 2008; Rosas-Guerrero et al., 2014; Wessinger et al., 2014; Abrahamcyk et al., 2017; Johnson and Wester, 2017). Minnaar et al. (2019) give an overview over the most important plant traits in flower biology, listing e.g. display size (Harder and Barrett, 1995; Karron et al., 2012), shape of the corolla tube (Kulbaba and Worley, 2012, 2013), overall colour (Stanton et al., 1986), colour patterns (de Jager et al., 2016; Kemp et al., 2019), brightness and contrast (Sletvold et al., 2016), shape and symmetry (e.g. Møller, 1995; Gómez et al., 2006) and nectar as a reward (Zimmerman, 1983; Thomson, 1986; Klinkhamer et al., 1991; Klinkhamer and de Jong, 1993; Hodges, 1995; Jersáková and Johnson, 2006). Floral functional traits further include pollen and ovule production, the ratio between the two, and breeding system. These data are informative on both the likely pollination syndrome, i.e. pollen vector, and on breeding system (compare Cruden, 1977; Cruden and Jensen, 1979; Cruden and Miller-Ward, 1981; Cruden and Lyon, 1985; Small, 1988; Kirk, 1993; Lopez et al., 1999; Cruden, 2000; Michalski and Durka, 2009; Alarcón et al., 2011; Abrahamcyk et al., 2017; Lozada-Gobilard et al., 2019).

In the present study we compile data on pollen and ovule numbers, nectar-reward (nectar amount, concentration and sugar content) and optical flower signal (visual and UV light spectrum) in addition to flower architecture of 18 species of *Streptocarpus subgenus Streptocarpus*, covering five major flower types and three subtypes described by Möller et al. (2019). P/o ratios are calculated as a proxy for breeding systems, and compared to the results of hand-pollination experiments. Additionally growth habit, life cycle and habitat preference (light exposure in habitat) are assessed due to possible influence

on the flower variables. Further, the interplay of floral functional traits is analysed via ordination and vector fitting, and retrieved clusters are presented and discussed phylogenetically.
We aim to address the following research questions:
Are the commonly described floral types of *Streptocarpus* indicative for pollination syndromes, and supported by the retrieved floral functional clusters (gamete production, nectar reward, optical flower signal and floral architecture)?
Are the different subsets of floral functional traits correlated to some degree, generally supporting the idea of pollination syndromes that are characterised by a particular interplay of floral characters?
Are there any evident phylogenetic pattern for flower types, floral functional traits and pollination syndromes? Is pollinator-driven speciation supported by phylogenetic shifts between floral functional plant traits?

V.3 Materials and Methods

Streptocarpus species were cultivated in a pollinator-protected greenhouse at Botanische Gärten, Universität Bonn (Bonn University Botanic Gardens), with 18 species selected for analysis: *S. bindseili* Eb.Fisch., *S. burundianus* Hilliard & B.L.Burtt, *S. cooperi* C.B.Clarke, *S. cyaneus ssp. polackii* (B.L.Burtt) Weigend & T.J.Edwards, *S. denticulatus* Turrill, *S. dunnii* Mast., *S. fasciatus* T.J.Edwards & C.Kunhardt, *S. gardenii* Hook., *S. johannis* L.L.Britten, *S. liliputana* Bellstedt & T.J.Edwards, *S. longiflorus* (Hilliard & B.L.Burtt) T.J.Edwards, *S. modestus* L.L.Britten, *S. pole-evansii* I.Verd., *S. polyanthus* ssp. *verecundus* Hook., *S. pusillus* Harv. ex C.B.Clarke, *S. rexii* (Bowie ex Hook.) Lindl., *S. roseoalbus* Weigend & T.J.Edwards and *S. wilmsii* Engl.

V.3.1 Floral types

Floral types after Möller et al. (2019) are represented as follows: The small-pouch type is represented by *S. burundianus*. The keyhole flower type is represented by *S. johannis*, *S. pusillus* and *S. polyanthus*. The personate flower type is represented by *S. pole-evansii*. The bird-pollination type is represented by *S. dunnii*. The open tube flower type with three subtypes is represented as follows: The open cylindrical tube type with predominatly straight tubes is represented by *S. modestus*, *S. wilmsii* and *S. roseoalbus*. While Möller et al. assign *S. modestus* and *S. wilmsii* to further subgroups ("broad" and "narrow cylindrical tube" subtypes) we do not differentiate between these species, due to similar height and width of the flower opening and tube length. The open tube flower type with pollinator chamber is represented by *S. bindseili*, *S. cooperi*, *S. cyaneus ssp. polackii*, *S. denticulatus*, *S. fasciatus*, *S. gardenii*, *S. longiflorus* and *S. rexii*. *S. liliputana* as only representative of the Acanth flower type is also part of the analysis.

V.3.2 Assessment of plant traits

For these species data on pollen and ovule numbers, nectar reward, the optical signal of flowers and leaves as well as floral architecture were measured. Growth habit and life cycle were assessed and habitat preferences were checked by literature review. Additionally, breeding systems were tested experimentally: 100 flowers per species (except *S. dunnii,* $n = 20$) were tracked for spontaneous autogamy, and the effect of cross pollination was tested by pollination within (10 flowers) and between

individuals (10 flowers) of each species. The bulk of the data was collected in 2015, some datasets were complemented in 2016 (*S. bindseili* and *S. burundianus*).

UV images were generated for frontal and lateral flower displays and leaf material under UV-light. Pictures were taken with a single-lens reflex camera (Nikon R D300s) and an infrared neutralizer (OPTIK MAKARIO IR NG 52D), in combination with a UV light filter (OPTIK MAKARIO SP 400 UV 52D). Photos were created with a fixed aperture, but different shutter speeds (1/5, 1/4, 1/3, 1/2, 1/1.6, 1, 1.6, 2, 3, 4, 5 sec.). 1 sec. UV light exposure showed the clearest differentiation between species and was therefore used as standard for coding of the floral UV signal.

Floral architecture was measured from living material. For assessment of flower display sizes, photographed flowers were measured with Adobe® Photoshop® CS6 imaging software and Magnetic Lasso Tool function. In total, 17 variables were recorded: frontal and lateral display size in cm^2, ratio of frontal to lateral display size, height and width of the frontal display, height and width of the corolla opening, height and width of the upper left corolla lobe, height and width of the lower central corolla lobe, dorsal flower length (from base of corolla to incision between the upper corolla lobes), ventral flower length (from base of corolla to the tip of the lower central corolla lobe), anther length, filament length, pistil length and the distance from anther to stigma. Sample size was 10 per variable and species: In total, 180 flowers were imaged and analysed for display size and 180 additional flowers measured for the remaining variables of floral architecture.

Nectar reward was measured by inserting microcapillaries (0.5 and 1 µl minicaps®; 5 µl ringcaps® – Hirschmann Laborgeräte, Germany) into the corolla tube and agitated slightly on the receptacle to quantiatively extract the nectar. Sugar concentration was measured in degrees Brix with a hand-held refractometer (neoLab, type 'universal'). Nectar sugar production per flower was calculated from volume and concentration based on the formula by Galetto and Bernadello (2005), with *x* being the concentration measured:

$$\frac{mg}{\mu l} = 0.00226 + (0.00937x) + (0.0000585x^2)$$

Usually, nectar was measured for 25 flowers per species, with data points usually equalling single flowers. Nectar from several flowers (usually 2 to 6) had to be pooled and averaged for *S. burundianus*, *S. johannis*, *S. polyanthus*, *S. pusillus* and *S. wilmsii* due to very low nectar production. For some species only a smaller number of measurements were possible: Nectar amount could be measured, but not concentration and therefore sugar content for *Streptocarpus burundianus*. Only 3 respectively 5 valid data points could be obtained for *S. cooperi* and *S. wilmsii* for nectar concentration and derived nectar sugar content. In total, 640 flowers were measured for nectar amount, yielding a total of 450 data points.

For assessment of **gamete production**, pollen and ovule numbers per flower were obtained by counting a total of 12 flowers per species, and the p/o ratio was derived afterwards. Pollen counting was performed with a hemocytometer. The two closed anthers of individual flowers were cut off and dried in Eppendorf tubes for at least three days. After drying 600 µl of a glycerol-water (1:1) solvent was added and the samples were mixed for 5 minutes in a laboratory mill, and then placed into an ultrasonic

bath for 15 minutes to dislodge pollen grains from the anther walls. Samples were then vortexed to provide a homogeneous suspension before counting. Pollen was counted on five squares (each 1 x 1 mm) of the hemocytometer chamber and the total pollen amount of the sample was calculated with the following formula (Neuendorf, 2013):

$$\text{pollen grains per sample} = \frac{\text{counted pollen grains}}{\text{counted surface (mm}^2) \cdot \text{ chamber depth (mm)} \cdot \text{ dilution (1} \div \text{amount of diluent)}}$$

Ovule numbers were counted directly under a stereomicroscope by opening the gynoecium lengthwise with a scalpel and wetting it with water to prevent ovules from drying out during counting. Pollen and ovule counts were obtained for a total of 216 flowers.

V.3.3 Phylogenetic Analysis

Two chloroplast markers and one nuclear DNA region were selected to evaluate the phylogenetic relationships of the *Streptocarpus*-species here studied, based on Nishii et al. (2015). The chloroplast DNA regions included the gene encoding for the ribosomal protein L20 and the *rpl*20-*rps*12 intergenic spacer (*rp*L20), as well as the tRNA-Leu gene (*trn*LF), including the *trn*L intron, the tRNA-Phe gene (*trn*F) and the *trn*L-*trn*F intergenic spacer (altogether referred to as *trn*L-F). The nuclear DNA region included the internal transcribed spacer of nuclear ribosomal DNA (ITS), including ITS 1, ITS 2 and the 5.8S ribosomal RNA gene. Existing sequence matrices (Nishii et al., 2015; Möller & Cronk, 2001; de Villiers et al., 2013) were downloaded from GenBank for most of the ingroup species analysed here, as well as for the outgroup species *Didymocarpus citrinus* and *Streptocarpus papangae* (subgenus *Streptocarpella*, see also Jong et al., 2012). GenBank accession numbers are presented in the appendix (table A X.4.8). New sequences were generated for *S. burundianus*, *S. polyanthus* subsp. *verecundus*, *S. cyaneus* subsp. *polackii* and *S. johannis*, using universal primers and protocols described in Nishii et al. (2015). DNA sequence assembly was done in Geneious v. 8.1.2 (Kearse et al., 2012) and were aligned in MAFFT v. 7 (Katoh and Standley, 2013) using the FFT-NS-i option (Katoh et al., 2002). Maximum Likelihood (ML) phylogenetic analyses were performed in RAxML (Stamatakis, 2014) using the "per-partition branch length" option. Statistical support for nodes was assessed with 1000 ML bootstrap replicates.

V.3.4 Statistical Analysis

Data exploration and basic statistics were carried out with the IBM software package SPSS, version 24. Statistical procedures beyond data exploration were performed with arithmetic means on species level due to unequal sample sizes and non-normality of data. Cluster analysis and multivariate ordination were performed in order to structure, analyse and visualise the datasets for gamete production, nectar reward, optical flower signal and floral architecture of *Streptocarpus*. Both methods were performed with the arithmetic means of ratio scaled variables and coding of categorical variables with computing software R.

Categorical variables were assigned via R factor function, and factor levels were labelled as unordered (nominal) or ordered (ordinal). Categorical and nominal variables are floral types (assigned after Möller et al. 2019), predominant corolla colour, growth habit, life cycle and habitat preference. Floral types were coded 1 for open cylindrical tube type, 2 for open tube type with pollinator chamber, 3 for Acanth

type, 4 for keyhole type, 5 for personate type and 6 for bird-pollination type. Predominant corolla colour was coded 1 for white, 2 for bluish and 3 for reddish tones. As for growth habit, unifoliate species only produce a single phyllomorph and were coded 1. Plurifoliate species, seldom producing more than two to three phyllomorphs at a time (one of them usually dominant), were coded 2. In some species a rosulate growth habit is realised via iterative production of additional phyllomorphs, coded with three (compare Hilliard and Burtt, 1971; Möller and Cronk, 2001). Life cycle was coded 1 for monocarpic and 2 for polycarpic/perennial species. Habitat preference was categorised on basis of a literature analysis (appendix, table A X.4.3), and coded 1 for shady habitats and 2 for half shade or sun-exposed habitats.
Categorial but ordinal variables are the degree of outcrossing of the observed breeding systems (coded 1 for autogamous, 2 for facultative xenogamous and 3 for xenogamous), presence of an anther dummy signal in form of greenish-yellow to yellow floral guides (coded 0 for absence and 1 for presence) and UV-light reflection patterns of the frontal and lateral display (coded 0 for complete absorption, 1 for diffuse absorption, 2 for weak reflection, 3 for medium reflection and 4 for strong reflection).
Metric, ratio scaled variables are all variables related to gamete production (pollen production, ovule numbers and p/o ratios), nectar reward (amount of nectar production per flower in µl, nectar sugar concentration in percent and total sugar amount per flower in mg), as well as all 17 variables regarding floral architecture (in cm^2 for variables related to display size, others in mm).
Hierarchical clustering of (a) gamete production, (b) nectar reward, (c) optical flower signal and (d) flower architecture was performed in R via package cluster, version 2.0.5 (Mächler et al., 2019), and package vegan, version 2.5-1 (Oksanen, 2013). Best number of clusters was determined via Elbow method and visual inspection of cluster dendrograms (see appendix, figures A X.4.2 and A X.4.3). As for optical flower signal, decision on best number of clusters is solely based on visual inspection due to exclusive use of categorical information. Cluster analyses were conducted with average linkage, and dissimilarity matrices based on metric variables were produced via Bray Curtis index (gamete production, nectar reward, floral architecture), while Gower distances were used for optical flower signal. Clustering of nectar reward as well as all ordinations were performed without *S. burundianus*, due to missing information on nectar concentration and produced amount of sugar.
Non-Metric Multidimensional Scaling (NMDS, R package vegan) was used for ordination in order to handle different measuring scales and non-normality of data, with same dissimilarity matrices applied as for hierarchical clustering. Function metaMDS was used for iterative testing and selection of the solution with smallest stress. Data were standardised by square root transformation and Wisconsin double standardisation. NMDS was performed separately for each of the four datasets (gamete production, nectar reward, optical flower signal, floral architecture; combined analysis presented in the appendix, figures A X4.4 and A X.4.5). Afterwards variables of the other three datasets as well as breeding system, growth habit, life cycle and habitat preference were vector-fitted onto the respective ordinations to test for explanatory value. For this purpose, R function envfit from package vegan was performed with 999 permutations in each case. Model stresses between .05 and .1 were interpreted as good, and below .05 as excellent (McCune et al., 2002).

V.4 RESULTS

In the following section the observed data bandwidth, retrieved clusters and NMDS ordinations for the four analysed datasets are presented: Floral architecture, optical flower signal, nectar reward and gamete production.

V.4.1 FLORAL ARCHITECTURE

Frontal display size is 4.4 ± 3.4 cm^2 in average (min.: .7 cm^2, *S. pole-evansii*; max.: 10.7 cm^2, *S. roseoalbus*). Lateral display size is 2.6 ± 1.8 cm^2 (min.: .4 cm^2, *S. pole-evansii* and *S. burundianus*; max.: 5.9 cm^2, *S. cyaneus ssp. polackii* and *S. fasciatus*). The ratio of frontal to lateral display size is 1.9 ± .8 on average (min.: .4, *S. dunnii*; max.: 3.2, *S. johannis*). Please compare table V.1.

Species-specific profiles for other variables of floral architecture are presented in the appendix, table A X.4.1: All variables are strongly and positively intercorrelated (appendix, table A X.4.2), representing flower size in general, and are therefore not presented in detail here. Please compare figures V.2 to V.4 for depictions of floral architecture.

TABLE V.1: DISPLAY SIZE MEASUREMENTS OF *STREPTOCARPUS* SPECIES

Display sizes for the *Streptocarpus*-species analysed, exemplary for other variables of floral architecture due to high intercorrelation (see appendix). The table shows the total number of samplings (*n*), the average frontal display in cm^2, the average lateral display in cm^2 and the average display ratio (frontal : lateral) for each species, with corresponding standard deviation. Clusters derived from variables related to floral architecture are indicated: ○ = cluster 1, funnel shaped open tube types (cylindrical or with pollinator chamber), larger in size, pronounced frontal display, including Acanth-type of *S. liliputana*; ● = cluster 2, flowers of small or moderate intrageneric size and usually of more sophisticated corolla architecture, including keyhole flower types (*S. johannis*, *S. polyanthus*, *S. pusillus*), curved corollas of *S. denticulatus* (open tube with pollinator chamber) and *S. pole-evansii* (approximating masked flower type) as well as small-pouch flower type of *S. burundianus*; ● = cluster 3, roughly cylindric flower types of *S. dunnii* and *S. wilmsii*, without pronounced frontal display (display ratio < 1).

species	cluster	*n*	frontal display in cm^2	lateral display in cm^2	display ratio (frontal:lateral)
Streptocarpus bindseili Eb.Fisch.	○	10	7.4 ± 0.7	2.9 ± 0.3	2.6 ± 0.3
Streptocarpus burundianus Hilliard & B.L.Burtt	●	10	1.0 ± 0.2	0.4 ± 0.0	2.7 ± 0.4
Streptocarpus cooperi C.B.Clarke	○	10	3.9 ± 1.3	3.0 ± 0.3	1.3 ± 0.3
Streptocarpus cyaneus ssp. polackii (B.L.Burtt) Weigen & T.J.Edwards	○	10	10.2 ± 2.0	5.9 ± 1.1	1.7 ± 0.4
Streptocarpus denticulatus Turrill	●	10	2.3 ± 0.4	1.0 ± 0.2	2.3 ± 0.3
Streptocarpus dunnii Mast.	●	10	1.1 ± 0.2	2.5 ± 0.3	0.4 ± 0.1
Streptocarpus fasciatus T.J.Edwards & C.Kunhardt	○	10	9.9 ± 1.0	5.9 ± 0.9	1.7 ± 0.3
Streptocarpus gardenii Hook.	○	10	3.9 ± 0.8	3.2 ± 0.3	1.2 ± 0.2
Streptocarpus johannis L.L.Britten	●	10	3.5 ± 0.9	1.1 ± 0.4	3.2 ± 0.3
Streptocarpus liliputana Bellstedt & T.J.Edwards	○	10	3.8 ± 1.1	3.0 ± 0.3	1.3 ± 0.2
Streptocarpus longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	○	10	4.3 ± 0.7	3.8 ± 0.3	1.1 ± 0.1
Streptocarpus modestus L.L.Britten	○	10	5.7 ± 0.8	2.5 ± 0.4	2.3 ± 0.3
Streptocarpus pole-evansii I.Verd.	●	10	0.7 ± 0.1	0.4 ± 0.0	1.7 ± 0.2
Streptocarpus polyanthus Hook.	●	10	1.2 ± 0.2	0.5 ± 0.1	2.4 ± 0.3
Streptocarpus pusillus Harv. ex C.B.Clarke	●	10	1.3 ± 0.2	0.5 ± 0.1	2.8 ± 0.2
Streptocarpus rexii (Bowie ex Hook.) Lindl.	○	10	7.5 ± 1.0	5.5 ± 0.7	1.4 ± 0.2
Streptocarpus roseoalbus Weigend & T.J.Edwards	○	10	10.7 ± 1.6	3.5 ± 0.4	3.1 ± 0.3
Streptocarpus wilmsii Engl.	●	10	1.3 ± 0.2	1.5 ± 0.1	0.9 ± 0.1

Hierarchical clustering of floral architecture variables retrieved three clusters:

Cluster 1 represents generally larger, more or less funnel shaped flowers with a large frontal display (display ratio > 1). It includes the open tube type with pollinator chamber (*S. bindseili*, *S. cooperi*, *S. cyaneus ssp. polackii*, *S. fasciatus*, *S. gardenii*, *S. longiflorus*, *S. rexii*), the open cylindrical tube type (*S. modestus*, *S. roseoalbus*) and *S. liliputana* with its Acanth flower type.

Cluster 2 includes smaller to moderate flowers with more complex corolla architecture and a large frontal display (display ratio ≥ 1.7): Flowers of the keyhole type (*S. johannis*, *S. polyanthus*, *S. pusillus*) are part of this cluster, as well as the smaller and curved corollas of *S. denticulatus* (open tube type with pollinator chamber) and *S. pole-evansii* (personate flower type). Moreover, *S. burundianus* (pouch flower type) is also part of this cluster.
Cluster 3 includes the cylindrical flowers of *S. dunnii* (bird-pollination type) and *S. wilmsii* (open cylindrical tube), moreover characterised by a small frontal display (display ratio < 1).

V.4.2 Optical signal

Optical signal of *Streptocarpus* flowers is presented in table V.2 and figures V.2 to V.4. Regarding corolla colour, seven species have predominantly white flowers: *S. bindseili*, *S. burundianus*, *S. gardenii*, *S. polyanthus*, *S. pusillus*, *S. rexii* and *S. wilmsii*. Six species have flowers with a predominantly bluish tone, ranging from skyblue to medium violet: *S. cooperi*, *S. cyaneus ssp. polackii*, *S. johannis*, *S. liliputana*, *S. longiflorus* and *S. modestus*. Reddish flower colouration is present in five species, ranging from pale rose and pinkish to red: *S. denticulatus*, *S. dunnii*, *S. fasciatus*, *S. pole-evansii* and *S. roseoalbus*. Predominant corolla colour is usually identical in frontal and lateral displays. However, lateral display of *S. gardenii* is white with greenish shading, and lateral display of *S. roseoalbus* shows a chalky white to pinkish lateral view.
Seven species show anther dummy signals in the form of greenish-yellow to yellow floral guides: A clearly demarcated yellow stripe placed at the interior, ventral side of the corolla tube is found in *S. cyaneus ssp. polackii*, *S. longiflorus* and *S. roseoalbus*. A greenish-yellow blotch is placed at the interior, ventral side of the keyhole flower entrance of *S. polyanthus* and *S. pusillus*. Interior colour of the flower tube of *S. gardenii* is greenish, strongly contrasting the flower entrance of the otherwise white frontal display. In *S. denticulatus* a greenish-yellow blotch is placed at interior, ventral side of the corolla. Occassional occurrence of yellow floral guides in flowers of *S. johannis*, *S. modestus* and *S. wilmsii* is mentioned as an aspect of within-population variation by Hilliard and Burt (1971): Our accessions lacked anther dummy signals during the experimental phase, and species were labeled accordingly for the calculations. However, identical plant individuals of *S. modestus* showed a weak yellow floral guide at the interior, ventral side of the corolla opening during the following flowering season.
A broad spectrum of UV signals was observed for *Streptocarpus*-flowers. For the frontal display, strong UV light absorption was observed for *S. bindseili*, *S. burundianus*, *S. liliputana* and *S. longiflorus*. A rather diffuse but noticeable absorption was observed for S. *johannis*, *S. modestus, S. polyanthus*, *S. rexii* and *S. wilmsii.* A weak UV reflection was observed for *S. cooperi*, *S. cyaneus ssp. polackii*, *S. dunnii*, *S. gardenii* and *S. pusillus*, while moderate reflection was observed for *S. denticulatus* and *S. pole-evansii*. Strong UV reflection was observed for *S. fasciatus* and *S. roseoalbus*. For the lateral display side, UV reflection is either identical with the frontal display, or more reflective (observed for *S. cooperi*, *S. gardenii*, *S. johannis*, *S. liliputana*, *S. longiflorus*, *S. modestus* and *S. rexii*).

Hierarchical clustering of optical flower signal sorted the species studied into three clusters:

Cluster 1 represents species with white or bluish corollas, usually combined with UV light absorption of the frontal display (at maximum weak reflection), while an anther dummy signal is missing (*S. bindseili*, *S. burundianus*, *S. cooperi*, *S. johannis*, *S. liliputana*, *S. modestus*, *S. rexii* and *S. wilmsii*).
Cluster 2 is indentical to cluster 1 in regard to flower colour and UV reflection, but an anther dummy signal is present (*S. cyaneus ssp. polackii*, *S. gardenii*, *S. longiflorus*, *S. polyanthus* and *S. pusillus*).
Cluster 3 comprises species with red or reddish corollas which are UV-reflective. Anther dummy signal is present or missing.

In general, all *Streptocarpus* flowers contrast to the leaves: Corollas are coloured white, bluish or red and are uniformly UV absorbent or reflective, while leaves are green and have a mixed UV pattern: Leaf surfaces are generally UV reflective and leaf veins are absorbent (compare appendix, figure A X.4.1).

TABLE V.2: CATEGORISATION OF OPTICAL FLOWER SIGNAL OF *STREPTOCARPUS* SPECIES
The table shows the predominant flower colour (coded as white ○, bluish ● or reddish ●), the presence of an anther dummy signal as well as patterns of UV-light signal (complete absorption ●, diffuse absorption ⊙, weak reflection ○, medium reflection ○○ and strong reflection ○○○) for the frontal and lateral display. Please compare photographic documentation, figures V.2 to V.4. Clusters indicated: ◇ = cluster 1, white or bluish flower colour, missing anther dummy signal, UV light absorbent, diffuse signal or only weak UV reflection; ◆ = cluster 2, colouration and UV light pattern like cluster 1, but anther dummy signal present; ◆ = cluster 3, reddish flower colour, anther dummy signal present or absent, always UV light reflecting, ranging from weak to strong UV reflection. * = If possible, corolla colour was labelled after Hilliard and Burtt (1971).

species	cluster	flower colour		anther dummy	UV-signal frontal display	UV-signal lateral display
Streptocarpus bindseili Eb.Fisch.	◇	white	○		●	●
Streptocarpus burundianus Hilliard & B.L.Burtt	◇	white	○		●	●
Streptocarpus cooperi C.B.Clarke	◇	medium violet*	●		○	○○
Streptocarpus cyaneus ssp. polackii (B.L.Burtt) Weigend & T.J.Edwards	◆	skyblue	●	✓	○	○
Streptocarpus denticulatus Turrill	◆	pale violet to pinkish-red*	●	✓	○○	○○
Streptocarpus dunnii Mast.	◆	pink to reddish*	●		○	○
Streptocarpus fasciatus T.J.Edwards & C.Kunhardt	◆	pale rose	●		○○○	○○○
Streptocarpus gardenii Hook.	◆	white form* (others possible)	○	✓	○	○○
Streptocarpus johannis L.L.Britten	◇	liliac form (variations possible)	●		⊙	○
Streptocarpus liliputana Bellstedt & T.J.Edwards	◇	pale to medium violet	●		●	⊙
Streptocarpus longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	◆	medium violet to blue	●	✓	●	⊙
Streptocarpus modestus L.L.Britten	◇	pale to medium violet*	●		⊙	○
Streptocarpus pole-evansii I.Verd.	◆	pale-violet*, reddish tone	●		○○	○○
Streptocarpus polyanthus Hook.	◆	white form* (others possible)	○	✓	⊙	⊙
Streptocarpus pusillus Harv. ex C.B.Clarke	◆	white*	○	✓	○	○
Streptocarpus rexii (Bowie ex Hook.) Lindl.	◇	white form * (others possible)	○		⊙	○
Streptocarpus roseoalbus Weigend & T.J.Edwards	◆	pale pink to pink	●	✓	○○○	○○○
Streptocarpus wilmsii Engl.	◇	white*	○		⊙	⊙

FIGURE V.2: CLOSE-UP OF *STREPTOCARPUS* FLOWERS, ARCHITECTURE AND SIGNAL – I
Frontal and lateral flower displays, showing floral architecture and optical flower signal for visual light spectrum (columns 1 and 2) and in regard to UV light (1 sec. exposure time, columns 3 and 4), as well as longitudinal section showing architecture of reproductive organs and inner side of corolla (column 5). A: *Streptocarpus binseili* (open tube type with pollinator chamber); B: *S. burundianus* (small-pouch type); C: *S. cooperi* (open tube type with pollinator chamber); D: *S. cyaneus ssp. polackii* (open tube type with pollinator chamber); E: *S. denticulatus* (open tube type with pollinator chamber); F: *S. dunnii* (bird-pollination type). Scaling bars indicating 1 cm. Pictures by Andreas W. Mues.

FIGURE V.3: CLOSE-UP OF *STREPTOCARPUS* FLOWERS, ARCHITECTURE AND SIGNAL – II

Frontal and lateral flower displays, showing flower architecture and optical flower signal for visual light spectrum (columns 1 and 2) and in regard to UV light (1 sec. exposure time, columns 3 and 4), as well as longitudinal section showing architecture of reproductive organs and inner side of corolla (column 5). G: *S. fasciatus* (open tube type with pollinator chamber); H: *S. gardenii* (open tube type with pollinator chamber); I: *S. johannis* (keyhole type); J: *S. liliputana* (Acanth type); K: *S. longiflorus* (open tube type with pollinator chamber); L: *S. modestus* (open cylindrical tube type). Scaling bars indicating 1 cm. Pictures by Andreas W. Mues.

FIGURE V.4: CLOSE-UP OF *STREPTOCARPUS* FLOWERS, ARCHITECTURE AND SIGNAL – III
Frontal and lateral flower displays, showing flower architecture and optical flower signal for visual light spectrum (columns 1 and 2) and in regard to UV light (1 sec. exposure time, columns 3 and 4), as well as longitudinal section showing architecture of reproductive organs and inner side of corolla (column 5). M: *S. pole-evansii* (personate type); N: *S. polyanthus* (keyhole type); O: *S. pusillus* (keyhole type); P: *S. rexii* (open tube type with pollinator chamber); Q: *S. roseoalbus* (open cylindrical tube type); R: *S. wilmsii* (open cylindrical tube type). Scaling bars indicating 1 cm. Pictures by Andreas W. Mues.

V.4.3 NECTAR REWARD

Average nectar production in the species studied is 2.95 ± 3.15 µl (min.: .01 µl, *S. burundianus*; max.: 10.61 µl, *S. longiflorus*), average concentration is 28.7 ± 12.7 % (min.: 16.4 %, *S. longiflorus*; max.: 57.0 %, *S. pole-evansii*) and average sugar production per flower is .77 ± .69 mg (min.: .06 mg, *S. wilmsii*; max.: 2.07 mg, *S. denticulatus*). Data bandwidth and retrieved clusters are presented in table V.3.

TABLE V.3: NECTAR REWARD MEASUREMENTS OF *STREPTOCARPUS* SPECIES

The table shows the total number of samplings (*n*), average number of µl nectar per flower, average nectar concentration in percent and average amount of sugar per flower in mg, with corresponding standard deviation. Clusters indicated: ☐ = cluster 1, nectar production and total sugar content lower, nectar concentration below 27 %; ■ = cluster 2, intermediate nectar production and total sugar content, nectar concentration higher, ranging between 34.1 and 57.0 %; ■ = cluster 3, higher nectar amount and total sugar content, but nectar concentration low (16.4 and 16.9 %). * = Nectar concentration and sugar content could not be retrieved for *S. burundianus* with our method. As for *S. cooperi* and *S. wilmsii*, only 3 and 5 valid data points were retrieved for nectar concentration and sugar content, respectively.

species	cluster	*n*	µl nectar per flower	concentration in %	sugar per flower in mg
Streptocarpus bindseili Eb.Fisch.	☐	25	2.29 ± 1.57	26.2 ± 8.3	0.56 ± 0.51
Streptocarpus burundianus Hilliard & B.L.Burtt	*	25 (0)*	0.01 ± 0.01	*	*
Streptocarpus cooperi C.B.Clarke	☐	25 (3)*	0.10 ± 0.29	16.7 ± 2.9	0.15 ± 0.03
Streptocarpus cyaneus ssp. polackii (B.L.Burtt) Weigend & T.J.Edwards	☐	25	7.28 ± 6.75	25.3 ± 8.4	1.67 ± 0.20
Streptocarpus denticulatus Turrill	■	25	5.05 ± 3.49	47.5 ± 16.4	2.48 ± 0.28
Streptocarpus dunnii Mast.	■	25	6.67 ± 4.76	34.8 ± 16.8	2.19 ± 0.24
Streptocarpus fasciatus T.J.Edwards & C.Kunhardt	■	25	7.63 ± 6.39	16.9 ± 6.9	1.34 ± 0.25
Streptocarpus gardenii Hook.	☐	25	1.57 ± 1.44	19.7 ± 7.0	0.28 ± 0.04
Streptocarpus johannis L.L.Britten	☐	25	0.30 ± 0.14	25.3 ± 8.4	0.08 ± 0.01
Streptocarpus liliputana Bellstedt & T.J.Edwards	☐	25	1.71 ± 1.01	21.1 ± 7.2	0.36 ± 0.04
Streptocarpus longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	■	25	10.61 ± 6.60	16.4 ± 6.9	1.59 ± 0.17
Streptocarpus modestus L.L.Britten	■	25	0.91 ± 0.46	34.1 ± 9.8	0.33 ± 0.03
Streptocarpus pole-evansii I.Verd.	■	25	2.51 ± 1.59	57.0 ± 10.3	1.69 ± 0.18
Streptocarpus polyanthus Hook.	☐	25	0.85 ± 0.20	20.2 ± 2.3	0.18 ± 0.01
Streptocarpus pusillus Harv. ex C.B.Clarke	■	25	0.54 ± 0.19	32.6 ± 3.6	0.20 ± 0.02
Streptocarpus rexii (Bowie ex Hook.) Lindl.	☐	25	2.56 ± 1.39	20.6 ± 7.0	0.53 ± 0.06
Streptocarpus roseoalbus Weigend & T.J.Edwards	■	25	2.25 ± 1.50	52.1 ± 23.0	1.20 ± 0.10
Streptocarpus wilmsii Engl.	☐	25 (5)*	0.17 ± 0.21	20.6 ± 8.4	0.07 ± 0.04

Hierarchical clustering retrieved three clusters for the nectar data:

Cluster 1 is characterised by lower nectar amount, sugar production and nectar concentration, and consists of nine species: *Streptocarpus binseili*, *S. cooperi*, *S. cyaneus ssp. polackii*, *S. gardenii*, *S. johannis*, *S. liliputana*, *S. polyanthus*, *S. rexii* and *S. wilmsii*. For cluster 1, nectar production is 1.87 ± 2.22 µl (min.: .01 µl, *S. burundianus*; max.: 7.28 µl, *S. cyaneus ssp. polackii*), nectar sugar concentration is 21.7 ± 3.2 % (min.: 16.7 %, *S. cooperi*; max.: 26.2 %, *S. bindseili*) and sugar production per flower is .40 ± .46 mg (min.: .06 mg, *S. wilmsii*; max.: 1.54 mg, *S. cyaneus ssp. polackii*) in average.

Cluster 2 shows intermediate intrageneric nectar amount and sugar production, but higher concentration, and consists of six species: *S. denticulatus*, *S. dunnii*, *S. modestus*, *S. pole-evansii*, *S. pusillus* and *S. roseoalbus*. For cluster 2, nectar production is 2.99 ± 2.41 µl (min.: .54 µl, *S. pusillus*; max.: 6.67 µl, *S. dunnii*), nectar sugar concentration is 43.0 ± 10.5 % (min.: 32.6 %, *S. pusillus*; max.: 57.0 %, *S. pole-evansii*) and sugar production per flower is 1.1 ± .80 mg (min.: .18 mg, *S. pusillus*; max.: 2.07 mg, *S. denticulatus*) in average.

Cluster 3 is represented by two species, *S. fasciatus* and *S. longiflorus*, which are characterised by very similar profiles: nectar amount and sugar production per flower is higher (*S. fasciatus*: 7.63 µl, 1.34 mg sugar; *S. longiflorus*: 10.61 µl, 1.59 mg sugar), but nectar concentration is low (16.9 and 16.4 %).

V.4.4 Gamete production

Average pollen production per flower is 276 322 ± 140 646 pollen grains (min.: 90 750, *S. johannis*; max.: 506 450, *S. denticulatus*), average ovule number is 2 794 ± 1 730 (min.: 574, *S. burundianus*; max.: 6 797, *S. cooperi*) and average p/o ratio is 128 ± 76 (min.: 43, *S. johannis*; max.: 299, *S. denticulatus*) for the species analysed. Data bandwidth and retrieved clusters are presented in table V.4.

Hierarchical clustering retrieved three clusters for gamete production:
Cluster 1 contains species with higher pollen production, namely: *S. binseili*, *S. cooperi*, *S. cyaneus ssp. polackii*, *S. denticulatus*, *S. dunnii*, *S. fasciatus*, *S. longiflorus* and *S. roseoalbus*. For this cluster, average pollen production per flower is 417 750 ± 58 757 pollen grains (min.: 339 300, *S. cyaneus ssp. polackii*; max.: 506 450, *S. denticulatus*). Average ovule number is 3 812 ± 1 668 (min.: 1 715, *S. denticulatus*; max.: 6 797, *S. cooperi*), and average p/o ratio for this cluster is 139 ± 82 (min.: 58, *S. cooperi*; max.: 299, *S. denticulatus*).
Cluster 2 is formed by three species, *S. gardenii*, *S. pole-evansii* and *S. rexii*, and intrageneric pollen production is intermediate: Average number of pollen grains per flower is 231 517 ± 7 667 (min.: 223 550, *S. gardenii*; max. 238 850, *S. pole-evansii*), ovule production is 3 150 ± 1 985 (min.: 1 059, *S. pole-evansii*; max.: 5 008, *S. rexii*) and p/o ratio is 117 ± 101 (min.: 49, *S. rexii*; max.: 233, *S. pole-evansii*).

TABLE V.4: POLLEN AND OVULE PRODUCTION AS WELL AS P/O RATIO OF *STREPTOCARPUS* SPECIES
The table shows the total number of samplings (*n*), average number of ovules, average pollen amount per flower and average p/o ratio for the species analysed, with corresponding standard deviation. Clusters indicated: △ = cluster 1, species with highest pollen production, ranging from 339 300 (*S. cyaneus*) to 506 450 pollen grains per flower (*S. denticulatus*); ▲ = cluster 2, intermediate intrageneric pollen production, approx. 230 000 pollen grains per flower; ▲ = cluster 3, usually lower ovule numbers and pollen production below 200 000, lowest in *S. johannis* (90 750). For all clusters, p/o ratio shows no clear pattern.

species	cluster	*n*	ovules per flower			pollen per flower			p/o ratio		
Streptocarpus bindseili Eb.Fisch.	△	12	3 482	±	381	453 800	±	59 587	132	±	25
Streptocarpus burundianus Hilliard & B.L.Burtt	▲	12	574	±	119	116 950	±	40 869	212	±	87
Streptocarpus cooperi C.B.Clarke	△	12	6 797	±	705	386 950	±	58 674	58	±	11
Streptocarpus cyaneus ssp. polackii (B.L.Burtt) Weigend & T.J.Edwards	△	12	5 076	±	1 151	339 300	±	56 036	68	±	10
Streptocarpus denticulatus Turrill	△	12	1 715	±	222	506 450	±	70 838	299	±	51
Streptocarpus dunnii Mast.	△	12	2 402	±	348	483 800	±	55 975	206	±	43
Streptocarpus fasciatus T.J.Edwards & C.Kunhardt	△	12	4 574	±	787	411 100	±	62 332	92	±	19
Streptocarpus gardenii Hook.	▲	12	3 382	±	697	223 550	±	46 500	70	±	25
Streptocarpus johannis L.L.Britten	▲	12	2 180	±	597	90 750	±	26 757	43	±	14
Streptocarpus liliputana Bellstedt & T.J.Edwards	▲	12	636	±	130	136 600	±	28 434	222	±	63
Streptocarpus longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	△	12	2 452	±	336	398 800	±	72 740	163	±	22
Streptocarpus modestus L.L.Britten	▲	12	1 992	±	392	163 800	±	27 831	86	±	31
Streptocarpus pole-evansii I.Verd.	▲	12	1 059	±	189	238 850	±	31 465	233	±	57
Streptocarpus polyanthus Hook.	▲	12	1 203	±	266	139 350	±	17 862	120	±	26
Streptocarpus pusillus Harv. ex C.B.Clarke	▲	12	1 725	±	442	127 050	±	25 349	79	±	31
Streptocarpus rexii (Bowie ex Hook.) Lindl.	▲	12	5 008	±	1 061	232 150	±	40 804	49	±	13
Streptocarpus roseoalbus Weigend & T.J.Edwards	△	12	4 001	±	837	361 800	±	42 162	93	±	16
Streptocarpus wilmsii Engl.	▲	12	2 031	±	278	162 750	±	24 243	81	±	13

Cluster 3 is formed by the remaining seven species, *S. burundianus*, *S. johannis*, *S. liliputana*, *S. modestus*, *S. polyanthus*, *S. pusillus* and *S. wilmsii*, generally showing lower intrageneric pollen and ovule production: Average pollen production is 133 893 ± 25 689 pollen grains (min.: 90 750, *S. johannis*; max.: 163 800, *S. modestus*). Average ovule number is 1 477 ± 674 (min.: 574, *S. burundianus*; max.: 2 180, *S. johannis*), and average p/o ratio for this cluster is 120 ± 70 (min.: 43, *S. johannis*; max.: 222, *S. liliputana*).

As can be seen, p/o ratio is ranging freely, showing higher and lower values in all three clusters.

V.4.5 Growth habit, life cycle, habitat preference and breeding system

Species of unifoliate growth habit are *S. bindseili*, *S. cooperi*, *S. denticulatus*, *S. dunnii* and *S. wilmsii*. Plurifoliate growth habit is present in *S. burundianus*, *S. pole-evansii*, *S. polyanthus* and *S. pusillus*. All other species show rosulate habitus.

Moncarpic species, ending their life cycle after fruiting, are *S. cooperi*, *S. denticulatus* and *S. dunnii*. All other analysed species are polycarpic and perennial.

Species growing in shady conditions, namely in forests or deep rock crevices, are *S. bindseili*, *S. burundianus*, *S. cooperi*, *S. denticulatus*, *S. fasciatus*, *S. gardenii*, *S. johannis*, *S. liliputana* and *S. wilmsii*. Other species are growing at least in half shade or even sun-exposed, usually plants of the lowveld, on rock outcrops only slightly shaded by rocks, or along forest margins.

For detailed description of these variables see table A X.4.3 in the appendix.

Experimental results from hand-pollination and selfing and retrieved breeding systems are presented in table V.5. Most species showed a facultative xenogamous breeding system, being capable of self-fertilisation. Spontaneous autogamy was observed for four species: *S. bindseili*, *S. gardenii*, S. *polyanthus* and *S. pusillus*. Obligate xenogamy was only observed for *S. pole-evansii*.

TABLE V.5: BREEDING SYSTEMS OF *STREPTOCARPUS* SPECIES

Breeding systems as experimentally determined. To identify autogamy, 100 flowers were tracked under pollinator exclusion: 100 % fruit set is categorised as autogamy. All other species showed no fruit set without hand pollination. To test for self-compatibility and allogamy, 10 emasculated flowers each were hand-pollinated within and between individuals of a species. All hand-pollinated species were capable of geitonogamy as well as allogamy, therefore labelled facultatively xenogamous, except *S. pole-evansii* showing no fruit set at all. Later species was therefore labelled obligate xenogamous, with an incompatibility mechanism preventing seed set between the tested plant individuals. Only one plant of *S. dunnii* was flowering, which showed no spontaneous autogamous fruit set, but self-fertilisation was possible.

species	breeding system		
Streptocarpus bindseili Eb.Fisch.	autogamous		
Streptocarpus burundianus Hilliard & B.L.Burtt		facultative xenogamous	
Streptocarpus cooperi C.B.Clarke		facultative xenogamous	
Streptocarpus cyaneus ssp. polackii (B.L.Burtt) Weigend & T.J.Edwards		facultative xenogamous	
Streptocarpus denticulatus Turrill		facultative xenogamous	
Streptocarpus dunnii Mast.		facultative xenogamous	
Streptocarpus fasciatus T.J.Edwards & C.Kunhardt		facultative xenogamous	
Streptocarpus gardenii Hook.	autogamous		
Streptocarpus johannis L.L.Britten		facultative xenogamous	
Streptocarpus liliputana Bellstedt & T.J.Edwards		facultative xenogamous	
Streptocarpus longiflorus (Hilliard & B.L.Burtt) T.J.Edwards		facultative xenogamous	
Streptocarpus modestus L.L.Britten		facultative xenogamous	
Streptocarpus pole-evansii I.Verd.			obligate xenogamous
Streptocarpus polyanthus Hook.	autogamous		
Streptocarpus pusillus Harv. ex C.B.Clarke	autogamous		
Streptocarpus rexii (Bowie ex Hook.) Lindl.		facultative xenogamous	
Streptocarpus roseoalbus Weigend & T.J.Edwards		facultative xenogamous	
Streptocarpus wilmsii Engl.		facultative xenogamous	

V.4.6 Reciprocal NMDS ordinations and vector fitting of trait subsets

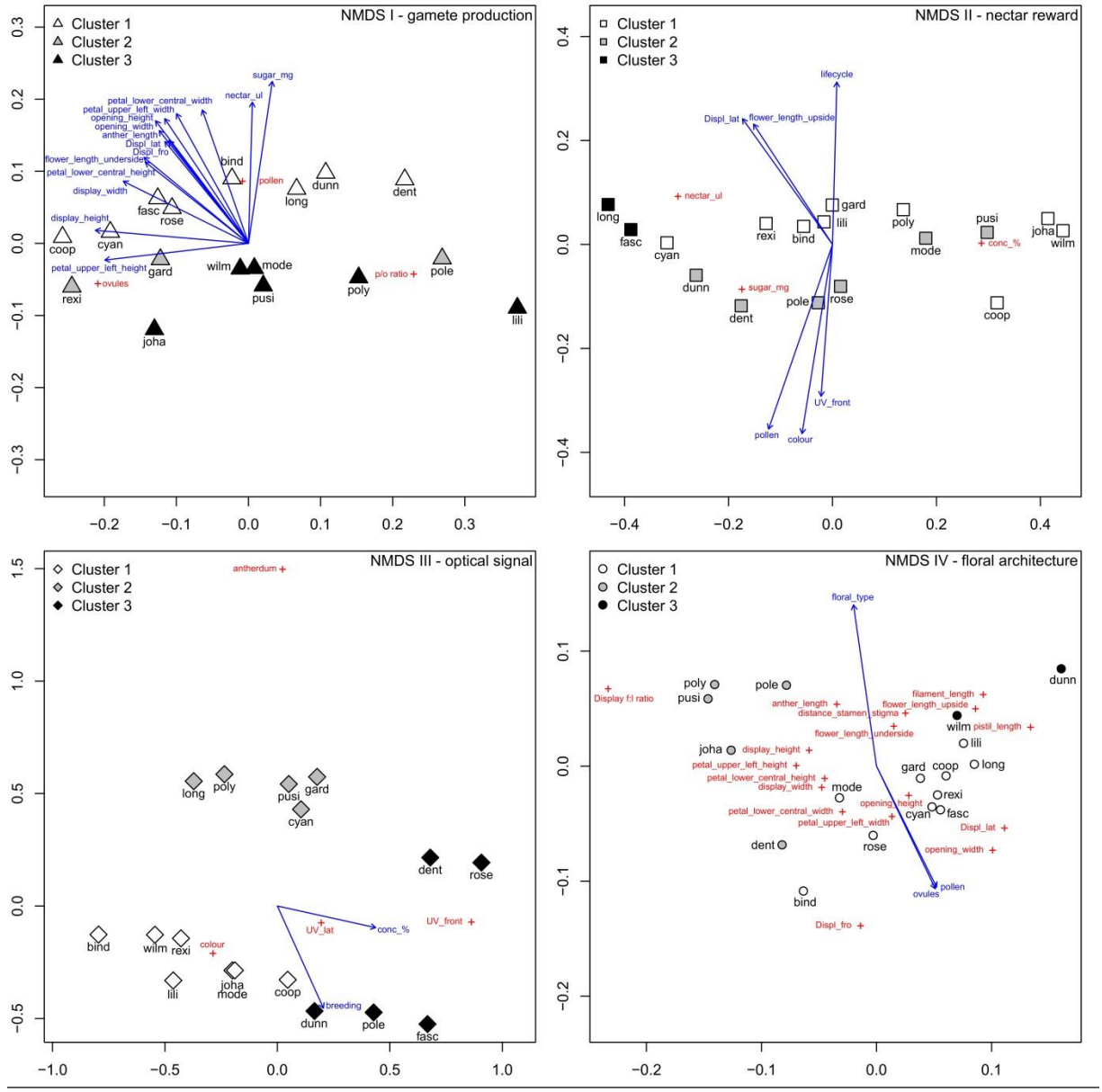

FIGURE V.5: RECIPROCAL NMDS ORDINATIONS AND VECTOR FITTING OF ANALYSED TRAIT SUBSETS

NMDS results for gametic variables (upper left), nectar reward (upper right), optical signal (lower left) and floral architecture (lower right). For each NMDS, ordinated variables are shown in red (+) and retrieved species clusters are indicated, with species names abbreviated. Other subsets of floral form and function (ordinated via separate NMDS), as well as additional variables (growth habit, life cycle, habitat preference and breeding system) were vector-fitted onto the respective ordinations to test for explanatory value. Only significant vectors ($p \leq .05$) are shown. Vectors show the direction of most rapid change in the variable within the dataset. Stress of NMDS for gametic (stress value: 0.020) and nectar variables (0.010) are indicating excellent fit, and good fit for optical signal (0.068) and floral architecture (0.074). In all cases two convergent solutions were found after 20 tries.

NMDS ordinations of floral form and function and reciprocal vector fitting of significant vectors are shown in figure V.5, and detailed information on significance and explained variance of all fitted vectors is given in the appendix, tables A X.4.4 to A X.4.7.

As can be seen, NMDS ordination of gamete production is strongly explained by floral architecture, most of the 17 intercorrelated variables are significant. Explained variance is ranging from r^2 = .38 for lateral display size to .53 for the height of the flower opening. Variables of no explanatory value are the ratio of frontal to lateral display size (r^2 = .05, p = .708), dorsal flower length (r^2 = .26, p = .112) and three out of four variables regarding reproductive organs: filament length (r^2 = .14, p = .362), pistil length (r^2 = .23, p = .139) and distance between anthers and stigma (r^2 = .33, p = .059, however a trend), only anther length is significant (r^2 = .46, p = .017). Produced nectar amount (r^2 = .45, p = .017) and total sugar production (r^2 = .60, p = .002) are correlated with gamete production, but nectar concentration is not (r^2 = .19, p = .250). No variable of the optical flower signal, breeding system, growth habit, life cycle or habitat preference are of any explanatory value for gamete production.

Floral type is of no explanatory value for gamete production (r^2 = .20, p = .227).

Regarding NMDS ordination of nectar reward, pollen production is the only gametic variable of explanatory value (r^2 = .66, p = .002), but p/o ratio does show a trend (r^2 = .34, p = .064). As for optical flower signal, flower colour is highly significant and strongly explanatory for nectar reward (r^2 = .64, p = .002). UV signal of the frontal flower display is also of explanatory value (r^2 = .40, p = .030), and UV signal of the lateral display does show a trend (r^2 = .31, p = .090). As for floral architecture, lateral display size (r^2 = .42, p = .028) and dorsal flower length (r^2 = .36, p = .039) are of explanatory value for nectar reward. Two other variables also related to corolla tube length show a trend, ventral flower length (r^2 = .30, p = .077) and pistil length (r^2 = .32, p = .054). In regard to the remaining variables, only life cycle is explanatory and significant (r^2 = .46, p = .014), but a trend is present for breeding system (r^2 = .33, p = .057) and growth habit (r^2 = .29, p = .085). Other variables are non-significant.

Non-significance of floral type for nectar reward has to be highlighted (r^2 = .02, p = .887).

NMDS ordination of optical flower signal is only explained by breeding system (r^2 = .44, p = .011) and nectar concentration (r^2 = .36, p = .047), but nectar sugar production does show a trend (r^2 = .32, p = .066).

Floral type is of no explanatory value for optical flower signal (r^2 = .04, p = .778).

As for NMDS ordination of floral architecture, pollen production (r^2 = .38, p = .026) and ovule number (r^2 = .38, p = .035) are of explanatory value.

No other variable is of significance, except floral type - for the first time (r^2 = .55, p = .004).

A combined cluster analysis and NMDS ordination of all floral functional traits and floral type was also performed (see appendix, figures A X.4.4 and A X.4.5): No clear patterns are emerging, only keyhole flower type appears somewhat more homogeneous in regard to floral functional traits. Permutational Multivariate Analysis of Variance shows that floral types are of no explanatory value for the combined ordination of floral functional traits (PERMANOVA: r^2 = .01, p = .756).

V.4.7 Phylogenetic pattern of floral functional traits and flower types

The retrieved phylogeny of the analysed species in general replicates the results of Nishii et al. (2015), see figure V.6: Species of the so called *S. cyaneus* group or 'Cape primrose clade' (Weigend and Edwards, 1994; de Villiers et al., 2013) are forming a polytomy, which is further forming a trichotomy with *S. gardenii* and *S. polyanthus*. Most of the other species analysed are part of a sister clade. Only *S. pusillus* differs phylogenetically, as well as newly analysed *S. burundianus*, being sister to outgroup-species *S. papangae*.

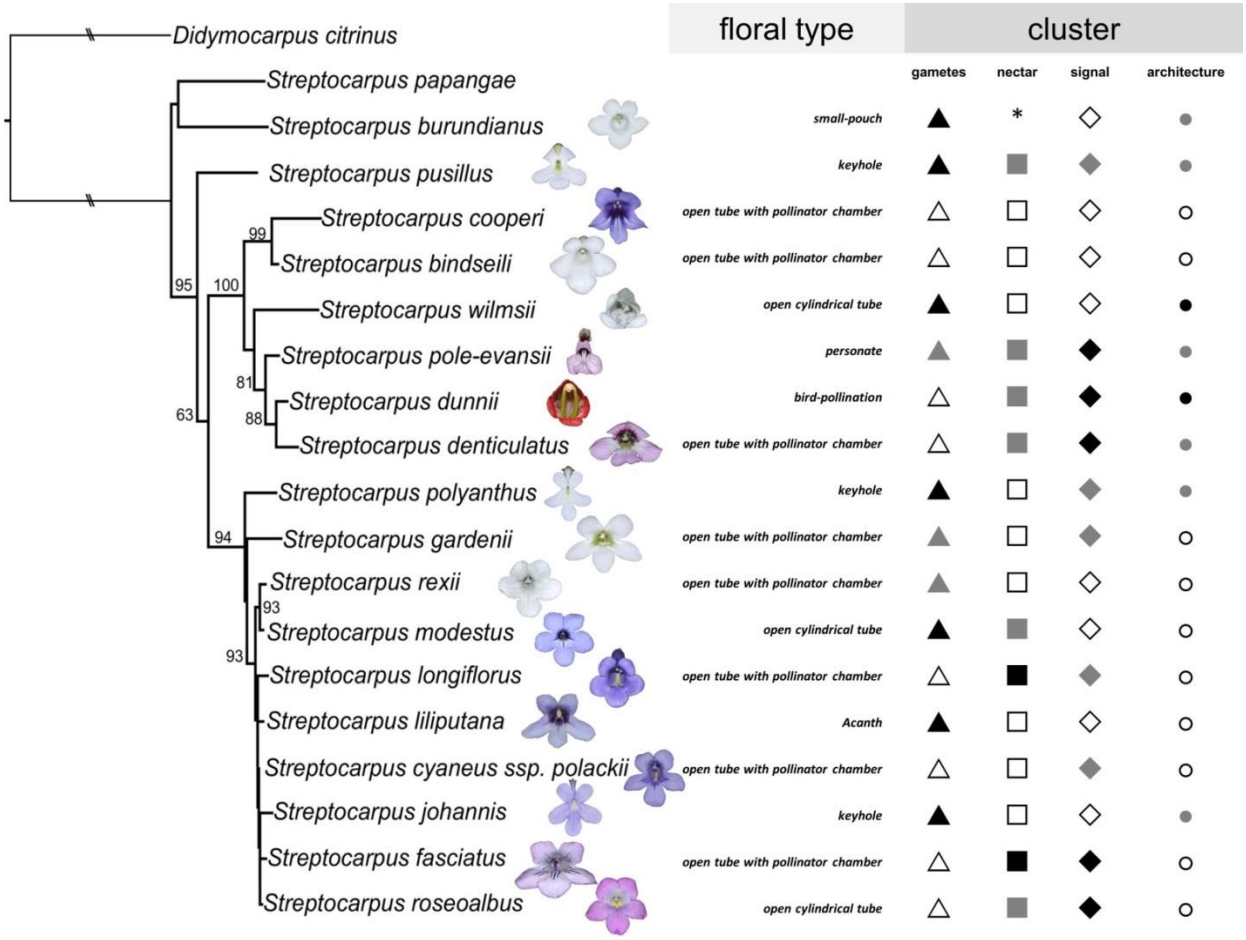

FIGURE V.6: PHYLOGENY OF *STREPTOCARPUS* SPECIES AND MAPPING OF FLORAL FUNCTIONAL TRAITS AND FLOWER TYPES

ITS, rpl20 and trnLF tree for the *Streptocarpus* species analysed, branch support indicated. Retrieved clusters for gamete production, nectar reward, optical signal and floral architecture are plotted onto the phylogeny, indicated as before. **Gamete production:** △ = cluster 1, species with highest pollen production, ranging from 339 300 (*S. cyaneus*) to 506 450 pollen grains per flower (*S. denticulatus*); ▲ = cluster 2, intermediate intrageneric pollen production, approx. 230 000 pollen grains per flower; ▲ = cluster 3, usually lower ovule numbers and pollen production below 200 000, lowest in *S. johannis* (90 750). No clear pattern of p/o ratio. **Nectar reward:** □ = cluster 1, nectar production and total sugar content lower, nectar concentration below 27 %; ■ = cluster 2, intermediate nectar production and total sugar content, nectar concentration higher, ranging between 34.1 and 57.0 %; ■ = cluster 3, higher nectar amount and total sugar content, but nectar concentration low (16.4 and 16.9 %). **Optical flower signal:** ◇ = cluster 1, white or bluish flower colour, missing anther dummy signal, UV light absorption, diffuse signal or only weak UV reflection; ◆ = cluster 2, colouration and UV light pattern like cluster 1, but anther dummy signal present; ◆ = cluster 3, reddish flower colour, anther dummy signal present or absent, always UV light reflecting, ranging from weak to strong UV reflection. **Floral architecture:** ○ = cluster 1, long- or short tubed flowers, larger in size, pronounced frontal display, including Acanth-type of *S. liliputana*; ● = cluster 2, flowers of small or moderate intrageneric size and usually of more sophisticated corolla architecture, including keyhole flower types (*S. johannis*, *S. polyanthus*, *S. pusillus*), strongly curved corollas of *S. denticulatus* and *S. pole-evansii* (approximating masked flower type) and campanulate flower of *S. burundianus*; ● = cluster 3, roughly cylindric flower types of *S. dunnii* and *S. wilmsii*, without pronounced frontal display (display ratio < 1).

In general, distribution of the retrieved clusters of floral form and function reveal high phylogenetic plasticity and almost free combination in *Streptocarpus*. Only two exceptions can be highlighted: First, floral architecture of the Cape primrose polytomy appears to be vaguely homogenous, with predominantly open tubed flowers (cluster 1 of floral architecture). However, three different flower subtypes of the open tube flower type (after Möller et al., 2019) are represented within the polytomy: Open cylindrical tube type, open tube type with pollinator chamber and Acanth type. Moreover, *S. johannis* shows a keyhole flower type within the polytomy. The other three subsets of floral functional traits, gamete production, nectar reward and optical signal, show even greater variation than floral architecture: The complete bandwidth of observed clusters is represented within the polytomy.
Second, the exotic floral architecture of *S. denticulatus* and *S. pole-evansii* as well as the cylindric flowers of *S. dunnii* and *S. wilmsii* are closely related. This trend for unusual intrageneric flower forms is accompanied by a reddish optical flower signal and higher nectar sugar concentration in *S. pole-evansii*, *S. dunnii* and *S. denticulatus*. As for the commonly described flower types, these species represent three very different main flower types realised in close phylogenetic proximity: personate (*S. pole-evansii*), bird-pollination (*S. dunnii*) and open-tube flower type (*S. denticulatus, S. wilmsii*).
All in all, flower types are distributed almost arbitrary: Open tube flowers constitute the basic floral type, with cylindrical tube flowers and open tube flowers with pollinator chamber alternating irregularly along the phylogenetic three. The other floral types analysed here are popping up in between, e.g. representatives of the keyhole flower type (*S. johannis*, *S. polyanthus* and *S. pusillus*) are spanning almost the whole spread of the phylogenetic three.

V.5 Discussion

V.5.1 Floral types reported for *Streptocarpus* only correlate to floral architecture

Surprisingly, the established classification of floral types in *Streptocarpus* is not supported by the retrieved pattern of gamete production, nectar reward and optical flower signal. Only representatives of the keyhole flower type appear somewhat more homogeneous (see also ordination of combined data in the appendix, figure A X.4.5), showing lower pollen and ovule production and less nectar reward than most – but not all – other species, and no extreme differences in optical signal. For all other flower types, pattern of gamete production, nectar reward and optical signal appear to be recombined quite freely and floral types are of no explanatory value for these subsets of floral functional traits (demonstrated via vector fitting of separate NMDS ordinations, PERMANOVA results of the combined data and phylogenetic mapping). Floral types are only explanatory for floral architecture, underlining the fact that the established classification is mainly based on flower shape. Therefore, the assumption of flower types as indicators of pollinator syndromes is not supported by uniform pattern of floral functional traits.

V.5.2 Floral traits are largely uncorrelated

Our in-depth analysis of floral plant traits in *Streptocarpus subgenus Streptocarpus* shows extreme plasticity of flower function and strong disjunctness of the analysed suites of floral functional traits. Ordination of gamete production is significantly explained by several variables of floral architecture, which all could be subsumed to a single factor of influence due to strong intercorrelations – flower size.

The solitary influence of the size and stoutness of flowers on pollen and ovule production is further supported by the missing correlations between gamete production and three out of four variables measured for reproductive organs (filament length, carpel length, distance between anthers and stigma). Only anther length is of explanatory value. Moreover, experimentally derived true breeding systems are of no explanatory value for gamete production in this dataset. P/o ratios as commonly accepted indicators of breeding systems even appear to be a free floating variable, without any explanatory value here at all: Several facultative xenogamous species (*S. cooperi*, *S. cyaneus ssp. polackii*, *S. johannis*, *S. rexii*) show lower p/o ratios than the autogamous species analysed (*S. bindseili*, *S. gardenii*, *S. polyanthus*, *S. pusillus*). Moreover, no variable of the optical flower signal as well as life cycle and habitat preference are of any explanatory value for gamete production. Produced nectar amount and sugar content are explanatory for gamete production: Both nectar variables might be linked to higher gamete production by a general need for higher resource investment. However, nectar concentration, strongly influencing pollinator interaction via uptake of nectar and handling time of flowers, is not linked to gamete production.

Ordination of floral architecture is reciprocally explained by pollen and ovule production in a significant way. Beside the already mentioned link between floral architecture and floral types, no other variable is of any explanatory value for floral architecture, revealing the extreme plasticity of floral architecture in regard to combination with other floral functional traits. As for the ordination of nectar reward, only few variables of the other subsets of floral functional traits are of explanatory value: Floral architecture is represented by lateral display size, flanked by trends for three other variables related to flower length – dorsal and ventral flower length as well as filament length. Therefore, the already well researched link between flower length and investment in nectar reward (see i.e. Plowright, 1981; Harder and Cruzan, 1990; Dafni, 1991; Kaczorowski et al., 2005; Fenster et al., 2006; Ornela et al., 2007; Gomez et al., 2008; Willmer, 2011) is replicated for *Streptocarpus*. The amount of pollen is linked to the reward system, but no other gametic variable (p/o ratio shows a trend however). This points again to the link of general resource investment already seen for the gametic ordination. Flower colour is strongly linked to nectar reward pattern, representing higher investment in nectar concentration when flowers are reddish, which is additionally flanked by higher frontal UV reflection (lateral UV reflection as a trend). As for other variables assessed, only life cycle is explanatory, again showing higher investment in nectar concentration for the monocarpic plants that are flowering reddish: *S. denticulatus* and *S. dunnii*.

Regarding the ordination of optical flower signal, only two out of the pool of all analysed variables are explanatory: First, the five species with reddish flower colour and UV light reflection are characterised by higher nectar concentration. This link between signal and reward already became obvious via ordination of the nectar system and vector fitting. Second, optical flower signal is the only set of floral functional traits that is explained by experimentally derived breeding strategies: Autogamy is missing amongst the five species with reddish flower colour, and *S. pole-evansii* as only species with verified obligate xenogamous breeding system is counting amongst them.

All in all, ordinations and vector fitting procedures predominantly show a decoupling between the four subsets of floral functional traits: Only the correlation of flower size and pollen and ovule production is linking floral architecture to gamete production, and only the correlation of higher nectar amount and sugar production to higher pollen production is linking the gamete production to nectar reward. These links can be subsumed under a general need for higher resource investment, possibly linked to

involvement of larger animal vectors in the pollination process with higher nutritional needs, larger pollen bearing areas, etc. Moreover, optical flower signal and nectar reward are generally correlated by higher nectar concentration for reddish flowers with stronger UV reflection. Beside these trends, flower traits are combined quite freely amongst each other.

Instead of rejecting pollination syndromes for the genus, the data might be best interpreted as supportive for two different evolutionary driving forces shaping plant-animal interaction in *Streptocarpus*: Floral architecture controlling for plant-pollinator fit and pollen placement on the one hand, and the interplay of attraction and reward on the other hand, pointing to a coupling between the sensory capacities and preferences of the specific pollinator guilds and their nutritional needs.

V.5.3 Floral traits show almost free recombination along the course of evolution

Clusters are also recombined freely along the phylogentic tree, only two trends are present: First, the Cape primrose polytomy shows a trend for open tubed flowers – however three subtypes are realised (open cylindrical, open tube with pollinator chamber, Acanth type). Second, the link between reddish flower colour accompanied by higher investment in nectar reward can be observed for two species of the polytomy, *S. roseoalbus* and *S. fasciatus*, as well as for monophyletic *S. pole-evansii*, *S. deticulatus* and *S. dunnii*. Roalson and Roberts (2016) have shown the strong evolutionary driving force of reddish flower colour for New World Clades of Gesneriaceae, but not for Old World Clades like *Streptocarpus*. Our study of plant traits might be interpreted in such a way that the shift to reddish flowers in *Streptocarpus* is in its evolutionary emergence.

The strong plasticity of *Streptocarpus* in regard to flower types and floral architecture as well as growth habit was already presented in previous studies: Möller and Cronk (2001) showed plasticity of growth habit via ITS phylogeny, showing that the three different growth forms have originated several times independently. As for flower types, Harrison et al. (1999) and Hughes et al. (2006) showed plasticity of six floral types also via ITS phylogenies. In this paper we have replicated the phylogenetic plasticity of floral architecture and flower types, and we have demonstrated phylogenetic plasticity for two other quantitatively assessed subsets of floral functional traits not shown before: Gamete production and nectar reward pattern. The cause for this phylogenetic plasticity of flower traits and growth habit might either reflect independent origins (homoplasy) or the "capture" of molecular markers from dissimilar parent species (de Villiers et al., 2013; Pirie et al., 2009). Hybridisation has to be assumed as a powerful evolutionary operative force in *Streptocarpus* (Hilliard and Burtt, 1971).

V.5.4 Floral plasticity and pollinator-driven speciation

It remains unresolved in how far phylogenetic shifts between floral functional plant traits are pollinator-driven: Due to the absence of clear-cut pattern amongst the floral functional traits, combined (!) with rapid alteration along the phylogenetic tree, pollinator-driven speciation is either not very successful (not leading to adaptive radiation), or too young to have realised its full potential. The data are rather supportive for the strong impact of hybridisation on floral plasticity and speciation. Large parts of genus *Streptocarpus* are supposedly forming a underlying, shared single gene pool (Hilliard and Burtt, 1971): It is possible that freak pollination events between genetically insufficiently segregated species keep the gene flow within the genus ongoing, making *Streptocarpus* an "active melting pot" of speciation and

floral plasticity. If a newly established hybrid swarm with a new combination of functional floral traits is fitting to sensory capacities and nutritional needs of a potential animal vector, a new species might be established in a short time frame. This theory might be supported by lowered conflict between ITS and plastid gene trees demonstrated for keyhole flowers (de Villiers et al., 2013): If such a flower type is once established, it exerts much higher control on pollinators (and therefore on gene flow) due to highly restrictive flower access and pollen placement, and therefore a stronger segregation of a part of the complete gene pool of the genus. All in all, the extreme plasticity of flower function and decoupling of floral functional traits observed for *Streptocarpus* link to latest findings on floral plasticity, plant-animal interactions and evolution that show the strong interplay and malleability of these levels, e.g.: Kemp et al. (2019) show pollinator influence on plant community level already working via non-random assembly of complex flower colour patterns in these communities. Parachnowitsch et al. (2019) present that signal-reward associations are suggesting correlational selection and evolutionary shaping of nectar traits, and pollinator responses to nectar traits may drive selection. Tong et al. (2019) suggest that gain (or loss) of nectar quickly results in adaptive behavioural shifts in the pollinator. Zych et al. (2019) even report 'adaptive wandering' in floral characters, meaning observed variation between communities for *Angelica sylvestris*, a plant otherwise well adapted to (floral) ecological generalisation.

In this sense, the floral ecological plasticity here presented for *Streptocarpus* is another example for the ease evolution is shaping and changing plant-animal interactions and therewith speciation. We agree with Möller et al. (2019), that "living collections play a key part in this research, helping to unravel the mechanisms by which plant diversity is established". Nevertheless, future research should complement the presented findings on floral plant traits in *Streptocarpus* by pollinator observations in the field.

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V.7 Literature

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VI. VIGOROUS HYBRID SEED SET BETWEEN THREE CLOSELY RELATED SPECIES OF *STREPTOCARPUS* SUBGENUS *STREPTOCARPUS* INDICATE POSTZYGOTIC BARRIERS OF NO RELEVANCE FOR REPRODUCTIVE ISOLATION

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VI.1 ABSTRACT

Background and aims: The Cape primrose clade or *Streptocarpus cyaneus* group represents a closely allied species group within the genus *Streptocarpus* (Gesneriaceae). Floral architecture is considered as the crucial factor for reproductive isolation between these closely allied species. We here investigate the presence of reproductive isolation between three species of the group (*S. rexii, S. rosealbus* and *S. johannis*) by performing crossing experiments.

Methods: Crossing experiment, seed counts, imaging technique, Generalised Linear Modeling (GLM), Generalised Estimating Equations (GEE).

Key results: F1 hybrids are fertile. Seed set is generally high in interspecific crosses, comparable to intraspecific siring success. Reduced seed set was primarily observed for experimental groups involving *Streptocarpus johannis* as pollen donor. Nevertheless, average seed set per fruit is at least in the hundreds, often showing round about 1,000 seeds, and in some cases even approximating 2,000 seeds or more.

Conclusion: The theory of a single large gene pool underlying the Cape primrose clade is strongly supported, allowing for hybrid speciation due to absence of postzygotic crossing barriers. Prezygotic barriers such as eco-geographical isolation, including differences in floral architecture and optical signal, appear to maintain the species distinct in nature.

Key words: *Streptocarpus*, Cape primrose clade, hybridisation, crossing experiment, seed counting, ImageJ, Generalised Estimating Equations.

VI.2 INTRODUCTION

Streptocarpus Lindl. comprises round about 179 species, predominantly distributed in southern Africa and Madagascar. The main diversification in the genus appears to be recent and has been dated to ca. 1.5 million years ago in the eastern South Africa (Möller et al., 2019; Möller and Cronk, 2001). The genus was recently expanded to include all representatives of Gesneriaceae subfamily Didymocarpoideae in Africa and Madagascar, including those formerly assigned to other genera such as *Saintpaulia* H.Wendl. and *Linnaeopsis* Engl. (Möller et al., 2019, citing Darbyshire and Massingue, 2014; Nishii et al., 2015; Randrianasolo et al., 2018).

Streptocarpus shows considerable morphological diversity (cf. Hilliard and Burtt, 1971): Vegetative growth patterns are sometimes eccentric, with some plants only developing a single leaf, and inflorescences are sprouting from the basal part of the midrib, while other species show more or less orthodox rosulate or caulescent growth patterns. Flower morphology is even more diverse. Classically, the flowers of *Streptocarpus* were classified into open-tube, keyhole, personate, small-pouch, flat-faced *Saintpaulia* and bird-pollination flowers (Harrison et al., 1999; Hughes et al., 2006; Nishii et al., 2015). Möller et al. (2019) further subdivide the open tube type by classification of six subtypes.

Beyond interspecific differences, there is also considerable intraspecific diversity in details of shape and colour, with many different forms and subtypes described. Natural hybrids do occur in the field that might be the cause of this variance, and cross-pollination is reported to be frequent, though clearly not obligatory. Hilliard and Burtt (1971) report 28 instances of suspected hybridisation in their hybrid catalogue (p. 77 ff.). They conclude that hybridisation "has been powerfully operative in the evolution of subgenus *Streptocarpus*" (p. 77), and that "a very large part of (the) subgenus (…) belongs potentially to a single gene pool (…)" (p. 76). Their claim is supported by the high degree of crossability within the subgenus. Crossability of *Streptocarpus* is also of economic importance, with numerous interspecific hybrids commercialised worldwide, usually involving i.a. *S. rexii*. However, detailed data on interspecific fertility and F1 fertility are largely missing (Hilliard and Burtt, 1971: 74–77).

Current research on *Streptocarpus* is coming back to the possible prevalence of hybridisation first proposed by Hilliard and Burtt (1971): de Villiers et al. (2013) state that the extreme plasticity of floral architecture between closely allied species in the ITS phylogenies of *Streptocarpus* found by Harrison et al. (1999) and Hughes et al. (2006) could be indicative either of independent origins of similar morphologies (i.e. homoplasy) or might reflect the 'capture' of a molecular marker from morphologically dissimilar parent species (de Villiers et al., 2013, citing Pirie et al., 2009) and thus go back to hybridisation. However, de Villiers et al. (2013) also show for closely related species of subgenus *Streptocarpus*, the so called 'Cape primrose clade', that growth form shifts rather than floral plasticity might be interpreted as evidence for hybrid origin, providing evidence for this based on gene tree conflicts for *S. bolusii*, *S. vandeleurii*, *S. grandis*, *S. fanniniae* (with *S. gardenii* being an additional candidate). The historical relevance of hybridisation was therefore demonstrated by de Villiers et al. (2013), but it is unaddressed whether it is still an active force, shaping diversity in the genus as Hilliard and Burtt (1971) suggested.

We therefore investigate here whether florally divergent taxa are reproductively isolated beyond ecogeographical isolation, i.e. whether additional pre- or postmating barriers are present. We use the classical approach of experimental crossing. To test this hypothesis, three members of the Cape primrose clade were selected for the crossing and seed counting experiments, all showing different floral architectures and optical flower signals (see figure VI.1): *S. rexii* (Bowie ex Hook.) Lindl. with its long-tubed and white corolla colour, *S. roseoalbus* Weigend & T.J.Edwards with its short-tubed reddish flower and *S. johannis* L.L.Britten as representative of the keyhole flower type, with bluish corolla. All species show rosulate growth pattern. *Streptocarpus rexii* and *S. johannis* are sympatric, while *S. roseoalbus* is allopatric. Flowering time of the species is overlapping: October to December for *S. johannis*, October to April for *S. rexii* (Hilliard and Burtt, 1971), and December to March for *S. roseoalbus* (Weigend and Edwards, 1994).

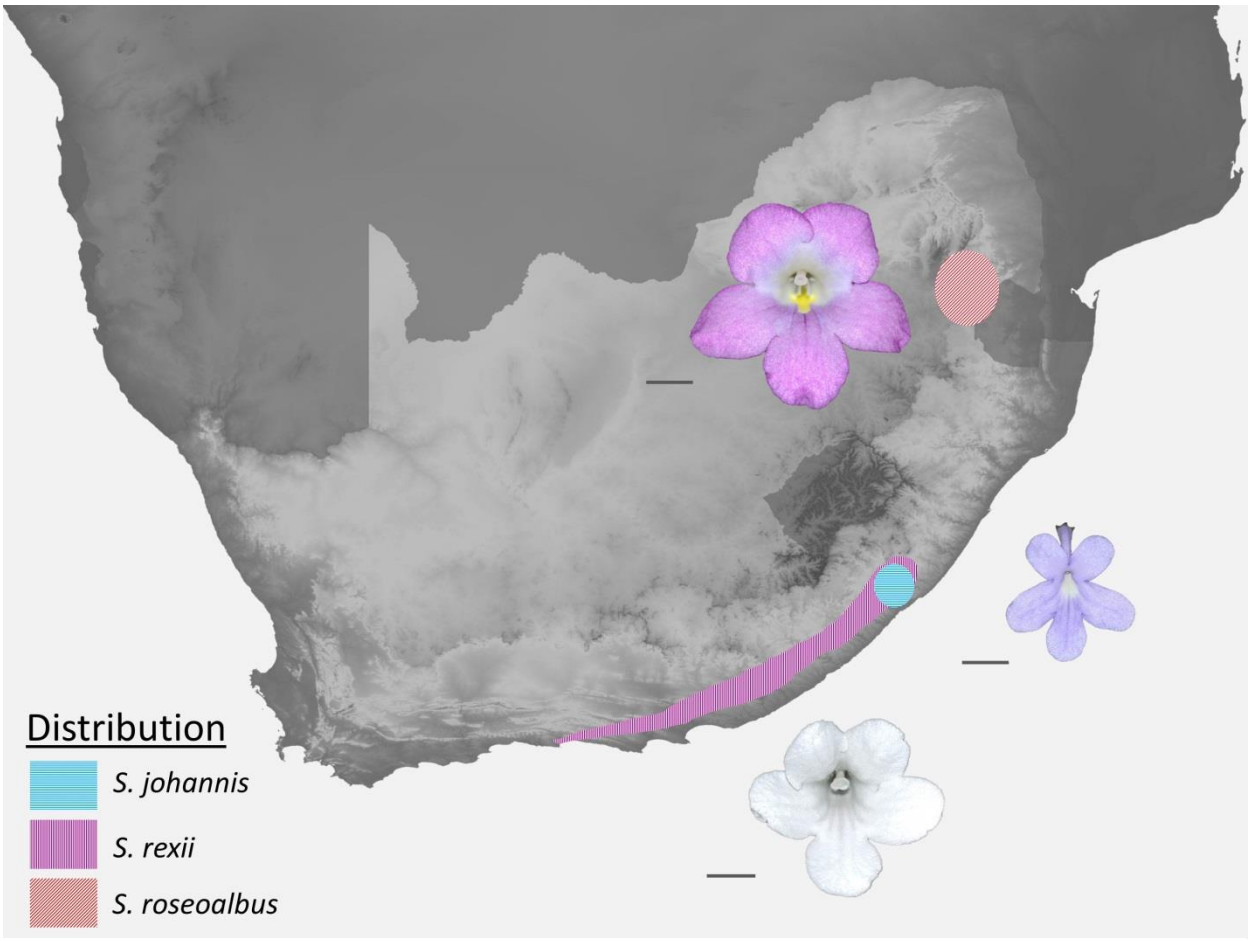

FIGURE VI.1: DISTRIBUTION OF *STREPTOCARPUS JOHANNIS*, *S. REXII* AND *S. ROSEOALBUS* IN SOUTH AFRICA
S. johannis L.L.Britten is distributed at the Eastern Cape, from Port St. Johns through Lusikisiki to the Ngeli slopes on the Cape-Natal border, growing in forests on rocks and earth banks (Hilliard and Burtt, 1971). *S. rexii* (Bowie ex Hook.) Lindl. is distributed from George, Cape province, eastward to southernmost Natal, along the coastline in forests (Hilliard and Burtt, 1971). *S. roseoalbus* Weigend & T.J.Edwards is distributed in the Eastern Transvaal, close to Barberton, occurring in lowveld vegetation (Weigend and Edwards, 1994). Phylogenetically, all species are belonging to a polytomy within *Streptocarpus* subgenus *Streptocarpus* (Nishii et al., 2015). While *S. johannis* and *S. rexii* are sympatric, *S. roseoalbus* is allopatric to the other species. Flower scaling bars indicate 1 cm. Topographical map by SFC9394, electronically retrieved 04.03.2019 from https://commons.wikimedia.org/wiki/File:South_Africa_topo_continent.png, modified to grey scale, Creative Commons 2.5 (https://creativecommons.org/licenses/by/2.5/deed.en). Flower pictures by A. W. Mues.

VI.3 MATERIALS AND METHODS

All plants were cultivated in a pollinator-proof greenhouse at Botanische Gärten der Universität Bonn (Bonn University Botanic Gardens).

Crossing experiments were performed in 2015 and 2017. In 2015, reciprocally crossed F1 hybrid plants between *S. johannis*, *S. roseoalbus* and *S. rexii* were raised to prepare the core crossing experiment. F1 hybrids were obtained for all possible combinations (first epithet indicating the maternal strain): *johannis-rexii, rexii-johannis, rexii-roseoalbus, roseoalbus-rexii, roseoalbus-johannis* and *roseoalbus-rexii*. In 2017, parental species were pollinated allogamously and geitonogamously as control groups, and reciprocal crossings between species were repeated in order to obtain F1 hybrid seed material. The existing F1 hybrids were also pollinated allogamously and geitonogamously, and reciprocal hybrids were crossed with each other (e.g. *rexii-roseoalbus* with *roseoalbus-rexii*) to obtain F2 seed material.

Pollination was carried out with complete pollen material of both anthers of the respective pollen donor to ensure a sufficient pollen load.
For allogamous pollination and crossing, corollas of newly opened flowers were carefully but completely removed to prevent self-pollination: Stamens are fused to the ventral part of the corolla, and anthers were covered with the corolla before removal and pulled away from the gynoecium to prevent contamination of the stigma. Spontaneous autogamy was not observed in pre-experiments, but emasculation was deemed necessary to prevent accidental selfing during handling of the plants.
Fruits were harvested at incipient dehiscence, indicated by colour change towards greenish-brown. Fruit length and ripening time from the day of pollination to the day of harvest were recorded.
All in all, 30 experimental groups based on variation of maternal and paternal contribution, pollination procedure and generational level were run in 2017, with each group represented by 5 fruits, totalling $N = 150$ fruits.

VI.3.1 Seed Count

Due to the high seed production in *Streptocarpus*, seed number was assessed by photographic documentation and application of freely available image processing software, ImageJ (National Institute of Health, USA). Counting technique was adjusted from Costa and Yang (2009) as described below. Harvested fruits were dried separately in paper sachets for ca. three months. Fruits were afterwards opened on a sheet of paper by twisting the spiralised fruit until seed material was released completely. As a first cleaning step, seed material was carefully dropped onto a second paper, with lighter debris sticking electrostatically to the first paper. As a second cleaning step, heavier debris (larger fibres etc.) was removed with a pair of tweezers, and seed material was distributed evenly on the paper: Attention was paid to separate seeds from each other in order to prevent miscounts. Pictures were taken with a single-lens camera (EOS Digital 600D) and stored in jpg format.
Before counting, pictures were trimmed in paint (© Microsoft) and colour inverted to create a monochromatic, dark background. Processed pictures were loaded into ImageJ and converted to 8-bit format. Using threshold applications and redfilter function, seeds were recognised as target objects and remaining debris was filtered out. Subsequently, thresholds were switched to black and white to create a final, bicromatic picture. Counting of seed material was performed by means of the "analyse particle" function, with no restrictions placed on parameters size and circularity due to the spindle shape of the seeds.

VI.3.2 Statistical Analysis

Statistics were carried out with IBM software package SPSS, version 24. The dependent and ratio scaled variable (DV) to be explained is seed production. Explanatory, independent and nominal variables (IVs) are "paternal" and "maternal contribution" to siring success (representing pollen donors and pollen recipients), "generational level" and "pollination procedure". Additionally, "fruit length" and "ripening time" are ratio scaled covariates, analysed in regard to possible impact on the DV.
Regarding maternal and paternal contribution to siring success, every individual strain involved in a pollination procedure was labelled conservative as a single unit: Parental species were labelled according to their species epithets "joha", "rexi" and "rose". Maternal and paternal strains of pollination

within the F1 generation were labelled according to the crossing direction they originated from (first abbreviation always indicating the maternal hybrid origin): "joha-rexi", "joha-rose", "rexi-joha", "rexi-rose", "rose-joha" and "rose-rexi". Three levels were selected to describe generational level: Pollinations carried out between plant individuals of the same original species constitute the control group and were coded "parental". Crossing *between* parental species was coded "F1" to label fruits and seed material that constitute the first filial generation. Pollinations, fruit- and seed set between F1 hybrids were coded "F2". Pollination procedure has also three levels: "Geitonogamy" describes pollination within plant individuals. "Allogamy" describes pollination between plant individuals of the same genetic makeup. "Crossing" describes recombination of genetic makeup between original species or between different strains of the F1.

Statistical analysis of siring success accounts for the nested design of the crossing experiment: Every combination of maternal and paternal contribution for each generational level and each pollination procedure represents a separate experimental group. Moreover, every experimental group is represented by five fruits and measurements of their length, ripening time and seed set (repeated measurements, nested within the experimental groups). Exploratory analysis of normal distribution of metric variables was generally verified via Shapiro Wilks test for most of the experimental groups, but not for the overall distribution of the variables across the groups. Analysis was therefore performed via Generalised Estimating Equations (GEE, Liang and Zeger, 1986). GEEs are offering the full flexibility of Generalised Linear (Mixed) Modelling in combination with highly robust estimation procedures (Baltes-Götz, 2016; Swan, 2006), allowing for estimation of non-normal, nested data structures including repeated measurements.

In a first step, an Intercepts-Only Model without introduction of predictors was calculated to check for necessity of multilevel modelling (Tabachnick and Fidell, 2006). Group means for the different treatments are differing highly significant from the grand mean of seed set (Wald χ^2 (1, N = 150) = 90,137, p = .000), validating the application of Generalised Linear Modelling techniques. In a second step, a Covariate Interaction Model was calculated to check for interactions between predictors and the covariates fruith length and ripening time. Interaction terms were checked for significance in order to remove insignificant interactions from the final model (Engqvist, 2005). Predictors with interactions were introduced to the model according to following working hypothesis (model building): First, seed set is depending on the maternal contribution, due to the general dependence of the seed set from ovule production of the maternal strain. Second, paternal contribution influences seed set via genetic fit to the maternal strain, pollen viability and fertility. Third, seed set is assumedly influenced by generational level, with higher seed set expected for pollination within species (only parental, control groups) than for F1 hybrid seed material, due to genetic mismatch. Seed set is expected to rise again for F2 seed material, due to genetic recombination of parental strains. Fourth, pollination procedure is assumed to be of additional explanatory value, with higher seed set expected for allogamous outcrossing than geitonogamous pollination or even true crossing. For the final model presented here, significant interaction terms were introduced, insignificant interaction terms were removed and predictors without interactions were placed as main effects. Intercepts-Only Model, Covariate Interaction Model and the complete Final Model are presented in the appendix (A X.5.1 to A X.5.3).

VI.4 RESULTS

F1 hybrids appeared homogenous between reciprocal crossings of parental species (figure VI.2), with fully functional reproductive organs. Siring success was observed for every experimental group and usually for every pollinated flower. However, in 10 cases fruits were aborted and new flowers had to be pollinated to create a balanced experimental design. Abortions never happened between maternal and paternal strains of *S. rexii* and *S. roseoalbus* and their descendants, but always with involvement of *S. johannis*. Abortions were characterised by poorly developed fruits (average length 1.38 cm; min.: 0.5 cm, max.: 2.9 cm) and missing seed production. Interspecific crosses with *S. johannis* aborted three fruits after pollination with *S. rexii*, and two after pollination with *S. roseoalbus*. During breeding of F2, maternal strain joha-rexi aborted two fruits after geitonogamous pollination, and two additional fruits after crossing with the reciprocal strain rexi-joha. Another abortion was observed for allogamous pollination of strain rose-joha.

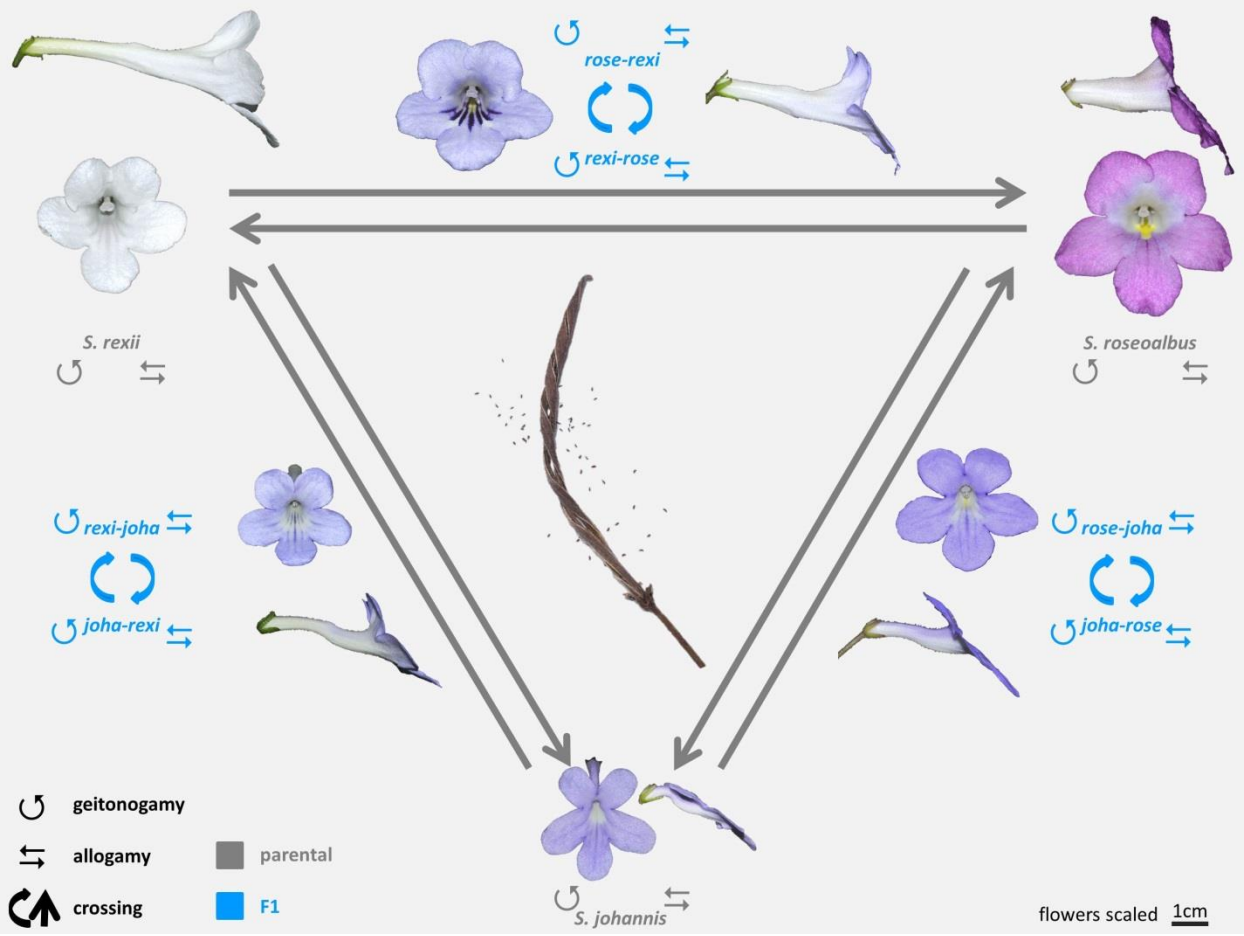

FIGURE VI.2: CROSSING PLAN FOR *S. JOHANNIS*, *S. REXII* AND *S. ROSEOALBUS* AND DEPICTION OF THE RESULTING F1
Hybrids appear homogenous for each reciprocal crossing of parental species, and are all fertile. The figure also shows a typical spirally twisted fruit and seed material. Pictures by A. W. Mues.

Table VI.1 presents arithmetic means and standard deviations for seed set, fruit length and ripening time for all experimental groups. Grand mean of seed set across all experimental groups is 1461.27. When neglecting differentiation between allogamous and geitonogamous crossing procedure within

parental species, *S. johannis* produced 751.2 seeds on average, *S. roseoalbus* 2162.9 and *S. rexii* 3437.3 seeds. Average fruit length was 3.5 cm for *S. johannis*, 5.7 cm for *S. roseoalbus* and 9.3 cm for *S. rexii*. Ripening time was shortest for *S. rexii* with 69.3 days on average, followed by *S. johannis* with 75.1 days and *S. roseoalbus* with 92.3 days.

TABLE VI.1: SEED SET, FRUIT LENGTH AND RIPENING TIME OF *S. JOHANNIS*, *S. REXII*, *S. ROSEOALBUS* AND HYBRID OFFSPRING
Means and standard deviations for 30 experimental treatments, each containing five measured fruits, in total 150 measurements per variable.

parental, allogamy / geitonogamy	*n*	seed number			fruit length in cm			ripening time in days		
Streptocarpus johannis, allogamy	5	694.60	±	595.18	3.50	±	0.90	74.60	±	4.67
Streptocarpus johannis, geitonogamy	5	807.80	±	476.61	3.40	±	0.61	75.60	±	5.50
Streptocarpus rexii, allogamy	5	3672.20	±	539.22	9.70	±	0.55	69.80	±	6.94
Streptocarpus rexii, geitonogamy	5	3202.40	±	797.51	8.94	±	1.04	68.80	±	5.02
Streptocarpus roseoalbus, allogamy	5	2050.40	±	679.75	5.54	±	0.59	91.40	±	3.58
Streptocarpus roseoalbus, geitonogamy	5	2275.40	±	634.72	5.88	±	0.53	93.20	±	9.23
parental →F1, crossing										
johannis ♀ x rexii ♂	5	572.20	±	336.30	3.34	±	0.58	82.80	±	3.49
johannis ♀ x roseoalbus ♂	5	924.00	±	234.84	3.78	±	0.26	83.80	±	12.91
rexii ♀ x johannis ♂	5	228.40	±	105.93	5.54	±	0.25	57.60	±	6.69
rexii ♀ x roseoalbus ♂	5	2885.40	±	482.40	8.96	±	0.46	68.60	±	6.02
roseoalbus ♀x johannis ♂	5	946.20	±	221.17	5.66	±	0.11	90.80	±	4.02
roseoalbus ♀x rexii ♂	5	2956.60	±	1446.53	6.26	±	1.05	86.20	±	9.09
F1 → F2, allogamy / geitonogamy										
joha-rexi, allogamy	5	1016.00	±	752.55	6.28	±	1.26	55.20	±	16.74
joha-rexi, geitonogamy	5	959.80	±	900.29	5.98	±	1.08	46.80	±	11.08
joha-rose, allogamy	5	794.80	±	509.16	5.24	±	1.76	66.00	±	14.11
joha-rose, geitonogamy	5	963.80	±	767.84	5.52	±	1.27	75.20	±	7.22
rexi-joha, allogamy	5	1471.60	±	630.95	7.10	±	1.17	62.60	±	14.31
rexi-joha, geitonogamy	5	1538.80	±	385.67	6.92	±	0.82	64.00	±	12.92
rexi-rose, allogamy	5	806.00	±	698.60	6.52	±	1.64	80.00	±	2.74
rexi-rose, geitonogamy	5	850.20	±	330.54	6.58	±	0.82	75.60	±	5.22
rose-joha, allogamy	5	1595.40	±	856.70	5.28	±	1.35	71.60	±	13.61
rose-joha, geitonogamy	5	2008.60	±	1086.66	5.32	±	1.52	73.40	±	2.61
rose-rexi, allogamy	5	665.60	±	667.95	5.78	±	1.77	65.20	±	13.12
rose-rexi, geitonogamy	5	926.60	±	720.45	6.12	±	1.54	67.80	±	17.40
F1 → F2, crossing										
joha-rexi ♀ x rexi-joha ♂	5	972.60	±	646.18	6.08	±	1.30	57.40	±	29.49
joha-rose ♀ x rose-joha ♂	5	2233.20	±	748.96	7.06	±	0.76	80.60	±	2.88
rexi-joha ♀ x joha-rexi ♂	5	1231.80	±	442.61	6.42	±	0.44	65.40	±	3.13
rexi-rose ♀ x rose-rexi ♂	5	1366.80	±	1212.01	6.66	±	2.81	79.20	±	3.77
rose-joha ♀ x joha-rose ♂	5	1855.60	±	685.73	5.88	±	0.46	74.00	±	5.52
rose-rexi ♀ x rexi-rose ♂	5	1365.40	±	981.35	7.56	±	1.63	71.00	±	11.31

Interspecific seed set showed characteristic profiles of different experimental groups with regards to seed number, fruit length and ripening time. The Covariate Interaction Model (cf. Appendix A X.5.2) showed significant interactions between covariate ripening time and maternal contribution, and between covariate fruith length and maternal as well as paternal contribution. Covariates showed no interaction with generational level and crossing procedure. Significant interactions were introduced to the final GEE model, presented in table VI.2: The final model explains seed set under prediction of maternal contribution (interacting with fruit length and ripening time), paternal contribution (interacting with fruit length), generational level and crossing procedure.

The **intercept** of the model is negative, showing that all analysed factors are contributing strongly to the observed grand mean of seed set, turning negative when all parameters are set to zero.
Maternal contribution in interaction with fruit length is highly significant and strongly positive for seed set in all involved maternal strains (cf. table VI.2). Therefore, seed number is predominantly controlled by the maternal strain, with longer fruits containing more seed material. Interaction terms are strongest for the pure strains joha and rose.

Contrary to this, **paternal contribution in interaction with fruit length** of *S. johannis*, strain joha-rose and strain rexi-rose show a highly significant, but negative influence on seet set, compare tables VI.1 and VI.2. Seed number is reduced when *S. rexii* and *S. roseoalbus* are pollinated with *S. johannis* (rexi x joha: $\bar{x}$ = 228.4 seeds and 5.54 cm fruit length, compare rexi x rose: $\bar{x}$ = 2885.4 seeds, 8.96 cm // rose x joha: $\bar{x}$ = 946.2 seeds, 5.66 cm, compare rose x rexi: $\bar{x}$ = 2956.6 seeds, 6.26 cm). Compared to allogamous as well as geitonogamous pollination of other F1 strains, paternal contribution of strain joha-rose in interaction with smaller fruit length is correlated to lower seed set (allogamous: $\bar{x}$ = 794.8, min.: 145 seeds, 3.2 cm, max.: 1453 seeds, 7.2 cm; geitonogamous: $\bar{x}$ = 963.8, min.: 133 seeds, 3.9 cm, max.: 1994 seeds, 6.9 cm). Similarly, paternal contribution of strain rexi-rose during allogamous as well as geitonogamous pollination showed reduced seed set in correlation to shorter fruits (allogamous: $\bar{x}$ = 806.0, min.: 232 seeds, 5.6 cm, max.: 1726 seeds, 9.2 cm; geitonogamous: $\bar{x}$ = 850.2, min.: 441 seeds, 5.3 cm, max.: 1358 seeds, 7.0 cm).

Regarding **ripening time and maternal contribution**, significant and positive interactions become evident for hybrid strains joha-rexi and joha-rose, showing higher seed set across experimental groups for these maternal strains when ripening time is longer (joha-rexi$_{maternal}$: $\bar{x}$ = 982.8, min.: 171 seeds, 37 days (a case of allogamous pollination); max.: 2441 seeds, 64 days (a case of geitonogamous pollination) // joha-rose$_{maternal}$: $\bar{x}$ = 1330.6, min.: 133 seeds, 69 days (a case of geitonogamous pollination); max.: 2972 seeds, 81 days (a case of crossing with rose-joha)). Contrary to this, the maternal contribution of *S. roseoalbus* across experimental groups shows faster ripening when seed number is higher (rose$_{maternal}$: $\bar{x}$ = 2057.2, 1359 seeds at maximum ripening time, 104 days (a case of allogamous pollination); 2421 seeds at minimum ripening time, 74 days (pollinated with *S. rexii*)).

Regarding **generational level**, production of F1 hybrid seed material constitutes a marked bottleneck: The effect is highly significant and strongly negative. Comparison of arithmetic means of seed

production between experimental groups shows lowest F1 seed production in all cases *S. johannis* is involved (compare tables VI.1 and VI.2).

As for **procedures** applied, geitonogamous pollination and true crossing are of no further explanatory value. However, seed set is reduced significantly and negatively for allogamous pollination between members of a strain. This effect can be observed for all allogamous pollinations within F1 when compared with geitonogamy, expect for hybrid joha-rexi. Within parental species, allogamous pollination shows higher seed set for *S. rexii*, but lowered seed set for the other two species (compare tables VI.1 and VI.2).

TABLE VI.2: FINAL GEE MODEL FOR SEED SET OF *S. JOHANNIS*, *S. REXII*, *S. ROSEOALBUS* AND HYBRID OFFSPRING
Dependend variable is seed set. Significant covariate interaction terms are introduced, other factors (generational level, procedure) are main effects. Only significant parameter estimates presented, full analysis presented in the appendix (table A X.5.3).

parameter	B	std. error	95% Wald confidence interval		hypothesis test		
			lower	upper	Wald Chi-Square	df	sig.
test of model effects							
maternal_contribute * fruit_length					49.455	5	**0.000**
paternal_contribute * fruit_length					124.927	5	**0.000**
maternal_contribute * ripening_time					27.861	9	**0.001**
generation					21.949	2	**0.000**
procedure					10.580	2	**0.005**
parameter estimates							
(Intercept)	**-1906.793**	586.6517	-3056.610	-756.977	10.564	1	**0.001**
[maternal_contribute=joha] * fruit_length	**790.503**	96.3856	601.591	979.415	67.264	1	**0.000**
[maternal_contribute=joha_rexi] * fruit_length	**444.427**	34.2932	377.213	511.640	167.952	1	**0.000**
[maternal_contribute=joha_rose] * fruit_length	**444.398**	73.9015	299.554	589.242	36.161	1	**0.000**
[maternal_contribute=rexi] * fruit_length	**516.925**	32.3862	453.449	580.401	254.763	1	**0.000**
[maternal_contribute=rexi_joha] * fruit_length	**493.220**	104.3217	288.753	697.686	22.353	1	**0.000**
[maternal_contribute=rexi_rose] * fruit_length	**420.369**	13.3608	394.182	446.555	989.916	1	**0.000**
[maternal_contribute=rose] * fruit_length	**1125.519**	100.2713	928.991	1322.048	125.995	1	**0.000**
[maternal_contribute=rose_joha] * fruit_length	**608.222**	61.4997	487.685	728.759	97.809	1	**0.000**
[maternal_contribute=rose_rexi] * fruit_length	**479.820**	37.0723	407.160	552.480	167.517	1	**0.000**
[paternal_contribute=joha] * fruit_length	**-128.817**	22.3602	-172.642	-84.992	33.189	1	**0.000**
[paternal_contribute=joha_rose] * fruit_length	**-74.418**	10.4023	-94.806	-54.030	51.180	1	**0.000**
[paternal_contribute=rexi_rose] * fruit_length	**-43.479**	8.0554	-59.268	-27.691	29.133	1	**0.000**
[maternal_contribute=joha_rexi]*ripening_time	**5.071**	2.5885	-0.003	10.144	3.838	1	**0.050**
[maternal_contribute=joha_rose]*ripening_time	**13.035**	6.0766	1.125	24.945	4.601	1	**0.032**
[maternal_contribute=rose] * ripening_time	**-24.466**	7.4755	-39.118	-9.815	10.712	1	**0.001**
[generation=F1]	**-458.140**	104.2706	-662.507	-253.774	19.305	1	**0.000**
[procedure=allogamy]	**-82.819**	41.8887	-164.919	-0.718	3.909	1	**0.048**

VI.5 DISCUSSION

In general, our analysis shows that hybrid seed set between selected members of the Cape primrose clade is only moderately variable across different crossing directions, generational levels and pollination procedures, consistently resulting in hundreds to thousands of seeds per fruit.

Reduced seed set was primarily observed for experimental groups involving *Streptocarpus johannis* as pollen donor, leading to a decline of F1 hybrid seed production. This result is underlined by the observation of aborted fruits, which were restricted to *S. johannis* and its descendants.

Apart from some effects on ripening time and fruit length, seed set is generally high in all crossing groups. Interestingly, lowest seed set between all crossing groups was observed for the two sympatric crossing scenarios in nature, pollination of *S. rexii* with *S. johannis* ($\bar{x}$ = 228.40) and *S. johannis* with *S. rexii* ($\bar{x}$ = 572.20). Nevertheless, seed set for these two scenarios is still in the hundreds: When imagining a freak pollination event between these two species with very different flower architecture and colour signal, resulting seed numbers would be easily high enough to cause the establishment of a hybrid swarm. Possibly, reinforcement (or Wallace effect) is an ongoing process for the sympatric scenario of *S. rexii* and *S. johannis*: From the perspective of the crossing experiments, both species are incipient and hybrid formation is possible.

Although strains joha-rose and rexi-rose showed a negative paternal effect on F2 seed set, seed number is high, roughly approximating round about 1,000 seeds per fruit. Other experimental groups aiming at F2 seed set are easily surpassing this threshold, with some crossings leading to more than 2,000 seeds per fruit.

Our results strongly support the theory of Hilliard and Burtt (1971) of a single large gene pool for the Cape primrose clade, still allowing for hybridisation and speciation due to missing postmating or specific postzygotic crossing barriers. Our results further support the findings of de Villiers et al. (2013): They demonstrated the historical relevance of hybridisation within genus *Streptocarpus*, and showed that growth form shifts rather than floral plasticity might be interpreted as evidence for hybrid origins. Highly specialised keyhole flower types like of *S. baudertii*, *S. polyanthus* and *S. johannis* (often rumoured to be of hybrid origin) were only present in stable taxa without gene tree conflict, giving support to the hypothesis that floral architecture has been maintaining reproductive isolation in a reticulate evolutionary scenario.

Taken together, ecogeographical isolation and prezygotic pollination barriers such as floral architecture and optical flower signal are likely the crucial factors to guide and control pollinators and keep the species separate in nature.

VI.6 ACKNOWLEDGEMENTS

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VII. FUNCTIONAL FLORAL ARCHITECTURE, OPTICAL SIGNAL AND NECTAR REWARD IN *STREPTOCARPUS* HYBRIDS, ALLOWING FOR HOMOPLOID HYBRID SPECIATION

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VII.1 ABSTRACT

Background and aims: Large parts of genus *Streptocarpus* are assumed to form a single gene pool. Hybridisation as a possible cause of speciation within the genus has been discussed for some time in scientific literature. Here we present crossing experiments to analyse hybridisability of selected species of *Streptocarpus* subgenus *Streptocarpus*, representing the floral diversity of the genus to a large extent. Obtained F1 hybrids are analysed in regard to floral architecture, optical flower signal, nectar reward and the heredity of these floral plant traits. Respective factors are of high importance for attraction, control and sorting of pollinators, and therefore determine hybrid fitness and possible establishment of hybrid swarms in the field.

Methods: Crossing experiments, refractometer nectar analysis, UV light photography, hierarchical clustering, Non-metric Multidimensional Scaling (NMDS).

Key results: Hybridisation within the subgenus is often achieved with ease. Flowers of F1 hybrids are predominantly fully functional: Only floral architecture showed moderate instability within hybrids, while nectar reward and optical flower signal appeared stable. Only 5 out of 40 hybrids showed severe malfunctions of the reproductive system. Heredity of floral plant traits showed dominant-recessive pattern, and F1 phenotypes appeared more homogenous than the parental generation.

Conclusion: Establishment of fully functional hybrid swarms and onset of homoploid hybrid speciation is possible in nature, if an initial freak pollination event is able to circumvent the evolutionary established prezygotic crossing barriers (attraction of pollinators via flower signal, control of pollinator access via floral architecture, suitable nectar reward, etc.), and a compatible pollinator is present.

Key words: *Streptocarpus*, Cape primrose clade, floral architecture, nectar reward, optical flower signal, hybridisation, crossing experiment, NMDS, hierarchical clustering, coefficient of variation, plant-pollinator interaction.

VII.2 INTRODUCTION

Streptocarpus Lindl. describes a genus of about 179 species accounted for in the wild, commonly known as Cape Primroses. Recently, *Streptocarpus* was expanded to include all African and Malagasy Gesneriaceae genera of subtribe Streptocarpinae, in tribe Trichosporeae, subfamily Didymocarpoideae (Möller et al., 2019, citing Darbyshire and Massingue, 2014; Nishii et al., 2015; Randrianasolo et al., 2018). Many species evolved very recently round about 1.5 million years ago, via radiation in the eastern South Africa (Möller et al., 2019; Möller and Cronk, 2001).

Floral diversity in *Streptocarpus* has been of scientific and commercial interest for a long time: Extensive breeding efforts during the last centuries have created a wide array of cultivars of high economic importance (Buta et al., 2010; Nishii et al., 2015). From a scientific perspective, *Streptocarpus* is remarkable for the great proportion of possible floral diversity realised in only one genus (Hilliard and Burtt, 1971). Latest illustration of floral diversity was presented by Möller et al. (2019), based on the general framework of open-tube, keyhole, personate, small-pouch, flat-faced *Saintpaulia* and bird-pollination flower types of Harrison et al. (1999), Hughes et al. (2006), and modifications presented by Nishii et al. (2015).

Hybridisation as a possible cause of floral diversity has already been discussed by Hilliard and Burtt (1971). They concluded that large parts of subgenus *Streptocarpus* might form a single gene pool, and reported 28 instances of suspected hybridisation (p. 77 ff.). The possibility of hybridisation in nature is further supported by the fact that many species share the same habitat, and have overlapping flowering time (cf. Hilliard and Burtt, 1971). Recent scientific research pays attention to hybridisation in genus *Streptocarpus* again: Gene three conflicts and possible capture of foreign genetic information via hybridisation are rampant in *Streptocarpus*, correlating to growth form shifts, as evidenced by gene tree conflicts for *S. bolusii*, *S. vandeleurii*, *S. grandis*, *S. fanniniae*, and *S. gardenii* being an additional candidate (de Villiers et al., 2013).

The importance of hybridisation for speciation has been outlined thoroughly by Buerkle and Rieseberg (2008), with focus on homoploid hybrid speciation. In this process, independent lineages are formed by combination of parental genomes without increase in ploidy level. Homoploid hybrid speciation has been confirmed for some plant and animal taxa (e.g. Rieseberg, 1997; Gross and Rieseberg, 2005; Howarth and Baum, 2005; Schwarz et al., 2005; Gompert et al., 2006, Mir et al., 2006), but it is considered to be rare due to possibility of backcrossing with parental lines and missing separation of gene pools (Rieseberg, 1997; Gross and Rieseberg, 2005; Coyne and Orr, 2007). However, recent and convincing experimental demonstration for homoploid speciation in *Helianthus* sunflowers (Rieseberg, 2000; Buerkle and Rieseberg, 2008) and *Heliconius* butterfly species (Mavárez et al., 2006) showed that novel ecological or intrinsic, genetic factors can contribute quickly to gene pool separation and stabilisation, within tens of generations. Rapidity of this process is further supported by simulation models of hybrid speciation (compare McCarthy et al., 1995; Buerkle et al., 2000).

In *Streptocarpus* sugenus *Streptocarpus*, hybridisation as possible aspect of the speciation process appears to be entirely at the homoploid level: Hilliard and Burtt (1971) already assigned acaulescent species with a basic chromosome number of $x = 16$ into subgenus *Streptocarpus* and caulescent species with $x = 15$ into subgenus *Streptocarpella*. Nishii et al. (2015) generally confirmed this division in their recent redefinition of *Streptocarpus* , stating that among all characters analysed only basic chromosome

number is fully congruent with the topology of the retrieved tree. Moreover, polyploidy in subgenus *Streptocarpus* is seldom, and absent in the 'Cape primose clade' or *Streptocarpus cyaneus* group (Weigend and Edwards, 1994), with all species being diploid and showing 32 chromosomes. The Cape primrose clade is of special interest in regard to homoploid hybrid speciation: Members of this group form a phylogenetically not well resolved polytomy, repeatedly shown for chloroplastic and/or nuclear markers (Harrison et al., 1999; Hughes et al., 2006; de Villiers et al., 2013; Nishii et al., 2015). Therefore, at least members of this clade might be connected by an underlying shared gene pool that allows for hybrid speciation.

For zoophilous *Streptocarpus*, the bottleneck for exchange of genetic information across species and possible hybrid speciation is the moment of interaction between plant and pollinator. Unfortunately, pollinator information is scarce for *Streptocarpus*. Möller et al. (2019) only report tabanid and/or nemistrinid long-proboscid flies for *S. primulifolius* and *S. formosus* in their review, and Potgier and Edwards (2006) report *S. formosus* to be part of the pollination guild of long-proboscid fly *Stenobasipteron wiedemanni*, along with 18 other plant species of six different families. Although information on specific plant-pollinator interaction for *Streptocarpus* is rare, the described pattern for *Stenobasipteron* is common for Southern African pollination systems, which are characterised by a high level of asymmetric ecological specialisation, convergent floral evolution and specialisation of plant guilds for particular pollinators (compare Johnson, 2010).

Floral specialisation towards pollinators or pollinator guilds is manifested via specific floral traits and their interplay. Groups of floral phenotypes that represent specialised plant-animal interactions are described by the concept of pollination syndromes (c.f. Delpino, 1868–1875; Vogel, 1954; van der Pijl, 1961; Fenster et al., 2004; Vogel, 2006, etc.). As for floral phenotypes recognised in *Streptocarpus*, Harrison et al. (1999) stated e.g. that open tube and personate flower types are indicative for bee pollination, keyhole types are indicative for moth or butterfy pollination, and *S. dunnii* with its brightly red flowers shows a bird pollination syndrome.

Homoploid hybrid speciation requires that the isolating mechanisms of floral specialisation of the original species are circumvented in a first step (cf. Grant, 1949), i.e. by a freak pollination event and initial hybrid formation. After that, successful homoploid hybrid speciation and establishment of hybrid swarms in the field depends on the fit between floral plant traits and the pollinators present in the habitat, like mechanical fit via floral architecture, fit to sensory capacities via floral signal, and fit to nutritional needs via reward.

In the present chapter a crossing experiment is presented as a classical, experimental approach for better understanding of hybrid formation in genus *Streptocarpus*. We investigate general crossability of selected species of *Streptocarpus* subgenus *Streptocarpus*, some of them members of the Cape primrose clade. Moreover, floral architecture, optical flower signal and nectar reward of parental plants and obtained hybrids are described and analysed. Clustering and ordination of data is performed in order to detect correspondence of patterns between hybrids and parental species. Moreover, instability of pattern is analysed for metric variables (floral architecture and nectar reward) in order to rate possible loss of hybrid fitness, i.e. via loss of floral symmetry or reliability of produced nectar reward.

VII.3 MATERIALS AND METHODS

Streptocarpus species and hybrids were cultivated in a pollinator-protected greenhouse at Botanische Gärten, Universität Bonn (Bonn University Botanic Gardens). For crossing and analysis of floral plant traits, three to five plant individuals per species and hybrid were used, and data were pooled. Nine species were selected for the crossing experiment in order to represent basic pattern of floral architecture, optical flower signal and nectar reward of the subgenus: *S. cooperi* C.B.Clarke (in abbreviation: coop), *S. dunnii* Mast. (dunn), *S. johannis* L.L.Britten (joha), *S. longiflorus* (Hilliard & B.L.Burtt) T.J.Edwards (long), *S. modestus* L.L.Britten (mode), *S. pole-evansii* I.Verd. (pole), *S. polyanthus* ssp. *verecundus* Hook. (poly), *S. rexii* (Bowie ex Hook.) Lindl. (rexi) and *S. roseoalbus* Weigend & T.J.Edwards (rose). For clarity and conciseness, abbreviations will be used to refer to species and hybrids in the following. Abbreviations for hybrids refer to the parental lines involved in the crossing, with the maternal crossing partner named in the first place (i.e. coop-rexi referring to *S. cooperi* as maternal and *S. rexii* as paternal crossing partner). Accession and voucher numbers are presented in the appendix (table A X.6.6).

VII.3.1 CROSSING PROCEDURES

Crossings between selected species as well as allogamous and geitonogamous pollinations were performed in 2015. Flower buds intended as maternal crossing partner were emasculated with a tweezer to prevent accidental self-pollination. Hand-pollinations were performed by placement of the complete pollen material of the selected paternal flower onto the stigma of the maternal crossing partner. Tweezers were cleaned with ethanol between handling of different flowers.

At least five hand pollinations were performed per crossing direction. In sum, 485 between-species pollinations were performed, of them 110 pollinations within the Cape primrose clade (long, rose, joha, mode, rexi). All possible reciprocal crossings were performed, with both parental lines applied as maternal as well as paternal partner.

Geitonogamous and allogamous pollination was each performed with 10 pollinations per species. Allogamy could not be tested for *S. dunnii* due to only a single flowering individual. All in all, 170 additional within-species pollinations were performed.

VII.3.2 ASSESSMENT OF FLORAL PLANT TRAITS

Analysis of floral architecture, optical signal and nectar reward was also performed in 2015 for original species, with limited re-examination in 2016 in order to complete data sets. Analysis of floral architecture of hybrids was performed in 2017, with limited re-examination in 2018. Analysis of optical flower signal and nectar reward of hybrids was accomplished in 2018.

For analysis of **floral architecture**, individual flowers were measured and display sizes calculated. For assessment of display sizes flowers were photographed and measured via Adobe® Photoshop® CS6 imaging software and Magnetic Lasso Tool function. In total, 16 variables regarding flower architecture were assessed: frontal and lateral display size in cm^2, display size ratio (frontal:lateral), height and width of the frontal display, height and width of the corolla opening, height and width of the unfused part of the upper left petal of the gamopetalous corolla, height and width of the unfused part of the lower central petal, dorsal flower length (from base of corolla to incision between the upper petals), ventral

flower length (from base of corolla to the tip of the lower central petal), anther length, filament length and carpel length.
Sample size is usually 10 per variable for each species and hybrid. Exceptions for display size variables are hybrids joha-coop, mode-coop, poly-pole and rose-mode (only single data points), mode-long and mode-rexi (5 data points per variable), rexi-mode (8) and poly-rose (9). Data points for other variables of floral architecture are lowered for hybrids mode-rexi (6 data points) and joha-rexi (9).
In total, 441 flowers were pictured and analysed for display sizes (parental: 90; F1: 351), and 485 flowers measured for floral architecture (parental: 90; F1: 395).

For **assessment of nectar reward**, microcapillaries were inserted into the corolla tube onto the receptacle of the flower and circled around the nectar disk at the base of the gynoecium to collect nectar (0.5 and 1 µl minicaps®; 5 µl ringcaps® – Hirschmann Laborgeräte, Germany). Concentration of nectar sugar was measured in degrees Brix with a hand-held refractometer (neoLab, type 'universal'). Nectar sugar production per flower was derived from nectar volume and nectar concentration after Galetto and Bernadello (2005), with *x* being the measured concentration:

$$\frac{mg}{\mu l} = 0.00226 + (0.00937x) + (0.0000585x^2)$$

Analysis of nectar reward is therefore based on three variables: nectar amount in µl, nectar sugar concentration in percent and sugar production in mg. If nectar production of single flowers was not sufficient for assessment, nectar from several flowers had to be pooled and averaged. In general, number of data points is 25 per variable and for each species and hybrid. Exceptions are *S. cooperi* with only 3 valid data points per nectar variable, as well as hybrids coop-rose (10 data points per variable) and rexi-joha (3).
In total, 1188 data points per nectar variable (parental: 225; F1: 963) were used for nectar analysis.

For analysis of **UV signal**, flowers were illuminated with UV light and pictures were taken for frontal and lateral flower displays with a single-lens reflex camera (Nikon R D300s), and an infrared neutralizer (OPTIK MAKARIO IR NG 52D) in combination with a UV light filter (OPTIK MAKARIO SP 400 UV 52D). Photos were created with a fixed aperture, but different shutter speeds (1/5, 1/4, 1/3, 1/2, 1/1.6, 1, 1.6, 2, 3, 4, 5 sec.). Pictures were analysed in regard to UV light responsiveness of the corollas: 1 sec. UV light exposure showed highest differentiation between species and was therefore used as basis for coding of the floral UV signal.

VII.3.3 Assessment of instability of floral architecture and nectar reward

Metric, ratio scaled variables analysed for floral architecture and nectar reward further allowed for assessment of instability of the observed pattern: Coefficients of variation (CV) were calculated in order to obtain dimensionless numbers, allowing for comparison between different levels of analysis. CVs were calculated as follows:

$$CV\ in\ \% = \frac{standard\ deviation\ \sigma}{arithmetic\ mean\ \overline{x}} \times 100$$

As for floral architecture, all variables except display sizes and variables related to the male reproduction system were suitable for calculation of CVs. Display size variables had to be excluded due to single data points for four hybrids, and anther and filament length had to be excluded due to floral dysfunction in some hybrids, see results. All three variables of nectar reward were suitable for calculation. CVs were further averaged for variables and retrieved clusters, in order to calculate superordinate CVs.

VII.3.4 Statistical Analysis

Data exploration and basic statistics were carried out with the IBM software package SPSS, version 24. Outliers in regard to observed data bandwidth were labelled according to standard conventions. Statistical procedures beyond data exploration were performed with aggregated data on species level (arithmetic means, CVs) due to unequal sample sizes and non-normality of data.

Cluster analysis and multivariate ordination were performed in order to structure, analyse and visualise floral architecture, optical signal and nectar reward of original species and hybrids. Both methods were performed with computing software R: a) with the arithmetic means of continuous variables and nominal coding of discrete variables, and b) with CVs for floral architecture and nectar reward. Analysed categorical, nominal variables are the predominant colour tone of the frontal corolla display (coded 1 for white, 2 for bluish and 3 for reddish tones) and the presence of an anther dummy signal in form of greenish-yellow to yellow floral guides (coded 0 for absence and 1 for presence). Categorical but ordinal variables are the UV light reflection patterns of the frontal and lateral display (coded 0 for complete absorption, 1 for diffuse absorption, 2 for weak reflection, 3 for medium reflection and 4 for strong reflection). Variables assessed for analysis of floral architecture and nectar reward are metric and ratio scaled.

Hierarchical clustering of (a) optical flower signal, (b) nectar reward and (c) floral architecture were performed in R via packages cluster (version 2.0.5; Mächler et al., 2019), NbClust (version 3.0; Charrad et al., 2014) and vegan (version 2.5-1; Oksanen, 2013). Best number of clusters was determined via a combination of visual inspection, Elbow method as well as use of multiple indices and selection according to majority rule. Cluster analyses were conducted with complete linkage to account for the interrelations between hybrids and parental species, and dissimilarity matrices based on metric variables were produced via Bray Curtis index (nectar, floral architecture), while Gower distances were used for optical signal (see appendix, figures A X.6.1 to A X.6.3).

Non-Metric Multidimensional Scaling (NMDS, R package vegan) was used for ordination of arithmetic means and CVs of variables of floral architecture and nectar reward in order to handle different measuring scales and non-normality of data. Same dissimilarity matrices were applied as for hierarchical clustering. Function metaMDS was used for iterative testing and selection of the solution with smallest stress. Data was standardised by square root transformation and Wisconsin double standardisation.

Model stresses below .2 were interpreted as acceptable, below .1 as good and below .05 as excellent (McCune et al. 2002).

VII.4 RESULTS

VII.4.1 CROSSABILITY

Siring success between and within parental species is presented in detail in figure VII.1. In general, successful hybridisation is common between the selected *Streptocarpus* species. Pollination between members of the Cape primrose polytomy showed vigorous hybrid fruit set, often up to 100 %, especially when *S. rexii* and *S. roseoalbus* were involved as paternal or maternal partner. Only pollinations between *S. longiflorus* and *S. johannis* were unsuccessful within members of the polytomy, and fruit seed was generally lower for *S. johannis* (maternal and paternal line).

♀ \ ♂	*S. cooperi*	*S. pole-evansii*	*S. dunnii*	*S. polyanthus*	*S. longiflorus*	*S. roseoalbus*	*S. johannis*	*S. modestus*	*S. rexii*
S. cooperi	↺ 10/10 ⇆ 10/10	0/10	7/10	0/10	3/10	8/10	2/5	2/5	3/5
S. pole-evansii	0/10	↺ 0/10 ⇆ 0/10	0/10	0/10	0/10	0/10	0/5	0/5	0/5
S. dunnii	2/5 Ω	0/5	↺ 10/10	0/5	3/5	3/5	0/5	0/5	3/5
S. polyanthus	1/10	3/10	0/10	↺ 10/10 ⇆ 10/10 ↻	0/10	1/10	0/5	0/5	1/5
S. longiflorus	5/10	0/10	8/10	0/10	↺ 10/10 ⇆ 10/10	9/10	0/5	3/5 Ω	4/5
S. roseoalbus	3/10	0/10	7/10	0/10	1/10	↺ 10/10 ⇆ 10/10	4/5	5/5	5/5
S. johannis	2/5	0/5	1/5	2/5	0/5	2/5	↺ 10/10 ⇆ 10/10	2/5	2/5
S. modestus	1/5	1/5 Ω	0/5	1/5	3/5	5/5	2/5	↺ 10/10 ⇆ 10/10	3/5
S. rexii	3/5	0/5	3/5	0/5	5/5	4/5	5/5	5/5	↺ 10/10 ⇆ 10/10

FIGURE VII.1: *STREPTOCARPUS* CROSSING GRID AND SIRING SUCCESS FOR CROSSPOLLINATION, ALLOGAMY AND GEITONOGAMY
Maternal and paternal lines during pollination procedures are indicated by the vertical (♀) and horizontal (♂) headers. Results for the Cape primrose polytomy (long, rose, joha, mode, rexi) are presented in the lower right corner of the grid. Siring success is indicated by an index (x/y), showing successful fruit set (x) in relation to the number of pollinated flowers (y), further highlighted by a heatmap, representing siring success from 10 to 100 % via colour intensity. At least five hand pollinations were performed per crossing direction. Results regarding allogamous (⇆) and geitonogamous (↺) siring success are presented by the diagonal line of the grid. Siring success was highest within the Cape primrose polytomy. All species except *S. pole-evansii* showed vigourous fruit seet after allogamous and geitonogamous pollination. Allogamy could not be tested for *S. dunnii* due to only a single flowering plant. Spontaneous autogamy (↻) was only observed for *S. polyanthus*. In three cases (dunn-coop, long-mode, mode-pole) hybrid seed material did not germinate (Ω). Crossing experiment performed by A. W. Mues. Flower pictures by A. W. Mues, scaling bars representing 1 cm.

Regarding species outside of the Cape primrose clade, *S. cooperi* and *S. dunnii* have to be highlighted in their capability of producing hybrid fruit set as maternal as well as paternal crossing partner. For *S. cooperi*, pollen material of all species except *S. pole-evansii* and *S. polyanthus* led to hybrid fruit seed

(maternal siring success ranging between 40 and 80 %), while pollen material of *S. cooperi* led to successful hybridisation with all analysed species except *S. pole-evansii* (paternal siring success ranging from 10 to 60 %).

Pollen material of *S. dunnii* only missed siring success when *S. pole-evansii*, *S. polyanthus* and *S. modestus* were pollinated with it (paternal siring success for remaining species: 20 to 80 %), while pollen material of the same species plus *S. johannis* led to no hybrid fruit set of *S. dunnii* (maternal siring success for remaining species ranging between 40 and 60 %).

Siring success was much lower when *S. polyanthus* was involved: As paternal crossing partner, only pollination of *S. johannis* and *S. modestus* showed limited fruit set (20 and 40 % siring success). As maternal crossing partner, only pollen material of *S. cooperi*, *S. rexii*, *S. roseoalbus* and *S. pole-evansii* led to limited fruit seed of *S. polyanthus* (10 to 30 percent siring success).

Regarding *S. pole-evansii*, pollen material of no other species was able to hybridise with it (maternal siring success: 0 %), and paternal siring success of *S. pole-evansii* was low, too: Only pollination of *S. polyanthus* and *S. modestus* led to limited siring success (30 and 20 %, seed material obtained from *S. modestus* not viable).

Regarding within-species-pollination, *S. pole-evansii* differed again from other species, with no fruit seed observed. Geitonogamous and allogamous pollination were always successful for all other species (100 % siring success). *S. polyanthus* showed spontaneous autogamy.

Obtained hybrid seed material did not germinate in three cases (dunn-coop, long-mode, mode-pole). Hybrid coop-dunn did not flower during the experimental phase.

The remaining 40 hybrids were analysed in regard to optical flower signal, floral architecture and nectar reward. Individual hybrid plants of each successful crossing appeared homogenous. Results are described below.

VII.4.2 Optical flower signal

Optical flower signal visible to the human eye is presented in figure VII.2, while retrieved pattern of UV light absorption or reflection are presented in figure VII.3.

Categorisation of data is summarised in table VII.1, also showing assignment of retrieved clusters for each species and hybrid.

VII.4.2.1 Data bandwidth and clustering of optical flower signal

Five clusters were retrieved for optical flower signal (cf. appendix, figure A X.6.3): Cluster I contains parental species *S. cooperi*, *S. johannis*, *S. modestus* and *S. rexii* and 10 of their hybrids, plus hybrid poly-pole. Corolla colour is bluish, seldom white (rexi), anther dummy signal is missing and UV light is usually (diffusely) absorbed.

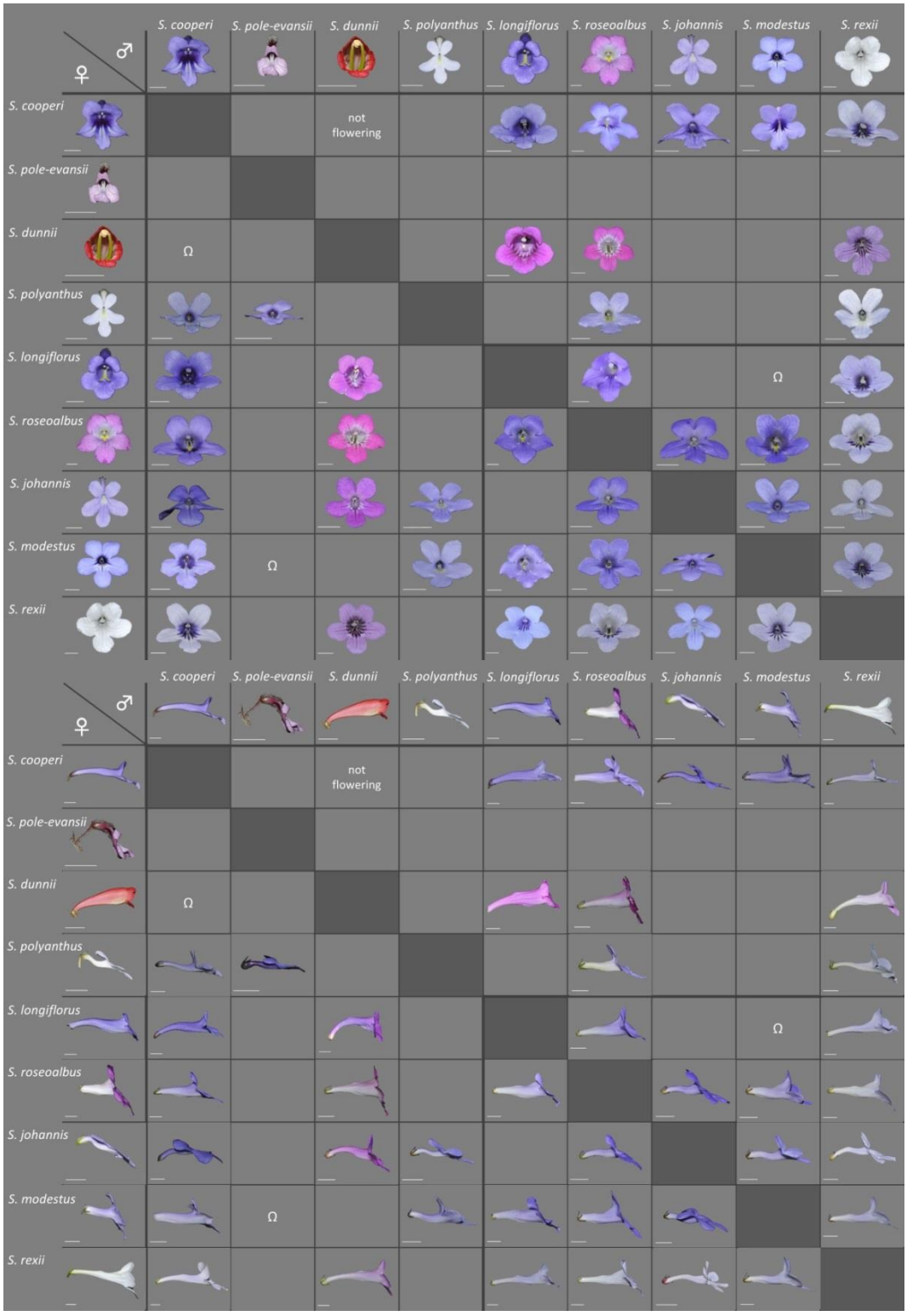

FIGURE VII.2: FLORAL ARCHITECTURE AND COLOUR SIGNAL IN VISIBLE LIGHT SPECTRUM OF SPECIES AND HYBRIDS
Top: Frontal displays; bottom: Lateral displays. Scaling bar = 1 cm. Picture credits: Parental species, rose-long (frontal) and mode-coop by A. W. Mues, long-dunn and dunn-long (front, lat.) as well as frontal pictures of coop-mode, coop-rose, long-rose, rexi-joha and rexi-long by L. Hoff, others by L. Nicolin.

Cluster II contains parental species *S. dunnii* and *S. pole-evansii* as well as three descendents from *S. dunnii* (dunn-rexi, rexi-dunn, joha-dunn). Corolla colour is reddish and anther dummy signal is missing. Regarding UV signal, (diffuse) absorption is the predominant pattern, especially among hybrids. Moderate reflection was observed at maximum (*S. pole-evansii*). Cluster III contains *S. roseoalbus* as parental species and three hybrids (coop-rose, dunn-long, dunn-rose). Corolla colour is usually reddish (except coop-rose, bluish corolla), anther dummy is present and UV light is reflected moderately or strongly (*S. roseoalbus*). Cluster IV only contains *S. polyanthus* and its hybrid poly-rexi. Corolla colour is white, anther dummy is present and UV light is absorbed diffusely. Cluster V is the largest cluster and contains *S. longiflorus* as parental species as well as 22 hybrids (8 of them stemming from *S. longiflorus*). Corolla colour is predominantly bluish, seldom reddish (hybrids long-dunn and rose-dunn), and anther dummy is present. Frontal and lateral displays usually show complete or diffuse UV light absorption, seldom weak (lateral) reflection. It must be noted that all retrieved clusters are representing parental species as well as hybrids: Thus, the F1 generation does not show any patterns that are absent in the field. Inheritance patterns are in favour of cluster V, however.

VII.4.2.2 INHERITANCE OF OPTICAL FLOWER SIGNAL

White corolla colour of parental species (poly, rexi) is inherited recessively, only the hybrid between both white parental species (poly-rexi) showed white corolla colour. However, involvement of white flowering parental lines is linked to hybrids with less intense flower colours (light-blue and light-red tones). Blue corolla colours (parentals coop, long, joha, mode) are inherited dominantly over white corolla colour, and also over pinkish-red corolla colour stemming from *S. pole-evansii* and *S. roseoalbus*. Hybrids stemming from both pinkish-red parental lines showed bluish corolla colour in case the other parental line showed white corolla colour, compare hybrids poly-pole, poly-rose, rose-rexi and rexi-rose. The intense red corolla colour of *S. dunnii* is inherited dominantly however, and led to pinkish corolla colours when crossed with white or bluish flowering crossing partners.

Anther dummy signal is inherited dominantly: Whenever parental lines with anther dummy signal (long, poly, rose) were involved, hybrid offspring also showed an anther dummy signal, only exception is hybrid poly-pole. Interestingly, hybrids coop-mode and mode-coop showed anther dummy signal in their corollas, although not observed in both parental lines (cf. Hilliard and Burt (1971) for varying presence of anther dummy signal in *S. modestus*).

In case reciprocal crossings were successful, the resulting hybrids showed no difference in optical colour signal in visible light spectrum.

Regarding UV light pattern, the broad bandwidth of parental species is not reflected in the F1. UV light signal of parental species is ranging from full absorption (long) to strong reflection (rose) with various intermediate patterns. Hybrids never showed strong reflection, even moderate and weak reflection were seldom. The F1 predominantly showed diffuse UV light absorption, in some cases full absorption. When UV reflecting species *S. dunnii* and *S. roseoalbus* were involved as parental lines, hybrids showed a tendency for higher UV reflection in some but not all cases. Contrary to optical signal visible to the human eye, reciprocal crossings were not always identical in regard to their UV light patterns (i.e. coop-rose and rose-coop, long-rose and rose-long, dunn-long and long-dunn, etc.).

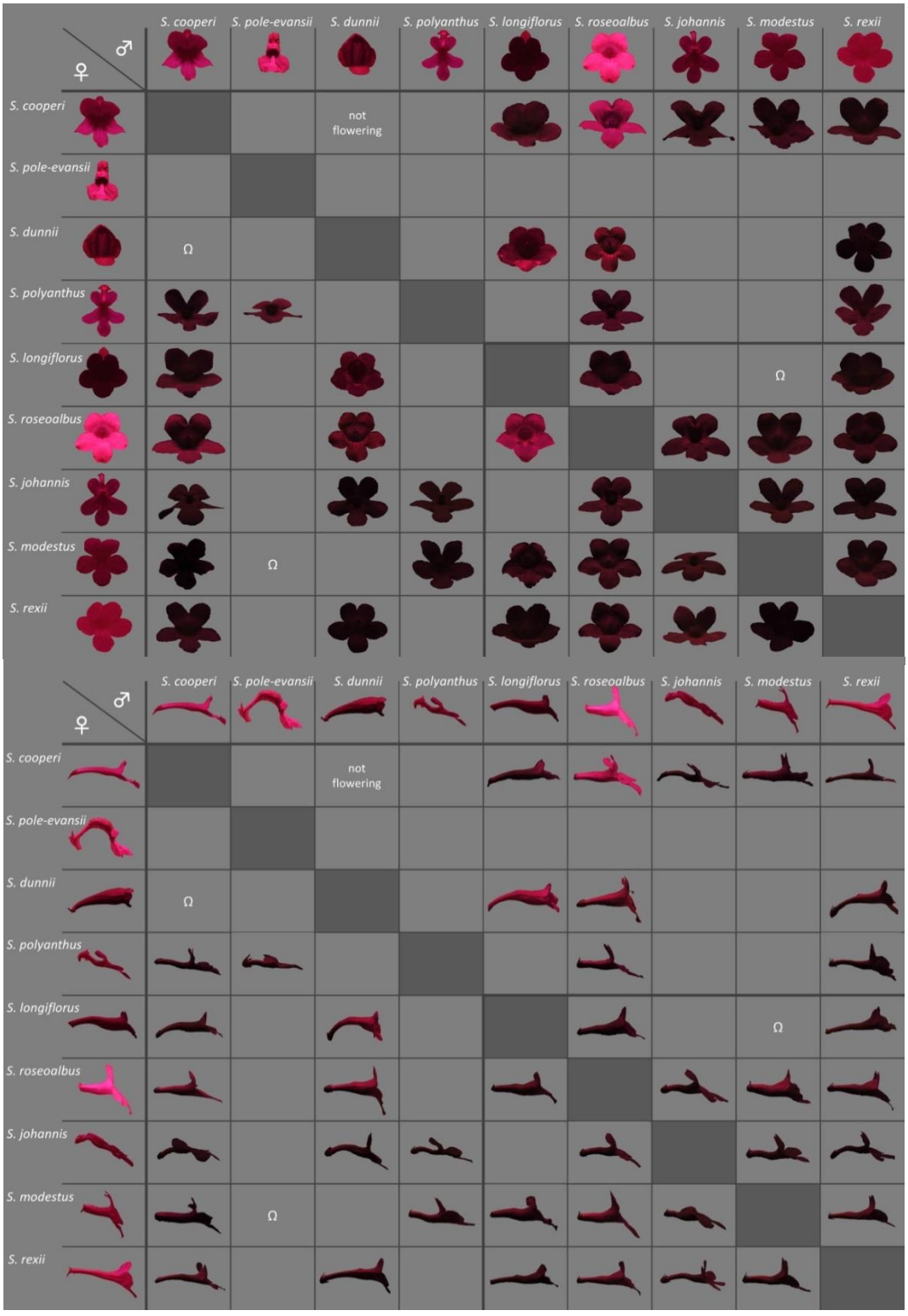

FIGURE VII.3: FLORAL UV SIGNAL OF SPECIES AND HYBRIDS, AFTER 1 SEC. UV LIGHT EXPOSURE
Picture credits: Parental species, rose-long (frontal) and mode-coop by A. W. Mues, long-dunn and dunn-long by L. Hoff, others by L. Nicolin.

TABLE VII.1: OVERVIEW OF OPTICAL FLOWER SIGNAL OF NINE *STREPTOCARPUS* SPECIES AND FORTY HYBRIDS

The table shows the observed predominant colour tone of the corolla (coded as white ○, bluish 🔵 or reddish 🔴), the presence of an anther dummy signal as well as patterns of UV light signal after 1 sec. UV light exposure (complete absorption ●, diffuse absorption ⊙, weak reflection ○, medium reflection ○○ and strong reflection ○○○, compare figure VII.3). Results of hierarchical clustering are indicated. *) While mentioned as an aspect or possible variation by Hilliard and Burt (1971), our accessions of S. *johannis* and *S. modestus* did not show signs of an anther dummy signal in 2015. However, flowers of *S. modestus* showed a weak yellow floral guide during flowering season in 2016, and hybrids between *S. modestus* and *S. cooperi* showed a pronounced anther dummy signal in 2017 and 2018.

species and hybrids	cluster	predominant colour	anther dummy	UV frontal	UV lateral
Streptocarpus cooperi C.B.Clarke	I	🔵		○	○○
Streptocarpus dunnii Mast.	II	🔴		○	○
Streptocarpus johannis L.L.Britten	I	🔵	*	⊙	○
Streptocarpus longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	V	🔵	✓	●	⊙
Streptocarpus modestus L.L.Britten	I	🔵	*	⊙	○
Streptocarpus pole-evansii I.Verd.	II	🔴		○○	○○
Streptocarpus polyanthus Hook.	IV	○	✓	⊙	⊙
Streptocarpus rexii (Bowie ex Hook.) Lindl.	I	○		⊙	○
Streptocarpus roseoalbus Weigend & T.J.Edwards	III	🔴	✓	○○○	○○○
S. cooperi x *S. johannis*	I	🔵		⊙	●
S. cooperi x *S. longiflorus*	V	🔵	✓	⊙	⊙
S. cooperi x *S. modestus*	V	🔵	✓*	⊙	⊙
S. cooperi x *S. rexii*	I	🔵		⊙	⊙
S. cooperi x *S. roseoalbus*	III	🔵	✓	○	○○
S. dunnii x *S. longiflorus*	III	🔴	✓ (2 small dots)	○	○○
S. dunnii x *S. rexii*	II	🔴		●	⊙
S. dunnii x *S. roseoalbus*	III	🔴	✓	○	○○
S. johannis x *S. cooperi*	I	🔵		⊙	●
S. johannis x *S. dunnii*	II	🔴		●	⊙
S. johannis x *S. modestus*	I	🔵		⊙	⊙
S. johannis x *S. polyanthus*	V	🔵	✓	⊙	●
S. johannis x *S.rexii*	I	🔵		⊙	●
S. johannis x *S. roseoalbus*	V	🔵	✓	⊙	⊙
S. longiflorus x *S. cooperi*	V	🔵	✓	⊙	⊙
S. longiflorus x *S. dunnii*	V	🔴	✓ (2 small dots)	⊙	○
S. longiflorus x *S. rexii*	V	🔵	✓ (weak)	⊙	⊙
S. longiflorus x *S. roseoalbus*	V	🔵	✓	⊙	⊙
S. modestus x *S.cooperi*	V	🔵	✓*	●	⊙
S. modestus x *S. johannis*	I	🔵		⊙	⊙
S. modestus x *S. longiflorus*	V	🔵	✓	⊙	⊙
S. modestus x *S. polyanthus*	V	🔵	✓	●	⊙
S. modestus x *S. rexii*	I	🔵		⊙	⊙
S. modestus x *S. roseoalbus*	V	🔵	✓	⊙	○
S. polyanthus x *S. cooperi*	V	🔵	✓	⊙	⊙
S. polyanthus x *S. pole-evansii*	I	🔵		⊙	⊙
S. polyanthus x *S. rexii*	IV	○	✓	⊙	●
S. polyanthus x *S. roseoalbus*	V	🔵	✓	⊙	⊙
S. rexii x *S. cooperi*	I	🔵		●	⊙
S. rexii x *S. dunnii*	II	🔴		●	⊙
S. rexii x *S. johannis*	I	🔵		⊙	⊙
S. rexii x *S. longiflorus*	V	🔵	✓(weak)	●	⊙
S. rexii x *S. modestus*	I	🔵		●	⊙
S. rexii x *S. roseoalbus*	V	🔵	✓	⊙	⊙
S. roseoalbus x *S. cooperi*	V	🔵	✓	⊙	○
S. roseoalbus x *S. dunnii*	V	🔴	✓	⊙	○
S. roseoalbus x *S. johannis*	V	🔵	✓	⊙	⊙
S. roseoalbus x *S. longiflorus*	V	🔵	✓	○	⊙
S. roseoalbus x *S. modestus*	V	🔵	✓	⊙	○
S. roseoalbus x *S. rexii*	V	🔵	✓	⊙	⊙

VII.4.3 Floral architecture

For depictions of floral architecture see figure VII.2.

VII.4.3.1 Data bandwidth, clustering and NMDS of floral architecture

In the following, hierarchical clustering and NMDS ordination of arithmetic means are presented for floral architecture, compare figure VII.4 (top)**.** Frontal and lateral display size and width of the flower opening are highlighted for description of data bandwidth: Detailed information for all variables of floral architecture, and for all species and hybrids are presented in the appendix (tables A X.6.1 and A X.6.2).

Frontal display size ranges between .70 cm^2 (pole) and 10.66 cm^2 (rose) for parental species ($\bar{x}$: 4.29 cm^2), and between .51 cm^2 (poly-pole) and 10.64 cm^2 (rexi-rose) for hybrids ($\bar{x}$: 5.18 cm^2). Frontal display size shows no outliers amongst parental species or hybrids.

Lateral display size ranges between .41 cm^2 (pole) and 5.53 cm^2 (rexi) for parental species ($\bar{x}$: 2.52 cm^2), and between .60 cm^2 (poly-pole) and 6.31 cm^2 (coop-rose) for hybrids ($\bar{x}$: 3.76 cm^2). Lateral display size shows no outliers amongst parental species or hybrids.

Opening width ranges between 1.0 mm (poly) and 19.6 mm (rose) for parental species (arithmetic mean: 9.8 mm), and between 2.2 mm (poly-pole) and 17.0 mm (coop-rose) for hybrids (arithmetic mean: 8.5 mm). Opening width shows no outliers amongst parental species or hybrids.

As for hierarchical clustering of arithmetic means, three clusters were retrieved for floral architecture. Cluster 1 contains six of nine parental species, namely *S. cooperi*, *S. dunnii*, *S. longiflorus*, *S. modestus*, *S. rexii* and *S. roseoalbus*, as well as 31 of 40 assessed hybrids. Common denominator of this cluster is the presence of larger, funnel shaped flowers with larger flower openings. Average frontal display size of this cluster is 5.82 cm^2 (min.: 1.09 cm^2, dunn; max.: 10.66 cm^2, rose), average lateral display size is 4.04 cm^2 (min.: 1.93 cm^2, mode-joha; max.: 6.31 cm^2, coop-rose), and average opening width is 10.3 mm (min.: 3.2 mm, rexi-joha; max.: 19.6 mm, rose).

Cluster 2 only contains *S. johannis* as parental species, and eight hybrid descendants of it and/or *S. polyanthus* (joha-coop, joha-dunn, joha-poly, mode-joha, mode-poly, poly-coop, poly-rexi, poly-rose). Flowers are of moderate size with smaller flower openings. Average frontal display size is 2.77 cm^2 (min.: 1.38 cm^2, joha-poly; max.: 3.49 cm^2, joha), average lateral display size is 2.26 cm^2 (min.: 1.08 cm^2, joha; max.: 3.37 cm^2, poly-coop), and average opening width 3.6 mm (min.: 2.1 mm, joha; max.: 4.8 mm, poly-coop). Hybrids of cluster 2 therefore resemble key-hole flowers of *S. johannis* and *S. polyanthus*.

Cluster 3 only contains *S. polyanthus* and *S. pole-evansii* as well as their offspring (poly-pole). Flower size is small and floral architecture is complex, subsuming the key-hole flower type of *S. polyanthus* and the masked flower type of *S. pole-evansii*. Average frontal display size is .81 cm^2 (min.: .51 cm^2, poly-pole; max.: 1.23 cm^2, poly), average lateral display size is .51 cm^2 (min.: .41 cm^2, pole; max.: .60 cm^2, poly-pole), and average opening width is 2.1 mm (min.: 1.0 mm, poly; max.: 3.10 mm, pole).

Fit of NMDS is good (stress: 0.099; no convergent solutions, best solution after 20 tries). Clusters 1 to 3 appear in a layered order in NMDS ordination. Moreover, most of the parental species are placed further apart from hybrids, except *S. rexii*. Due to preservation of ordering relationships in NMDS between objects, hybrids appear closer together, while distinctive flower types of parental species show stronger dissimilarity and are placed apart. Nevertheless, all retrieved clusters contain parental species. The F1 thus does not show any floral architecture that deviates strongly from species in the field.

VII.4.3.1 INHERITANCE OF FLORAL ARCHITECTURE

Regarding inheritance of floral architecture, larger flower size is inherited dominantly over smaller flower sizes. Due to this, cluster 1 contains the bunch of hybrids and parental species, all large-flowered. Most descendants of small-flowered *S. polyanthus* are assigned to cluster 2, showing moderate flower size. Small flower size is only preserved in hybrid poly-pole due to small flower size in both parental lines. Key-hole flower structure is also a strong hereditary factor: The pronounced key-hole flower type of *S. polyanthus* is preserved to some degree in all its descendents, represented by key-hole flower clusters 2 and 3. In *S. johannis* the key-hole structure is not as strongly pronounced as in *S. polyanthus*, but nevertheless a somewhat compressed, smaller flower opening is present in all its descendents. However, six descendents of *S. johannis* (coop-joha, joha-mode, joha-rexi, joha-rose, rexi-joha, rose-joha) are assigned to cluster 1 due to larger flower size, while four other descendents (joha-coop, joha-dunn, joha-poly, mode-joha) are assigned to cluster 2.

VII.4.3.2 INSTABILITY OF FLORAL ARCHITECTURE

Hierarchical clustering and NMDS ordination of coefficients of variation (CV) for floral architecture are presented in figure VII.4 (bottom). Two clusters were retrieved in regard to instability of floral architecture (see appendix for detailed information, table A X.6.3 and figures A X.6.1 and A X.6.2): Cluster 1 contains six out of nine parental species (coop, joha, long, mode, poly, rexi) as well as five hybrids (coop-rexi, coop-rose, dunn-rose, mode-long and rose-dunn). The average CV of floral architecture is 6.2 % for this cluster (min.: 3.5 % for flower length, underside; max.: 8.6 % for opening width). Cluster 2 contains three parental species (dunn, pole, rose) and the remaining 35 out of 40 hybrids. The average CV for cluster 2 is 11.6 %, and thus 5.4 % higher than in cluster 1 (min.: 5.4 % for carpel length; max.: 15.5 % for the width of the non-fused part of the lower central petal of the gamopetalous corolla). Floral architecture therefore shows higher instability in hybrids than in most species.

Stress level for NMDS ordination of CVs of floral architecture is too high, due to this interpretation of results is limited (stress: 0.246; no convergent solutions, best solution after 20 tries). NMDS ordination of the observed variance does not show any clear structure: Instability of floral architecture does not seem to be restricted to specific species, hybrids or variables.

It must be noted that hybrids not only showed less stable floral architecture, but also severe deformations of the male reproductive system in some cases. Hybrid long-coop missed anthers in 4 out of 10 flowers, while hybrid coop-long showed no anthers at all, and flower openings were not accessible in 3 out of 10 cases due to deformation of petals. Two other hybrids of *S. longiflorus* (long-rexi, long-rose) showed deformed or missing anthers in all flowers. Hybrid coop-mode showed deformations in 3 out of 10 cases (1x no anthers, 1x no stigma, 1x both missing). All in all, 5 out of 40 hybrids showed severe malfunctions of the (male) reproductive system. Further, these hybrids are part of CV-cluster 2: Although CVs could not be calculated for the partly or completely absent male reproductive system, sexual dysfunction is accompanied by general instability of other variables of floral architecture.

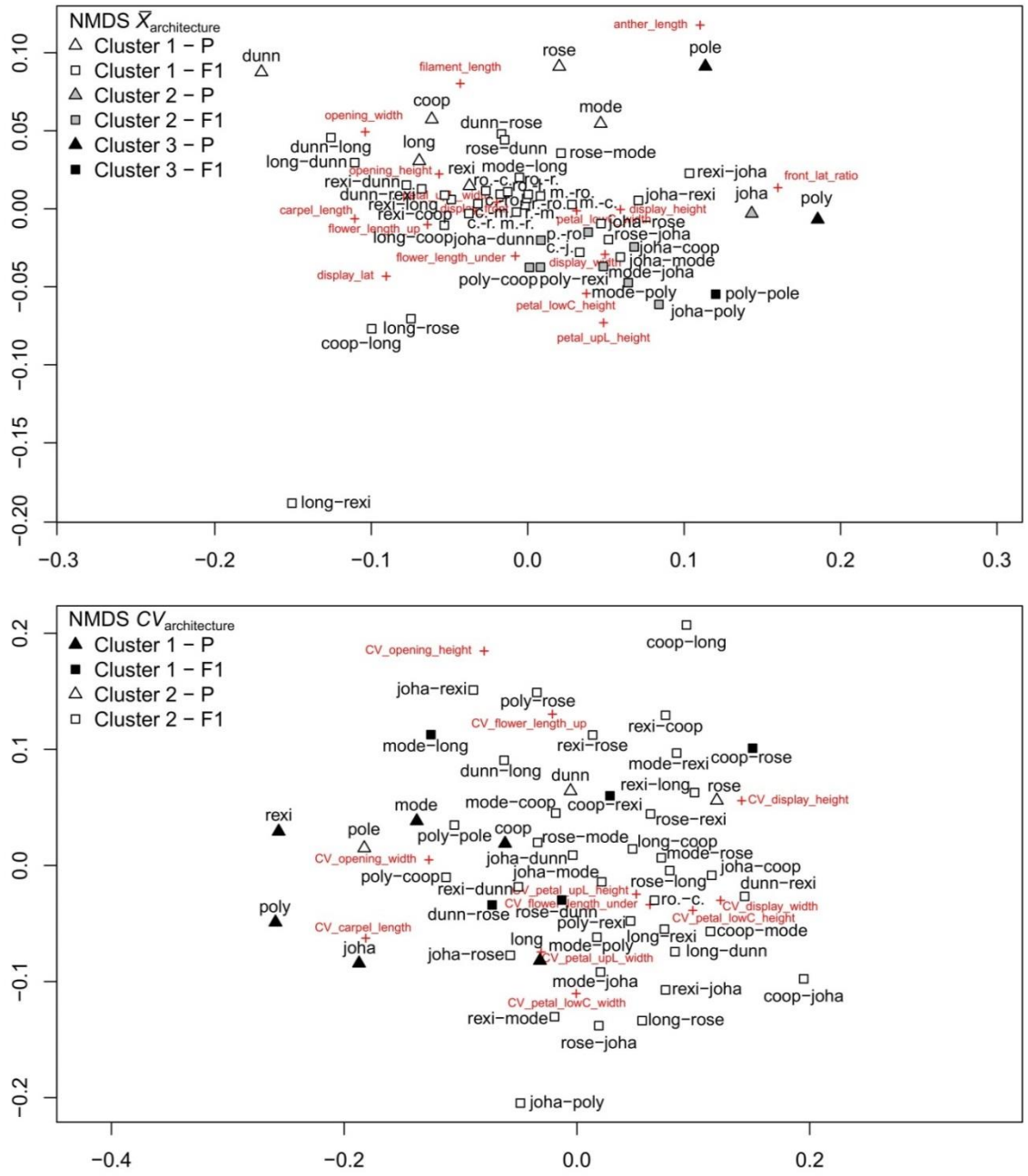

FIGURE VII.4: RESULTS OF NMDS ORDINATION AND HIERARCHICAL CLUSTERING OF FLORAL ARCHITECTURE

Top: Results for arithmetic means of floral architecture, with three clusters retrieved. NMDS stress value of 0.099 is indicating good fit (no convergent solutions, best solution after 20 tries). Cluster 1 (△ parental, ☐ F1): Parentals coop, dunn, long, mode, rexi, rose and 31 hybrids; larger, funnel shaped flowers with larger flower opening, average frontal display 5.82 cm^2, lateral display 4.04 cm^2, opening width 10.3 mm. Cluster 2 (▲ parental, ■ F1): *S. johannis* and eight hybrids of it and *S. polyanthus*; flowers of moderate size with smaller flower opening, average frontal display 2.77 cm^2, lateral display 2.26 cm^2, opening width 3.6 mm. Cluster 3 (▲parental, ■ F1): *S. polyanthus* and *S. pole-evansii* as well as their offspring (poly-pole); small flowers with complex floral architecture, average frontal display .81 cm^2, lateral display .51 cm^2, opening width 2.1 mm. Bottom: Results for the observed coefficients of variation of floral architecture. NMDS stress value of 0.246 is high (no convergent solutions, best solution after 20 tries). Two clusters were retrieved: Cluster 1 (▲parental, ■ F1): Average $CV_{architecture}$ 6.2 %, containing six out of nine parental species and only five out of forty hybrids. Cluster 2 (△ parental, ☐ F1), remaining species and hybrids: Average $CV_{architecture}$ 11.6 %.

VII.4.4 NECTAR REWARD

Measured data bandwidth of nectar reward is shown in figure VII.5, and presented further in the appendix (table A X.6.4).

VII.4.4.1 DATA BANDWIDTH, CLUSTERING AND NMDS OF NECTAR REWARD

For all species and hybrids analysed here, nectar reward could be measured. Produced nectar amount per flower is ranging between .30 µl (*S. johannis*) and 10.61 µl (*S. longiflorus*) for parental species, and between .15 µl (mode-poly) and 6.45 µl (long-dunn) for hybrids. Among parental species, the maximal production represented by *S. longiflorus* appears as extreme value, and *S. dunnii* (6.67 µl) as outlier. Among hybrids, the maximal production of long-dunn appears as extreme value, and dunn-long (4.79 µl), long-rexi (4.24 µl), long-coop (3.70 µl) as well as coop-long (3.50 µl) appear as outliers. Arithmetic mean of nectar amount is 3.05 µl for parental species, and 1.26 µl for hybrids.

Nectar sugar concentration is ranging between 16.4 % (*S. longiflorus*) and 57.0 % (*S. pole-evansii*) for parental species, and between 11.7 % (long-coop) and 48.8 % (rose-long) for hybrids. Nectar sugar concentration of *S. pole-evansii* appears as outlier among parental species, while no outliers were observed for hybrids. Arithmetic mean of nectar sugar concentration is 30.8 % for parental species, and 31.9 % for hybrids.

Sugar production per flower is ranging between .08 mg (*S. johannis*) and 2.19 mg (*S. dunnii*) in parental species, and between .03 mg (rexi-joha) and 1.62 mg (long-dunn) for hybrids. Sugar production of parental species shows a broad spectrum without any outliers. For hybrids, long-dunn (1.62 mg) and dunn-long (1.53 mg) appear as outliers. Arithmetic mean of sugar production is .88 mg for parental species, and .39 mg for hybrids.

Results of NMDS ordination and hierarchical clustering of nectar reward are shown in figure VII.6 (top). Four clusters of nectar reward were retrieved: Cluster 1 contains parental species *S. cooperi*, *S. longiflorus*, *S. polyanthus* and *S. rexii* as well as 6 hybrids (coop-joha, coop-long, joha-coop, long-coop, long-rexi and rexi-joha). In average, cluster 1 is characterised by a nectar production of 2.72 µl (min.: .18 µl, rexi-joha; max.: 10.61 µl, long), 17.8 % nectar sugar concentration (min.: 11.7 %, long-coop; max.: 22.0 %, coop-long) and .47 mg sugar production per flower (min.: .03 mg, rexi-joha; max.: 1.59 mg, long).

Cluster 2 contains parental species *S. dunnii* and *S. modestus* as well as the majority of hybrids, 21 out of 40 (coop-mode, coop-rose, dunn-rose, joha-mode, joha-rose, mode-joha, mode-long, mode-poly, mode-rexi, mode-rose, poly-pole, poly-rexi, poly-rose, rexi-coop, rexi-dunn, rexi-mode, rexi-rose, rose-coop, rose-joha, rose-mode and rose-rexi). In average, cluster 2 is characterised by 0.89 µl nectar production (min.: .15 µl, mode-poly; max.: 6.67 µl, dunn), 36.9 % nectar sugar concentration (min.: 31.0 %, coop-rose; max.: 42.8 %, rexi-rose) and .36 mg sugar production per flower (min.: .07 mg, mode-poly; max.: 2.19 mg, dunn).

Cluster 3 contains *S. johannis* as parental species and 11 out of 40 hybrids (coop-rexi, dunn-long, dunn-rexi, joha-dunn, joha-poly, joha-rexi, long-dunn, long-rose, mode-coop, poly-coop and rexi-long). In average, cluster 3 shows 1.92 µl nectar production (min.: .30 µl, joha; max.: 6.45 µl, long-dunn), 26.8 % nectar sugar concentration (min.: 23.7 %, long-dunn; max.: 30.6 %, rexi-long) and .55 mg sugar production per flower (min.: .08 mg, joha; max.: 1.62 mg, long-dunn).

Cluster 4 contains parental species *S. pole-evansii* and *S. roseoalbus* as well as two hybrids (rose-dunn and rose-long). In average, cluster 4 is characterised by 1.79 µl nectar production (min.: .94 µl, rose-dunn; max.: 2.51 µl, pole), 51.1 % netar sugar concentration (min.: 46.6 %, rose-dunn; max.: 57.0 %, pole) and 1.08 mg sugar production per flower (min.: .56 mg, rose-dunn; max.: 1.69 mg, pole).
As is evident, clusters are predominantly ordered according to nectar sugar concentration, while bandwidth of nectar amount and sugar production still shows high variation within clusters. All retrieved clusters contain parental species; the F1 generation thus does not show any nectar profile that is strongly deviating from nectar reward in the field. NMDS ordination supports the results of hierarchical clustering, and species and hybrids appear in a layered structure according to the retrieved clusters of nectar concentration. Fit of NMDS for arithmetic means of nectar reward is excellent (stress: 0.017, two convergent solutions after 20 tries).

VII.4.4.2 INHERITANCE OF NECTAR REWARD

Hybrid nectar amount and sugar production appear more homogenous than reward of original species. While descandants of nectar rich species *S. dunnii* and *S. longiflorus* also showed higher nectar production, other hybrids seldom showed more than 1 µl nectar per flower. This holds true for descandants of species with low nectar production (< 1 µl, coop, joha, mode, poly), but also for offspring from species with moderate nectar production (> 2 µl, rexi, rose). Similarly, descendants of *S. dunnii* and *S. longiflorus* showed higher sugar production, while other hybrids produced less than .5 mg sugar per flower. This holds true for descandants of species with low sugar production (< 1 mg, coop, joha, mode, poly, rexi), but also for hybrids of *S. roseoalbus*, with moderate sugar production (1.2 mg). Regarding nectar sugar concentration, inheritance patterns are favoured as described by clusters 2 and 3: Nectar concentration of most hybrids is ranging between 20 and 40 %. Nevertheless, diversity of hybrid nectar concentration was much higher than for nectar amount and sugar production, reflecting the diversity of parental species.

VII.4.4.3 INSTABILITY OF NECTAR REWARD

Hierarchical clustering and NMDS of coefficients of variation (CV) for nectar reward are presented in figure VII.6 (bottom)**.** Two clusters were retrieved (see appendix for detailed information; table A X.6.5, figures A X.6.1 and A X.6.2): Cluster 1 contains parental species *S. cooperi*, *S. polyanthus* as well as 19 of 40 hybrids. Observed average CV for produced nectar amount is 33.8 %, sugar concentration shows an average variation of 18.6 % and CV for sugar production is 33.6 %. Cluster 2 contains the remaining seven out of nine parental species, as well as 21 out of 40 hybrids. Observed average CV for nectar amount is 62.6 %, sugar concentration shows an average variation of 27.8 % and CV for sugar production is 62.0 %. Therefore, hybrids are almost evenly distributed between cluster 1 and cluster 2, with cluster 1 showing lower variability within nectar variables than cluster 2. The majority of parental species is part of cluster 2, thus showing higher variability of nectar reward than many hybrids.
Fit of NMDS for CVs of nectar reward is excellent (stress: 0.031, two convergent solutions after 20 tries). NMDS ordination does not show any clear structure, pattern of instability are therefore not restricted to specific original species, hybrids or variables of nectar reward.

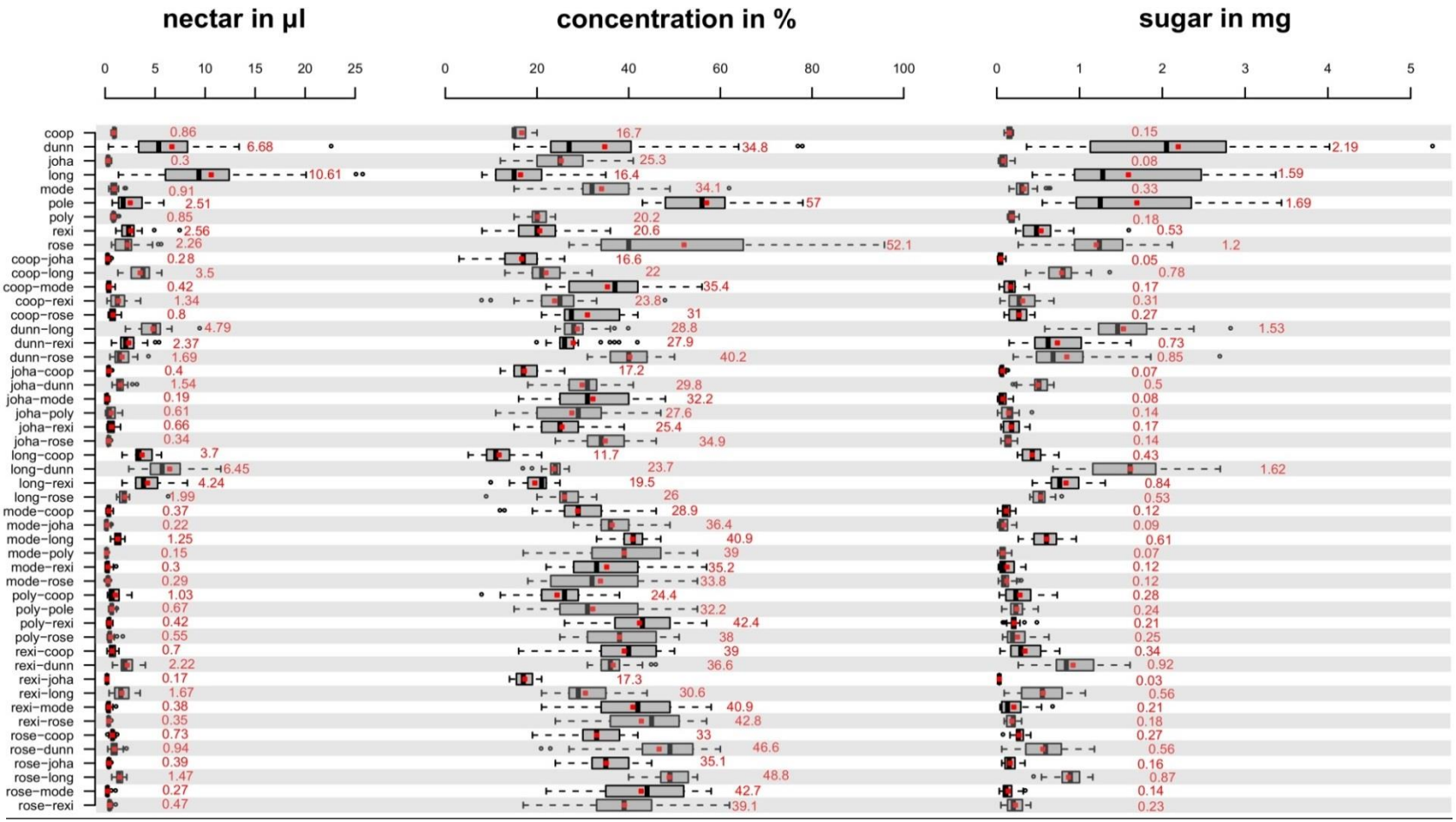

FIGURE VII.5: DATA BANDWIDTH OF NECTAR REWARD OF NINE *STREPTOCARPUS* SPECIES AND FORTY HYBRIDS

The table shows boxplots and arithmetic means (in red) for nectar production per flower in µl (left), nectar sugar concentration in percent (centre), and sugar production per flower in mg (right).

180

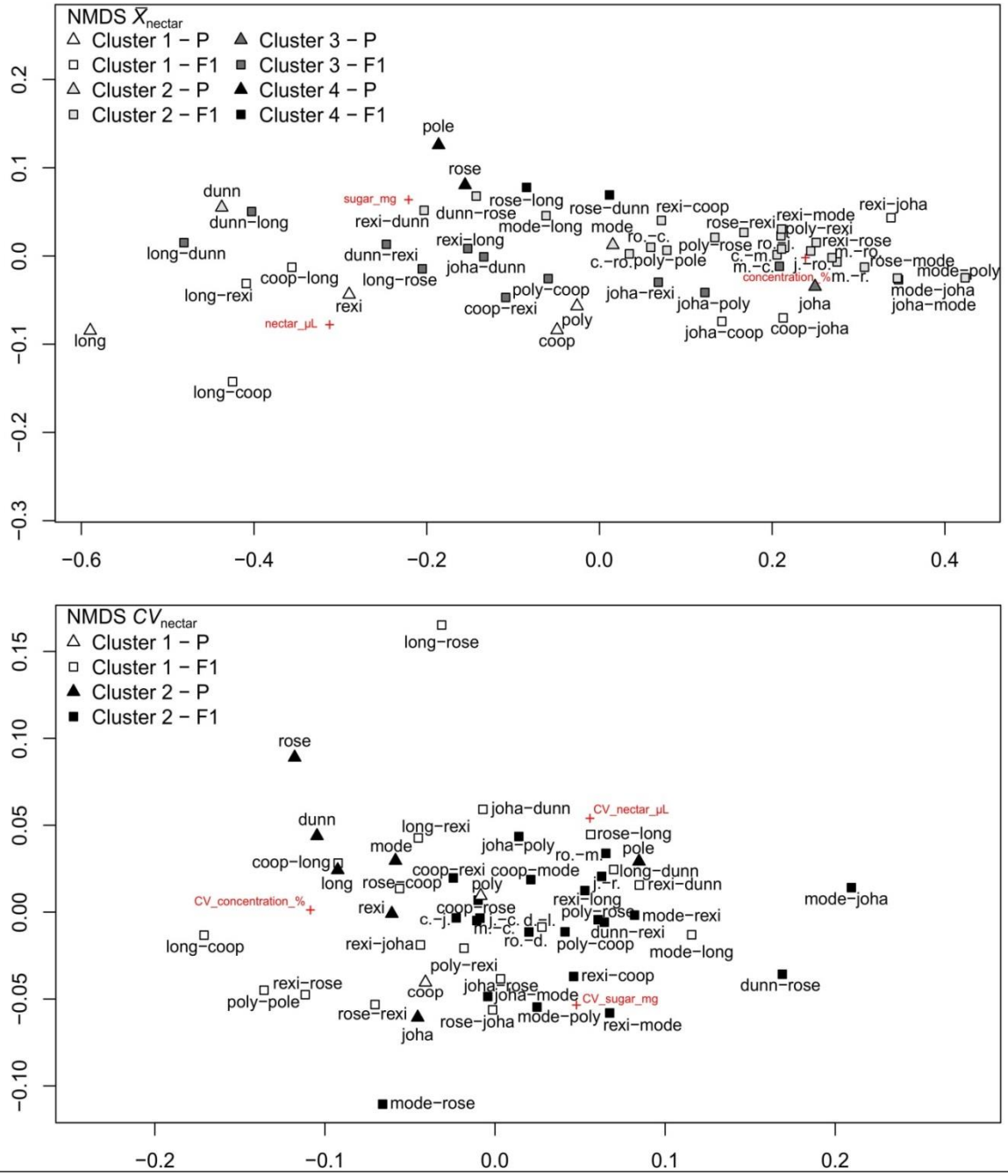

FIGURE VII.6: RESULTS OF NMDS ORDINATION AND HIERARCHICAL CLUSTERING OF NECTAR REWARD

Top: Results for arithmetic means of nectar data. NMDS stress value of 0.017 is indicating excellent fit (two convergent solutions after 20 tries). Four clusters were retrieved. Cluster 1 (△ parental, □ F1): 2.72 µl average nectar amount, 17.8 % concentration, .47 mg sugar. Cluster 2 (▲ parental, ▫ F1): 0.89 µl average nectar amount, 36.9 % concentration, .36 mg sugar. Cluster 3 (▲ parental, ■ F1): 1.92 µl average nectar amount, 26.8 % concentration, .55 mg sugar. Cluster 4 (▲ parental, ■ F1) 1.79 µl average nectar amount, 51.1 % concentration, 1.08 mg sugar. Bottom: Results for the observed CVs of nectar variables. NMDS stress value of 0.031 is indicating excellent fit (two convergent solutions after 20 tries). Two clusters were retrieved: Cluster 1 (△ parental, □ F1), coop, poly and 19 hybrids; 33.8 % average $CV_{nectar\ amount}$, 18.6 % $CV_{concentration}$, 33.6 % CV_{sugar}. Cluster 2 (▲parental, ■ F1), remaining seven parentals and 21 out of 40 hybrids, 62.6 % $CV_{nectar\ amount}$, 27.8 % $CV_{concentration}$, 62.0 % CV_{sugar}.

VII.5 DISCUSSION

VII.5.1 HYBRIDISATION IS ACHIEVED WITH EASE IN *STREPTOCARPUS SUBGENUS STREPTOCARPUS*

The results presented here show that hybridisation between *Streptocarpus* species and establishment of hybrid swarms is generally possible in nature, due to weak or absent postzygotic crossing barriers. Hilliard and Burt (1971) presented a list of 28 situations that are valid or at least very likely cases of hybridisation between different species and subspecies of *Streptocarpus* in the field. We did not replicate any of these situations, but used different parental plants, thus showing that gene flow between species is not restricted to hybrids already observed in the field. The ability to hybridise is rather a general phenomenon within *Streptocarpus* subgenus *Streptocarpus*, supporting the idea that large parts of the genus are potentially still belonging to a single gene pool (Hilliard and Burt, 1971).

Siring success does show differences between species however. While cross-pollination between members of the Cape primrose clade is largely successful, other species of the subgenus are less hybridisable.

Two patterns have to be highlighted: First, although *S. cooperi* and *S. dunnii* were able to produce hybrid offspring as maternal or paternal crossing partner with most of the species analysed here, fruit set was considerably lower than within the Cape primrose clade. Thus, establishment of hybrid swarms in the field is not impossible for these species, given the large number of seed material a single fruit can produce, however such a scenario is lowered in its probabilistic occurrence and magnitude due to lowered fruit set.

Second, species chosen as representatives of more complex floral architecture, namely *S. pole-evansii* for the approximated masked flower type as well as *S. polyanthus* and *S. johannis* for the keyhole type, showed (extremely) reduced crossability. For *S. johannis* fruit set is even lowered within the Cape primrose clade, and no hybrid seed material was produced with *S. longiflorus* as maternal and paternal crossing partner – the only incompatability observed within the clade. The observed lowered crossability of species with complex floral architecture supports and extends the conclusions of de Villiers et al. (2013) in regard to hybrid-origin of established *Streptocarpus*-species: Evolutionary shifts in floral architecture did not show gene tree conflict in their analysis, only growth form shifts did. Floral architecture was therefore assumed to maintain reproductive isolation in the genus. Further, highly specialised keyhole flower types (*S. baudertii*, *S. polyanthus* and *S. johannis*) were only present in stable taxa without gene tree conflict. Our results indicate that complex floral architecture is not the only mechanism to enhance reproductive isolation. Rather, complex floral architecture seems to be coupled with other aspects of incompatibility, thus preventing intrusion of genetic information from other species. This combined strategy possibly evolved and stabilised in environments with high probability of hybridisation due to shared pollinators, a plausible scenario for the "zoological desert" of the southern African habitats of *Streptocarpus*. *S. pole-evansii* has to be considered as a special case, even missing seed set for geitonogamous and allogamous pollination, pointing to a self-incompatibility mechanism and insufficient genetic variation between individuals used here for the within-species crosspollination experiment.

VII.5.2 FULLY FUNCTIONAL F1 HYBRIDS ALLOW FOR HOMOPLOID HYBRID SPECIATION

Analysed hybrids generally showed full floral function for all three levels of analysis (floral architecture, optical flower signal and nectar reward), and retrieved clusters of hybrid pattern were always represented by parental species, too. Therefore, functionality of hybrids in natural environments and compatibility to existing pollinators has to be assumed in general. Combined inheritance of parental flower traits, and therefore a possible fostering of pollination syndrome evolution, is not supported by the F1 hybrid data set. F1 hybrids instead showed a trend to homogeneity, due to dominant inheritance pattern: floral architecture was predominantly funnel shaped, flower size was generally larger (mind low crossability between large and small flowered species) and offspring of keyhole type species showed compressed flower openings, however not as pronounced as in the parental generation. Optical signal was predominantly bluish, anther dummy signal was often present and UV signal was usually diffusely absorbing. Nectar reward was observed for all hybrids: Nectar sugar concentration predominantly ranged between ca. 20 and 40 % (two largest nectar clusters), and hybrids showed a trend for lower nectar amount and sugar production when compared to parental plants.

Analysis of coefficients of variation showed a stabilised nectar production pattern for many of the hybrids, even more stable than most parental species. Therefore, reliability of pollinator reward is enhanced in many hybrids. Contrary to this, CVs of floral architecture show higher deviance in hybrids than in parental species. This might be interpreted as selective disadvantage, generally termed fluctuating asymmetry hypothesis (compare Neal et al., 1998; Møller, 1995; Møller et al., 1995). However, fluctuating overall asymmetry for the most hybrids is only 5.4 % higher than the low-asymmetry cluster representing six out of the nine parental species (coop, joha, long, mode, poly, rexi) as well as five hybrids. It seems unlikely that such a low increase in floral asymmetry might turn out as a severe selective disadvantage in the field.

All in all, only 5 out of 40 hybrids showed severe reproductive knock-out anomalies, predominantly loss of or severe deformity of anthers and filaments. For the remaining 35 hybrids floral form and function has to be considered suitable for successful plant-pollinator interactions in the field.

Given these facts, and keeping in mind the generally high seed production within the genus, our results underline the powerful operative force of selective pollinator attraction and control via flower signal and floral architecture of species found in the field: Although crossability is high within the subgenus, the named prezygotic crossing barriers seem to be sufficient to separate gene pools. However, in case of a freak pollination event, the establishment of a fully functional hybrid swarm and onset of homoploid hybrid speciation is quite likely, if a fitting pollinator population is present, too. Future research should include in depth pollinator observations of species and hybrids in natural habitats to foster our understanding of plant-pollinator interactions and of the maintenance of species boundaries. Moreover, analysis of inheritance and splitting up of trait combinations of F2 hybrids are necessary in order to assess the possible relevance of pollination syndrome evolution in *Streptocarpus*.

VII.6 ACKNOWLEDGMENTS

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VII.7 LITERATURE

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VIII. GENERAL CONCLUSIONS

The results here presented underline a general statement of Vogel (2006) about the nature of pollination syndromes: They are **superimposed**, and constitute a third level of floral organisation, beyond basal morphology and structural moulding of floral architecture. Pollination syndromes integrate more elusive floral plant traits like optical signal and nectar reward that are more variable between and within plant individuals. They are realised within the framework of phylogenetic constraints, but can display strong plasticity and recombination, i.e. via hybridisation. Only few plant traits are key elements of successful plant-pollinator interaction: The assemblage of traits represented in classical pollination syndromes represents different evolutionary driving forces, in combination creating a fully functional, more or less specialised, plant-pollinator interface. These statements are elaborated in the following sections, after a brief review of major results of this thesis.

VIII.1 MAJOR RESULTS

In **chapter II** floral symmetry in Geraniales appeared to be uncorrelated to quantitatively assessed gamete production (pollen, ovules, p/o ratio) and nectar reward (amount, concentration, sugar content). Therefore, resource efficiency via pollinator positioning (pollen position hypothesis, reward wastage hypothesis) cannot account for floral symmetry in Geraniales. Phylogenetic constraints are present for gamete production, nectar reward and pollinator guilds. While variables of nectar reward are correlated to pollinator guilds, representing important plant traits for plant-animal interaction, gamete production is uncorrelated to pollinator guilds. Breeding system related variables are therefore predominantly influenced by other factors than plant-pollinator interactions in Geraniales: Habitus is of explanatory value, with higher gamete production detected in plant species with stouter habitus (shrubs, small trees).

In **chapter III** clusters of anemophilous, zoophilous and ambophilous pollination syndromes were retrieved for Hamamelidaceae. Zoophily appears to be reinvented several times from a putatively ancestral anemophilous state in Hamamelidaceae, and is common in winter flowering species of the family. Flowering time is uncorrelated to the retrieved pollination modes. Moreover, ambophily was retrieved for two genera of the family at least dating back to the Eocene, and possibly even the Upper Cretaceous, arguing against an ephemeral transitional stage of mixed pollination modes.

In **chapter IV** analysis of floral plant traits of carnivorous active flypaper plants *Pinguicula* and *Drosera* proved absence of a pollinator-prey conflict. Pollinators and prey are separated by highly conserved patterns of optical flower signal (colour, anther dummies, UV signal) and optical contrast to trap leaves. In both genera p/o ratios are extremely low in xenogamous breeding systems, clearly arguing against pollen loss due to a pollinator-prey conflict.

In **chapter V** subsets of floral functional traits (floral architecture, optical signal, nectar reward, gamete production) were analysed in depth for members of *Streptocarpus* subgenus *Streptocarpus*. The subsets appeard to be largely uncorrelated from each other and show an arbitrary distribution on the phylogenetic tree. Reported flower types of the subgenus, commonly described as indicators for pollination syndromes, only correlate to floral architecture, but are disjunct from the other subsets of

floral function. Only few floral plant traits appear to be correlated at all, indicating two separate evoloutionary driving forces: 1. The link between flower size and gamete production indicates a general aspect of resource investment and mechanical fit to pollinators. 2. The link between variables of optical flower signal and nectar reward appears to address correlations between sensory systems and nutritional needs of pollinators.

In **chapter VI** crossing experiments between three closely related *Streptocarpus* species of the Cape primrose clade demonstrated vigorous seed set, with hundreds to thousands of seeds per fruit. The experiment proved the absence of postzygotic crossing barriers and supports the theory of a large, single gene pool underlying the subgenus. Prezygotic barriers such as eco-geographical isolation, including control of plant-animal interactions via floral architecture and optical attraction, appear to keep the species separated in the field.

In **chapter VII** a more extensive crossing experiment with nine parental species of *Streptocarpus* subgenus *Streptocarpus* was reported. Hybrids are established with ease within the subgenus, especially within the Cape primrose clade. 40 hybrids were retrieved and analysed in depth in regard to floral architecture, optical signal and nectar reward. Hybrids predominantly showed full floral function, only five hybrids had severe malfunctions of the (male) reproductive system. Floral plant traits showed dominant-recessive heredity patterns, and observed hybrid patterns always joined into patterns already present in parental species. Establishment of functional hybrid swarms in the field and onset of homoploid hybrid speciation is therefore possible and likely, if a freak pollination event circumvents established prezygotic crossing barriers (eco-geographical isolation, pollinator sorting via floral architecture, optical attraction, etc.), and a pollinator compatible to the hybrid is constantly present in the field.

VIII.2 INTERCORRELATION OF FLORAL PLANT TRAITS: BREEDING SYSTEM IS DISJUNCT

The analysis of datasets here reported demonstrated some general aspects of pollination syndromes that are discussed in the following. In general, pollination syndromes show clear cut pattern when compared on level of clearly abiotic vs. clearly biotic pollination syndromes, an often neglected aspect of the debate. The difference between abiotic and biotic pollination systems is well resolved in this thesis (objective 2, chapter III, Hamamelidaceae), especially by quantitative data assessment of gametic variables (hypotheses i, ii). However, the detection of ambophilous flowers in Hamamelidaceae, capable of both wind- and animal pollination, shows that even between these strong polarities functional pollination modes are possible and realised in nature.

The differentiation between biotic pollination systems is less clear-cut, in literature as well for the results here presented. In general, results support the grouping of animal taxa according to their functionality, either to differentiate between functional pollinator guilds according to Fenster et al. (2004), or between pollinator and prey taxa in carnivorous plants (objectives 1 and 3).

As for the subsets of floral function analysed here (floral structure and symmetry, optical signal, reward and gamete production), some major trends can be identified in regard to intercorrelation of floral plant traits:

- Pollen production and flower size often showed significant correlations, cf. objective 2 – carnivorous plants, and objective 4 – *Streptocarpus*. The correlation is putatively caused by mechanical fit to

compatible pollinators, or influence of growth habit. Correlations between pollen production and flower size are reported in literature (e.g. Young and Stanton, 1990; Worley and Barrett, 2000; Gómez et al., 2008).

- Significant correlations between corolla tube length or spur length and nectar production have been found in this thesis, cf. objective 2 – Hamamelidaceae, objective 3 – carnivorous plants, and objective 4 – *Streptocarpus*. Many studies have shown that long-tongued pollinators prefer to forage concealed nectar from deep corollas or nectar spurs, while short-tongued pollinators forage from openly accessible nectar (e.g. Inouye, 1980; Branquart and Hemptinne, 2000; Gómez et al., 2008).
- Nectar reward and optical flower signal show correlations, cf. objective 1 – Geraniales, objective 3 – carnivorous plants, and objective 4 – *Streptocarpus*. Nectar reward and optical flower signal are both plant presentation traits, adapted to address the sensory systems of animal taxa and their nutritional needs. Johnson and Steiner (2000) point out that the role of colour in filtering of flower visitors has been overemphasised, i.e. because red blindness of bees is doubtful, and flower colour itself is not significantly correlated to pollination systems. However, the results presented here are more in line with Fenster et al. (2004): Flower colour appears as important predictor at higher taxonomic scales (i.e. red colour and bird pollination), while nectar reward can further differentiate taxa that are more closely related (i.e. Hymenoptera with different tongue-lengths). All in all, the sheer presence of plant presentation traits, floral signal and reward in biotic pollination systems cannot be highlighted enough: They are responsible for attraction and guidance of animal pollinators, and shows functionality even in extreme and special cases like flowering at the fringe of the growth season (objective 2 – Hamamelidaceae), or by circumventing pollinator-prey conflict in carnivorous plants (objective 3 – carnivorous plants).
- As a major result of this thesis that is not reported in literature, **breeding systems and related variables of gamete production often appeared strongly disjunct from other subsets of floral functional traits**, cf. objective 1 – Geraniales, objective 3 – carnivorous plants, and objective 4 – *Streptocarpus*. Breeding systems and related variables might be controlled more strongly by other factors, such as phylogenetic signal or life cycle. In line with this, the often assumed correlation between zygomorphic flower symmetry and higher resource efficiency due to controlled pollen placement cannot be confirmed by the Geraniales dataset (objective 1). Even **p/o ratios** in itself, often used as indicator for breeding systems since Cruden (1977), were of less predictive value in this thesis whenever true breeding systems were experimentally retrieved (cf. objective 3 – carnivorous plants, objective 4 – *Streptocarpus*).

VIII.3 HEREDITY AND GENE FLOW BETWEEN POLLINATION SYNDROMES

Multitrait studies addressing natural variation in syndrome characters are scarce, and usually are unable to detect selection acting on trait combinations (cf. Fenster et al. 2004). Contrary to this trend, Wessinger et al. (2014) present evidence that suites of floral traits suitable to bee- or hummingbird pollination are genetically linked in *Penstemon*, which could facilitate pollination syndrome evolution.
In this thesis, combination of floral traits and their heredity were tested explicitly for members of *Streptocarpus* subgenus *Streptocarpus* (objective 6). No genetic linkage of floral suites could be

detected. However, dominant-recessive inheritance pattern were common, and the F1 generation showed a trend towards homogenisation. Crossing of an F2 generation and analysis of feature splitting would be necessary to draw final conclusions about genetic linkage of floral traits in *Streptocarpus*. At the moment, the data at hand does not indicate genetic linkage of suites of floral plant traits and heredity of pollination syndromes. The data rather supports Vogel´s stand on this issue: A syndrome can become diffuse by hybridisation (Vogel, 2006), e.g. via homogenisation of the F1 and dominant-recessive inheritance pattern.

As for gene flow between syndromes in the field, results presented here emphasise the high importance of pollinator fidelity and specificity for the prevention of gene flow and hybridisation via praezygotic crossing barriers (objectives 4 to 6). Members of the Cape primrose clade showed high crossability and no postzygotic pollination barriers.

All in all, quantitative assessment of gamete production and nectar reward as well integrative analysis of floral function appear as a promising approach for better understanding of pollination syndrome evolution (hypothesis iv).

VIII.4 PHYLOGENETIC CONSTRAINTS AND SYNDROME EVOLUTION

In general, research on plant and animal evolution shows that plants have adapted to insect pollinators, and not the other way round (cf. Neal et al., 1998, and references therein). Van der Niet and Johnson (2012) argue that co-adaption, co-evolution and pollinator-driven speciation are influenced by "intrinsic factors" that constrain selection (plant traits such as zygomorphy, nectar spurs etc.) and "extrinsic factors that provide the selection regime" (i.e. the local pollinator assemblage, hebrivores etc.). This interplay between intrinsic and extrinsic factors is not strictly directed by species to species interaction: Vogel (2006) stated that pollination syndromes are the most superficial and evolutionary most labile organisational level of the flower, and pollination syndromes evolved diffusely between floral guilds and pollinator guilds. This idea is further supported by statements of Fenster et al. (2004) on trait lability within species: Fluctuations of pollinator assemblages and gene flow between populations undermines consistent specialisation, favouring functional groups of animal taxa instead of single pollinating species. The presence of pollination ecotypes and floral polymorphisms within populations are also evidence for divergent, pollinator-driven selection (Fenster et al., 2004). This also leads back to the general question of generalisation vs. specialisation in plant-pollinator interactions. Johnson and Steiner (2000) state that the dichotomy between generalisation and specialisation is a simplification, plant-pollinator interactions rather show a continuum. In general, it appears that pollinator-saturated ecosystems tend to specialise, while unsaturated ecosystems tend to generalise amongst pollinators in order to secure outcrossing (Willmer, 2011; Waser et al., 1996).

Van der Niet and Johnson (2012) showed that at least 25 percent of documented divergence events support the idea that pollinator shifts contributed to angiosperm evolution. However, that also leaves 75 percent of divergence events that are not attributable to pollinator shifts, and pollinators are not the only drivers of angiosperm speciation. Strauss and Whitall (2006) present an overview about biotic and abiotic factors that influence floral diversity, besides pollinators.

Considering these statements, hypothesis (iii) has to be judged on the individual case: Integrative assessment of floral plant traits in combination with latest phylogenies were of different explanatory

value for Geraniales, Hamamelidaceae (showing stronger phylogenetic constrains) and *Streptocarpus* (almost no phylogenetic pattern evident).

VIII.5 OUTLOOK

All in all, the quantitative and qualitative, integrative assessment of floral plant traits appears as a promising approach for better understanding of floral function, and is an improvement compared to the widespread assessment of pollination syndromes only based on floral colours and shapes.

Further research should try to complement the findings presented here by analysis of other floral functional traits such as odour or nectar sugar composition, as well as pollinator observations.

Data on pollinator assamblages involved in the pollination of Geraniales are very good, and moderate for carnivorous plants. Information on wild pollinators of Hamamelidaceae and *Streptocarpus* species is very limited however, and should be addressed in the future to complete our understanding of plant-pollinator interactions for these taxa in the field.

For better understanding of the heredity of pollination syndromes in *Streptocarpus*, crossing of the F2 generation and analysis of feature splitting is necessary.

To detect selection acting on floral trait combinations, phenotypic manipulation studies that vary traits should be conducted to test the importance of trait combinations (cf. Fenster et al. 2004), beside crossing experiments.

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IX. SUMMARY

Mues, Andreas Wilhelm (2020): Functional Integration of Floral Plant Traits: Shape and Symmetry, optical Signal, Reward and Reproduction in the Angiosperm Flower. Doctoral Thesis, Mathematisch-Naturwissenschaftliche Fakultät, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany.

Pollination syndromes represent floral suites that have originated and diversified in interaction with biotic and abiotic pollen vectors. Plant trait pattern that constitute respective syndromes have been used extensively to predict pollen vectors. However, research in this field has seemingly suffered from poor data quality, especially from overreliance on categorical data, and insufficient integration of important floral plant traits, especially breeding system related variables (pollen and ovule production, p/o ratio).

Main objective of this dissertation is to contribute to better understanding of the functional integration of angiosperm flowers and the concept of pollination syndromes via integrative and quantitative assessment of floral plant traits, aside with categorical information. The following research questions were selected to this purpose:

In chapter II diversification of floral plant traits and pollinator guilds are presented for members of order Geraniales. The order is small but florally diverse, and therefore particularly suitable for comparative studies. Floral symmetry in Geraniales appears to be uncorrelated to quantitatively assessed gamete production (pollen, ovules, p/o ratio) and nectar reward (amount, concentration, sugar content). Therefore, resource efficiency via pollinator positioning (pollen position hypothesis, reward wastage hypothesis) cannot account for floral symmetry. Phylogenetic constraints are present for gamete production, nectar reward and pollinator guilds. While variables of nectar reward are correlated to pollinator guilds, gamete production is uncorrelated.

In chapter III diversification of floral plant traits and flowering time are analysed for Hamamelidaceae, a small family known for the peculiar flowering time of some of its members in late autumn or winter. Clear clusters of anemophilous, zoophilous and ambophilous pollination syndromes are retrieved. Zoophily appears to be reinvented several times from a putatively ancestral anemophilous state and is common in winter flowering species. Flowering time is not correlated to the retrieved pollination modes. Moreover, ambophily is retrieved for two genera of the family at least dating back to the Eocene, and possibly even the Upper Cretaceous, arguing against an ephemeral transitional stage of mixed pollination modes.

In chapter IV floral plant traits of carnivorous active flypaper plants *Pinguicula* and *Drosera* are compared. Carnivorous plants are animal pollinated, and the potential capture of legitimate pollinators has long been researched under the term pollinator-prey conflict, usually focusing on sorting between pollinators and prey by the trap types. Analysis of floral plant traits proves the absence of such a conflict. Pollinators and prey are separated by highly conserved patterns of optical flower signal and contrast to trap leaves. In both genera p/o ratios are extremely low in species with xenogamous breeding systems, clearly arguing against pollen loss due to a pollinator-prey conflict.

In chapter V subsets of floral functional traits (floral architecture, optical signal, nectar reward, gamete production) are analysed for 18 members of *Streptocarpus* subgenus *Streptocarpus*. The subsets appear to be largely uncorrelated from each other and show an arbitrary distribution on the phylogenetic tree. Reported flower types of the subgenus, commonly described as indicators for pollination syndromes, only correlate to floral architecture, but are disjunct from the other subsets of floral function. Only few floral plant traits appear to be correlated at all, indicating two separate evolutionary driving forces: Floral architecture controlling for pollinator access, pollen placement and gamete production, and the interaction of floral signal and nectar reward as an adjustment to sensory capacities and nutritional needs of pollinator taxa.

In chapter VI crossing experiments between three closely related *Streptocarpus* species of the Cape primrose clade demonstrate vigorous seed set, with hundreds to thousands of seeds per fruit. The experiment proves the absence of postzygotic crossing barriers and supports the theory of a large, single gene pool underlying the subgenus. Prezygotic barriers such as eco-geographical isolation, including control of plant-animal interactions via floral architecture and optical attraction, appear to keep the species separated in the field.

In chapter VII a more extensive crossing experiment with nine parental species of *Streptocarpus* subgenus *Streptocarpus* is presented. Hybrids are established with ease within the subgenus, especially within the Cape primrose clade. Floral architecture, optical signal and nectar reward of 40 hybrids is presented. Hybrids predominantly show full floral function, only five hybrids show severe malfunctions of the (male) reproductive system. Floral plant traits are inherited dominant-recessively, and hybrid patterns always join into patterns already present in parental species. Establishment of functional hybrid swarms in the field and onset of homoploid hybrid speciation is therefore possible, if a freak pollination event circumvents established prezygotic crossing barriers (eco-geographical isolation, pollinator sorting via floral architecture, optical attraction, etc.), and a pollinator compatible to the hybrid is constantly present in the field.

Chapter VIII presents general conclusions of this thesis: Significant correlations across the different datasets were retrieved for pollen production and flower size, corolla tube length / spur length and nectar production, as well as between nectar reward and optical flower signal. Moreover, breeding systems and gamete production often appear disjunct from other subsets of floral functional traits. The results indicate a general split between plant traits relevant for pollinator attraction and interaction (optical signal, reward) and breeding system (pollen and ovule production, p/o ratio).

All in all, the quantitative and qualitative, integrative assessment of floral plant traits appears as a promising approach for better understanding of floral function, and is an improvement compared to the widespread assessment of pollination syndromes only based on floral colours and shapes. Further research should complement the presented results by additional analysis of other floral functional traits, such as odour or nectar sugar composition, and pollinator observations in the field.

X. APPENDICES

X.1 APPENDIX TO CHAPTER II

TABLE A X.1.1: DATA BANDWIDTH OF FLORAL MORPHOLOGY, LIFE CYCLE AND GROWTH HABIT OF GERANIALES SPECIES

The table presents floral morphological variables (anther number, secretory organs, floral symmetry), life cycle and growth habit of analysed Geraniales species. The number of secretory organs, their position (extra- or interstaminal, or both) and possible linkage between the vasculature of secretory organs and the main vasculature of the plants are presented after Jeiter et al. (2017b; see chapter II), and are extrapolated for the genus level. Flower symmetry is categorised into three groups, namely actinomorphic (⊕, *including cross symmetry), non-functional (·|·) and functional zygomorphic symmetry (Ψ). Life cycle is perennial (♃) for most species (only *P. grossularioides* is annual ☉), and was therefore not used for calculations. Growth habit is grouped into three categories: Herbs (including geophytes), subshrubs (woody plants < 1.5 m) and shrubs (woody plants > 1.5 m, including small trees).

species	anthers	secretory organs, number	secretory organs, position	secretory organs, vasculature	flower symmetry	life cycle	growth habit
Vivianiaceae							
Balbisia meyeniana Klotzsch	10	0	/	/	⊕	♃	subshrub
Balbisia peduncularis (Lindl.) D. Don	10	0	/	/	⊕	♃	subshrub
Balbisia verticillata Cav.	10	0	/	/	⊕	♃	subshrub
Viviania elegans (Poepp.) Reiche & Johow	10	5	ex./inter.	no link	⊕	♃	herb
Viviania marifolia Cav.	10	5	ex./inter.	no link	⊕*	♃	subshrub
Francoaceae							
Francoa appendiculata Cav.	8	8	inter.	no link	⊕	♃	herb
Francoa sonchifolia Cav.	8	8	inter.	no link	⊕	♃	herb
Tetilla hydrocotylaefolia DC.	8	8	inter.	no link	Ψ	♃	herb
Greyia flanaganii Bolus	10	10	ex./inter.	no link	·|·	♃	shrub
Greyia radlkoferi Szyszył.	10	10	ex./inter.	no link	·|·	♃	shrub
Greyia sutherlandii Hook. & Harv.	10	10	ex./inter.	no link	·|·	♃	shrub
Melianthaceae							
Melianthus comosus Vahl	4	1	ex.	linked	Ψ	♃	shrub
Melianthus dregeanus Kuntze	4	1	ex.	linked	Ψ	♃	shrub
Melianthus pectinatus Harv.	4	1	ex.	linked	Ψ	♃	shrub
Melianthus villosus Bolus	4	1	ex.	linked	Ψ	♃	shrub
Geraniaceae							
Erodium manescavi Coss.	5	5	ex.	linked	·|·	♃	herb
Erodium pelargoniflorum Boiss. & Heldr.	5	5	ex.	linked	·|·	♃	herb
Geranium reuteri Aedo & Muñoz Garm.	10	5	ex.	linked	⊕	♃	herb
Geranium sanguineum L.	10	5	ex.	linked	⊕	♃	herb
Geranium sylvaticum L.	10	5	ex.	linked	⊕	♃	herb
Geranium versicolor L.	10	5	ex.	linked	⊕	♃	herb
Geranium yunnanense Franch.	10	5	ex.	linked	⊕	♃	herb
Monsonia emarginata L'Hér.	15	5	ex.	linked	⊕	♃	herb
Monsonia marlothii (Engl.) F. Albers	15	5	ex.	linked	⊕	♃	subshrub
Pelargonium crispum (P.J.Bergius) L'Hér.	7	1	ex.	linked	Ψ	♃	subshrub
Pelargonium echinatum Curtis	6	1	ex.	linked	Ψ	♃	subshrub
Pelargonium fulgidum (L.) L'Hér.	7	1	ex.	linked	Ψ	♃	subshrub
Pelargonium grossularioides (L.) L'Hér.	7	1	ex.	linked	Ψ	☉	herb
Pelargonium myrrhifolium (L.) L'Hér.	5	1	ex.	linked	Ψ	♃	subshrub
Pelargonium odoratissimum (L.) L'Hér.	7	1	ex.	linked	Ψ	♃	subshrub
Pelargonium peltatum (L.) L'Hér.	7	1	ex.	linked	Ψ	♃	herb
Pelargonium tetragonum (L.f.) L'Hér.	7	1	ex.	linked	Ψ	♃	shrub
Pelargonium zonale (L.) L'Hér.	7	1	ex.	linked	Ψ	♃	shrub
Hypseocharitaceae							
Hypseocharis bilobata Killip	15	5	ex.	linked	⊕	♃	herb/ geophyte

TABLE A X.1.2: DATA BANDWIDTH OF POLLEN AND OVULE PRODUCTION AND P/O RATIO OF GERANIALES SPECIES

The table shows sample number (*n*) for 34 Geraniales species, average ovule number, pollen production per anther and flower as well as p/o ratio, if possible with standard deviation. Hierarchical clusters are indicated: △ = cluster 1, viable ovules fixed to 5, pollen production per flower < 10 000, usually much lower, p/o ratio seldom > 1 000; ▲ = cluster 2, ovule number fixed or variable, floral pollen production ca. 10 000 to 20 000, p/o ratio ca. 3 000 to 4 000, except for *Tetilla* (* = 12 data points for ovule number, only single pollen measurement); ▲ = cluster 3, ovule number fixed or variable, floral pollen production ca. 90 000 to 340 000, p/o ratio variable, extremely high for *Melianthus* (ca. 9 000 to 24 000); ▲ = cluster 4, ovule number variable and high, pollen production extremely high, ca. 600 000 to 930 000, p/o ratio ca. 5 000 to 11 000.

species	cluster	*n*	ovule number			pollen per anther			pollen per flower			p/o ratio		
Vivianiaceae														
Balbisia meyeniana Klotzsch	▲	8	92	±	9	61 080	±	10 384	610 800	±	103 837	6 658	±	1 217
Balbisia peduncularis (Lindl.) D. Don	▲	8	161	±	14	86 428	±	12 582	864 281	±	125 819	5 426	±	971
Balbisia verticillata Cav.	▲	8	98	±	26	92 778	±	25 777	927 775	±	257 771	10 584	±	5 572
Viviania elegans (Poepp.) Reiche & Johow	▲	7		6		2 340	±	650	23 400	±	6 495	3 900	±	1 083
Viviania marifolia Cav.	▲	7		6		2 083	±	993	20 827	±	9 934	3 471	±	1 656
Francoaceae														
Francoa appendiculata Cav.	▲	12	400	±	28	19 898	±	2 190	159 183	±	17 520	400	±	56
Francoa sonchifolia Cav.	▲	12	147	±	5	23 860	±	3 066	184 333	±	21 425	1 258	±	161
**Tetilla hydrocotylaefolia* DC.	▲	12(1)	252	±	40	1770			14 160			61		
Greyia flanaganii Bolus	▲	10	247	±	38	34 350	±	5 527	343 500	±	55 268	1 425	±	342
Greyia radlkoferi Szyszył.	▲	10	472	±	65	24 685	±	8 492	246 850	±	84 916	530	±	178
Greyia sutherlandii Hook. & Harv.	▲	10	231	±	10	33 360	±	7 949	333 600	±	79 488	1 449	±	361
Melianthaceae														
Melianthus comosus Vahl	▲	5		8		22 630	±	11 582	90 520	±	46 329	11 315	±	5 791
Melianthus dregeanus Kuntze	▲	5		8		47 880	±	2 281	191 520	±	9 123	23 940	±	1 140
Melianthus pectinatus Harv.	▲	5		8		41 760	±	15 974	167 040	±	63 895	20 880	±	7 987
Melianthus villosus Bolus	▲	5		16		36 590	±	13 160	146 360	±	52 641	9 148	±	3 290
Geraniaceae														
Erodium manescavi Coss.	△	10		5		1 716	±	439	8 580	±	2 196	1 770	±	525
Erodium pelargoniflorum Boiss. & Heldr.	△	10		5		1 302	±	720	6 510	±	3 602	1 302	±	720
Geranium reuteri Aedo & Muñoz Garm.	△	10		5		242	±	218	2 420	±	2 178	474	±	435
Geranium sanguineum L.	△	10		5		314	±	208	3 140	±	2 081	628	±	416
Geranium sylvaticum L.	△	10	5	±	1	512	±	205	5 120	±	2 047	994	±	419
Geranium versicolor L.	△	10		5		237	±	95	2 369	±	947	474	±	190
Geranium yunnanense Franch.	△	10		5		588	±	355	5 880	±	3 548	1 176	±	710
Monsonia emarginata L'Hér.	△	10		5		433	±	160	6 499	±	2 407	1 300	±	482
Monsonia marlothii (Engl.) F. Albers	▲	10		5		1 108	±	522	16 619	±	7 836	3 324	±	1 567
Pelargonium crispum (P.J.Bergius) L'Hér.	△	10		5		507	±	204	3 550	±	1 429	710	±	286
Pelargonium echinatum Curtis	△	10		5		644	±	199	3 864	±	1 196	773	±	239
Pelargonium fulgidum (L.) L'Hér.	△	10		5		492	±	175	3 446	±	1 226	689	±	245
Pelargonium grossularioides (L.) L'Hér.	△	10		5		243	±	43	1 700	±	303	340	±	61
Pelargonium myrrhifolium (L.) L'Hér.	△	10		5		309	±	42	1 547	±	211	309	±	42
Pelargonium odoratissimum (L.) L'Hér.	△	10		5		724	±	262	5 068	±	1 832	1 014	±	366
Pelargonium peltatum (L.) L'Hér.	△	10		5		672	±	279	4 701	±	1 954	940	±	391
Pelargonium tetragonum (L.f.) L'Hér.	△	10		5		468	±	167	3 272	±	1 173	654	±	235
Pelargonium zonale (L.) L'Hér.	△	10		5		547	±	220	3 829	±	1 544	766	±	309
Hypseocharitaceae														
Hypseocharis bilobata Killip	▲	8	48	±	4	6 908	±	4 463	110 150	±	72 617	2 222	±	1 395

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TABLE A X.1.3: DATA BANDWIDTH OF NECTAR REWARD OF GERANIALES SPECIES

Nectar data of 32 Geraniales species. Presented are averages and standard deviations for nectar production per flower in µl, nectar concentration in percent and sugar production per flower in mg, as well as total number of data points (*n*). Hierarchical clusters for nectar production are indicated (*Balbisia* excluded ☒): ☐ = cluster 1, low nectar and sugar production, concentration ranging from 13.7 % to 28.3 %; ▫ = cluster 2, low nectar and sugar production, concentration ranging from 32.1 % to 60.9 %; ▪ = cluster 3, considerably higher nectar and sugar production than clusters 1 and 2, nectar sugar concentration low (between 8 and 16 %); ■ = cluster 4, extremely high nectar and sugar production, nectar sugar concentration similar to cluster 3. * = species not used for calculations due to insufficient information on nectar production.

species	cluster	*n*	µl nectar per flower			concentration in %			mg sugar per flower		
Vivianiaceae											
Balbisia meyeniana Klotzsch	☒	10	0			0			0		
Balbisia peduncularis (Lindl.) D. Don	☒	10	0			0			0		
Balbisia verticillata Cav.	☒	10	0			0			0		
**Viviania elegans* (Poepp.) Reiche & Johow	*?*	25	0.73	±	1.05	*?*			*?*		
**Viviania marifolia* Cav.	*?*	10	*traces*			*?*			*?*		
Francoaceae											
Francoa appendiculata Cav.	▫	25	8.06	±	5.85	50.9	±	15.3	5.48	±	4.88
Greyia flanaganii Bolus	▪	25	40.78	±	23.01	10.9	±	3.1	4.72	±	4.10
Greyia radlkoferi Szyszył.	▪	25	33.18	±	20.86	10.1	±	2.8	3.29	±	2.27
Greyia sutherlandii Hook. & Harv.	▪	25	65.20	±	24.55	8.2	±	0.6	5.40	±	2.18
Melianthaceae											
Melianthus comosus Vahl	▪	25	68.52	±	20.99	16.0	±	7.0	11.54	±	6.03
Melianthus dregeanus var. insignis Kuntze	■	25	265.11	±	87.97	8.6	±	2.0	24.20	±	11.12
Melianthus pectinatus Harv.	▪	25	50.50	±	22.40	10.8	±	3.2	5.89	±	3.54
Melianthus villosus Bolus	■	25	166.32	±	44.23	14.4	±	4.1	25.47	±	10.35
Geraniaceae											
Erodium manescavi Coss.	▫	10	0.74	±	0.48	53.7	±	13.8	0.51	±	0.40
Erodium pelargoniflorum Boiss. & Heldr.	▫	10	0.43	±	0.19	60.9	±	6.4	0.34	±	0.17
Geranium reuteri Aedo & Muñoz Garm.	▫	10	9.77	±	2.73	58.2	±	6.9	7.19	±	1.60
Geranium sanguineum L.	▫	10	1.73	±	1.02	53.5	±	1.2	1.16	±	0.69
Geranium sylvaticum L.	▫	10	2.12	±	1.73	53.4	±	1.8	1.40	±	1.12
Geranium versicolor L.	☐	10	2.49	±	1.07	28.3	±	12.7	0.81	±	0.52
Geranium yunnanense Franch.	▫	10	9.55	±	6.25	47.7	±	10.2	5.29	±	3.40
Monsonia emarginata L'Hér.	☐	9	0.87	±	0.30	13.7	±	2.7	0.12	±	0.04
Monsonia marlothii (Engl.) F. Albers	▫	9	1.20	±	0.49	36.4	±	9.4	0.55	±	0.35
Pelargonium crispum (P.J.Bergius) L'Hér.	☐	10	2.02	±	0.55	16.2	±	2.7	0.34	±	0.08
Pelargonium echinatum Curtis	☐	10	2.16	±	0.54	16.9	±	4.1	0.38	±	0.11
Pelargonium fulgidum (L.) L'Hér.	☐	10	5.75	±	3.73	18.6	±	7.8	0.95	±	0.46
Pelargonium grossularioides (L.) L'Hér.	▫	10	0.23	±	0.13	42.8	±	11.2	0.12	±	0.08
Pelargonium myrrhifolium (L.) L'Hér.	▫	10	0.19	±	0.12	32.1	±	3.9	0.07	±	0.04
Pelargonium odoratissimum (L.) L'Hér.	▫	10	0.54	±	0.30	58.6	±	3.9	0.42	±	0.26
Pelargonium peltatum (L.) L'Hér.	☐	10	1.15	±	0.92	23.8	±	9.6	0.26	±	0.21
Pelargonium tetragonum (L.f.) L'Hér.	▫	10	9.86	±	2.77	33.5	±	4.8	3.72	±	0.90
Pelargonium zonale (L.) L'Hér.	☐	10	1.47	±	0.43	21.1	±	4.3	0.32	±	0.09
Hypseocharitaceae											
**Hypseocharis bilobata* Killip	*?*	10	0.55	±	0.34	*?*			*?*		

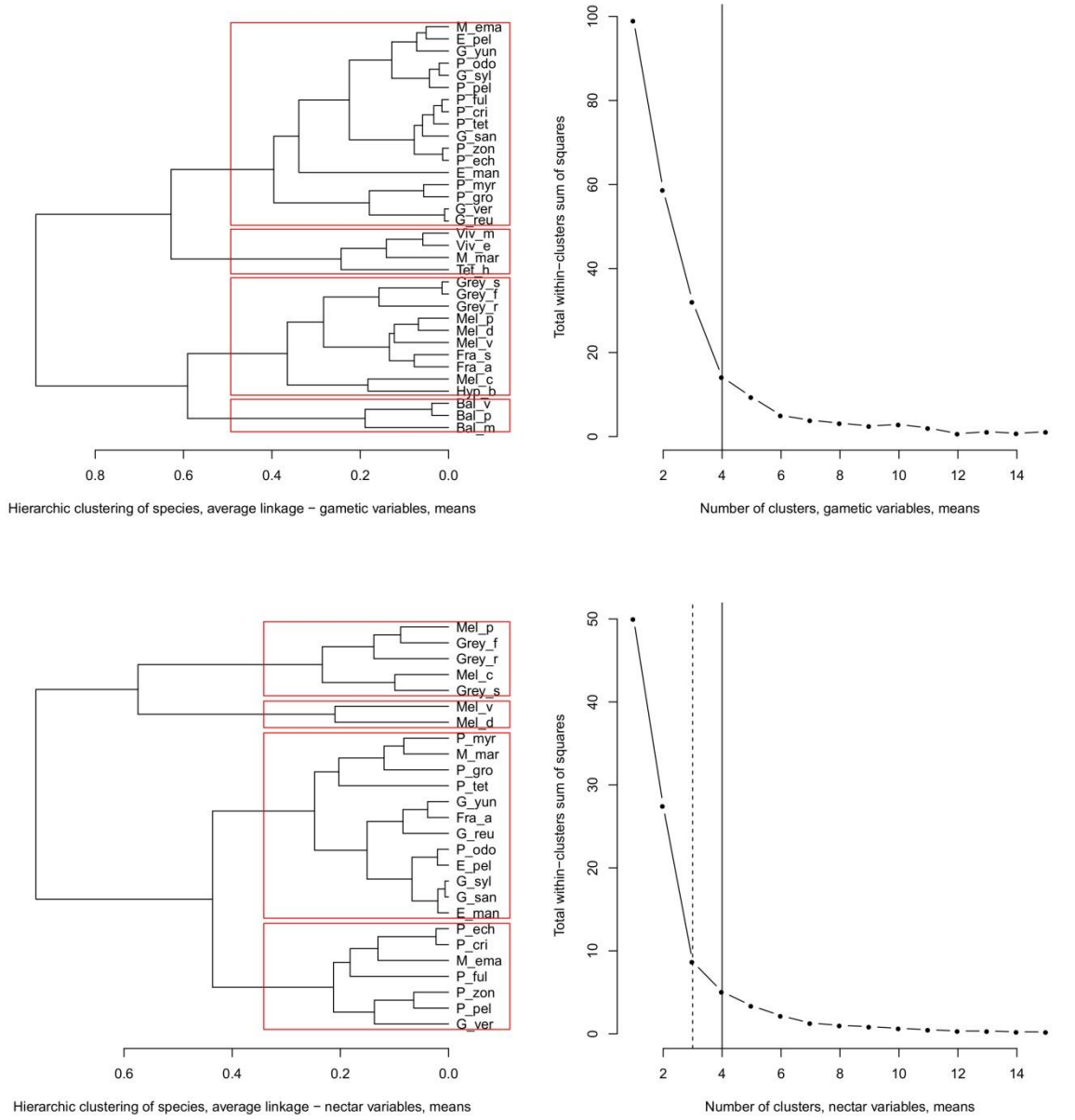

FIGURE A X.1.1: CLUSTER SELECTION AND HIERARCHICAL CLUSTERING OF NECTAR AND GAMETE PRODUCTION IN GERANIALES
Hierarchical clusters of species arithmetic means (left) and selection of appropriate clusters via Elbow method (right) for gametic variables (top row) and nectar reward (bottom row). Clustering performed via average linkage, Bray Curtis index applied. R function cophenetic (package vegan) was used to test for optimal linkage method by measuring the correlation between original dissimilarities and dissimilarities estimated from the created trees. Average linkage performed best for both dissimilarity matrices, for gametic variables: .96 (single linkage .93, complete linkage .92); for nectar reward: .93 (single linkage .86, complete linkage .92). Regarding selection of appropriate number of clusters via Elbow method, charts are showing total intra-cluster variation (y-axis: total within-cluster sum of squares) in dependence to k clusters (x-axis). The position of a bend in the plot is considered as indicator for the best number of clusters, with higher cluster numbers not adding substantially to the compactness of the clusters. For gametic variables as well as for nectar reward, four major clusters are selected (indicated by solid lines in Elbow-chart), and additional clusters are not adding substantially to compactness (monotonous, linear progress for the next clusters). Selected number of clusters is generally supported by R package NBClust and simultaneous testing of indices (number of genera involved was set as upper limit of possible clusters): As for gametic variables, 6 out of 23 indices propose 4 as the best number of clusters (2 indices propose 3 clusters, 1 index proposes 5 clusters). As for nectar reward, 10 out of 23 indices propose 3 as the best number of clusters (dashed line; 9 indices propose 2 clusters, 5 indices propose 7 clusters), however four clusters were selected on basis of Elbow method and visual inspection of the cluster dendrogram. By means of an additional fourth cluster, members of Geraniaceae s. str. are separated into two clusters of nectar concentration: Cluster 1 with lower concentration (13.7 to 28.3 %), and cluster 2 with higher concentration (32.1 to 60.9 %, including *Francoa*).

TABLE A X.1.4: OVERVIEW OF FLOWER VISITORS AND FUNCTIONAL POLLINATOR GUILDS FOR THE GERANIALES SPECIES ANALYSED
Three functional pollinator guilds are distinguished: Short-probiscid insect pollinators (S-P, including beetles), long-proboscid insect pollinators (L-P) and avian pollinators (B). Pollinator guilds are predominantly assigned on basis of flower visitors reported in literature (indexed, sources presented at the end of the table), and further supported by additional assessment criteria: Flower shape, visibility of nectar site [either rated as exposed (ex) or hidden (h)] and predominant flower colour. Regarding general flower shape, saucer- and bowl-types were rated as accessible for short-probiscid insect pollinators. Hidden nectar was rated as indicative for long-probiscid pollinators. As for flower colour, red was rated as indicative for bird pollination syndromes. In case of missing reports on flower visitors, pollinator guilds were primarily assigned on basis of the additional assessment criteria, and reports of similar species of the same genus were used as general guideline.

species	flower colour	flower shape	nectar visibility	reported flower visitors	pollinator guild
Vivianiaceae					
Balbisia meyeniana Klotzsch	yellow	saucer	-	?	S-P
Balbisia peduncularis (Lindl.) D. Don	yellow	saucer	-	Hymenoptera: Colletidae (*Bicolletes* sp.), Apidae (group Exomalopsini)[14]	S-P
Balbisia verticillata Cav.	yellow	saucer	-	?	S-P
Viviania elegans (Poepp.) Reiche & Johow	pink	bowl to hypocrateriform	h	L-P Flies: *Bombyliidae* (personal observation, A. W. Mues, Botanical Garden Bonn)	L-P
Viviania marifolia Cav.	red	hypocrateriform	h	Diptera: *Chaetodemoticus chilensis*[2]; Hymenoptera, Diptera, Lepidoptera[11]	L-P
Francoaceae					
Francoa appendiculata Cav.	white	hypocrateriform	ex	Hymenoptera: *Bombus dahlbomii*[10]; bees and bumblebees (pers. obs., A. W. Mues, Botanical Garden Bonn)	S-P
Francoa sonchifolia Cav.	white	hypocrateriform	ex	Hymenoptera: Bees and bumblebees (pers. obs., A. W. Mues, Botanical Garden Bonn)	S-P
Tetilla hydrocotylaefolia DC.	white	bilabiate, tubular	ex	?	S-P
Greyia flanaganii Bolus	red	urceolate	ex	Bees; Birds: Cape white-eyes, various kinds of sunbirds[12]	B
Greyia radlkoferi Szyszył.	red	bowl	ex	Birds, nectarivores: *Promerops gurneyi*[9]	B
Greyia sutherlandii Hook. & Harv.	red	bowl	ex	Birds, nectarivores: *Promerops gurneyi*, *Nectarinia famosa*, *Nectarinia afra*, *Nectarinia chalybea*[3]	B
Melianthaceae					
Melianthus comosus Vahl	red	bilabiate, tubular	ex	Birds, nectarivores: *Cinnyris chalybeus*, *Anthobaphes violacea*, *Cinnyris fuscus*[8]; Birds, occasional nectarivores: *Zosterops pallidus*, *Monticola rupestris*, *Ploceus velatus*, *Ploceus capensis*, *Colius striatus*, *Colius indicus*, *Onychognathus morio*, *Sturnus bicolour*, *Pycnonotus capensis*[8]; Birds, non-nectarivores: *Cossypha caffra*, *Onychognathus nabouroup*, *Passer melanurus*, *Serinus flaviventris*[8]	B
Melianthus dregeanus Sond.	red	bilabiate, tubular	ex	Birds, nectarivores: *Cinnyris chalybeus, Cinnyris afer*, *Nectarinia famosa*[8]; Birds, occasional nectarivores: *Zosterops pallidus*, *Ploceus capensis*, *Pycnonotus capensis*[8]	B
Melianthus pectinatus Harv.	red	bilabiate, tubular	ex	Birds, nectarivores: *Cinnyris chalybeus*, *Cinnyris fuscus*, *Nectarinia famosa*[8]; Birds, occasional nectarivores:	B

species	flower colour	flower shape	nectar visibility	reported flower visitors	pollinator guild
				Zosterops pallidus, *Colius striatus*, *Onychognathus morio*, *Pycnonotus capensis*[8]; Birds, non-nectarivores: *Onychognathus nabouroup*[8]	
Melianthus villosus Bolus	red	bilabiate, tubular	ex	Birds, nectarivores: *Cinnyris chalybeus*, *Nectarinia famosa*[5]; Birds, occasional nectarivores: *Zosterops pallidus*, *Ploceus capensis*, *Pycnonotus capensis*[5]	B
Geraniaceae					
Erodium manescavi Coss.	pink + white	saucer	ex	?	S-P
Erodium pelargoniflorum Boiss. & Heldr.	white + pink	saucer	ex	Coleoptera: Dasytidae[4]; Diptera: Syrphidae, Bombylidae (*Bombylius* sp.), and Muscidae[4]; Hymenoptera: Anthophoridae (*Anthophora* sp.); Apidae (*Psithyus sp.*), Sphecidae, Sapygidae[4]; Lepidoptera: Lycaenidae (*Lyssandra* sp.)[4]	S-P
Geranium reuteri Aedo & Muñoz Garm.	pink	hypocrateriform	h	?	L-P
Geranium sanguineum L.	magenta	saucer	ex	Diptera: Conopidae, Syrphidae[4]; Hymenoptera: Vespidae (*Polistes, Eumenes*), Megachilidae, Collettidae, Halictidae, Andrenidae[4]; Symphyta[7] (Proctor et al. 1996)	S-P
Geranium sylvaticum L.	violett	saucer	ex	Diptera: Muscidae (*Thricops aculeipes*, *T. nigritellus*), Syrphidae (*Platycheirus manicatus*), Dolichopodidae (*Dolichopus plumipes*), Anthomyiidae (*Pegoplata aestiva*)[4] (Totland 1993), Empididae[7]; Hymenoptera: Apidae (*Bombus lapponicus*)[4] (Totland 1993), Bees[7] (Proctor et al. 1996); Lepidoptera: Lycaenidae (*Albulina orbitulus*)[4] (Totland 1993)	S-P
Geranium versicolor L.	white + pink	bowl	ex	?	S-P
Geranium yunnanense Franch.	white/ pink	saucer	ex	?	S-P
Monsonia emarginata L'Hér.	white	saucer	ex	Beetles[1]	S-P
Monsonia marlothii (Engl.) F. Albers	pink	saucer	ex	Beetles[1]	S-P
Pelargonium crispum (P.J.Bergius) L'Hér.	white/ pink	funnel-shaped / "flag blossom"	h	Bees: *Anthophora* sp. (Anthophoridae)[13]	L-P
Pelargonium echinatum Curtis	white/ pink	funnel-shaped / "flag blossom"	h	L-P Flies: *Prosoeca peringueyi* (Nemestrinidae)[13]; Butterflies: *Tarsocerus cassus*[13]	L-P
Pelargonium fulgidum (L.) L'Hér.	red	funnel-shaped / "flag blossom"	h	Birds: *Nectarinia chalybea* (Nectariniidae) [13](Marloth 1925; F. Albers, pers. comm.)	B
Pelargonium grossularioides (L.) L'Hér.	pink	funnel-shaped / "flag blossom"	h	Bees: *Apis mellifera* cf. *capensis* (Apidae)[13]	L-P
Pelargonium myrrhifolium (L.) L'Hér.	white	funnel-shaped / "flag blossom"	h	L-P Flies: *Bombyliidae* gen. sp.[13] (Vogel 1954; C. F. Jacotguillarmod;	L-P

species	flower colour	flower shape	nectar visibility	reported flower visitors	pollinator guild
				Nemestrinidae, *Moegistorynchus longirostris*[6]	
Pelargonium odoratissimum (L.) L'Hér.	pale pink	funnel-shaped / "flag blossom"	h	?	L-P
Pelargonium peltatum (L.) L'Hér.	white/ pink	funnel-shaped / "flag blossom"	h	L-P Flies: *Pangonia* (=*Philoliche*) sp. [13] (Marloth 1925), *Philoliche* (*P.*) *formosa* (Austen)[13], *P.* (*Ommatiosteres*) *gulosa* (Wiedemann)[13]; Beetles: *Peritrichia capicola* Fabricius (Scarabaeidae: Hopliinae) [13]	L-P
Pelargonium tetragonum (L.f.) L'Hér.	cream/ pink	funnel-shaped / "flag blossom"	h	L-P Flies: Unidentified long-proboscid hovering fly [13] (E. van Jaarsveld, pers. comm.)	L-P
Pelargonium zonale (L.) L'Hér.	white/ pink	funnel-shaped / "flag blossom"	h	?	L-P
Hypseocharitaceae					
Hypseocharis bilobata Killip	white	bowl	h	Flies (pers. obs., M. Weigend)	S-P

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TABLE A X.1.5: ACCESSION AND VOUCHER NUMBERS FOR GERANIALES SPECIES

Accession- and voucher numbers for the analysed Geraniales species, according to their origin: Botanical Garden Bonn (Herbarium BONN) or Botanical Garden Berlin-Dahlem (Herbarium Berolinense, B). *) collector number instead of accession number, also voucher number.

species	Botanical Garden	accession number	voucher number
Vivianiaceae			
Balbisia meyeniana Klotzsch	Bonn	MW 9366*	MW 9366*
Balbisia peduncularis (Lindl.) D. Don	Berlin	314039110/001	37581
Balbisia verticillata Cav.	Bonn	MW 9367*	MW 9367*
Viviania elegans (Poepp.) Reiche & Johow	Bonn	33160	718
Viviania marifolia Cav.	Berlin	Kern 18	-
Francoaceae			
Francoa appendiculata Cav.	Berlin Bonn	288199270/002 MW 9353* 33098	32510 MW 9353* 2332
Francoa sonchifolia Cav.	Bonn	32174	1390
Tetilla hydrocotylaefolia DC.	Berlin	Kern & Belov 21	-
Greyia flanaganii Bolus	Bonn	32142	889
Greyia radlkoferi Szyszył.	Berlin	019897484/001 130080630/001	1156 -
Greyia sutherlandii Hook. & Harv.	Berlin	023-02-90-70 262-9-1980	- -
Melianthaceae			
Melianthus comosus Vahl	Berlin Bonn	151028414 MW 9182*	- MW 9182*
Melianthus dregeanus (var. insignis) Kuntze	Bonn	34341	2344
Melianthus pectinatus Harv.	Bonn	MW 9164*	MW 9164*
Melianthus villosus Bolus	Bonn	MW 9163*	MW 9163*
Geraniaceae			
Erodium manescavi Coss.	Berlin Bonn	215478214/001 3785	43398 1857
Erodium pelargoniflorum Boiss. & Heldr.	Bonn	MW 9312*	MW 9312*
Geranium reuteri Aedo & Muñoz Garm.	Bonn	14031	729
Geranium sanguineum L.	Bonn	5721	2615
Geranium sylvaticum L.	Bonn	22496	2616
Geranium versicolor L.	Bonn	MW 9313*	MW 9313*
Geranium yunnanense Franch.	Bonn	28913	1554
Monsonia emarginata L'Hér.	Berlin	072060670/001 072060673/001	44182 -
Monsonia marlothii (Engl.) F. Albers	Berlin	278998320/001	28138
Pelargonium crispum (P.J.Bergius) L'Hér.	Berlin	259199883/001 017149043/001	38646 -
Pelargonium echinatum Curtis	Berlin	008187483/001	8147
Pelargonium fulgidum (L.) L'Hér.	Berlin	008177484/001	-
Pelargonium grossularioides (L.) L'Hér.	Berlin	008057484/001 057050440/001 056090574/002	28165 42155 43962
Pelargonium myrrhifolium (L.) L'Hér.	Berlin	019048144/002 024028234/001	- -
Pelargonium odoratissimum (L.) L'Hér.	Berlin	259099883/001 008017483/001	45450 -
Pelargonium peltatum (L.) L'Hér.	Berlin	269238583/001 87350880	29829 -
Pelargonium tetragonum (L.f.) L'Hér.	Berlin	008117484/001	10140
Pelargonium zonale (L.) L'Hér.	Berlin	118640770 276018484	- -
Hypseocharitaceae			
Hypseocharis bilobata Killip	Bonn	33109	1890

X.2 APPENDIX TO CHAPTER III

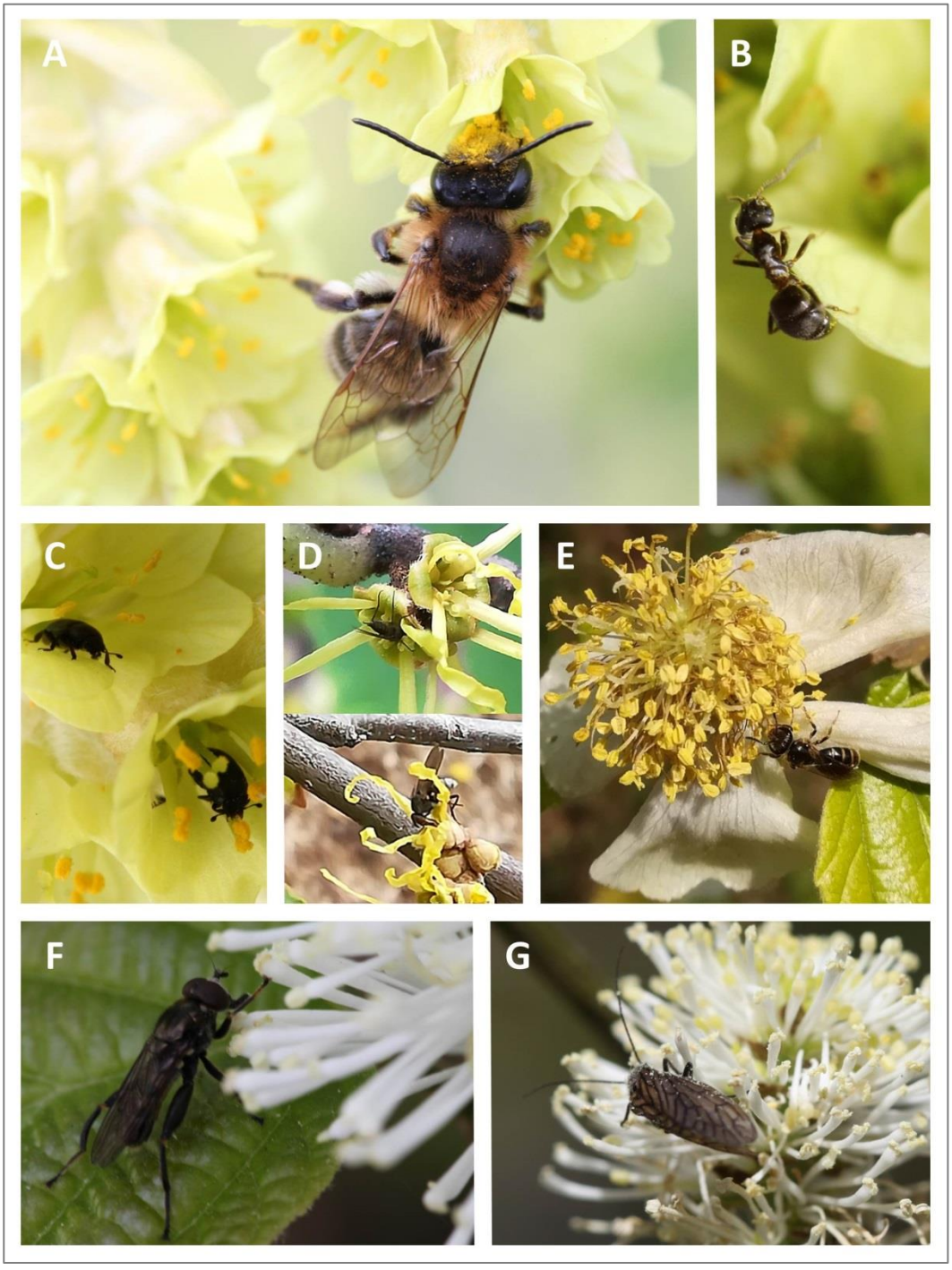

FIGURE A X.2.1: FLOWER VISITORS OF HAMAMELIDACEAE IN BONN UNIVERSITY BOTANICAL GARDENS
(A) Apidae (Hymenoptera) were often observed for genus *Corylopsis*, but flowers were also visited frequently by ants (B) and beetles (C). Diptera were observed as visitors of *Hamamelis virginiana* (D). Floral traits of genera *Parrotiopsis* and *Fothergilla* are indicative of ambophily, and insect flower visitors were observed: For *Parrotiopsis jacquemontiana*, solitary bees (E) as well as Plecoptera (Neoptera) were observed. (F) Brachycera (Diptera) and (G) Plecoptera (Neoptera) were observed for *Fothergilla major*. Pictures A, B, C, F and G by L. Hoff, D and E by A. W. Mues.

TABLE A X.2.1: DATA BANDWIDTH OF GAMETE PRODUCTION IN HAMAMELIDACEAE

The table shows arithmetic means and standard deviations for pollen production per anther, per flower and per inflorescence, ovule numbers and p/o ratios. Sample size is *n* = 12 inflorescences per species, and the share of inflorescences with perfect (☿), staminate (♂) or pistillate flowers (♀) is indexed (e.g. ♂ = 4/12 indicates presence of staminate flowers in 4 out of 12 inflorescences). In case of different flower types, arithmetic means and standard deviations are presented separately for flower types and the inflorescences bearing them, as well as combined, neglecting the flower type ($\bar{x}$).Ovule number is fixed to two functional ovules per flower (one per carpel), the only exception is *Disanthus* with six or occasionally seven ovules per carpel. The table further shows adjusted ovule numbers and p/o ratios after female anthesis of *Fortunearia*, *Parrotia* and *Sycopsis*, in italic. Results of hierarchical clustering are presented in column C (gametic clusters A and B).

species	C	*n*	pollen per anther (if applicable, per flower type)	pollen per flower (if applicable, per flower type)	pollen per inflorescence (if applicable, per flower type)	ovules per flower	ovules per infl. *- adjusted, anthesis -*	p/o ratio *- adjusted, anthesis -*
Corylopsis glabrescens Franch. & Sav.	B	☿ = 12	7 960.0 ± 1 751.2	39 800.0 ± 8 755.8	321 983.3 ± 79 306.8	2	16.2 ± 1.6	19 900.0 ± 4 377.9
Corylopsis pauciflora Siebold & Zucc.	B	☿ = 12	21 312.5 ± 5 732.2	124 233.3 ± 49 987.1	277 050.0 ± 119 537.8	2	4.5 ± 1.2	62 116.7 ± 24 993.6
Corylopsis sinensis Hemsl.	B	☿ = 12	6 685.0 ± 1 514.7	33 425.0 ± 7 573.5	376 908.3 ± 91 118.6	2	22.7 ± 2.7	16 712.5 ± 3 786.8
Corylopsis spicata Siebold & Zucc.	B	☿ = 12	8 514.4 ± 2 302.5	43 050.0 ± 10 990.3	314 516.7 ± 94 608.3	2	14.5 ± 1.2	21 525.0 ± 5 495.1
Corylopsis veitchiana Bean	B	☿ = 12	5 322.5 ± 1 327.8	26 400.0 ± 6 104.5	348 466.7 ± 73 716.7	2	26.7 ± 3.3	13 200.0 ± 3 052.3
Corylopsis willmottiae Rehder & E.H.Wilson	B	☿ = 12	10 713.3 ± 2 122.3	53 566.7 ± 10 611.4	643 650.0 ± 165 752.2	2	24.0 ± 3.2	26 783.3 ± 5 305.7
Disanthus cercidifolius Maxim.	B	☿ = 12	4233.3 ± 1 719.5	21 166.7 ± 8 597.6	42 333.3 ± 17 195.2	12.5 ± .9	25.0 ± 1.8	1 691.6 ± 680.9
Distyliopsis tutcheri (J.H.Hemsl.) Endress	B	☿ = 12/12 ♂ = 4/12 $\bar{x}$ (☿,♂)	7 450.0 ± 3 056.2 11 500.0 ± 4 815.3 8 495.2 ± 3 075.3	77 946.7 ± 30 407.6 69 795.0 ± 30 788.7 81 024.7 ± 26 806.7	104 076.7 ± 103 116.0 181 600.0 ± 104 709.2 164 610.0 ± 129 608.0	2	2.5 ± 1.7	69 240.0 ± 51 334.7
Distylium myricoides Hemsl.	B	☿ = 12/12 ♂ = 12/12 $\bar{x}$ (☿,♂)	8 953.3 ± 3 929.0 11 077.1 ± 3 638.1 9 734.6 ± 3 424.2	44 425.8 ± 19 376.1 37 339.8 ± 11 882.8 41 509.2 ± 14 610.4	240 573.3 ± 111 561.8 165 000.0 ± 67 584.6 405 573.3 ± 133 583.3	2	10.8 ± 1.4	38 248.9 ± 13 254.0
Distylium racemosum Siebold & Zucc.	B	☿ = 12/12 ♂ = 12/12 $\bar{x}$ (☿,♂)	11 232.4 ± 1 882.5 10 177.0 ± 2 693.3 10 986.8 ± 1 681.3	69 532.7 ± 12 930.9 44 605.3 ± 17 538.0 56 510.7 ± 14 760.4	218 520.0 ± 101 714.3 181 140.0 ± 103 099.8 399 660.0 ± 69 360.2	2	6.7 ± 3.7	88 906.4 ± 72 118.6
Fortunearia sinensis Rehder & E.H.Wilson	A	☿ = 12	15 163.3 ± 1 604.2	75 816.7 ± 8 021.1	2 326 750.0 ± 387 863.7	2	61.2 ± 6.1 *14.1* ± *1.4*	37 908.3 ± 4 010.5 *164 247.5* ± *17 376.7*
Fothergilla gardenii L.	A	☿ = 12	1 749.0 ± 841.0	39 180.8 ± 16 879.5	1 373 573.8 ± 563 159.3	2	70.3 ± 4.8	19 590.4 ± 8 439.7
Fothergilla major G.Lodd.	A	☿ = 12	3 070.0 ± 994.1	67 881.2 ± 24 233.9	1 642 711.7 ± 589 303.7	2	48.3 ± 2.5	33 940.6 ± 12 116.9
Hamamelis japonica Siebold & Zucc.	B	☿ = 12	3 479.2 ± 1 084.2	13 916.7 ± 4 336.7	42 558.3 ± 12 441.6	2	6.2 ± .6	6 958.3 ± 2 168.4
Hamamelis mollis Oliv. ex F.B.Forbes & Hemsl.	B	☿ = 12	8 041.7 ± 3 881.9	32 166.7 ± 15 527.4	101 350.0 ± 47 060.8	2	6.3 ± .8	16 083.3 ± 7 763.7
Hamamelis vernalis Sarg.	B	☿ = 12	4 760.4 ± 2 311.2	19 041.7 ± 9 245.0	58 208.3 ± 29 306.1	2	6.2 ± 1.0	9 520.8 ± 4 622.5
Hamamelis virginiana L.	B	☿ = 12	6 033.3 ± 2 082.9	24 133.3 ± 8 331.6	68 908.3 ± 27 129.1	2	5.7 ± .8	12 066.7 ± 4 165.8
Loropetalum chinense (R. Br.) Oliv.	B	☿ = 12	6 007.1 ± 1 524.0	25 750.0 ± 6 418.8	166 483.3 ± 51 650.3	2	12.8 ± 1.8	12 875.0 ± 3 209.4
Parrotia persica (DC.) C.A.Mey.	A	☿ = 12	12 840.0 ± 5 966.4	147 504.4 ± 70 918.9	795 213.3 ± 509 784.8	2	10.2 ± 2.5 *3.3* ± *6.1*	73 752.2 ± 35 459.4 *2 675 817.1* ± *255 043.6*
Parrotiopsis jacquemontiana (Decne.) Rehder	A	☿ = 12/12 ♂ = 1/12 $\bar{x}$ (☿,♂)	6 606.7 ± 1 910.4 3 920.0 6 597.5 ± 1 916.3	122 647.7 ± 35 791.0 78 400.0 122 487.4 ± 35 870.4	1 833 293.3 ± 679 037.7 78 400.0 1 839 826.7 ± 675 924.5	2	29.3 ± 3.2	61 557.2 ± 17 804.0
Sinowilsonia henryi Hemsl.	A	♂ = 12 ♀ = 12	10 580.0 ± 3 961.9	52 445.6 ± 19 716.3	3 430 566.7 ± 1 225 920.3	2	193.5 ± 34.9	17 683.3 ± 6 319.2
Sycopsis sinensis Oliv.	A	☿ = 12 ♂ = 2 $\bar{x}$ (☿,♂)	12 233.3 ± 5 971.3 4 850.0 ± 2 899.1 11 814.7 ± 5 611.1	100 765.6 ± 51 564.5 38 800.0 ± 23 193.1 97 710.7 ± 50 102.8	716 660.0 ± 423 230.8 50 000.0 ± 7 353.9 724 993.3 ± 421 340.1	2	14.0 ± 2.3 *5.3* ± *7.9*	51 084.7 ± 25 980.5 *1 725 748.5* ± *771 076.5*

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TABLE A X.2.2: COMPOSITION AND SIZE OF INFLORESCENCES AND SIZE OF REPRODUCTIVE ORGANS IN HAMAMELIDACEAE

The table shows arithmetic means and standard deviations for variables of inflorescence composition (anthers per flower, flowers and anthers per inflorescence, n_1 = 12 inflorescences per species analysed), as well as measures of inflorescence size and of reproductive organs (10 measurements per species and variable, 12 for inflorescence size of *Parrotia* and *Sycopsis*). The share of inflorescences with different flower types is indexed as in the previous table. Number of carpels is fixed to two for the species analysed. Anther length is highly conserved, and standard deviations are therefore not presented. Adjusted style length after female anthesis of *Fortunearia*, *Parrotia* and *Sycopsis* presented in italic. Non-gametic clusters presented in column C.

species	C	n_1	flowers per inflorescence (if applicable, per flower type)	anthers per flower (if applicable, per flower type)	anthers per inflorescence (if applicable, per flower type)	n_2	anther length in mm	filament length in mm	style length in mm	inflorescence frontal size in cm²	inflorescence lateral size in cm²
Corylopsis glabrescens Franch. & Sav.	2	☿ = 12	8.1 ± .8	5	40.4 ± 4.0	10	.5	3.4 ± .5	6.2 ± .6	2.0 ± .4	1.1 ± .2
Corylopsis pauciflora Siebold & Zucc.	2	☿ = 12	2.3 ± .6	5.8 ± 1.2	12.8 ± 3.8	10	1.8	7.4 ± .8	9.8 ± .4	1.2 ± .2	1.7 ± .2
Corylopsis sinensis Hemsl.	2	☿ = 12	11.3 ± 1.4	5	56.7 ± 6.9	10	1.4	7.8 ± .8	11.0 ± 1.2	3.3 ± .6	6.3 ± 1.0
Corylopsis spicata Siebold & Zucc.	2	☿ = 12	7.3 ± .6	5.1 ± .3	36.8 ± 3.5	10	1	6.9 ± .9	7.9 ± .9	1.4 ± .3	2.7 ± .5
Corylopsis veitchiana Bean	2	☿ = 12	13.3 ± 1.7	5 ± .4	66.8 ± 10.7	10	.7	3.6 ± .5	5.5 ± .5	2.8 ± .3	4.6 ± .6
Corylopsis willmottiae Rehder & E.H.Wilson	2	☿ = 12	12.0 ± 1.6	5	60.0 ± 8.0	10	.6	3.4 ± .7	5.0 ± .7	1.5 ± .3	2.6 ± .6
Disanthus cercidifolius Maxim.	2	☿ = 12	2	5	10	10	1	.5	.5	.2 ± .03	.3 ± .1
Distyliopsis tutcheri (J.H.Hemsl.) Endress	3	☿ = 12/12 ♂ = 4/12 x̄ (☿,♂)	1.3 ± .9 2.5 ± .6 2.1 ± 1.4	10.6 ± 1.4 6.0 ± 1.0 9.7 ± 1.9	13.1 ± 8.3 15.5 ± 5.9 18.3 ± 10.2	10	2	5.8 ± .9	1	.3 ± .2	.4 ± .2
Distylium myricoides Hemsl.	3	☿ = 12/12 ♂ = 12/12 x̄ (☿,♂)	5.4 ± .7 4.5 ± 1.5 9.9 ± 1.4	5.0 ± .3 3.4 ± .3 4.3 ± .3	27.1 ± 4.0 15.2 ± 5.0 42.3 ± 5.2	10	2.9	.5	5.2 ± .4	.8 ± .1	1.6 ± .3
Distylium racemosum Siebold & Zucc.	3	☿ = 12/12 ♂ = 12/12 x̄ (☿,♂)	3.3 ± 1.8 4.1 ± 1.2 7.4 ± 2.0	6.2 ± .8 4.4 ± 1.6 5.1 ± .6	19.8 ± 10.1 17.0 ± 5.9 36.8 ± 7.2	10	2.1	2.1 ± .2	6.9 ± .6	.9 ± .2	1.8 ± .4
Fortunearia sinensis Rehder & E.H.Wilson	3	☿ = 12	30.6 ± 3.1	5	152.9 ± 15.3	10	2	0	2 *6.9* ± *.5*	1.3 ± .2	2.7 ± .3
Fothergilla gardenii L.	1	☿ = 12	35.2 ± 2.4	22.7 ± 1.5	800.3 ± 88.2	10	.5	12.1 ± 1.5	9.3 ± .9	6.7 ± .9	7.6 ± 1.1
Fothergilla major G.Lodd.	1	☿ = 12	24.2 ± 1.3	22.0 ± 1.7	529.8 ± 39.5	10	.5	13.4 ± 1.3	9.9 ± .7	6.7 ± .6	7.1 ± 1.2
Hamamelis japonica Siebold & Zucc.	2	☿ = 12	3.1 ± .3	4	12.3 ± 1.2	10	1	1.5	2.3 ± .3	1.5 ± .2	1.1 ± .2
Hamamelis mollis Oliv. ex F.B.Forbes & Hemsl.	2	☿ = 12	3.2 ± .4	4	12.7 ± 1.6	10	1	1.5 ± .2	1.5 ± .2	2.1 ± .3	1.7 ± .2
Hamamelis vernalis Sarg.	2	☿ = 12	3.1 ± .5	4	12.3 ± 2.1	10	1	1.4 ± .2	2.0 ± .2	1.1 ± .1	.9 ± .1
Hamamelis virginiana L.	2	☿ = 12	2.8 ± .4	4	11.3 ± 1.6	10	1.0	.5	.5	.8 ± .1	.5 ± .1
Loropetalum chinense (R. Br.) Oliv.	2	☿ = 12	6.4 ± .9	4.3 ± .5	27.7 ± 4.4	10	1	.1	.1	4.2 ± 1.0	3.3 ± .6
Parrotia persica (DC.) C.A.Mey.	3	☿ = 12	5.1 ± 1.2	11.5 ± 1.4	58.8 ± 17.4	10 (12)	3.2	8.9 ± .6	1 *3.7* ± *.5*	1.8 ± .4	1.6 ± .3
Parrotiopsis jacquemontiana (Decne.) Rehder	1	☿ = 12/12 ♂ = 1/12 x̄ (☿,♂)	14.7 ± 1.7 1 14.8 ± 1.6	18.6 ± .9 20 18.6 ± .9	272.3 ± 31.0 20 273.9 ± 31.6	10	1	5.0 ± .3	4.5 ± .2	4.8 ± 1.4	3.0 ± .4
Sinowilsonia henryi Hemsl.	3	♂ = 12 ♀ = 12	66.2 ± 7.2 96.8 ± 17.5	5.0 ± .02	327.9 ± 36.2	10	1.2	.2 ± .04	2.9 ± .3	1.1 ± .2 1.1 ± .3	4.4 ± .4 2.5 ± .6
Sycopsis sinensis Oliv.	3	☿ = 12 ♂ = 2 x̄ (☿,♂)	7.0 ± 1.1 1.5 ± .7 7.3 ± 1.0	8.2 ± 1.0 8 8.2 ± .9	57.4 ± 12.7 12.0 ± 5.7 59.4 ± 12.0	10 (12)	2.2	14.8 ± 1.9	.1 *5.7* ± *.8*	1.4 ± .5	1.6 ± .5

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TABLE A X.2.3: MEASUREMENTS OF PERIANTH ORGANS AND FLOWER SIGNAL OF HAMAMELIDACEAE

● = UV absorbing, ○ = UV reflecting; Non-gametic clusters presented in column C. * = for *Parrotiopsis jacquemontiana* data on bracts are presented, ** = Bogle, 1970; *** = filaments reflecting.

species	C	*n*	flower types	number of perianth organs	perianth organs: length in mm	perianth organs: width in mm	perianth colour	UV signal perianth	colouration of stamens	UV signal stamen	odour
Corylopsis glabrescens Franch. & Sav.	**2**	10	☿	5 petals	6.5 ± .5	4.1 ± .7	yellow	●	yellow anthers	●	strong sweet smell
Corylopsis pauciflora Siebold & Zucc.	**2**	10	☿	5 petals	10.4 ± .5	6.8 ± .8	yellow	●	yellow anthers	●	-
Corylopsis sinensis Hemsl.	**2**	10	☿	5 petals	10.7 ± 1.2	4.5 ± .5	yellow	●	red anthers	●	fresh, like citrus and mandarins
Corylopsis spicata Siebold & Zucc.	**2**	10	☿	5 petals	8.0 ± 1.9	4.4 ± 1.2	yellow	●	red anthers	●	faint sweet smell
Corylopsis veitchiana Bean	**2**	10	☿	5 petals	6.8 ± .8	4.5 ± .5	yellow	●	yellow anthers	●	-
Corylopsis willmottiae Rehder & E.H.Wilson	**2**	10	☿	5 petals	5.3 ± .7	3.1 ± .3	yellow	●	yellow anthers	●	faint sweet smell
Disanthus cercidifolius Maxim.	**2**	10	☿	5 petals	5.8 ± 1.3	1.8 ± .3	red	●	red anthers	●	- (though sometimes reported)
Distyliopsis tutcheri (J.H.Hemsl.) Endress	**3**	10	☿ ♂	atepalous	-	-	-	-	dark red anthers	●	-
Distylium myricoides Hemsl.	**3**	10	☿ ♂	atepalous	-	-	-	-	dark red anthers	●	-
Distylium racemosum Siebold & Zucc.	**3**	10	☿ ♂	atepalous	-	-	-	-	dark red anthers	●	-
Fortunearia sinensis Rehder & E.H.Wilson	**3**	10	☿	5 reduced petals	2	.1	(green)	○	dark red anthers	●	-
Fothergilla gardenii L.	**1**	10	☿	reduced, 5 to 7 irregular tepals	1	1	(green)	-	white filaments anthers yellow	●	faint sweet smell
Fothergilla major G.Lodd.	**1**	10	☿	reduced, 5 to 7 irregular tepals	1	1	(green)	-	white filaments anthers yellow	●	faint sweet smell
Hamamelis japonica Siebold & Zucc.	**2**	10	☿	4 petals	11.7 ± .7	1.3 ± .3	yellow-orange	●	thecas yellow (other: reddish)	●	woody and acidic, a little bit sweet
Hamamelis mollis Oliv. ex F.B.Forbes & Hemsl.	**2**	10	☿	4 petals	15.1 ± .9	1.4 ± .2	yellow	●	red stamens	●	woody and acidic, a little bit sweet
Hamamelis vernalis Sarg.	**2**	10	☿	4 petals	11.1 ± .6	1.3 ± .3	yellow-orange	●	thecas yellow (other: reddish)	●	woody and acidic, a little bit sweet
Hamamelis virginiana L.	**2**	10	☿	4 petals	9.8 ± .8	1	yellow	●	yellow stamens	●	heavy, nutty smell, woody and acidic
Loropetalum chinense (R. Br.) Oliv.	**2**	10	☿	4 to 5 petals	18.1 ± 2.1	2.1 ± .2	white (red)	○	greenish yellow stamens	●	faint, sweet smell (resembling lime trees)
Parrotia persica (DC.) C.A.Mey.	**3**	10	☿	5 to 9 (10) very reduced tepals**	-	-	(green)	-	dark red anthers	●	-
*Parrotiopsis jacquemontiana** (Decne.) Rehder	**1**	10	☿ ♂	apetalous; samples showed 4 bracts	16.6 ± 3.8 *	14.3 ± 2.2 *	white*	●	yellow stamens	●	- (though sometimes reported)
Sinowilsonia henryi Hemsl.	**3**	10	♂ ♀	5, extremely reduced 5 reduced organs	- 1	- .5	(green)	○	yellow stamens	●	♂: strange, fungus-like smell ♀: faint smell, "green" and nutty
Sycopsis sinensis Oliv.	**3**	10	☿ ♂	extremely reduced**	-	-	-	-	yellow/ orange anthers	●/○***	-

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FIGURE A X.2.2: UV LIGHT SIGNAL OF HAMAMELIDACEAE FLOWERS AND INFLORESCENCES

The plate shows the patterns of UV light reflection or absorption for selected species of Hamamelidaceae. A. *Corylopsis willmottiae* (inflorescence, frontal view, UV exposure: 5 sec.); B. *Corylopsis sinensis* (inflorescence, lateral view, UV exp.: 1 sec.); C. *Hamamelis mollis* (single flower, UV exp.: 1.3 sec.); D. *Loropetalum chinense* (inflorescence, frontal view, UV exp.: 1.3 sec.); E. *Parrotiopsis jacquemontiana* (inflorescence, frontal view, UV exp.: 1.3 sec.); F. *Sinowilsonia henryi* (male inflorescence, lateral view, UV exp.: 1 sec.); G. *Distylium racemosum* (inflorescence, lateral view, UV exp.: 1.6 sec.); H. *Fothergilla major* (inflorescence, frontal view, UV exp.: 2 sec.). Pictures A, B and H by L. Hoff. Pictures C, D, E, F and G by A. W. Mues.

TABLE A X.2.4: NECTAR PRODUCTION OF HAMAMELIDACEAE SPECIES

The table shows the number of data points (n_1), the average number of flowers probed in order to retrieve a data point for the respective species (n_2), as well as the average production of nectar in µl, nectar concentration in % and sugar production in mg, with corresponding standard deviation. For *Corylopsis veitchiana, Disanthus cercidifolius* and all species of *Hamamelis* nectar could not be analysed with the technique applied: Only minute amounts of nectar could be detected, and fast crystallisation indicates higher nectar concentrations. All species with nectar reward belong to non-gametic cluster 2.

taxon	n_1	n_2	nectar production per flower (µl)	concentration per flower (%)	sugar production per flower (mg)
Corylopsis glabrescens Franch. & Sav.	5	58.0 ± 26.5	.03 ± .01	32.4 ± 2.9	.010 ± .005
Corylopsis pauciflora Siebold & Zucc.	10	13.2 ± 2.2	.07 ± .02	38.8 ± 9.9	.033 ± .016
Corylopsis sinensis Hemsl.	10	56.5 ± 28.4	.07 ± .05	27.6 ± 5.3	.020 ± .010
Corylopsis spicata Siebold & Zucc.	10	10.5 ± 4.4	.16 ± .07	40.8 ± 4.6	.076 ± .025
Corylopsis veitchiana Bean			✓		
Corylopsis willmottiae Rehder & E.H.Wilson	10	10.7 ± 7.0	.19 ± .09	22.6 ± 5.8	.047 ± .031
Disanthus cercidifolius Maxim.			✓		
Hamamelis Gronov. ex L.			✓		

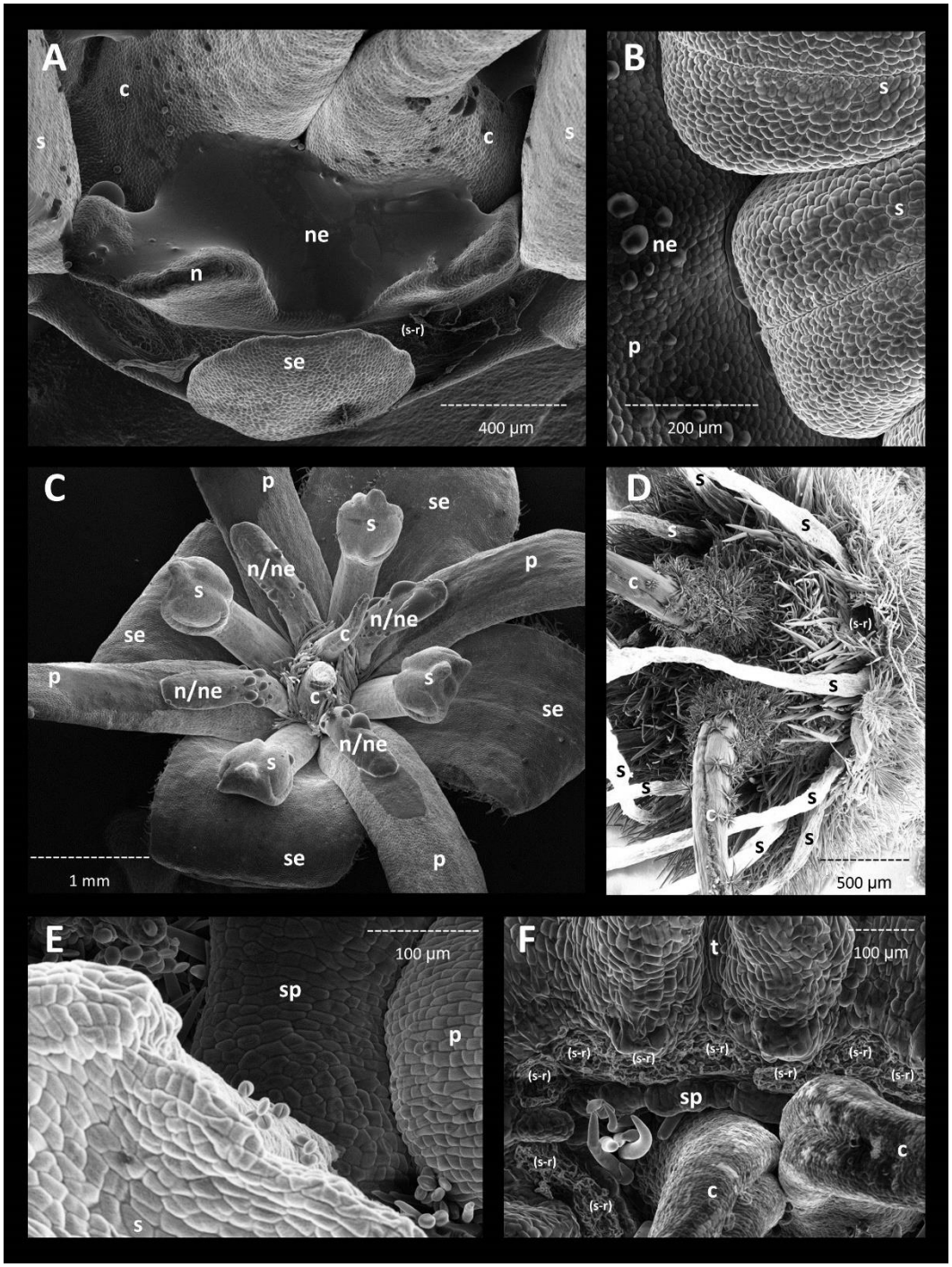

FIGURE A X.2.3: CSEM PICTURES OF HAMAMELIDACEAE FLOWERS AND PRESENCE OF NECTAR
(A) Substantial amounts of nectar are produced by nectaries of genus *Corylopsis* (here: *Corylopsis pauciflora*). (B) Minute amounts of nectar are secreted from petals of *Disanthus cercidifolius*. (C) In genus *Hamamelis* minute amounts of nectar are produced by nectar stamens (here: *Hamamelis virginiana*). No nectar is present in showy flowers of *Parrotiopsis* (D), *Loropetalum* (E) and *Fothergilla* (F, *Fothergilla major*). Legend: c = carpel, n = nectary, ne = nectar, p = petal, s = stamina, s-r = stamina removed, se = sepal, sp = sterile phyllome – not secreting, t = tepals (reduced). Pictures A and F by L. Hoff and H.-J. Ensikat; pictures B, C, D and E by A. W. Mues and H.-J. Ensikat.

TABLE A X.2.5: CLASSIFICATION OF FLOWERING TIME FOR HAMAMELIDACEAE SPECIES

The table presents the flowering time in Bonn Botanical Gardens for the Hamamelidaceae species analysed, as well as the distribution and flowering time reported in literature (sources are presented at the end of the table). In general, observed flowering times are in line with reported phenology. For some species differences between reported and observed flowering time are evident, which can be attributed to habitats of higher elevation and specific climatic conditions in the distribution area. Labels for flowering time were therefore predominantly assigned after observed flowering time in Bonn, and labels were chosen bold and simple: Winter flowering species are blooming noticeably earlier, and autumn flowering species noticeably later than spring flowering species, marking the marginal extremes of the growth period.

species	distribution (habitat, altitude, localities)	reported flowering time for habitat in literature	flowering onset in Bonn BGB, 2016 (2015 for *H. virginiana*)	succession of leaves and flowers in Bonn BGB	classification of flowering time
Corylopsis glabrescens Franch. & Sav.	Japan: Mts. Kirishima, Kyushu and in limestone areas in western Shikoku (Yamanaka, 1986)	April (FoJ, 1965)	April	flowers before leaves	**spring**
Corylopsis pauciflora Siebold & Zucc.	Slopes and forests; 200 - 300 m (FoC, 2003) China: Taiwan, Korea (FoC, 2003) Japan: Honshu (n. Kinki Distr. and Kaga, Echizen, and Mino Prov.) (FoJ, 1965)	May – July (FoC, 2003) March – April (FoJ, 1965)	March	flowers before leaves	**spring**
Corylopsis sinensis Hemsl.	Forests, mountains; 1000 – 1500 m China: Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangxi, Sichuan, Zhejiang (FoC, 2003)	May – July (FoC, 2003)	April	flowers before leaves	**spring**
Corylopsis spicata Siebold & Zucc.	Japan: endemic to Kochi Prefecture. Shikoku (Yamanaka, 1986) Mountains, Shikoku (FoJ, 1965)	March – April (FoJ, 1965)	March	flowers before leaves	**spring**
Corylopsis veitchiana Bean	Forests; ca. 1200 m China: Anhui, Hubei, East Sichuan (FoC, 2003)	April – June (FoC, 2003)	April	flowers before leaves	**spring**
Corylopsis willmottiae Rehder & E.H.Wilson	Forests; ca. 1200 m China: Western Sichuan (FoC, 2003)	March – June (FoC, 2003)	April	flowers before leaves	**spring**
Disanthus cercidifolius Maxim.	Mixed evergreen and deciduous broad-leaved forests; 450 - 1200 m China: Hunan, Jiangxi, Zhejiang; Japan (FoC, 2003) Japan: Honshu (sw. part of centr. distr. and Aki Prov.), Shikoku (FoJ, 1965)	October – November (FoC, 2013) September – November (Xiao et al., 2009)	October	flowers together with leaf senescence	**autumn**
Distyliopsis tutcheri (J.H.Hemsl.) Endress	Mountains in evergreen forests; 800 - 1000 m China: Fujian, Guangdong, Hainan (FoC, 2003)	April – June (FoC, 2003)	February	evergreen	**winter**
Distylium myricoides Hemsl.	Montane evergreen forests; 500 - 800 m China: Anhui, Fujian, Guangdong, Guangxi, East Guizhou, Hunan, Jiangxi, Sichuan, South East Yunnan (Funing Xian), Zhejiang (FoC, 2003)	April – June (FoC, 2003) Genus *Distylium*: "Spring-flowering evergreen trees or shrubs (…) in subtropical and warm temperate eastern and southeastern Asia (…)." (Walker, 1944, p. 323)	May	evergreen	**spring**
Distylium racemosum Siebold & Zucc.	Forests; 1000 - 1300 m China: Fujian, Hainan, Taiwan, Zhejiang. Japan: Ryukyu Islands; Korea (FoC, 2003) Japan: Honshu (s. Kantō Distr. and westw.); Shikoku, Kyushu (FoJ, 1965)	April – June (FoC, 2003) March – May (FoJ, 1965) Genus *Distylium*: "Spring-flowering evergreen trees or shrubs (…) in subtropical and warm temperate eastern and southeastern Asia (…)." (Walker, 1944, p. 323)	May	evergreen	**spring**
Fortunearia sinensis Rehder & E.H.Wilson	Forests; 800 - 1000 m China: Anhui, Henan, Hubei, Jiangxi, Shaanxi, Sichuan, Zhejiang (FoC, 2003)	March – April (FoC, 2003)	April	flowers together with leaves	**spring**
Fothergilla gardenii L.	Restricted to the Atlantic and Gulf Coastal Plains from northeastern North Carolina (where most abundant) to the western panhandle of Florida and adjacent Alabama (Weaver, 1971) Native to margins of swamps and pocosins on the Atlantic and Gulf Coastal Plains from Virginia to Alabama (Weaver, 1976)	Peak of flowering in North Carolina: second and third week of April, few other shrubs blooming (Weaver, 1971) Flowers appear before the leaves (Weaver, 1971)	April	flowers together with leaves	**spring**
Fothergilla major G.Lodd.	Occurs in scattered localities from northwestern North Carolina and northeastern Tennessee along the Appalachians into north-central Alabama, very few isolated populations in the Piedmont of central North Carolina. Growing at elevations several thousand feet higher than *F. gardenii* (Weaver, 1971) Native to the southern Appalachians, growing on dry, sunny ridges (Weaver, 1976)	Peak of Flowering in North Carolina: late April to early May (Weaver, 1971) Flowers appear with the leaves (Weaver, 1971)	April	flowers together with leaves	**spring**
Hamamelis japonica Siebold & Zucc.	Hokkaido, Honshu, Shikoku, Kyushu (FoJ, 1965)	March – April (FoJ, 1965)	February	flowers before leaves	**winter**

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species	distribution (habitat, altitude, localities)	reported flowering time for habitat in literature	flowering onset in Bonn BGB, 2016 (2015 for *H. virginiana*)	succession of leaves and flowers in Bonn BGB	classification of flowering time
Hamamelis mollis Oliv. ex F.B.Forbes & Hemsl.	Thickets, forests; 300 - 800 m China: Anhui, Guangxi, Hubei, Hunan, Jiangxi, Sichuan, Zhejiang (FoC, 2003) 1300 - 2500 m; China: Hubei and Jiangxi (Harms, 1930)	April – May (FoC, 2003) End of March to beginning of April (Harms, 1930)	January	flowers before leaves	**winter**
Hamamelis vernalis Sarg.	Confined to gravelly beds and rocky banks of streams. USA: Interior Highlands of Missouri, Arkansas, and eastern Oklahoma (Bradford and Marsh, 1977)	Early spring-blooming: January to mid-March; some flowers were found opening in late November (Bradford and Marsh, 1977)	February	flowers before leaves	**winter**
Hamamelis virginiana L.	Occurs in open woodlands. Canada, USA: Canada to Florida and the Gulf Coast, and from the Atlantic Coast to Iowa, Missouri, eastern Oklahoma, and eastern Texas (Bradford and Marsh, 1977)	Fall-blooming: mainly October – November, occasional early flowering in September or persisting until late December (Bradford and Marsh, 1977) Late September to late November. Little else in flower, blooming at the marginal extreme of the growing season (Anderson and Hill, 2002)	September	flowers together with leaf senescence	**autumn**
Loropetalum chinense (R. Br.) Oliv.	Forests, sunny hills; 1000 - 1200 m China: Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangsu, Jiangxi, Sichuan, Yunnan, Zhejiang. East and North India, Japan (FoC, 2003) Japan: Honshu (Ise Prov.) (FoJ, 1965)	March – April (FoC, 2003) May (FoJ, 1965)	May	leaves before flowers	**spring**
Parrotia persica (DC.) C.A.Mey.	Moist and deciduous Hyrcanian forest region south and south-west of the Caspian Sea. Western limit: Talish Mountains in Azerbaijan; eastern limit Gorgan province in northern Iran; one small, disjunct population in forests southeast of the Great Caucasus (Nicholson, 1989; Safarov, 1977, cited from Sefidi et al., 2010) Native to northern Iran and endemic to the Alborz Mountains (Sefidi et al., 2010) The weather is humid and mild, with a relatively limited range of temperature fluctuations. Spring is the driest part of the year, and fall and winter the wettest (Nicholson, 1989) In Iran limited to elevations ranging from 150 to 700 m (Mahjoob, 2006, cited from Sefidi et al., 2010)	(Philadelphia, USA: March; Nicholson 1989) (Arnold Arboretum, Massachusetts, USA: April – May; Weaver, 1976) Iran: Region Asalem, 600 m altitude: Flowering March 5-10 Leaf onset: April 5-10 Region Galangrud, 200 m altitude: Flowering March 20-25 Leaf onset: March 27 to April 3 (Mirbadin and Dastmalchi, 2001)	January, both male and female phase	flowers before leaves	**winter**
Parrotiopsis jacquemontiana (Decne.) Rehder	Kashmir, West Afghanistan, Pakistan (Bogle, 1970) Pakistan: Hills, 1.200 - 2.800 m Kashmir, Murree, Hazara, Swat, Kuram (FoP, 2013)	Mar – May (FoP, 2013) May (Wendelbo, 1968)	April	flowers before leaves	**spring**
Sinowilsonia henryi Hemsl.	Forests; 1000 - 1500 m China: Gansu, Henan, Hubei, Shaanxi, Shanxi, Sichuan (FoC, 2003)	March – May (FoC, 2003)	April, both male and female inflorescences	flowers together with leaves	**spring**
Sycopsis sinensis Oliv.	Mountain thickets, evergreen forests; 1300 – 1500 m China: Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangxi, Shaanxi, Sichuan, Taiwan, Yunnan, Zhejiang (FoC, 2003)	April – June (FoC, 2003) Genus *Sycopsis*: "Spring-flowering evergreen trees or shrubs (…)" (Walker, 1944, p. 335)	Male phase: January Female phase: March	evergreen	**winter**

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C. sinensis: http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200010520;

C. veitchiana: http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200010525;

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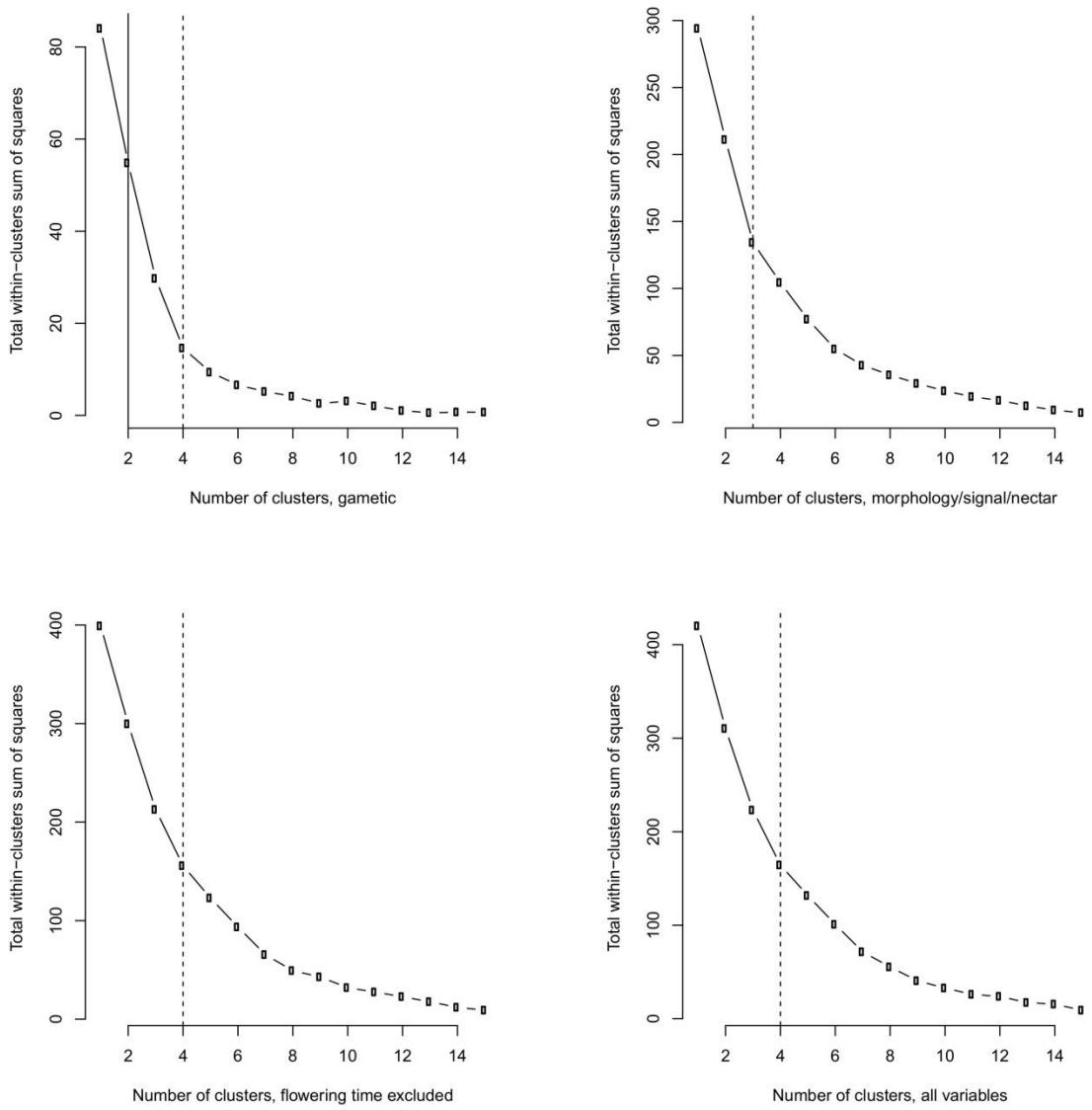

FIGURE A X.2.4: SELECTION OF CLUSTERS FOR DATASETS OF HAMAMELIDACEAE

Presented are the appropriate numbers of clusters, as retrieved via Elbow method. Charts are showing total intra-cluster variation (y-axis: total within-cluster sum of squares) in dependence to k clusters (x-axis). The position of a bend in the plot is considered as indicator for the best number of clusters, with higher cluster numbers not adding substantially to the compactness of the clusters. Top left: Only gametic variables. Elbow-method suggests four clusters, but two clusters (solid line) were selected after visual inspection of the cluster dendrogram, representing a general split between higher and lower gamete production. When considering four clusters, *Sycopsis* and *Parrotia* are forming an additional cluster amongst species showing higher gamete production, and *Disanthus* together with *Hamamelis virginiana*, *H. japonica* and *H. vernalis* are forming a separate cluster within species of lower gamete production (compare figure A X.2.5). Top right: Only non-gametic variables (morphology, signal, reward, without flowering time). Three clusters selected, additional clusters are not adding substantially to compactness (monotonous, linear progress for the next three clusters). Bottom left: All variables except flowering time. Four clusters selected, additional clusters are not adding substantially to compactness (monotonous, linear progress for the next three clusters). Bottom right: All variables, including flowering time. Four clusters selected, additional clusters are not adding substantially to compactness (monotonous, linear progress for the next three clusters).

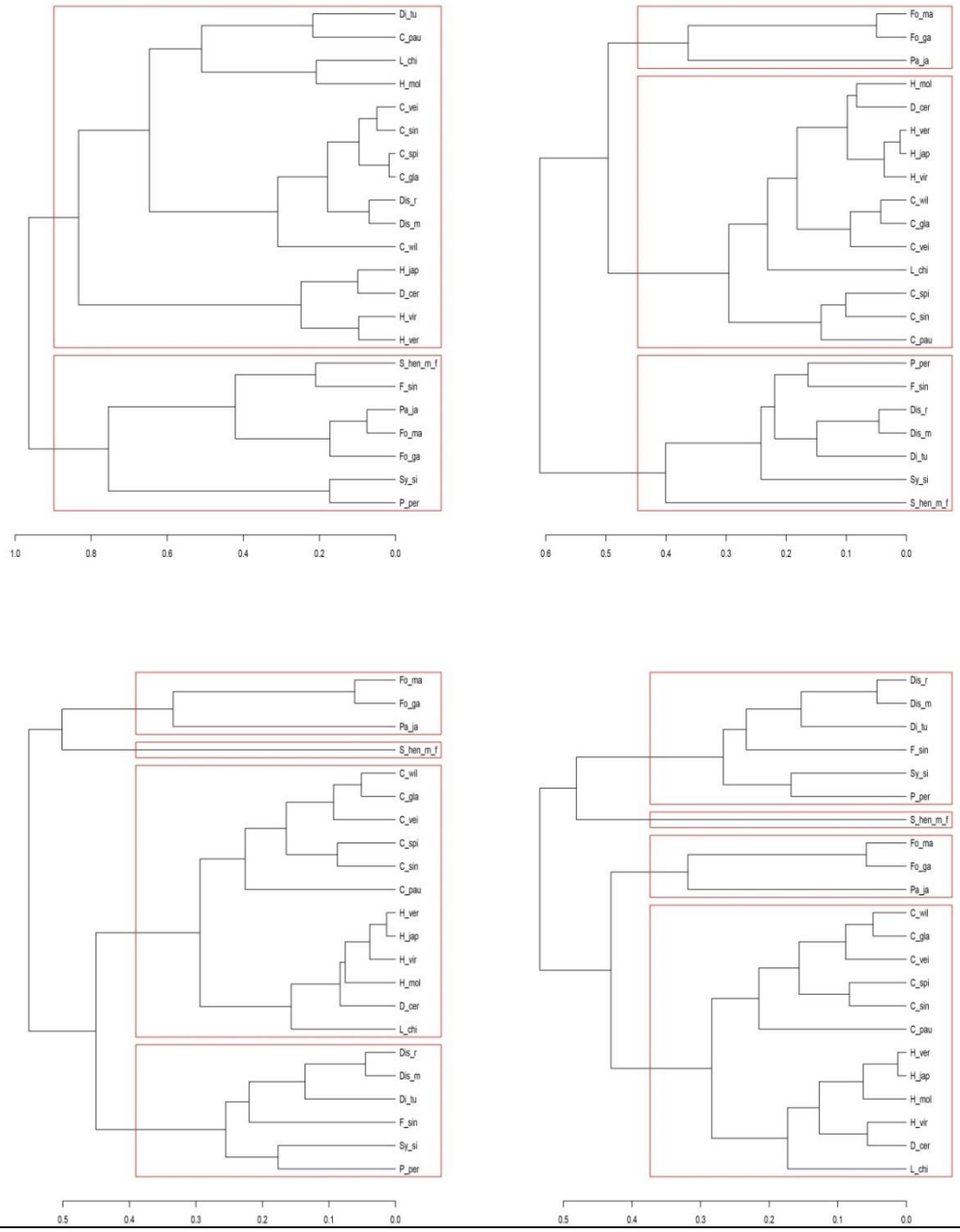

FIGURE A X.2.5: HIERARCHICAL CLUSTERING OF HAMAMELIDACEAE DATASETS

Hierarchical clustering of species arithmetic means for a) gametic variables (top - left), b) non-gametic variables of morphology, signal and nectar reward (top - right), c) combined clustering of gametic and non-gametic variables without flowering time (bottom - left) and d) after adding flowering time (bottom - right). Bray Curtis index applied for clustering of gametic variables, otherwise Gower index was used. R function cophenetic (package vegan) was used to test for performance of linkage method by measuring the correlation between original dissimilarities and dissimilarites estimated from the created trees. Linkage methods usually performed similar and with good results for all dissimilarity matrices: a) complete linkage .86, average linkage .88; single linkage .71; for b) complete linkage .90, average linkage .93; single linkage .92; for c) complete linkage .89, average linkage .92; single linkage .91; for d) complete linkage .87, average linkage .89; single linkage .90. Clustering finally performed via complete linkage to account for monophyly of the family. Two major clusters were selected for gametic variables, three for non-gametic variables and four for the combination of gametic and non-gametic information (compare figure A X.2.4). In general, combined clustering replicates the result of non-gametic clustering. However, *Sinowilsonia* (S_hen; data from male (m) and female inflorescences (f)) emerges as a separate cluster in the combined clustering of gametic and non-gametic data, either aligning to genera *Fothergilla* and *Parrotiopsis* (without flowering time introduced) or aligning to genera *Distylium*, *Distyliopsis*, *Fortunearia*, *Sycopsis* and *Parrotia* (after adding flowering time).

TABLE A X.2.6: CORRELATIONS BETWEEN RATIO SCALED AND ORDINAL VARIABLES OF HAMAMELIDACEAE

Presented are Kendalls´s τ_b correlations for arithmetic means of ratio scaled variables, as well as correlations between ratio scaled and ordinal variables (presence of staminate flowers, a showy colour signal and nectar reward). The number of asterisks shows the significance of the observed correlation (2-tailed; *p = .05; ** p = .01). Correlations from .1 to .3 are rated as low, from .3 to .5 as moderate, from .5 and above as strong (Cohen, 1988).

τ_b	pollen per anther	anthers per flower	pollen per flower	flowers per infl.	anthers per infl.	pollen per infl.	ovules per infl.	p/o ratio	anther length	filament length	style length	petal length	petal width	infl. size frontal	infl. size lateral	pres. stam. flowers	pres. colour signal	pres. nectar reward
pollen per anther	1.000	.127	**.576****	-.017	.087	**.307***	**-.321***	**.576****	**.523****	.013	.170	-.258	-.147	-.247	-.100	.298	**-.533****	-.016
anthers per flower		1.000	**.572****	.209	**.410****	**.400***	.146	**.481****	.077	**.545****	**.401***	**-.322***	.014	.236	.254	.114	-.148	-.247
pollen per flower			1.000	.182	**.321***	**.506****	-.087	**.827****	.303	**.319***	.230	**-.401***	-.174	.022	.100	.289	**-.443***	-.252
flowers per inflorescence				1.000	**.791****	**.677****	**.661****	.165	-.156	.114	**.357***	-.130	.045	**.408****	**.625****	.161	-.109	-.252
anthers per inflorescence					1.000	**.781****	**.504****	**.321***	-.046	**.306***	**.340***	-.237	.027	**.408****	**.625****	.174	-.187	-.306
pollen per inflorescence						1.000	**.338***	**.489****	.101	.223	.274	**-.321***	-.103	.273	**.455****	.289	**-.366***	**-.369***
ovules per inflorescence							1.000	-.174	**-.413****	.000	.183	.040	.286	**.347***	**.529****	-.019	.264	-.038
p/o ratio								1.000	**.403***	.301	.265	**-.472****	-.272	.004	.048	.340	**-.559****	-.294
anther length									1.000	-.037	-.046	-.255	**-.364***	**-.321***	-.220	**.462***	**-.687****	-.312
filament length										1.000	**.439****	-.153	.113	**.319***	.231	-.058	.071	.081
style length											1.000	-.152	.094	.257	**.439****	-.039	.006	.102
petal length												1.000	**.630****	.258	.071	**-.390***	**.654****	**.358***
petal width													1.000	.281	.219	**-.378***	**.696****	**.608****
inflorescence size frontal														1.000	**.558****	-.276	**.366***	-.037
inflorescence size lateral															1.000	-.045	.135	-.112
presence staminate flowers																1.000	**-.676****	**-.524****
presence colour signal																	1.000	**.612****

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TABLE A X.2.7: GENBANK ACCESSION NUMBERS FOR OUTGROUP SPECIES

species	ITS	ETS	trnH-psbA	trnL-trnF	atpB-rbcL	rps16
Exbucklandia tonkinensis (Lecomte) H.T.Chang	GU576650	GU576616	GU576753	GU576821	GU576581	GU576786
Altingia yunnanensis Rehder & E.H.Wilson	GU576644	GU576610	GU576748	GU576815	GU576576	GU576781
Exbucklandia populnea (R.Br. ex Griff.) R.W.Br.	GU576649	GU576615	GU576752	GU576820	GU576580	GU576785
Liquidambar styraciflua L.	GU576670	GU576636	GU576773	GU576841	GU576601	GU576806

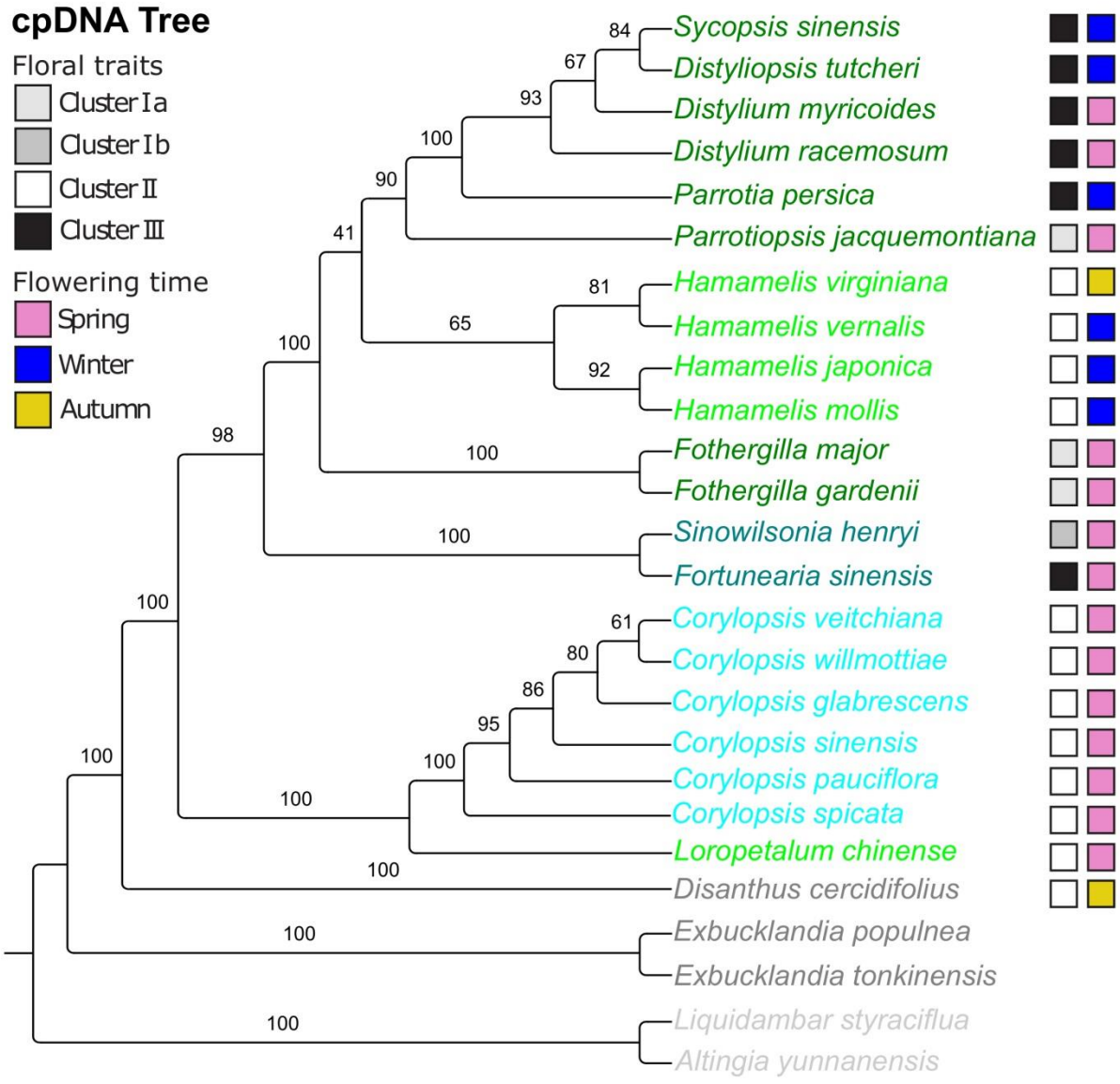

FIGURE A X.2.6: CPDNA MAXIMUM LIKELIHOOD TREE FOR HAMAMELIDACEAE
Tree rooted with the Altingiaceae. Values corresponding to bootstrap support are presented above branches. Flowering time and retrieved clusters of floral plant traits indicated. Species names coloured according to taxonomic classification: Altingiaceae in light grey; Hamamelidaceae, subfamily Exbucklandioideae (including Disanthoideae) in dark grey. Hamamelidaceae, subfamily Hamamelidoideae: Tribe Hamamelideae in light green, tribe Fothergilleae in dark green, tribe Eustigmateae in bluish green, tribe Corylopsideae in aqua.

TABLE A X.2.8: ANALYSIS OF PHYLOGENETIC SIGNAL FOR HAMAMELIDACEAE SPECIES

Phylogenetic signal was tested via pagel´s lambda for performed NMDS ordinations and species coordinates (a), for retrieved clusters of gametic, non-gametic and combined datasets of analysed variables (b), as well as for isolated plant trait variables (c). Presented are results for chloroplast as well as for nuclear markers, and presence of phylogenetic signal is further highlighted by colour. Phylogenetic signal is detected more often via nuclear markers. Aggregated information of NMDS and hierarchical clustering shows presence of phylogenetic signal in all cases tested for the nuclear tree. For isolated variables, phylogenetic signal could be detected in most cases via nuclear markers, except for gametic variables represented on the single flower level (pollen per anther and flower, p/o ratio), filament length, presence of (functional) unisexual inflorescences and showy colour signal.

analysis of phylogenetic signal (pagel´s lambda)	chloroplast tree	nuclear tree
a) NMDS, species coordinates		
NMDS gametic, x-axis coordinates	0.99	0.98
NMDS gametic, y-axis coordinates	0.00	1.00
NMDS morphology/signal/reward, x-axis coordinates	1.00	1.00
NMDS morphology/signal/reward, y-axis coordinates	1.00	0.99
NMDS all except flowering time, x-axis coordinates	0.99	0.99
NMDS all except flowering time, y-axis coordinates	1.00	0.98
NMDS all variables, x-axis coordinates	0.99	0.99
NMDS all variables, y-axis coordinates	0.00	0.98
b) retrieved clusters		
clusters – gametic variables	1.00	0.94
clusters – morphology/signal/reward	1.00	1.00
clusters – gametic and non-gametic (m/s/r) combined	1.00	1.00
c) plant traits, isolated		
anther length	1.00	0.85
filament length	0.00	0.00
style length	0.00	0.70
anthers per flower	1.00	1.00
flowers per inflorescence	1.00	1.00
anthers per inflorescence	1.00	1.00
presence/absence of staminate flowers	1.00	0.94
bi- vs. (funct.) unisexual inflorescences	0.00	0.00
petal height	0.00	0.99
petal width	0.00	1.00
inflorescence size, frontal	0.00	1.00
inflorescence size, lateral	0.00	0.95
showy colour signal	0.00	0.00
anther colour	1.00	1.00
nectar reward	1.00	1.00
pollen per anther	0.00	0.00
pollen per flower	0.00	0.00
pollen per inflorescence	1.00	1.00
ovules per inflorescence	1.00	1.00
p/o ratio	0.00	0.00
flowering time	1.00	1.00

TABLE A X.2.9: ACCESSION AND VOUCHER NUMBERS FOR ANALYSED HAMAMELIDACEAE
Herbarium BONN, Nees Institut für Biodiversität der Pflanzen, Germany.

species (floral traits and phylogenetic analysis)	accession number	voucher number
Corylopsis glabrescens Franch. & Sav.	28911	1605
Corylopsis pauciflora Siebold & Zucc.	8602	555
Corylopsis sinensis Hemsl.	31329	1779
Corylopsis spicata Siebold & Zucc.	4964	2656
Corylopsis veitchiana Bean	31497	556
Corylopsis willmottiae Rehder & E.H.Wilson	36869	1428
Disanthus cercidifolius Maxim.	37187	1600
Distyliopsis tutcheri (J.H.Hemsl.) Endress	36795	1764
Distylium myricoides Hemsl.	28912	1621
Distylium racemosum Siebold & Zucc.	22135	1595
Fortunearia sinensis Rehder & E.H.Wilson	5717	934
Fothergilla gardenii L.	37361	1506
Fothergilla major G.Lodd.	9845	940
Hamamelis japonica Siebold & Zucc.	6062	2625
Hamamelis mollis Oliv. ex F.B.Forbes & Hemsl.	6063	565
Hamamelis vernalis Sarg.	23044	1466
Hamamelis virginiana L.	32739	1602
Loropetalum chinense (R. Br.) Oliv.	37976	1777
Parrotia persica (DC.) C.A.Mey.	12241	534
Parrotiopsis jacquemontiana (Decne.) Rehder	8766	935
Sinowilsonia henryi Hemsl.	31503	1593
Sycopsis sinensis Oliv.	11578	600

X.3 APPENDIX TO CHAPTER IV

TABLE A X.3.1: DATA BANDWIDTH OF MORPHOLOGICAL VARIABLES OF DROSERACEAE AND *PINGUICULA*

The table shows the number of analysed samples (*n*), arithmetic means for frontal and lateral display size (in cm^2) and, only for *Pinguicula*, the size of the flower opening (in cm^2) and spur length (cm). For display size ratio (frontal:lateral) only arithmetic means are presented, otherwise corresponding standard deviations are shown.*) only single measurement.

species	*n*	frontal display in cm^2			lateral display in cm^2			f:l ratio	opening in cm^2			spur length in cm		
Droseraceae														
Dionaea muscipula J.Ellis	10	2.29	±	.55	.83	±	.26	2.9	-			-		
Drosera adelae F.Muell.	10	0.19	±	.03	.04	±	.01	4.6	-			-		
D. aliciae Raym.-Hamet	10	0.79	±	.12	.47	±	.05	1.7	-			-		
D. anglica Huds.	1	.41	*		.17	*		2.4	-			-		
D. binata Labill.	10	6.34	±	.84	2.29	±	.47	2.9	-			-		
D. callistos N.G.Marchant & Lowrie	10	1.41	±	.47	.44	±	.19	4.1	-			-		
D. capensis L.	5	3.18	±	.67	1.15	±	.29	2.8	-			-		
D. capensis f. alba L.	10	.67	±	.18	.42	±	.13	1.6	-			-		
D. capensis f. rubra L.	10	1.62	±	.80	.38	±	.15	4.2	-			-		
D. dichrosepala Turcz.	5	.13	±	.03	.08	±	.01	1.7	-			-		
D. filiformis Raf.	10	1.56	±	.51	.67	±	.18	2.3	-			-		
D. intermedia Hayne	10	.12	±	.04	.06	±	.02	1.9	-			-		
D. leioblastus N.G.Marchant & Lowrie	1	.06	*		.02	*		2.5	-			-		
D. menziesii ssp. menziesii R.Br. ex DC.	5	3.85	±	1.36	1.25	±	.54	3.3	-			-		
D. paleacea subsp. roseana (N.G.Marchant & Lowrie) Schlauer	10	.14	±	.04	.05	±	.02	3.2	-			-		
D. paradoxa Lowrie	1	.49	*		.11	*		4.5	-			-		
D. platystigma Lehm.	1	1.79	*		0.36	*		4.9	-			-		
D. regia Stephens	5	4.27	±	1.43	1.54	±	.41	2.9	-			-		
D. rotundifolia L.	10	.18	±	.03	.06	±	.01	2.8	-			-		
D. spatulata Labill.	5	.24	±	.03	.08	±	.01	3.1	-			-		
D. spatulata (*var. lovellae*) Labill.	10	.31	±	.05	.10	±	.09	3.8	-			-		
Pinguicula														
P. agnata Casper	5	5.43	±	.67	1.63	±	.40	3.4	.10	±	.02	.5	±	.1
P. cyclosecta Casper	10	3.83	±	.36	1.14	±	.20	3.4	.02	±	.00	2.4	±	.2
P. ehlersiae Speta & F. Fuchs	10	2.92	±	.24	.75	±	.17	4.0	.02	±	.00	2.1	±	.2
P. emarginata Zamudio & Rzed.	10	.48	±	.14	.22	±	.06	2.3	.01	±	.00	.7	±	.1
P. esseriana B. Kirchn.	10	1.46	±	.24	.58	±	.07	2.5	.04	±	.01	1.6	±	.1
P. gigantea Luhrs	10	7.10	±	1.08	2.43	±	.49	3.0	.06	±	.02	.8	±	.1
P. gracilis Zamudio	5	1.00	±	.13	.31	±	.04	3.2	.06	±	.01	.5	±	.0
P. gypsicola Brandegee	10	1.71	±	.42	.76	±	.14	2.2	.02	±	.00	1.9	±	.2
P. hemiepiphytica Zamudio & Rzed.	5	6.13	±	2.57	1.74	±	.58	3.5	.07	±	.02	3.3	±	.4
P. hirtiflora var. hirtiflora Ten.	5	.56	±	.20	.24	±	.09	2.4	.06	±	.01	.6	±	.2
P. ibarrae Zamudio	10	4.24	±	.65	1.98	±	.38	2.2	.13	±	.03	.6	±	.1
P. jaumavensis Debbert	10	1.63	±	.24	.72	±	.14	2.3	.04	±	.01	1.5	±	.1
P. laueana Speta & F. Fuchs	5	4.70	±	1.57	1.65	±	.32	2.8	.08	±	.02	4.5	±	.7
P. medusina Zamudio & Studničk̆a	5	.70	±	.11	.39	±	.10	1.9	.05	±	.02	.3	±	.1
P. mirandae Zamudio & A. Salinas	5	1.66	±	.12	.82	±	.25	2.2	.06	±	.01	.4	±	.0
P. moctezumae Zamudio & R.Z. Ortega	10	9.55	±	2.15	2.64	±	.73	3.8	.05	±	.02	3.7	±	.2
P. moranensis Kunth	10	5.83	±	2.32	1.98	±	.65	3.0	.04	±	.01	2.9	±	.3
P. moranensis f. alba Kunth	10	6.91	±	1.40	1.69	±	.36	4.1	.04	±	.01	2.4	±	.2
P. potosiensis Speta & F.Fuchs	1	4.99	*		2.61	*		1.9	.01	*		3.3	*	
P. rectifolia Speta & F.Fuchs	10	2.39	±	.75	1.29	±	.42	1.9	.02	±	.01	2.4	±	.3

TABLE A X.3.2: CATEGORISATION OF OPTICAL FLOWER AND LEAF SIGNAL OF DROSERACEAE AND *PINGUICULA*

The table shows the categorisation of optical signal of flowers and leaves for the analysed Droseraceae and *Pinguicula*: Predominant colour of the frontal display (white: ○, also white with pink nectar guides ◎; reddish colours: violet to pink ●, orange ●, red ●), nectar spur colour (green ●, violet to pink ●), anther colour (if openly visible; whitish ○ or yellow ●), anther dummy signal (greenish-yellow ■, yellow ■ or whitish □) and UV signal of respective flower parts (absorption ●, diffuse ●, reflection with darker flower centre ⊙, full reflection ○). Leaf colour is usually green (■), however secreting hairs of *Drosera* are often reddish or the leaf shows a mixed green to red pattern (■, variable, rarely completely reddish ■). Leaves reflect UV light in few cases (□), but usually show a mixed pattern (⊡).

species	flower colour	UV front	UV lat	spur colour	UV spur	anther colour	anther dummy	UV anther (dummy)	leaf colour	UV leaf
Droseraceae										
Dionaea muscipula J.Ellis	○	●	●	-	-	●	-	●	■	⊡
Drosera adelae F.Muell.	●	●	●	-	-	●	-	●	■	□
D. aliciae Raym.-Hamet	●	●	●	-	-	●	-	○	■	⊡
D. anglica Huds.	○	●	●	-	-	●	-	●	■	⊡
D. binata Labill.	○	●	●	-	-	●	-	○	■	⊡
D. callistos N.G.Marchant & Lowrie	●	⊙	○	-	-	●	-	●	■	⊡
D. capensis L.	●	⊙	○	-	-	●	-	●	■	⊡
D. capensis f. alba L.	○	⊙	○	-	-	●	-	●	■	⊡
D. capensis f. rubra L.	●	⊙	○	-	-	●	-	○	■	□
D. dichrosepala Turcz.	○	●	●	-	-	●	-	●	■	⊡
D. filiformis Raf.	●	●	●	-	-	●	-	●	■	⊡
D. intermedia Hayne	○	●	●	-	-	●	-	●	■	⊡
D. leioblastus N.G.Marchant & Lowrie	○	●	●	-	-	○	-	●	■	⊡
D. menziesii ssp. menziesii R.Br. ex DC.	●	⊙	○	-	-	●	-	○	■	⊡
D. paleacea subsp. roseana (N.G.Marchant & Lowrie) Schlauer	○	●	●	-	-	●	-	○	■	⊡
D. paradoxa Lowrie	●	●	●	-	-	●	-	●	■	⊡
D. platystigma Lehm.	●	⊙	○	-	-	●	-	○	■	⊡
D. regia Stephens	○	●	●	-	-	●	-	●	■	⊡
D. rotundifolia L.	○	●	●	-	-	○	-	○	■	⊡
D. spatulata Labill.	●	●	○	-	-	●	-	●	■	⊡
D. spatulata (*var. lovellae*) Labill.	○	●	●	-	-	●	-	●	■	⊡
Pinguicula										
P. agnata Casper	◎	●	○	●	●	-	■	●	■	⊡
P. cyclosecta Casper	●	●	○	●	○	-	□	●	■	⊡
P. ehlersiae Speta & F. Fuchs	●	⊙	○	●	○	-	■■	●	■	⊡
P. emarginata Zamudio & Rzed.	◎	●	●	●	○	-	■	●	■	⊡
P. esseriana B. Kirchn.	●	⊙	○	●	○	-	■	●	■	⊡
P. gigantea Luhrs	●	●	○	●	●	-	■■	●	■	⊡
P. gracilis Zamudio	○	●	●	●	●	-	■	●	■	⊡
P. gypsicola Brandegee	●	●	○	●	○	-	□	●	■	⊡
P. hemiepiphytica Zamudio & Rzed.	●	⊙	○	●	○	-	□	●	■	⊡
P. hirtiflora var. hirtiflora Ten.	●	●	●	●	●	-	■	●	■	⊡
P. ibarrae Zamudio	○	●	○	●	○	-	■	●	■	⊡
P. jaumavensis Debbert	●	⊙	○	●	○	-	■■	●	■	⊡
P. laueana Speta & F. Fuchs	●	●	○	●	○	-	-	-	■	⊡
P. medusina Zamudio & Studnička	●	●	●	●	○	-	■	●	■	⊡
P. mirandae Zamudio & A. Salinas	●	●	○	●	○	-	■	●	■	⊡
P. moctezumae Zamudio & R.Z. Ortega	●	●	○	●	○	-	■□	●	■	⊡
P. moranensis Kunth	●	●	○	●	○	-	■□	●	■	⊡
P. moranensis f. alba Kunth	○	●	○	●	○	-	■	●	■	⊡
P. potosiensis Speta & F.Fuchs	●	●	○	●	●	-	□	●	■	⊡
P. rectifolia Speta & F.Fuchs	●	●	○	●	○	-	■□	●	■	⊡

TABLE A X.3.3: DATA BANDWIDTH OF NECTAR REWARD OF DROSERACEAE AND *PINGUICULA*

The table shows the total number of flowers sampled for the measurement of nectar production (n_1, first showing the total number of sampled flowers, and second the share of nectariferous flowers), the average nectar production in µl for nectariferous flowers, the number of samples useable for measurement of nectar concentration with the technique applied (n_2), the average nectar concentration in percent and the average amount of sugar per flower in mg. Results are presented with corresponding standard deviation (- = no nectar present; x = not detectable with applied method).

species	n_1	nectar amount per nectariferous flower in µl			n_2	concentration in %			sugar production per flower in mg		
Droseraceae											
Dionaea muscipula J.Ellis	17/17	.340	±	.251	10	28.2	±	7.6	.10	±	.07
Drosera adelae F.Muell.	10/0		-		-		-			-	
D. aliciae Raym.-Hamet	10/0		-		-		-			-	
D. anglica Huds.	10/0		-		-		-			-	
D. binata Labill.	10/0		-		-		-			-	
D. callistos N.G.Marchant & Lowrie	10/0		-		-		-			-	
D. capensis L.	10/0		-		-		-			-	
D. capensis f. alba L.	10/0		-		-		-			-	
D. capensis f. rubra L.	10/0		-		-		-			-	
D. dichrosepala Turcz.	10/0		-		-		-			-	
D. filiformis Raf.	10/0		-		-		-			-	
D. intermedia Hayne	10/0		-		-		-			-	
D. leioblastus N.G.Marchant & Lowrie	10/0		-		-		-			-	
D. menziesii ssp. menziesii R.Br. ex DC.	10/0		-		-		-			-	
D. paleacea subsp. roseana (N.G.Marchant & Lowrie) Schlauer	10/0		-		-		-			-	
D. paradoxa Lowrie	10/0		-		-		-			-	
D. platystigma Lehm.	10/0		-		-		-			-	
D. regia Stephens	10/0		-		-		-			-	
D. rotundifolia L.	10/0		-		-		-			-	
D. spatulata Labill.	10/0		-		-		-			-	
D. spatulata (*var. lovellae*) Labill.	10/0		-		-		-			-	
Pinguicula											
P. agnata Casper	10/0		-		-		-			-	
P. cyclosecta Casper	15/15	1.747	±	.485	15	11.3	±	1.1	.20	±	.06
P. ehlersiae Speta & F. Fuchs	24/22	.789	±	.449	15	15.5	±	4.8	.14	±	.06
P. emarginata Zamudio & Rzed.	15/0		-		-		-			-	
P. esseriana B. Kirchn.	25/25	.557	±	.252	20	11.3	±	6.0	.07	±	.03
P. gigantea Luhrs	25/25	.189	±	.190	5	38.2	±	18.4	.15	±	.15
P. gracilis Zamudio	5/5	.149	±	.071	x		x			x	
P. gypsicola Brandegee	12/12	.260	±	.107	10	9.3	±	2.0	.02	±	.01
P. hemiepiphytica Zamudio & Rzed.	11/11	3.934	±	3.083	10	9.2	±	2.2	.39	±	.27
P. hirtiflora var. hirtiflora Ten.	15/10	.003	±	.003	x		x			x	
P. ibarrae Zamudio	20/12	.036	±	.012	x		x			x	
P. jaumavensis Debbert	34/34	.319	±	.205	15	12.3	±	9.2	.03	±	.03
P. laueana Speta & F. Fuchs	10/10	2.248	±	1.739	10	8.1	±	1.7	.18	±	.16
P. medusina Zamudio & Studnička	10/10	.022	±	.015	x		x			x	
P. mirandae Zamudio & A. Salinas	5/5	.009	±	.012	x		x			x	
P. moctezumae Zamudio & R.Z. Ortega	23/23	9.658	±	3.558	23	9.0	±	4.3	.97	±	.72
P. moranensis Kunth	18/18	1.092	±	.861	18	7.3	±	2.5	.08	±	.06
P. moranensis f. alba Kunth	20/20	4.322	±	4.457	20	7.9	±	2.3	.28	±	.21
P. potosiensis Speta & F.Fuchs	10/10	2.124	±	.878	10	5.1	±	1.1	.11	±	.06
P. rectifolia Speta & F.Fuchs	27/27	1.179	±	1.360	23	7.3	±	2.4	.09	±	.06

TABLE A X.3.4: DATA BANDWIDTH OF GAMETE PRODUCTION OF DROSERACEAE AND *PINGUICULA*

The table shows the number of analysed samples (*n*), the arithmetic means and corresponding standard deviations for anther number, pollen dispersal units (PDU) per anther, PDU per flower, ovule number and PDU to ovule ratio. As for *Dionaea* and *Drosera*, PDUs are pollen tetrads (T), and values for pollen grains (P) are presented for comparison. As for *Pinguicula*, PDU number is pollen grain number.

species	*n*	PDU	anthers	PDU per anther	PDU per flower	ovules	PDU:O ratio
Droseraceae							
Dionaea muscipula J.Ellis	12	T	12.9 ± 2.0	95.6 ± 76.8	1 333.3 ± 1 278.0	29.3 ± 14.5	74.7 ± 94.5
		P		382.5 ± 307.2	5 333.3 ± 5 112.1		298.6 ± 377.9
Drosera adelae F.Muell.	12	T	5.1 ± .3	123.1 ± 100.2	622.9 ± 499.0	61.3 ± 14.3	10.0 ± 7.0
		P		492.5 ± 401.0	2 491.7 ± 1 996.1		39.9 ± 28.2
D. aliciae Raym.-Hamet	12	T	5.1 ± .3	45.4 ± 35.6	235.4 ± 193.8	225.0 ± 55.3	1.0 ± .8
		P		181.7 ± 142.6	941.7 ± 775.1		4.1 ± 3.1
D. anglica Huds.	6	T	5	120.0 ± 83.9	600.0 ± 419.5	112.2 ± 24.6	5.6 ± 4.4
		P		480.0 ± 335.6	2 400.0 ± 1 678.1		22.4 ± 17.4
D. binata Labill.	12	T	5	1 018.3 ± 615.6	5 091.7 ± 3 078.2	327.9 ± 43.6	15.0 ± 7.8
		P		4 073.3 ± 2 462.6	20 366.7 ± 12 312.8		59.9 ± 31.1
D. callistos N.G.Marchant & Lowrie	12	T	4.8 ± .4	166.5 ± 173.8	812.5 ± 874.2	28.0 ± 3.0	28.6 ± 31.0
		P		665.8 ± 695.1	3 250.0 ± 3 496.6		114.6 ± 123.9
D. capensis L.	12	T	5.1 ± .3	332.5 ± 183.0	1 670.8 ± 903.6	299.7 ± 56.3	5.3 ± 2.5
		P		1 330.0 ± 731.8	6 683.3 ± 3 614.6		21.2 ± 10.0
D. capensis f. alba L.	12	T	5	112.5 ± 130.3	562.5 ± 651.3	432.6 ± 45.2	1.3 ± 1.4
		P		450.0 ± 521.0	2 250.0 ± 2 605.1		5.1 ± 5.7
D. capensis f. rubra L.	12	T	5	137.5 ± 140.3	687.5 ± 701.7	250.3 ± 32.4	2.8 ± 2.7
		P		550.0 ± 561.3	2 750.0 ± 2 806.6		11.1 ± 10.9
D. dichrosepala Turcz.	12	T	4.8 ± .4	39.8 ± 33.4	190.6 ± 162.3	8.2 ± 1.5	22.3 ± 16.9
		P		159.4 ± 133.7	762.5 ± 649.2		89.2 ± 67.6
D. filiformis Raf.	12	T	5	107.9 ± 187.0	539.6 ± 934.9	112.9 ± 33.3	5.5 ± 10.3
		P		431.7 ± 747.9	2 158.3 ± 3 739.6		21.9 ± 41.0
D. intermedia Hayne	12	T	5	95.0 ± 64.9	475.0 ± 324.4	100.7 ± 10.7	4.6 ± 3.1
		P		380.0 ± 259.5	1 900.0 ± 1 297.6		18.4 ± 12.3
D. leioblastus N.G.Marchant & Lowrie	12	T	5	35.4 ± 32.4	177.1 ± 162.1	8.8 ± 2.0	20.6 ± 16.8
		P		141.7 ± 129.7	708.3 ± 648.7		82.5 ± 67.3
D. menziesii ssp. menziesii R.Br. ex DC.	12	T	5.4 ± .8	947.2 ± 600.7	5 170.8 ± 3 343.6	166.5 ± 47.4	33.4 ± 23.8
		P		3 788.8 ± 2 402.6	20 683.3 ± 13 374.3		133.4 ± 95.2
D. paleacea subsp. roseana (N.G.Marchant & Lowrie) Schlauer	12	T	5	38.8 ± 33.9	193.8 ± 169.3	6.3 ± 1.0	30.1 ± 26.7
		P		155.0 ± 135.4	775.0 ± 677.1		120.4 ± 106.9
D. paradoxa Lowrie	12	T	4.9 ± .3	119.2 ± 63.7	581.3 ± 308.6	56.2 ± 12.6	10.4 ± 5.3
		P		476.7 ± 255.0	2 325.0 ± 1 234.4		41.8 ± 21.2
D. platystigma Lehm.	6	T	4.8 ± .4	39.2 ± 38.1	193.8 ± 192.3	13.3 ± 3.3	13.0 ± 10.0
		P		156.7 ± 152.4	775.0 ± 769.3		52.1 ± 40.1
D. regia Stephens	12	T	4.4 ± .7	579.9 ± 790.8	2 666.7 ± 3 928.5	136.2 ± 22.2	19.3 ± 26.5
		P		2 319.4 ± 3 163.3	10 666.7 ± 15 714.1		77.0 ± 105.9
D. rotundifolia L.	12	T	4.9 ± .3	53.3 ± 35.2	262.5 ± 177.3	108.7 ± 25.6	2.6 ± 1.8
		P		213.3 ± 141.0	1 050.0 ± 709.0		10.5 ± 7.3
D. spatulata Labill.	12	T	5	81.7 ± 33.5	408.3 ± 167.6	91.8 ± 23.1	4.5 ± 1.7
		P		326.7 ± 134.1	1 633.3 ± 670.6		18.1 ± 6.7
D. spatulata (*var. lovellae*) Labill.	12	T	5	55.0 ± 22.8	275.0 ± 113.8	70.5 ± 11.7	4.0 ± 1.8
		P		220.0 ± 91.1	1 100.0 ± 455.3		16.1 ± 7.3

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species	*n*	PDU	anthers	PDU per anther	PDU per flower	ovules	PDU:O ratio
Pinguicula							
P. agnata Casper	12	P	2	5 316.7 ± 1 846.3	10 633.3 ± 3 692.6	235.3 ± 41.9	47.4 ± 20.8
P. cyclosecta Casper	12	P	2	6 808.3 ± 2 587.5	13 616.7 ± 5 175.1	290.8 ± 54.8	49.7 ± 25.3
P. ehlersiae Speta & F. Fuchs	12	P	2	6 983.3 ± 3 049.2	13 966.7 ± 6 098.5	322.5 ± 37.2	43.9 ± 19.3
P. emarginata Zamudio & Rzed.	12	P	2	1 466.7 ± 1 052.6	2 933.3 ± 2 105.1	66.3 ± 10.7	46.8 ± 38.0
P. esseriana B. Kirchn.	12	P	2	4 900.0 ± 1 492.4	9 800.0 ± 2 984.8	250.7 ± 53.3	41.2 ± 18.9
P. gigantea Luhrs	12	P	2	5 425.0 ± 3 737.6	10 850.0 ± 7 475.1	327.83 ± 46.6	35.2 ± 26.6
P. gracilis Zamudio	12	P	2	2 316.7 ± 913.4	4 633.3 ± 1 826.7	121.3 ± 10.2	38.4 ± 16.0
P. gypsicola Brandegee	12	P	2	6 041.7 ± 2 066.4	12 083.3 ± 4 132.8	238.0 ± 76.5	65.3 ± 51.3
P. hemiepiphytica Zamudio & Rzed.	6	P	2	10 250.0 ± 4 838.9	20 500.0 ± 9 677.8	304.0 ± 38.0	70.2 ± 37.2
P. hirtiflora var. hirtiflora Ten.	12	P	2	2 591.7 ± 929.8	5 183.3 ± 1 859.5	66.2 ± 17.7	84.1 ± 40.6
P. ibarrae Zamudio	12	P	2	5 191.7 ± 2 351.2	10 383.3 ± 4 702.4	485.0 ± 122.7	21.4 ± 11.7
P. jaumavensis Debbert	12	P	2	6 091.7 ± 1 818.3	12 183.3 ± 3 636.6	281.4 ± 64.0	43.5 ± 8.7
P. laueana Speta & F. Fuchs	3	P	2	13 300.0 ± 1 997.5	26 600.0 ± 3 995.0	364.7 ± 23.2	73.0 ± 10.1
P. medusina Zamudio & Studničkа	6	P	1.7 ± .5	7 475.0 ± 2 458.6	11 550.0 ± 2 729.7	267.8 ± 58.8	45.6 ± 18.6
P. mirandae Zamudio & A. Salinas	6	P	2	4 675.0 ± 1 372.9	9 350.0 ± 2 745.7	76.2 ± 20.0	133.9 ± 64.3
P. moctezumae Zamudio & R.Z. Ortega	12	P	2	9 591.7 ± 4 464.7	19 183.3 ± 8 929.4	428.3 ± 71.6	44.0 ± 18.1
P. moranensis Kunth	12	P	2	10 858.3 ± 9 259.4	21 716.7 ± 18 518.9	367.8 ± 49.3	61.1 ± 52.1
P. moranensis f. alba Kunth	12	P	2	16 458.3 ± 8 946.5	32 916.7 ± 17 892.9	426.9 ± 47.4	78.1 ± 43.1
P. potosiensis Speta & F.Fuchs	6	P	2	10 783.3 ± 5 596.2	21 566.7 ± 11 192.4	448.3 ± 71.4	48.5 ± 25.5
P. rectifolia Speta & F.Fuchs	12	P	2	7 775.0 ± 4 331.1	15 550.0 ± 8 662.2	313.3 ± 78.1	52.5 ± 32.2

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TABLE A X.3.5: BREEDING SYSTEMS OF DROSERACEAE AND *PINGUICULA*

The table shows the total number of bagged, unmanipulated flowers (n_1) analysed for absence (-) or presence (+) of spontaneous autogamy. In case of absence of spontaneous autogamy, the table also shows the total number of flowers bagged and hand pollinated (n_2) in order to test for self-compatibility. For the self-compatibility test, seed set was classified in three categories: vigorous (+), strongly impaired (only few seeds, +/-) or no seed set (-). Derived breeding systems are classified in three categories: *autogamous* (in case of spontaneous autogamy present), *facultative xenogamous* (if fruit set only occurred after hand pollination), and *obligate xenogamous* (if plants showed self-incompatibility). *) As for *Dionaea*, two plant individuals showed spontaneous autogamy (∑ 29 flowers), two other not (∑ 36 flowers).

species	n_1	autogamy	n_2	self-compatibility			breeding system
				+	+/-	-	
Droseraceae							
Dionaea muscipula J.Ellis	29; 36*	+/-*	-				autogamous
Drosera adelae F.Muell.	40	-	8			8	obligate xenogamous
D. aliciae Raym.-Hamet	20	+	-				autogamous
D. anglica Huds.	5	+	-				autogamous
D. binata Labill.	27	-	50			50	obligate xenogamous
D. callistos N.G.Marchant & Lowrie	15	-	5			5	obligate xenogamous
D. capensis L.	25	+	-				autogamous
D. capensis f. alba L.	38	+	-				autogamous
D. capensis f. rubra L.	26	+	-				autogamous
D. dichrosepala Turcz.	16	-	5			5	obligate xenogamous
D. filiformis Raf.	37	+	-				autogamous
D. intermedia Hayne	24	+	-				autogamous
D. leioblastus N.G.Marchant & Lowrie	30	-	10			10	obligate xenogamous
D. menziesii ssp. menziesii R.Br. ex DC.	18	-	7			7	obligate xenogamous
D. paleacea subsp. roseana (N.G.Marchant & Lowrie) Schlauer	50	-	5			5	obligate xenogamous
D. paradoxa Lowrie	15	-	3			3	obligate xenogamous
D. platystigma Lehm.	15	-	3			3	obligate xenogamous
D. regia Stephens	5	-	4	4			facultative xenogamous
D. rotundifolia L.	15	+	-				autogamous
D. spatulata Labill.	18	+	-				autogamous
D. spatulata (*var. lovellae*) Labill.	26	+	-				autogamous
Pinguicula							
P. agnata Casper	15	-	10		4	6	facultative xenogamous
P. cyclosecta Casper	33	-	14			14	obligate xenogamous
P. ehlersiae Speta & F. Fuchs	25	-	14			14	obligate xenogamous
P. emarginata Zamudio & Rzed.	17	-	12		3	9	facultative xenogamous
P. esseriana B. Kirchn.	39	-	9			9	obligate xenogamous
P. gigantea Luhrs	15	-	14	4		10	facultative xenogamous
P. gracilis Zamudio	31	-	5			5	obligate xenogamous
P. gypsicola Brandegee	19	-	17		6	11	facultative xenogamous
P. hemiepiphytica Zamudio & Rzed.	15	-	4	1		3	facultative xenogamous
P. hirtiflora var. hirtiflora Ten.	10	-	6		2	4	facultative xenogamous
P. ibarrae Zamudio	21	-	10	7		3	facultative xenogamous
P. jaumavensis Debbert	26	-	16	11		5	facultative xenogamous
P. laueana Speta & F. Fuchs	6	-	5	4		1	facultative xenogamous
P. medusina Zamudio & Studnička	10	-	5	4		1	facultative xenogamous
P. mirandae Zamudio & A. Salinas	10	-	4		1	3	facultative xenogamous
P. moctezumae Zamudio & R.Z. Ortega	15	-	5			5	obligate xenogamous
P. moranensis Kunth	15	-	6	1	3	2	facultative xenogamous
P. moranensis f. alba Kunth	17	-	12	11		1	facultative xenogamous
P. potosiensis Speta & F.Fuchs	5	-	1		1		facultative xenogamous
P. rectifolia Speta & F.Fuchs	15	-	12	2	4	6	facultative xenogamous

TABLE A X.3.6: ACCESSION NUMBERS FOR DROSERACEAE AND *PINGUICULA*
Bonn University Botanical Gardens.

species	accession number
Droseraceae	
Dionaea muscipula J.Ellis	27212, 27214, 9014, 14515
Drosera adelae F.Muell.	1830
D. aliciae Raym.-Hamet	15112
D. anglica Huds.	28347
D. binata Labill.	1833
D. callistos N.G.Marchant & Lowrie	11816
D. capensis L.	28300
D. capensis f. alba L.	10163
D. capensis f. rubra L.	8565
D. dichrosepala Turcz.	34543
D. filiformis Raf.	25906
D. intermedia Hayne	28376
D. leioblastus N.G.Marchant & Lowrie	11817
D. menziesii ssp. menziesii R.Br. ex DC.	30038
D. paleacea subsp. roseana (N.G.Marchant & Lowrie) Schlauer	34544
D. paradoxa Lowrie	28382, 16949
D. platystigma Lehm.	11815
D. regia Stephens	1859
D. rotundifolia L.	28383
D. spatulata Labill.	30120
D. spatulata (*var. lovellae*) Labill.	1855, 30120
Pinguicula	
P. agnata Casper	9161, 17651
P. cyclosecta Casper	16420
P. ehlersiae Speta & F. Fuchs	17644, 17645
P. emarginata Zamudio & Rzed.	16421
P. esseriana B. Kirchn.	8330, 17643
P. gigantea Luhrs	17648, 25530
P. gracilis Zamudio	16563, 17159, 17649
P. gypsicola Brandegee	12820
P. hemiepiphytica Zamudio & Rzed.	17702
P. hirtiflora var. hirtiflora Ten.	38764
P. ibarrae Zamudio	25876
P. jaumavensis Debbert	13663, 17655
P. laueana Speta & F. Fuchs	13664
P. medusina Zamudio & Studnička	14448
P. mirandae Zamudio & A. Salinas	27379
P. moctezumae Zamudio & R.Z. Ortega	17653, 17160
P. moranensis Kunth	17161, 17162, 17163
P. moranensis f. alba Kunth	13665
P. potosiensis Speta & F.Fuchs	16947
P. rectifolia Speta & F.Fuchs	16565

X.4 APPENDIX TO CHAPTER V

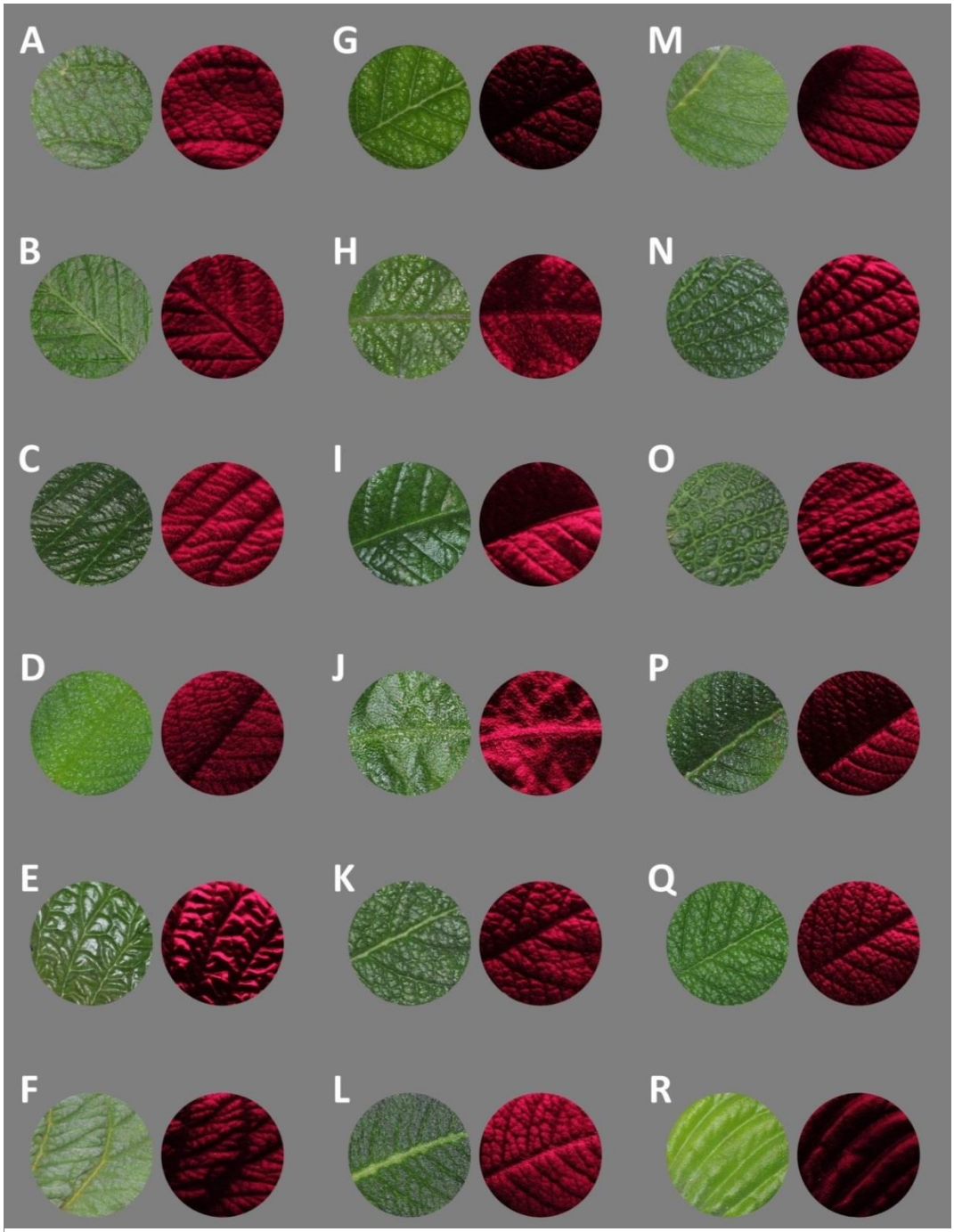

FIGURE A X.4.1: OPTICAL SIGNAL OF *STREPTOCARPUS* LEAVES IN DAYLIGHT AND UV LIGHT
Optical signal of leaves in full daylight spectrum (left) and UV light (right, 1 sec. exposure time), presented for 18 *Streptocarpus* species. All leaves greenish, showing a mixed pattern of UV absorption and reflection, therefore contrasting strongly to floral colour and UV signal. A: *Streptocarpus binseili* Eb.Fisch.; B: *S. burundianus* Hilliard & B.L.Burtt; C: *S. cooperi* C.B.Clarke; D: *S. cyaneus ssp. polackii* (B.L.Burtt) Weigend & T.J.Edwards; E: *S. denticulatus* Turrill; F: *S. dunnii* Mast.; G: *S. fasciatus* T.J.Edwards & C.Kunhardt; H: *S. gardenii* Hook.; I: *S. johannis* L.L.Britten; J: *S. liliputana* Bellstedt & T.J.Edwards; K: *S. longiflorus* (Hilliard & B.L.Burtt) T.J.Edwards; L: *S. modestus* L.L.Britten; M: *S. pole-evansii* I.Verd.; N: *S. polyanthus* Hook.; O: *S. pusillus* Harv. ex C.B.Clarke; P: *S. rexii* (Bowie ex Hook.) Lindl.; Q: *S. roseoalbus* Weigend & T.J.Edwards; R: *S. wilmsii* Engl. Pictures by Andreas W. Mues.

TABLE A X.4.1: DATA BANDWIDTH OF FLORAL ARCHITECTURE FOR *STREPTOCARPUS* SPECIES

The table shows the measured data bandwidth of floral architecture for 18 species of *Streptocarpus*. Presented are arithmetic means and standard deviations of 17 variables, with *n* = 10 for each species and variable. Measures of display size (frontal, lateral, f:l ratio) are presented in cm^2, others in mm. Variable name "distance between anther and stigma" abbreviated as "distance ant.-stig."

species	frontal display in cm^2	lateral display in cm^2	display ratio (f : l)	display height	display width	petal height, upper left	petal width, upper left	petal height, lower central	petal width, lower central
S. bindseili Eb.Fisch.	7.4 ± .7	2.9 ± .3	2.6 ± .3	30.6 ± 2.5	35.6 ± 2.4	11.0 ± 1.2	10.8 ± .8	15.6 ± 2.0	14.6 ± 1.1
S. burundianus Hilliard & B.L.Burtt	1.0 ± .2	.4 ± 0	2.7 ± .4	11.8 ± .9	11.9 ± 1.3	3.4 ± .6	3.2 ± .3	4.2 ± .7	4.3 ± .7
S. cooperi C.B.Clarke	3.9 ± 1.3	3.0 ± .3	1.3 ± .3	34.2 ± 1.7	31.8 ± 2.0	10.3 ± .8	10.7 ± .8	11.6 ± 1.0	8.2 ± .5
S. cyaneus ssp. polackii (B.L.Burtt) Weigend & T.J.Edwards	10.2 ± 2.0	5.9 ± 1.1	1.7 ± .4	51.8 ± 5.9	55.6 ± 5.3	16.5 ± 2.2	16.0 ± 1.6	19.4 ± 2.5	13.7 ± 1.4
S. denticulatus Turrill	2.3 ± .4	1.0 ± .2	2.3 ± .3	15.6 ± 1.2	23.4 ± 1.9	7.0 ± 1.1	8.0 ± .7	8.9 ± .9	9.6 ± 1.2
S. dunnii Mast.	1.1 ± .2	2.5 ± .3	.4 ± .1	13.5 ± 2.3	13.5 ± 2.3	3.9 ± .3	5.3 ± .8	6.8 ± .8	5.8 ± .6
S. fasciatus T.J.Edwards & C.Kunhardt	9.9 ± 1.0	5.9 ± .9	1.7 ± .3	44.7 ± 4.3	51.6 ± 5.6	14.3 ± 1.4	13.9 ± 1.0	18.8 ± 2.1	10.7 ± 1.3
S. gardenii Hook.	3.9 ± .8	3.2 ± .3	1.2 ± .2	31.7 ± 2.5	37.3 ± 2.5	11.7 ± .8	9.3 ± .5	15.1 ± 1.0	8.7 ± .7
S. johannis L.L.Britten	3.5 ± .9	1.1 ± .4	3.2 ± .3	29.1 ± 2.2	28.9 ± 1.8	10.6 ± .9	5.5 ± .9	11.0 ± 1.1	7.3 ± .9
S. liliputana Bellstedt & T.J.Edwards	3.8 ± 1.1	3.0 ± .3	1.3 ± .2	24.5 ± 3.7	28.1 ± 3.2	9.3 ± 1.3	8.0 ± .7	11.6 ± 1.3	7.0 ± .5
S. longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	4.3 ± .7	3.8 ± .3	1.1 ± .1	24.6 ± 1.6	28.2 ± 1.5	9.5 ± 1.3	9.5 ± 1.2	11.4 ± 1.1	13.1 ± 1.3
S. modestus L.L.Britten	5.7 ± .8	2.5 ± .4	2.3 ± .3	37.4 ± 1.2	40.8 ± 2.3	12.6 ± .6	8.2 ± .5	15.2 ± .7	10.0 ± .4
S. pole-evansii I.Verd.	.7 ± .1	.4 ± 0	1.7 ± .2	11.2 ± .8	11.1 ± 1.0	2.8 ± .3	2.9 ± .2	4.5 ± .2	3.2 ± .5
S. polyanthus Hook.	1.2 ± .2	.5 ± .1	2.4 ± .3	19.5 ± 1.2	17.1 ± 1.0	6.7 ± .4	2.4 ± .2	7.1 ± .5	3.7 ± .2
S. pusillus Harv. ex C.B.Clarke	1.3 ± .2	.5 ± .1	2.8 ± .2	18.3 ± 1.1	16.2 ± 1.1	7.1 ± .7	2.8 ± .5	5.8 ± .4	3.6 ± .2
S. rexii (Bowie ex Hook.) Lindl.	7.5 ± 1.0	5.5 ± .7	1.4 ± .2	41.6 ± 1.6	45.7 ± 1.4	14.8 ± .8	10.9 ± .6	16.4 ± .7	11.2 ± 1.8
S. roseoalbus Weigend & T.J.Edwards	10.7 ± 1.6	3.5 ± .4	3.1 ± .3	42.4 ± 4.1	46.2 ± 4.2	12.1 ± 1.6	12.4 ± .7	15.9 ± 1.9	11.9 ± .9
S. wilmsii Engl.	1.3 ± .2	1.5 ± .1	.9 ± .1	17.1 ± 2.8	18.4 ± 3.2	5.5 ± 1.2	6.7 ± 1.6	5.9 ± 1.7	5.3 ± .7

species (floral architecture, continued)	opening height	opening width	dorsal length	ventral length	anther length	filament length	pistil length	distance ant.-stig.
S. bindseili Eb.Fisch.	8.1 ± .3	8.1 ± .6	25.6 ± .8	47.6 ± 1.3	2.7 ± .3	4.7 ± .4	17.9 ± .7	.2 ± .4
S. burundianus Hilliard & B.L.Burtt	4.3 ± .5	4.6 ± .5	5.0 ± .5	11.4 ± 1.2	1.0 ± .1	3.4 ± .8	6.1 ± .4	2.1 ± .4
S. cooperi C.B.Clarke	12.4 ± 1.2	16.1 ± 1.2	43.6 ± 1.4	63.0 ± 1.6	2.5 ± .4	8.2 ± .3	42.0 ± 1.5	3.4 ± 1.3
S. cyaneus ssp. polackii (B.L.Burtt) Weigend & T.J.Edwards	19.9 ± 1.4	21.1 ± 2.0	55.2 ± 2.7	85.0 ± 4.6	3.9 ± .3	9.9 ± .6	51.1 ± 1.4	6.1 ± 2.3
S. denticulatus Turrill	7.2 ± .4	7.3 ± .5	10.1 ± .3	23.5 ± 1.0	1.5 ± 0	5.1 ± .3	10.7 ± 1.9	.9 ± .6
S. dunnii Mast.	7.4 ± 1.0	7.3 ± .9	37.8 ± 2.0	40.1 ± 2.2	2.0 ± 0	12.8 ± 1.4	41.3 ± 2.5	1.4 ± 2.1
S. fasciatus T.J.Edwards & C.Kunhardt	15.0 ± 2.7	20.8 ± 2.0	56.4 ± 2.7	81.8 ± 3.0	4.0 ± .2	9.2 ± .8	45.8 ± 2.1	6.3 ± 2.1
S. gardenii Hook.	10.8 ± 1.0	14.3 ± .8	40.7 ± 2.2	56.0 ± 3.0	2.5 ± .5	7.6 ± .5	35.0 ± 2.2	1.6 ± .8
S. johannis L.L.Britten	4.5 ± .5	2.1 ± .2	15.9 ± .9	34.9 ± 1.5	1.6 ± .3	3.4 ± .4	12.9 ± 1.3	2.2 ± .6
S. liliputana Bellstedt & T.J.Edwards	6.6 ± 1.0	8.0 ± .7	42.9 ± 2.0	52.9 ± 3.1	2.1 ± .2	10.8 ± .7	40.8 ± .9	2.3 ± 1.2
S. longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	10.3 ± .8	10.9 ± 1.1	45.6 ± 1.7	58.2 ± 2.6	2.4 ± .3	9.1 ± .6	40.3 ± 1.1	2.3 ± .9
S. modestus L.L.Britten	12.2 ± 1.0	11.6 ± .5	22.9 ± .7	43.8 ± 1.2	2.9 ± .3	7.0 ± .7	16.5 ± .7	3.6 ± .7
S. pole-evansii I.Verd.	2.2 ± .4	3.1 ± .4	11.2 ± .6	18.2 ± 1.0	1.4 ± .3	1.7 ± .3	5.8 ± .5	.6 ± .4
S. polyanthus Hook.	3.6 ± .2	1.0 ± .2	11.0 ± .6	25.4 ± 1.3	1.6 ± .2	2.6 ± .3	6.4 ± .6	1.4 ± .4
S. pusillus Harv. ex C.B.Clarke	3.6 ± .5	1.8 ± .4	9.5 ± .4	20.9 ± .9	1.7 ± .3	2.3 ± .4	5.4 ± .5	.9 ± .5
S. rexii (Bowie ex Hook.) Lindl.	13.8 ± 1.5	16.3 ± 1.7	49.2 ± 1.5	71.0 ± 2.7	3.0 ± 0	9.9 ± .7	42.3 ± 1.8	4.0 ± .8
S. roseoalbus Weigend & T.J.Edwards	15.9 ± 1.5	19.6 ± 1.1	29.4 ± 1.6	49.9 ± 3.3	3.3 ± .3	8.4 ± .7	27.0 ± .7	4.7 ± 1.0
S. wilmsii Engl.	6.9 ± 1.9	7.7 ± 1.8	24.2 ± .8	36.3 ± 2.7	2.2 ± .2	8.1 ± .9	19.6 ± .8	1.8 ± .8

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TABLE A X.4.2: KENDALLS´S T_B CORRELATIONS FOR FLORAL ARCHITECTURE OF *STREPTOCARPUS*

Except for display size ratio (frontal : lateral), all variables correlate strongly and significantly at p = .05 (*) or p = .01 (**).

τ_b	lateral display	display ratio	opening height	opening width	display height	display width	petal height (u. l.)	petal width (u. l.)	petal height (l. c.)	petal width (l. c.)	dorsal length	ventral length	anther length	filament length	pistil length	dist. ant.-stig.
frontal display	.699**	.085	.708**	.682**	.804**	.869**	.817**	.787**	.830**	.712**	.529**	.634**	.765**	.367*	.503**	.546**
lateral display	1	-.216	.734**	.734**	.660**	.699**	.621**	.721**	.765**	.621**	.804**	.908**	.725**	.643**	.752**	.559**
display ratio (f:l)		1	-.079	-.131	.098	.059	.085	-.039	.020	.007	-.307	-.203	-.046	-.446**	-.307	-.007
opening height			1	.849**	.708**	.748**	.656**	.796**	.708**	.669**	.590**	.721**	.761**	.559**	.643**	.607**
opening width				1	.721**	.734**	.656**	.862**	.669**	.577**	.643**	.721**	.748**	.533**	.643**	.607**
display height					1	.908**	.882**	.695**	.791**	.595**	.542**	.673**	.778**	.380*	.516**	.625**
display width						1	.895**	.761**	.856**	.686**	.529**	.660**	.791**	.393*	.529**	.599**
petal height (up., left)							1	.682**	.804**	.608**	.451**	.582**	.739**	.341*	.451**	.507**
petal width (up., left)								1	.748**	.721**	.630**	.708**	.734**	.493**	.630**	.568**
petal height (low., cen.)									1	.673**	.569**	.699**	.778**	.433*	.569**	.546**
petal width (low., cen.)										1	.451**	.556**	.582**	.393*	.451**	.441*
dorsal length											1	.869**	.608**	.630**	.843**	.520**
ventral length												1	.712**	.603**	.817**	.572**
anther length													1	.393*	.529**	.612**
filament length														1	.761**	.475**
carpel length															1	.533**

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TABLE A X.4.3: DISTRIBUTION, HABITAT, SOURCE, LIFE CYCLE AND GROWTH HABIT OF ANALYSED *STREPTOCARPUS* SPECIES

Presented are distribution, source of accession, habitat preference, life cycle and growth habit for 18 analysed species of genus *Streptocarpus*. Habitat preference is coded as ● for very shady growth conditions, like most forest plants, and ● for plants growing in half shade or even sun-exposed, usually plants of the lowveld, growing on rock outcrops only slightly shaded by rocks, or along forest margins. Life cycle is coded as ⊙ for monocarpic and ♃ for polycarpic/perennial. Growth habit is coded as unifoliate ♘, plurifoliate ♘♘ and rosulate ♘♘♘. *) Variations mentioned by (Hilliard and Burtt (1971) in grey, but not observed for the accessions at hand. Literature presented at the end of the table.

species	distribution	source of accession	habitat preference		life cycle	growth habit
Streptocarpus bindseili Eb.Fisch.	Mountains east of Ntaruka, Rwanda (Fischer, 1988: 387)	Rwanda	Growing in deep rock crevices of isolated quartzitic outcrops (Hinkel and Fischer, 1999: 205)	●	♃	♘
Streptocarpus burundianus Hilliard & B.L.Burtt	Burundi, north-east corner of Lake Tanganyika (Hilliard and Burtt, 1971: 227)	Bururi, Kumuyange, Burundi	On vertical rocks or on the soil at the foot of them, in very dark and shady places in montane forests (Hilliard and Burtt, 1971: 229)	●	♃	♘♘
Streptocarpus cooperi C.B.Clarke	Natal, from Qudeni across the Biggarsberg to the Drakensberg; probably mountainous parts of Orange Free State (Hilliard and Burtt, 1971: 176)	ex. hort.	forest plant, carpets the steep earth and rock banks bordering mountain streamlets (Hilliard and Burtt, 1971: 178)	●	⊙	♘
Streptocarpus cyaneus ssp. polackii (B.L.Burtt) Weigend & T.J.Edwards	Transvaal, Barberton (Weigend and Edwards, 1994: 372)	Kowyns Pass, Mpumalanga; South Africa	Common in montane forest patches (Weigend and Edwards, 1994: 373)	●	♃	♘♘♘
Streptocarpus denticulatus Turrill	Transvaal, restricted to two small areas around Barberton and Belfast (Hilliard and Burtt, 1971: 207)	Belfast, Stoffberg Rd., Mpumalanga, South Africa	Plant of rock outcrops, favouring shady cracks and overhangs (Hilliard and Burtt, 1971: 208)	●	⊙	♘
Streptocarpus dunnii Mast.	South-Eastern Transvaal and Swaziland, extending west to Leslie, particularly common in the Stenkampsbergen and the Drakensberg between Pilgrimsrest and Mbabande (Hilliard and Burtt, 1971: 211)	Transvaal, Swaziland	afromontane, exposed, rocky crags in open grassland, often growing at the base of large boulders or in rock clefts (Hughes et al., 2007: 1689)	●	⊙ (♃)*	♘ (♘♘)*
Streptocarpus fasciatus T.J.Edwards & C.Kunhardt	Eastern Transvaal (Edwards et al., 1992: 192)	Crocodile River Gorge, Mpumalanga, South Africa	Recorded from an enclave of woodland, Crocodile River Gorge. Growing under the protection of granite boulders (Edwards et al., 1992: 193)	●	♃	♘♘♘
Streptocarpus gardenii Hook.	Eastern Cape Province (Transkei districts of Tabankulu and Umzimkulu) and Natal (Hilliard and Burtt, 1971: 282)	Tugela River Gorge, Upper Drakensberg, Kwazulu-Natal, South Africa	Forest plant, often epiphytically or on rocks, along stream banks and other damp places, but sometimes rooted in soil on steeply sloping forest floor (Hilliard and Burtt, 1971: 282)	●	♃	♘♘♘
Streptocarpus johannis L.L.Britten	Eastern Cape and southern Natal, from Port St. Johns through Lusikisiki to the Ngeli slopes on the Cape-Natal border (Hilliard and Burtt, 1971: 283)	Magwa Falls, Transkei, Eastern Cape, South Africa	Forest plant, growing on rock and earth banks (Hilliard and Burtt, 1971: 284)	●	♃	♘♘♘
Streptocarpus liliputana Bellstedt & T.J.Edwards	Forested river gorges of the Laputana and Mkozi Rivers, Pondoland area (Bellstedt and Edwards, 2003)	Lupatana Gorge, South Africa	Grows only in deep shade in sparse colonies on rock seepages in forested areas	●	♃	♘♘♘

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species	distribution	source of accession	habitat preference		life cycle	growth habit
			(Bellstedt and Edwards, 2003)			
Streptocarpus longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	Transvaal, Blouberg, upper slopes (Edwards et al., 1992: 193)	Blouberg, Limpopo, South Africa	Exposed habitats, above the tree line in the shade of rocks (Edwards et al., 1992: 194)	○	♃	⚘⚘⚘
Streptocarpus modestus L.L.Britten	Cape, Pondoland, Magwa Falls near Lusikisiki (Hilliard and Burtt, 1971: 257)	Prince Albert Pass, Eastern Cape, ex. hort., South Africa	Grows wedged in cervices of rocky cliff faces along the lips of forested gorges (…) lightly shaded by other vegetation (Hilliard and Burtt, 1971: 258–259)	○	♃	⚘⚘⚘
Streptocarpus pole-evansii I.Verd.	Transvaal, apparently confined to a very limited area from the southern part of Kruger National park around Pretorius Kop across the valley of the Crocodile river to Barberton (Hilliard and Burtt, 1971: 209)	Silverhill Seeds, South Africa	Grows in shady crevices of granite boulders and also on rock outcrops in the Transvaal bushveld (Hilliard and Burtt, 1971: 209)	○	♃	⚘⚘
Streptocarpus polyanthus Hook.	Transvaal, Swaziland, Orange Free State and Natal (Hilliard and Burtt, 1971: 289)	Ifye Conservancy, Dalton, Kwazulu-Natal, South Africa	Growing in shelter of rock outcrops on steep, grassy hillslopes, or on the cliffs of forest margins (Hilliard and Burtt, 1971: 290)	○	♃ (☉)*	⚘⚘
Streptocarpus pusillus Harv. ex C.B.Clarke	Natal, Orange Free State, Lesotho and Eastern Cape, along the Drakensberg and its outliners (Hilliard and Burtt, 1971: 238)	ex. hort.	Favours cliff faces and is common in sheltered, damp cervices (Hilliard and Burtt, 1971: 238)	○	♃	⚘⚘
Streptocarpus rexii (Bowie ex Hook.) Lindl.	Cape province eastward from George to southernmost Natal (Hilliard and Burtt, 1971: 267)	Stutterheim Forests, Eastern Cape, South Africa	Coastal hills and forests (Hilliard and Burtt, 1971: 263)	○	♃	⚘⚘⚘
Streptocarpus roseoalbus Weigend & T.J.Edwards	Eastern Transvaal, Barberton, Agnes Mine (Weigend and Edwards, 1994: 368)	Malalotja Nature Reserve, Swaziland	The species occurs from 1000 to 1500 m, often in lowveld vegetation (Weigend and Edwards, 1994: 368)	○	♃	⚘⚘⚘
Streptocarpus wilmsii Engl.	Transvaal and Swaziland, along the Drakensberg from Mariepskop to Mbabane (Hilliard and Burtt, 1971: 235)	Gods Window, Mpumalanga, South Africa	Grows on the forest floor or as an epiphyte (Hilliard and Burtt, 1971: 235)	●	♃ (☉)*	⚘

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Hinkel, H., Fischer, E., 1999. Zur Flora und Fauna der Quarzitfelsen im südostlichen Ruanda/ Zentralafrika. Mainzer naturwiss. Archiv 37, 201–211.

Hughes, M., Möller, M., Edwards, T.J., Bellstedt, D.U., de Villiers, M., 2007. The impact of pollination syndrome and habitat on gene flow: a comparative study of two *Streptocarpus* (Gesneriaceae) species. American Journal of Botany 94, 1688–1695. https://doi.org/10.3732/ajb.94.10.1688

Hilliard O.M., Burtt B. L., 1971. *Streptocarpus*. An African plant study. University of Natal Press, Pietermaritzburg, South Africa.

Weigend, M., Edwards, T.J., 1994. Notes on *Streptocarpus cyaneus* and *S. parviflorus*. Sendtnera 2, 365–376.

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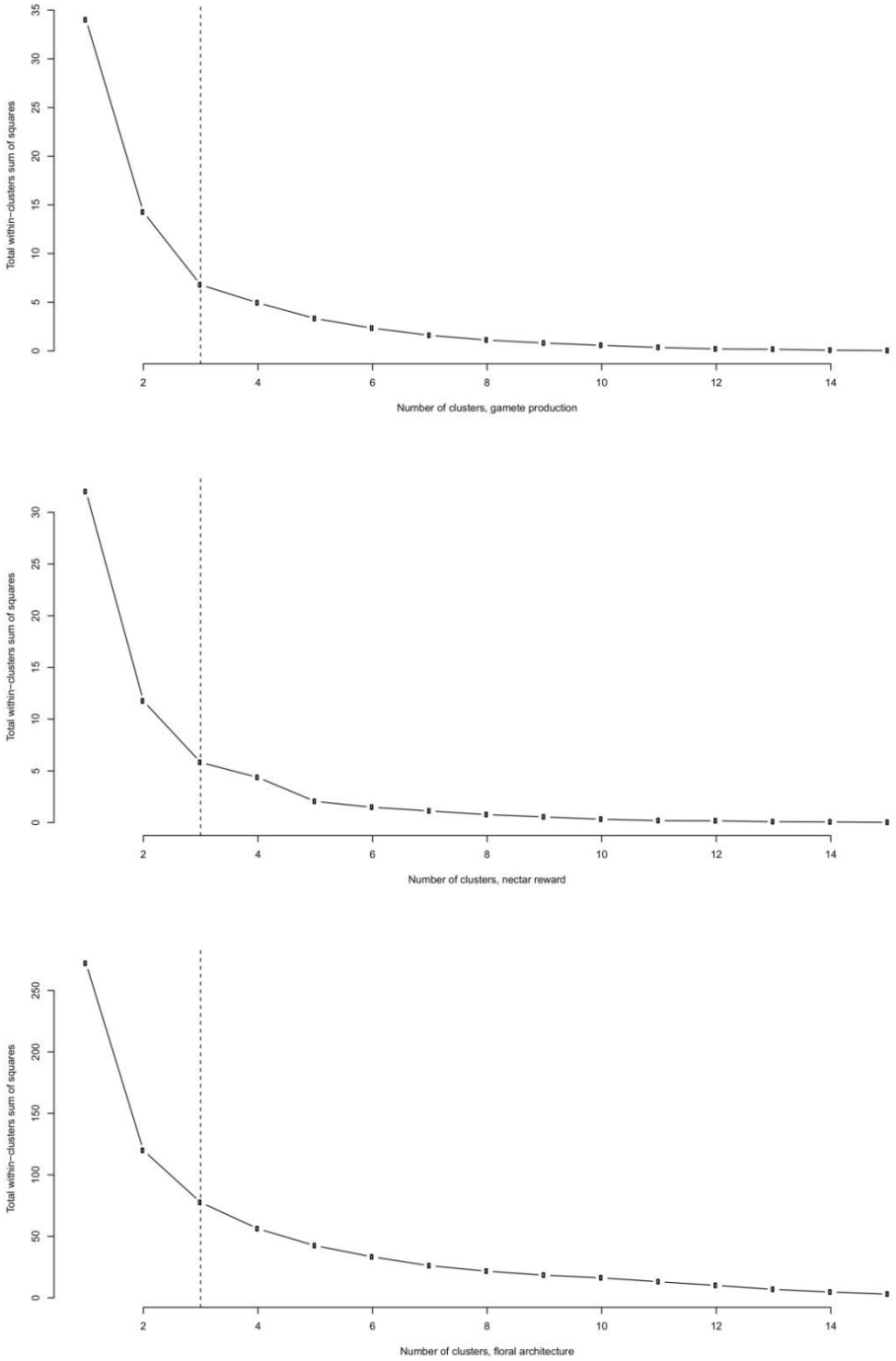

FIGURE A X.4.2: SELECTION OF CLUSTERS (GAMETE PRODUCTION, FLORAL ARCHITECTURE, REWARD) FOR *STREPTOCARPUS*

Selection of appropriate number of clusters via Elbow method for ratio scaled variables: Gamete production (top), nectar reward variables (centre) and floral architecture (bottom). Charts showing total intra-cluster variation (y-axis: total within-cluster sum of squares) in dependence to k clusters (x-axis). The position of a bend in the plot is considered as indicator for the best number of clusters, with higher cluster numbers not adding substantially to the compactness of the clusters. Best number of clusters is three in all cases: All plots show almost linear decline of total within-cluster sum of squares for higher number of clusters.

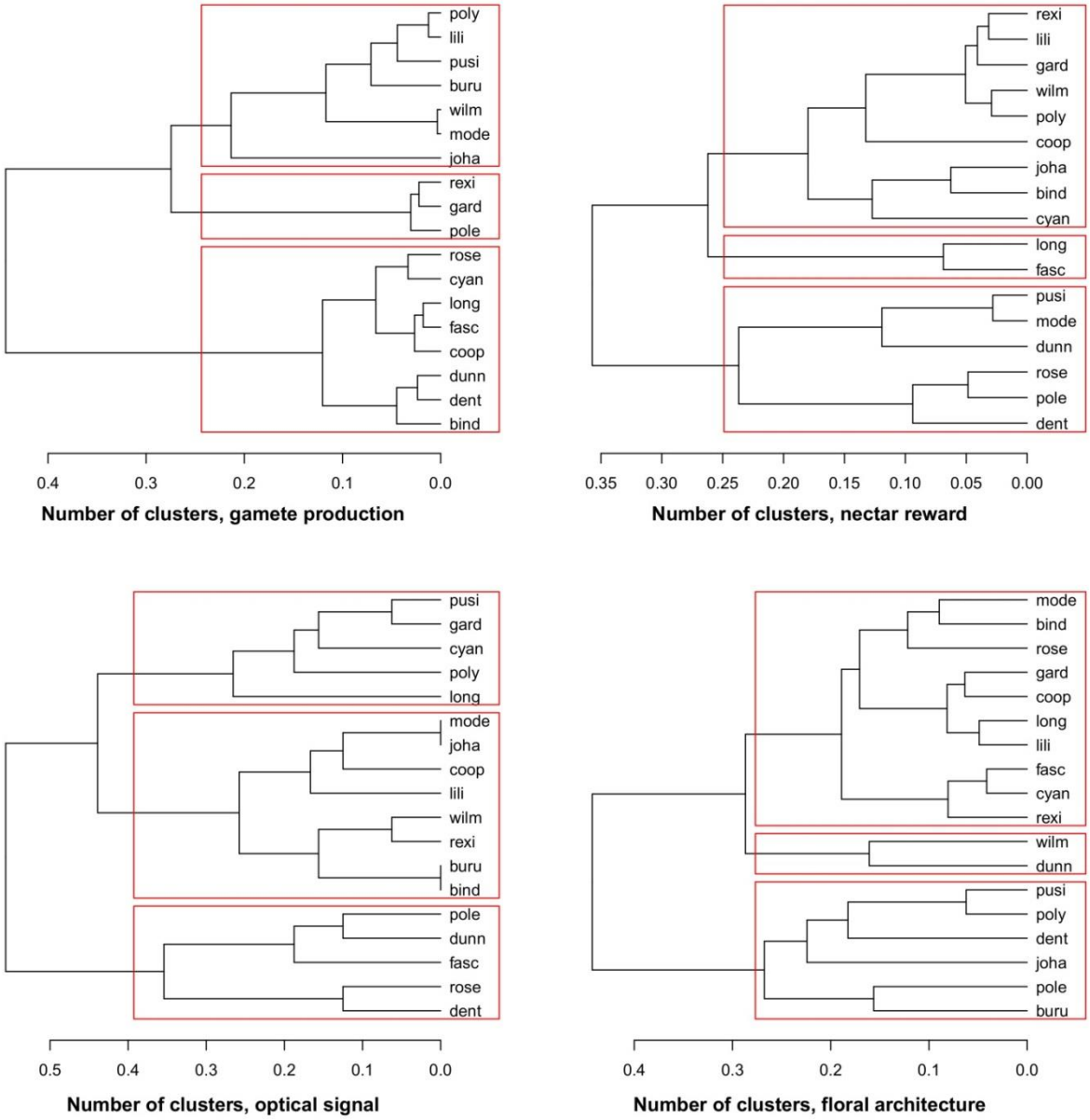

FIGURE A X.4.3: HIERARCHICAL CLUSTERING (GAMETES, REWARD, OPTICAL SIGNAL, FLORAL ARCHITECTURE) FOR *STREPTOCARPUS*
Hierarchical clustering of gamete production, nectar reward, optical flower signal and floral architecture for the *Streptocarpus* species analysed. Cluster analyses were conducted with average linkage. Performance of linkage method was tested via function cophenetic, R package vegan, with average linkage showing equal or better performance than single or complete linkage for the datasets: Gamete production, average linkage .85 (single: .82; complete: .85); nectar reward .79 (single: .75; complete: .69); optical signal .73 (single: .67; complete: .71); floral architecture .80 (single: .76; complete: .79). Dissimilarity matrices based on ratio scaled variables were produced via Bray Curtis index (gamete production, nectar reward, floral architecture), while Gower distances were used for optical signal. *S. burundianus* could not be included into clustering of nectar reward variables due to missing data. Exclusion of *S. burundianus* from the other datasets does not change the presented outcome. For all datasets, three clusters are of most explanatory value: Decision for cluster number is based on visual inspection of the retrieved cluster dendrograms and application of Elbow method (see figure A X.4.2). For optical flower signal, decision for three clusters is only based on visual inspection, due to solely categorial information.

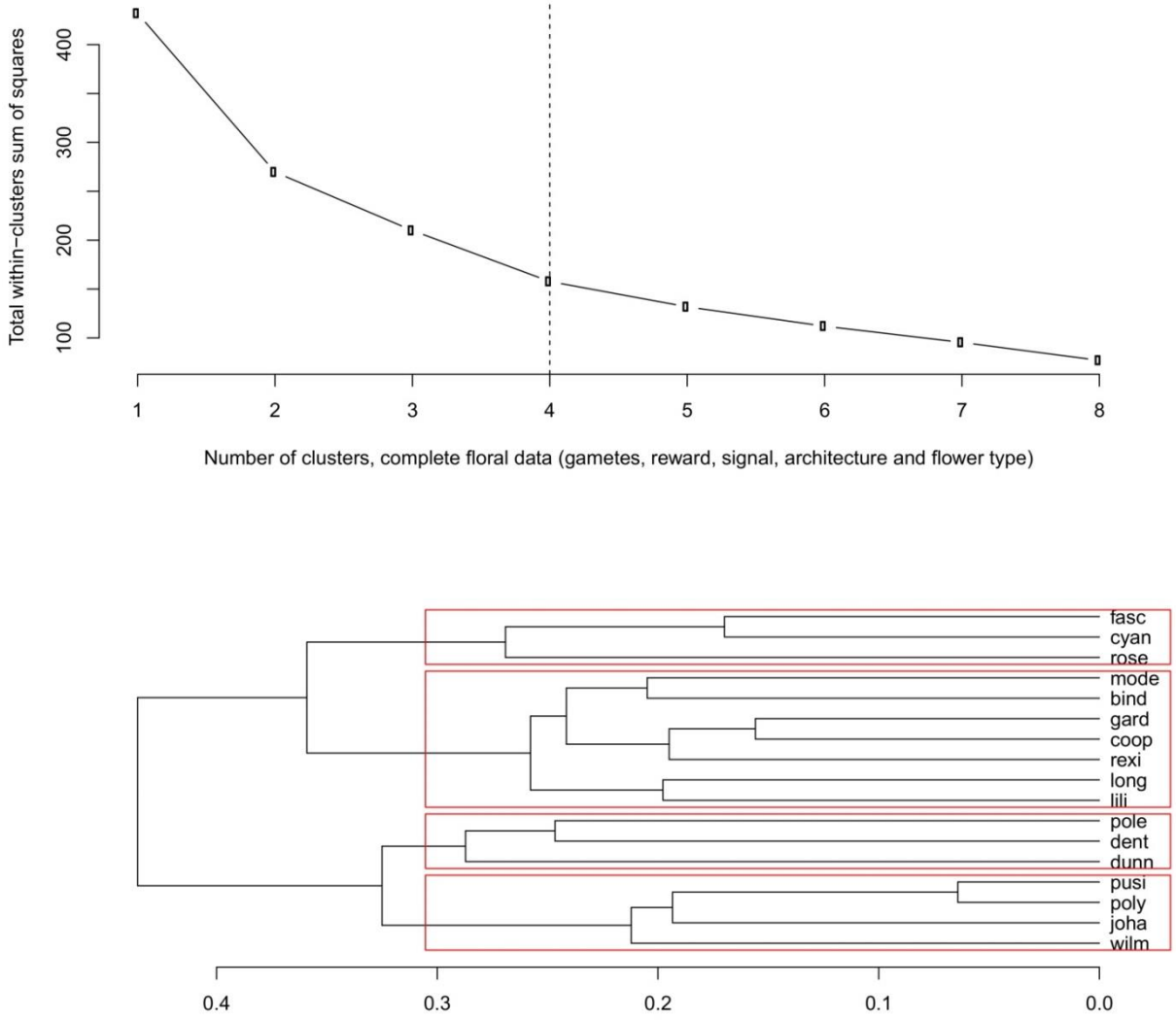

FIGURE A X.4.4: SELECTION OF CLUSTERS AND HIERARCHICAL CLUSTERING WITH ALL *STREPTOCARPUS* DATASETS ANALYSED AT ONCE
Selection of appropriate number of clusters by Elbow method (top) and retrieved cluster dendrogram (bottom) for all floral data, analysed at once (gamete production, nectar reward, optical flower signal and floral architecture). Best number of clusters is four: The Elbow plot only shows narrow and linear decline of total within-cluster sum of squares for higher number of clusters. *S. burundianus* excluded due to incomplete nectar data. Performance of linkage method was tested via function cophenetic, R package vegan, with average linkage showing equal or better performance than single or complete linkage (average linkage .68; single: .45; complete: .66). Dissimilarity matrix for hierarchical clustering produced via Gower index.

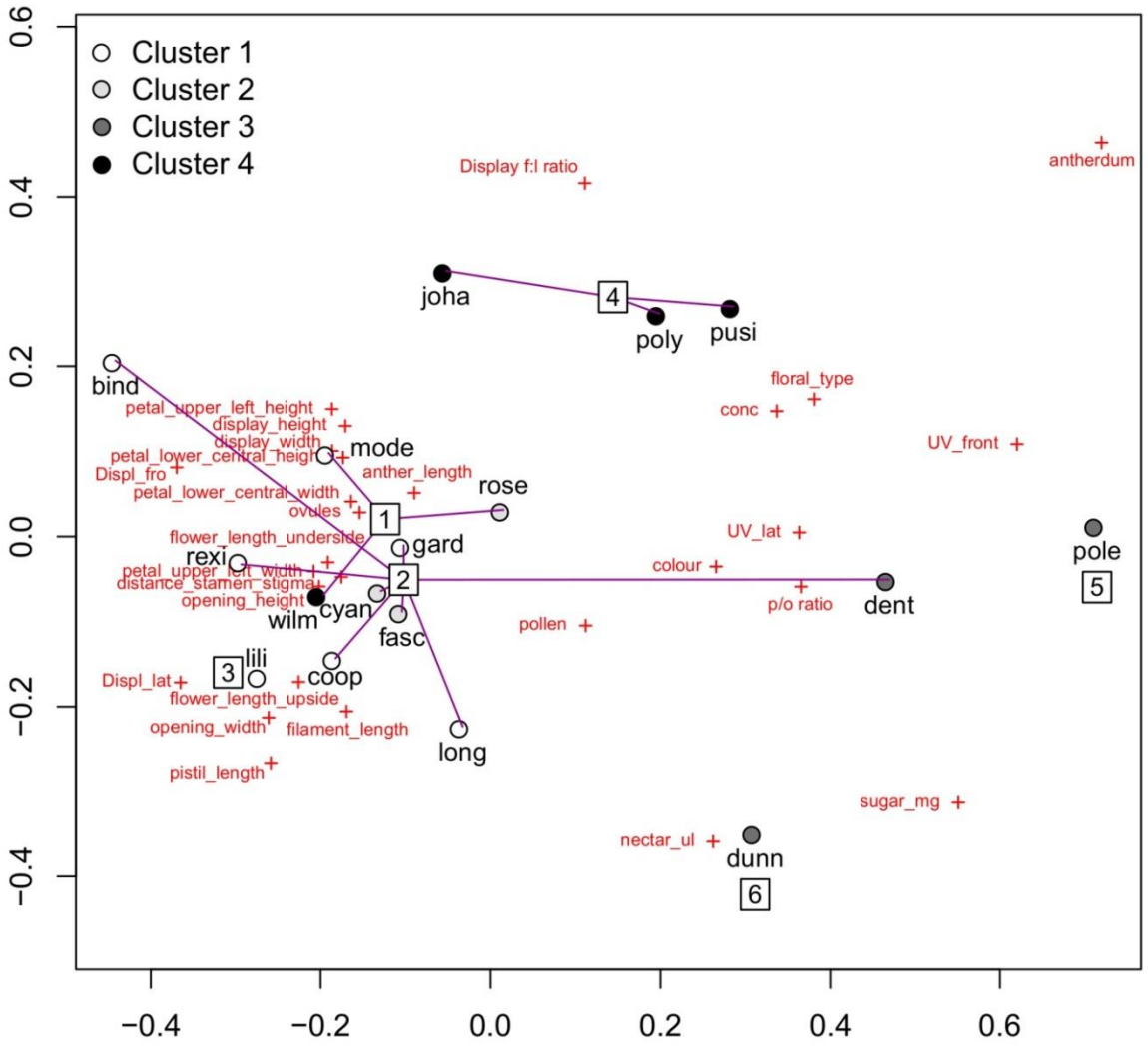

FIGURE A X.4.5: NMDS FOR ALL DATASETS OF *STREPTOCARPUS* ANALYSED AT ONCE
NMDS performed on all floral data at once, namely gamete production, nectar reward, optical flower signal, floral architecture and floral type after Möller et al. 2019 (compare chapter). Dissimilarity matrix produced via Gower index. NMDS stress value: 0.095, good fit (two convergent solutions after 20 tries). Plant trait clusters retrieved from hierarchical clustering indicated by colouration. Floral type marked by centroids (1 – open cylindrical tube, 2 – open tube with pollinator chamber, 3 – acanth type, 4 – keyhole type, 5 – personate type, 6 – bird-pollination type). PERMANOVA shows floral types disjunct from ordination and plant trait clusters (p = .76, non. sig.). Only keyhole flower type appears more homogenous, other floral types are not well resolved by NMDS and retrieved plant trait clusters. *S. burundianus* excluded due to missing nectar data. Species names abbreviated.

TABLE A X.4.4: VECTOR FITTING FOR NMDS OF GAMETE PRODUCTION OF *STREPTOCARPUS* SPECIES

Vector fitting for NMDS of gametic variables, performed via function envfit (R package vegan). Variables tested in order to explain the gametic ordination are floral type (Möller et al., 2019), breeding system, growth habit, life cycle, habitat preference as well as variables related to nectar reward, optical flower signal and floral architecture. The first two columns show the direction cosines of the vectors, r^2 gives the squared correlation coefficient; *p* values are based on 999 random permutations of the data. Significance codes: 0.01 = ** / 0.05 = * / 0.1 = .

vector	*NMDS1*	*NMDS2*	r^2	*p*	
floral type	0.79603	-0.60525	0.1989	0.227	
breeding system	0.89024	0.45549	0.0257	0.830	
growth habit	-0.18556	-0.98263	0.1841	0.225	
life cycle	-0.04704	-0.99889	0.2155	0.163	
habitat preference	0.57382	0.81898	0.0038	0.968	
nectar amount	0.02735	0.99963	0.4477	0.017	*
nectar concentration	0.56815	0.82293	0.1855	0.250	
nectar sugar production	0.14538	0.98938	0.6011	0.002	**
flower colour	0.19647	0.98051	0.2722	0.105	
UV signal, frontal	-0.18504	0.98273	0.1274	0.389	
UV signal, lateral	-0.54274	0.83990	0.1035	0.474	
anther dummy	0.05109	0.99869	0.0343	0.776	
display size, frontal	-0.63426	0.77312	0.3890	0.031	*
display size, lateral	-0.61173	0.79106	0.3799	0.039	*
display ratio, front./lat.	-0.14129	-0.98997	0.0478	0.708	
opening height	-0.60401	0.79698	0.5310	0.006	**
opening width	-0.55763	0.83009	0.5063	0.008	**
display height	-0.99638	0.08497	0.5296	0.009	**
display width	-0.89581	0.44443	0.4381	0.021	*
petal, upper left, height	-0.99334	-0.11525	0.4690	0.014	*
petal, upper left, width	-0.48553	0.87422	0.4939	0.008	**
petal, lower central, height	-0.78527	0.61915	0.3879	0.036	*
petal, lower central, width	-0.32704	0.94501	0.4473	0.018	*
flower length, upside	-0.60261	0.79804	0.2620	0.112	
flower length, underside	-0.77225	0.63532	0.4108	0.027	*
anther length	-0.62083	0.78394	0.4643	0.017	*
filament length	-0.25086	0.96802	0.1374	0.362	
pistil length	-0.50430	0.86353	0.2338	0.139	
distance anther-stigma	-0.97641	0.21591	0.3301	0.059	.

TABLE A X.4.5: VECTOR FITTING FOR NMDS OF NECTAR REWARD OF *STREPTOCARPUS* SPECIES

Vector fitting for NMDS of nectar variables, performed via function envfit (R package vegan). Variables tested in order to explain the nectar ordination are floral type (Möller et al., 2019), breeding system, growth habit, life cycle, habitat preference as well as variables related to gamete production, optical flower signal and floral architecture. The first two columns show the direction cosines of the vectors, r^2 gives the squared correlation coefficient; *p* values are based on 999 random permutations of the data. Significance codes: 0.01 = ** / 0.05 = * / 0.1 = .

vector	*NMDS1*	*NMDS2*	r^2	*p*	
floral type	-0.02769	-0.99962	0.0172	0.877	
breeding system	-0.08261	-0.99658	0.3250	0.057	.
growth habit	-0.12491	0.99217	0.2923	0.085	.
life cycle	0.02681	0.99964	0.4590	0.014	*
habitat preference	-0.76439	-0.64476	0.0614	0.624	
pollen amount	-0.32741	-0.94488	0.6633	0.002	**
ovule number	-0.25097	-0.96800	0.0528	0.683	
p/o ratio	-0.22762	-0.97375	0.3431	0.064	.
flower colour	-0.15890	-0.98729	0.6381	0.002	**
UV signal, frontal	-0.07495	-0.99719	0.4029	0.030	*
UV signal, lateral	-0.06371	-0.99797	0.3080	0.090	.
anther dummy	-0.53854	0.84260	0.0547	0.681	
display size, frontal	-0.74487	0.66720	0.1816	0.243	
display size, lateral	-0.58181	0.81332	0.4159	0.028	*
display ratio, front./lat.	0.98974	-0.14285	0.1151	0.439	
opening height	-0.98783	-0.15555	0.1919	0.201	

vector	*NMDS1*	*NMDS2*	r^2	*p*	
opening width	-0.81512	-0.57929	0.2096	0.167	
display height	-0.30572	0.95212	0.0934	0.494	
display width	-0.42673	0.90438	0.1591	0.284	
petal, upper left, height	-0.19305	0.98119	0.1796	0.244	
petal, upper left, width	-0.99619	0.08716	0.2338	0.141	
petal, lower central, height	0.38794	0.92168	0.2330	0.148	
petal, lower central, width	-0.71011	0.70410	0.3008	0.087	.
flower length, upside	-0.54834	0.83626	0.3600	0.039	*
flower length, underside	-0.45847	0.88871	0.2966	0.077	.
anther length	-0.59641	0.80268	0.2107	0.177	
filament length	-0.93928	0.34316	0.2232	0.156	
pistil length	-0.77184	0.63582	0.3196	0.054	.
distance anther-stigma	-0.92483	0.38037	0.1010	0.463	

TABLE A X.4.6: VECTOR FITTING FOR NMDS OF OPTICAL FLOWER SIGNAL OF *STREPTOCARPUS* SPECIES

Vector fitting for NMDS of optical flower signal, performed via function envfit (R package vegan). Variables tested in order to explain the optical signal ordination are floral type (Möller et al., 2019), breeding system, growth habit, life cycle, habitat preference as well as variables related to gamete production, nectar reward and floral architecture. The first two columns show the direction cosines of the vectors, r^2 gives the squared correlation coefficient; *p* values are based on 999 random permutations of the data. Significance codes: 0.01 = ** / 0.05 = * / 0.1 = .

vector	*NMDS1*	*NMDS2*	r^2	*p*	
floral type	0.25396	-0.96722	0.0351	0.778	
breeding system	0.41111	-0.91159	0.4426	0.011	*
growth habit	0.42203	0.90658	0.0376	0.760	
life cycle	-0.75990	0.65004	0.1424	0.362	
habitat preference	0.32782	0.94474	0.0890	0.523	
pollen amount	0.95885	-0.28392	0.1460	0.338	
ovule number	0.86974	-0.49351	0.0277	0.818	
p/o ratio	0.82634	-0.56317	0.0632	0.623	
nectar amount	0.88133	0.47250	0.0694	0.599	
nectar concentration	0.97662	-0.21498	0.3569	0.047	*
nectar sugar production	0.99611	-0.08806	0.3194	0.066	.
display size, frontal	0.99468	-0.10297	0.0396	0.766	
display size, lateral	0.43804	-0.89895	0.0074	0.955	
display ratio, front./lat.	0.59350	0.80483	0.0725	0.589	
opening height	0.96552	0.26034	0.0629	0.640	
opening width	0.99003	-0.14084	0.1074	0.438	
display height	0.97929	0.20246	0.0118	0.922	
display width	0.98489	0.17320	0.0207	0.869	
petal, upper left, height	-0.09216	-0.99574	0.0117	0.930	
petal, upper left, width	0.99029	-0.13903	0.0253	0.835	
petal, lower central, height	0.99384	0.11080	0.0049	0.974	
petal, lower central, width	-0.34959	0.93690	0.0153	0.880	
flower length, upside	-0.19395	-0.98101	0.0172	0.880	
flower length, underside	-0.17487	-0.98459	0.0059	0.970	
anther length	0.92046	-0.39083	0.0261	0.846	
filament length	-0.05688	-0.99838	0.0494	0.718	
pistil length	0.08609	-0.99629	0.0181	0.884	
distance anther-stigma	0.92346	-0.38370	0.1145	0.438	

TABLE A X.4.7: VECTOR FITTING FOR NMDS OF FLORAL ARCHITECTURE OF *STREPTOCARPUS* SPECIES

Vector fitting for NMDS of floral architecture, performed via function envfit (R package vegan). Variables tested in order to explain the ordination of floral architecture are floral type (Möller et al., 2019), breeding system, growth habit, life cycle, habitat preference as well as variables related to gamete production, nectar reward and optical flower signal. The first two columns show the direction cosines of the vectors, r^2 gives the squared correlation coefficient; *p* values are based on 999 random permutations of the data. Significance codes: 0.01 = ** / 0.05 = * / 0.1 = .

vector	*NMDS1*	*NMDS2*	r^2	*p*	
floral type	-0.14123	0.98998	0.5465	0.004	**
breeding system	0.82355	0.56725	0.1046	0.464	
growth habit	0.19271	-0.98125	0.0180	0.882	
life cycle	-0.97787	-0.20922	0.0641	0.625	
habitat preference	-0.09668	0.99530	0.1114	0.439	
pollen amount	0.44463	-0.89571	0.3756	0.026	*
ovule number	0.43232	-0.90172	0.3806	0.035	*
p/o ratio	0.07654	0.99707	0.0115	0.921	
nectar amount	0.87275	-0.48817	0.2431	0.155	
nectar concentration	-0.99967	-0.02550	0.1168	0.415	
nectar sugar production	0.79526	-0.60627	0.0768	0.569	
flower colour	0.89954	-0.43684	0.0435	0.733	
UV signal, frontal	-0.27307	-0.96199	0.0105	0.932	
UV signal, lateral	0.22453	-0.97447	0.0209	0.877	
anther dummy	-0.88468	-0.46619	0.0730	0.557	

TABLE A X.4.8: GENBANK ACCESSION NUMBERS FOR ANALYSED *STREPTOCARPUS* SPECIES

Downloaded GenBank accession numbers for analysed species of *Streptocarpus* subgenus *Streptocarpus*, as well as outgroup species *Didymocarpus citrinus* and *Streptocarpus papangae*. Presented are the accession numbers for sequence matrices of *rp*L20 (ribosomal protein L20 and the *rpl*20-*rps*12 intergenic spacer), *trn*L-F (tRNA-Leu gene, including the *trn*L intron, the tRNA-Phe gene and the *trn*L-*trn*F intergenic spacer) and ITS (internal transcribed spacer of nuclear ribosomal DNA, including ITS 1, ITS 2 and the 5.8S ribosomal RNA gene).

species	*rp*L20	*trn*L-F	ITS
Streptocarpus bindseili Eb.Fisch.	KR703852	KR703948	AF316960
Streptocarpus cooperi C.B.Clarke	-	-	AF316954
Streptocarpus denticulatus Turrill	HQ719108	HQ718915	HQ718991
Streptocarpus dunnii Mast.	HQ719105	HQ718912	HQ718988
Streptocarpus fasciatus T.J.Edwards & C.Kunhardt	KR703870	KR703970	KR704103
Streptocarpus gardenii Hook.	HQ719125	HQ718928	HQ719008
Streptocarpus liliputana Bellstedt & T.J.Edwards	HQ719132	HQ718935	HQ719015
Streptocarpus longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	HQ719137	HQ718940	HQ719020
Streptocarpus modestus L.L.Britten	HQ719177	HQ718967	HQ719060
Streptocarpus pole-evansii I.Verd.	-	KR704005	AF316950
Streptocarpus pusillus Harv. ex C.B.Clarke	HQ719100	HQ718907	HQ718983
Streptocarpus rexii (Bowie ex Hook.) Lindl.	HQ719205	KR704015	HQ719088
Streptocarpus roseoalbus Weigend & T.J.Edwards	-	KR704017	KR704137
Streptocarpus wilmsii Engl.	HE861730	HE956774	KR704151
outgroup			
Didymocarpus citrinus Ridl.	KR703821	AJ492293	DQ912669
Streptocarpus papangae Humbert	HQ719097	HQ718905	HQ718980

TABLE A X.4.9: ACCESSION AND VOUCHER NUMBERS FOR ANALYSED *STREPTOCARPUS* SPECIES
Herbarium BONN, Nees Institut für Biodiversität der Pflanzen, Germany.

species	accession number	voucher number
Streptocarpus bindseili Eb.Fisch.	33168	-
Streptocarpus burundianus Hilliard & B.L.Burtt	35164	-
Streptocarpus cooperi C.B.Clarke	36665	1313
Streptocarpus cyaneus ssp. polackii(B.L.Burtt) Weigend & T.J.Edwards	36668	1294
Streptocarpus denticulatus Turrill	36670	-
Streptocarpus dunnii Mast.	33901	-
Streptocarpus fasciatus T.J.Edwards & C.Kunhardt	36672	1284
Streptocarpus gardenii Hook.	36674	1285
Streptocarpus johannis L.L.Britten	36678	1297
Streptocarpus liliputana Bellstedt & T.J.Edwards	36681	-
Streptocarpus longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	36682	1251
Streptocarpus modestus L.L.Britten	36683	1288
Streptocarpus pole-evansii I.Verd.	10393	-
Streptocarpus polyanthus ssp. verecundus Hook.	36685	-
Streptocarpus pusillus Harv. ex C.B.Clarke	36687	1253
Streptocarpus rexii (Bowie ex Hook.) Lindl.	36688	1286
Streptocarpus roseoalbus Weigend & T.J.Edwards	36105	1254
Streptocarpus wilmsii Engl.	36694	1255

X.5 APPENDIX TO CHAPTER VI

TABLE A X.5.1: INTERCEPT-ONLY MODEL FOR SEED SET OF *STREPTOCARPUS* SPECIES AND HYBRIDS

Dependent variable is seed set. Grand mean of seed set for the whole dataset is 1461.273. Group means for the different treatments are differing highly significant from the grand mean (χ^2 (1, *N* = 150) = 90.137, p = .000), allowing for application of Generalized Linear Modelling techniques.

parameter	B	std. error	95% Wald confidence interval		hypothesis test		
			lower	upper	Wald Chi-Square	df	sig.
Intercept	**1461.273**	153.9146	1159.606	1762.940	90.137	1	**0.000**
Scale	1124939.878						
Goodness of Fit							
Quasi Likelihood under Independence Model Criterion (QIC)	167,616,048.15						
Corrected QIC (QICC)	167,616,043.79						

TABLE A X.5.2: COVARIATE INTERACTION MODEL FOR SEED SET OF *STREPTOCARPUS* SPECIES AND HYBRIDS

Dependent variable is seed set. For all factors of the model (maternal and paternal contribute, generation and procedure) interaction terms with covariates (fruit length, ripening time) are introduced to test for homogeneity of slopes among treatment groups. Maternal contribute shows significant interaction terms for fruit length and ripening time, paternal contribute only for fruit length. Interaction terms are nonsignificant for generation (seed set within parental species, F1 seed material and F2 seed material) and procedure (allogamy, geitonogamy and true crossing). Compared to the Intercept-Only Model, Goodness of Fit statistics improved substantially by introduction of interaction terms. [a]) Set to zero because parameter is redundant.

parameter	B	std. error	95% Wald confidence interval		hypothesis test		
			lower	upper	Wald Chi-Square	df	sig.
test of model effects							
maternal_contribute * fruit_length					60.871	5	**0.000**
paternal_contribute * fruit_length					64.441	5	**0.000**
maternal_contribute * ripening_time					21.167	5	**0.001**
paternal_contribute * ripening_time					7.535	5	0.184
generation * fruit_length					3.036	1	0.081
generation * ripening_time					3.700	1	0.054
procedure * fruit_length					0.155	2	0.925
procedure * ripening_time					0.895	2	0.639
parameter estimates							
(Intercept)	**-2126.555**	258.4657	-2633.139	-1619.972	67.694	1	**0.000**
[maternal_contribute=joha] * fruit_length	**649.561**	124.6795	405.193	893.928	27.142	1	**0.000**
[maternal_contribute=joha_rexi] * fruit_length	**452.590**	79.6880	296.405	608.776	32.257	1	**0.000**
[maternal_contribute=joha_rose] * fruit_length	**648.636**	231.3464	195.205	1102.067	7.861	1	**0.005**
[maternal_contribute=rexi] * fruit_length	**425.763**	32.9636	361.155	490.370	166.827	1	**0.000**
[maternal_contribute=rexi_joha] * fruit_length	**503.471**	101.0375	305.441	701.501	24.830	1	**0.000**
[maternal_contribute=rexi_rose] * fruit_length	**380.134**	58.2136	266.037	494.231	42.641	1	**0.000**
[maternal_contribute=rose] * fruit_length	**937.876**	74.1450	792.555	1083.198	160.003	1	**0.000**
[maternal_contribute=rose_joha] * fruit_length	**616.169**	44.8338	528.296	704.041	188.881	1	**0.000**

parameter	B	std. error	95% Wald confidence interval		hypothesis test		
			lower	upper	Wald Chi-Square	df	sig.
[maternal_contribute=rose_rexi] * fruit_length	**473.873**	30.9010	413.308	534.438	235.168	1	**0.000**
[paternal_contribute=joha] * fruit_length	**-143.190**	41.9100	-225.332	-61.048	11.673	1	**0.001**
[paternal_contribute=joha_rexi] * fruit_length	-15.360	80.5988	-173.331	142.611	0.036	1	0.849
[paternal_contribute=joha_rose] * fruit_length	-343.356	266.0902	-864.884	178.171	1.665	1	0.197
[paternal_contribute=rexi] * fruit_length	**100.310**	16.1651	68.627	131.993	38.506	1	**0.000**
[paternal_contribute=rexi_joha] * fruit_length	0[a]						
[paternal_contribute=rexi_rose] * fruit_length	24.594	49.8614	-73.133	122.320	0.243	1	0.622
[paternal_contribute=rose] * fruit_length	0[a]						
[paternal_contribute=rose_joha] * fruit_length	0[a]						
[paternal_contribute=rose_rexi] * fruit_length	0[a]						
[maternal_contribute=joha] * ripening_time	**14.367**	6.9349	0.775	27.959	4.292	1	**0.038**
[maternal_contribute=joha_rexi] * ripening_time	6.467	6.3257	-5.931	18.865	1.045	1	0.307
[maternal_contribute=joha_rose] * ripening_time	-2.760	20.3652	-42.675	37.156	0.018	1	0.892
[maternal_contribute=rexi] * ripening_time	**12.706**	5.0074	2.891	22.520	6.438	1	**0.011**
[maternal_contribute=rexi_joha] * ripening_time	1.683	9.5641	-17.063	20.428	0.031	1	0.860
[maternal_contribute=rexi_rose] *ripening_time	**11.709**	5.9018	0.141	23.276	3.936	1	**0.047**
[maternal_contribute=rose] * ripening_time	-10.752	5.7845	-22.089	0.586	3.455	1	0.063
[maternal_contribute=rose_joha] * ripening_time	9.617	5.2347	-0.643	19.877	3.375	1	0.066
[maternal_contribute=rose_rexi] * ripening_time	2.167	2.3041	-2.349	6.683	0.884	1	0.347
[paternal_contribute=joha] * ripening_time	0.974	2.4432	-3.814	5.763	0.159	1	0.690
[paternal_contribute=joha_rexi] * ripening_time	2.507	8.7765	-14.695	19.708	0.082	1	0.775
[paternal_contribute=joha_rose] * ripening_time	22.511	22.2220	-21.043	66.066	1.026	1	0.311
[paternal_contribute=rexi] * ripening_time	-2.429	1.5731	-5.512	0.654	2.384	1	0.123
[paternal_contribute=rexi_joha] * ripening_time	0[a]						
[paternal_contribute=rexi_rose] * ripening_time	-6.889	4.8867	-16.467	2.689	1.987	1	0.159
[paternal_contribute=rose] * ripening_time	0[a]						
[paternal_contribute=rose_joha] * ripening_time	0[a]						
[paternal_contribute=rose_rexi] * ripening_time	0[a]						
[generation=F1] * fruit_length	121.883	69.9542	-15.225	258.990	3.036	1	0.081
[generation=F2] * fruit_length	0[a]						
[generation=P] * fruit_length	0[a]						
[generation=F1] * ripening_time	-12.289	6.3891	-24.812	0.233	3.700	1	0.054
[generation=F2] * ripening_time	0[a]						
[generation=P] * ripening_time	0[a]						
[procedure=allogamy] * fruit_length	1.956	15.4487	-28.323	32.235	0.016	1	0.899
[procedure=crossing] * fruit_length	24.656	63.7871	-100.364	149.676	0.149	1	0.699
[procedure=geitonogamy] * fruit_length	0[a]						
[procedure=allogamy] * ripening_time	-1.375	1.5062	-4.328	1.577	0.834	1	0.361
[procedure=crossing] * ripening_time	-2.143	6.0005	-13.904	9.617	0.128	1	0.721
[procedure=geitonogamy] * ripening_time	0[a]						
(Scale)	145548.226						

parameter	B	std. error	95% Wald confidence interval lower	95% Wald confidence interval upper	hypothesis test Wald Chi-Square	hypothesis test df	hypothesis test sig.
Goodness of Fit							
Quasi Likelihood under Independence Model Criterion (QIC)	16,738,068.92						
Corrected QIC (QICC)	16,738,116.01						

TABLE A X.5.3: COMPLETE GEE MODEL FOR SEED SET OF *STREPTOCARPUS* SPECIES AND HYBRIDS

Dependent variable is seed set. Significant covariate interaction terms introduced, other factors (generation of seed material, procedure) as main effects. Maternal contribute in interaction with fruit length shows highly significant and positive influence on seed set for all analysed species and hybrid variants. Therefore, seed number is predominantly controlled by the maternal line, in interaction with longer fruits containing more seed material. Paternal contribute in interaction with fruit length shows highly significant and negative influence on seed set for pollen material of *S. johannis* and hybrids johannis-roseoalbus and rexii-roseoalbus. For hybrids johannis-rexii and johannis-roseoalbus, significant and positive interactions become evident with ripening time, showing higher seed set for these hybrids as maternal strains when ripening time is longer. Contrary to this, the maternal strain of *S. roseoalbus* shows faster ripening when seed number is higher. Regarding generation level, production of F1 hybrid seed material from the species analysed represents a bottleneck: The effect is highly significant and strongly negative. As for procedures applied, geitonogamous pollination or true crossing are of no further explanatory value, seed set is however reduced significantly in a negative way for allogamous pollination of plants. Compared to the Covariate Interaction Model, Goodness of Fit statistics are not substantially affected by simplification of the model to significant interaction terms and main factors. [a]) Set to zero because parameter is redundant.

parameter	B	std. error	95% Wald confidence interval lower	95% Wald confidence interval upper	hypothesis test Wald Chi-Square	hypothesis test df	hypothesis test sig.
test of model effects							
maternal_contribute * fruit_length					49.455	5	**0.000**
paternal_contribute * fruit_length					124.927	5	**0.000**
maternal_contribute * ripening_time					27.861	9	**0.001**
generation					21.949	2	**0.000**
procedure					10.580	2	**0.005**
parameter estimates							
(Intercept)	**-1906.793**	586.6517	-3056.610	-756.977	10.564	1	**0.001**
[maternal_contribute=joha] * fruit_length	**790.503**	96.3856	601.591	979.415	67.264	1	**0.000**
[maternal_contribute=joha_rexi] * fruit_length	**444.427**	34.2932	377.213	511.640	167.952	1	**0.000**
[maternal_contribute=joha_rose] * fruit_length	**444.398**	73.9015	299.554	589.242	36.161	1	**0.000**
[maternal_contribute=rexi] * fruit_length	**516.925**	32.3862	453.449	580.401	254.763	1	**0.000**
[maternal_contribute=rexi_joha] * fruit_length	**493.220**	104.3217	288.753	697.686	22.353	1	**0.000**
[maternal_contribute=rexi_rose] * fruit_length	**420.369**	13.3608	394.182	446.555	989.916	1	**0.000**
[maternal_contribute=rose] * fruit_length	**1125.519**	100.2713	928.991	1322.048	125.995	1	**0.000**
[maternal_contribute=rose_joha] * fruit_length	**608.222**	61.4997	487.685	728.759	97.809	1	**0.000**
[maternal_contribute=rose_rexi] * fruit_length	**479.820**	37.0723	407.160	552.480	167.517	1	**0.000**
[paternal_contribute=joha] * fruit_length	**-128.817**	22.3602	-172.642	-84.992	33.189	1	**0.000**
[paternal_contribute=joha_rexi] * fruit_length	6.064	9.9038	-13.347	25.475	0.375	1	0.540
[paternal_contribute=joha_rose] * fruit_length	**-74.418**	10.4023	-94.806	-54.030	51.180	1	**0.000**
[paternal_contribute=rexi] * fruit_length	18.541	19.1357	-18.965	56.046	0.939	1	0.333

parameter	B	std. error	95% Wald confidence interval		hypothesis test		
			lower	upper	Wald Chi-Square	df	sig.
[paternal_contribute=rexi_joha] * fruit_length	0[a]						
[paternal_contribute=rexi_rose] * fruit_length	**-43.479**	8.0554	-59.268	-27.691	29.133	1	**0.000**
[paternal_contribute=rose] * fruit_length	0[a]						
[paternal_contribute=rose_joha] * fruit_length	0[a]						
[paternal_contribute=rose_rexi] * fruit_length	0[a]						
[maternal_contribute=joha] * ripening_time	3.907	8.8162	-13.372	21.187	0.196	1	0.658
[maternal_contribute=joha_rexi]*ripening_time	**5.071**	2.5885	-0.003	10.144	3.838	1	**0.050**
[maternal_contribute=joha_rose]*ripening_time	**13.035**	6.0766	1.125	24.945	4.601	1	**0.032**
[maternal_contribute=rexi] * ripening_time	6.649	8.1891	-9.402	22.699	0.659	1	0.417
[maternal_contribute=rexi_joha]*ripening_time	0.876	10.1585	-19.034	20.787	0.007	1	0.931
[maternal_contribute=rexi_rose]*ripening_time	5.662	3.3657	-0.935	12.258	2.830	1	0.093
[maternal_contribute=rose] * ripening_time	**-24.466**	7.4755	-39.118	-9.815	10.712	1	**0.001**
[maternal_contribute=rose_joha]*ripening_time	8.604	6.6534	-4.437	21.644	1.672	1	0.196
[maternal_contribute=rose_rexi]*ripening_time	-0.083	2.5833	-5.147	4.980	0.001	1	0.974
[generation=F1]	**-458.140**	104.2706	-662.507	-253.774	19.305	1	**0.000**
[generation=F2]	-93.635	640.1929	-1348.390	1161.120	0.021	1	0.884
[generation=P]	0[a]						
[procedure=allogamy]	**-82.819**	41.8887	-164.919	-0.718	3.909	1	**0.048**
[procedure=crossing]	44.621	42.7260	-39.121	128.362	1.091	1	0.296
[procedure=geitonogamy]	0[a]						
(Scale)	146197.576						
Goodness of Fit							
Quasi Likelihood under Independence Model Criterion (QIC)	17,836,128.97						
Corrected QIC (QICC)	17,836,160.29						

X.6 APPENDIX TO CHAPTER VII

TABLE A X.6.1: DATA BANDWIDTH OF FRONTAL AND LATERAL DISPLAY SIZE FOR *STREPTOCARPUS* SPECIES AND HYBRIDS

Presented are the number of samplings (*n*), arithmetic means and standard deviations for display size variables. Retrieved clusters (display sizes plus additional variables, see table A X.6.2) are indicated. Cluster 1 (△ parental, □ F1): Larger, funnel shaped flowers with larger flower opening. Cluster 2 (▲ parental, ■ F1): Moderate size, smaller opening. Cluster 3 (▲parental, ■ F1): Small flowers, complex floral architecture. Abbreviations for hybrids refer to the parental lines involved in the crossing, with the maternal crossing partner named in the first place.

species and hybrids	*n*	cluster	frontal display, cm²			lateral display, cm²			display ratio (f:l)		
Streptocarpus cooperi C.B.Clarke	10	△	3.88	±	1.29	2.96	±	0.33	1.29	±	0.30
Streptocarpus dunnii Mast.	10	△	1.09	±	0.22	2.47	±	0.29	0.44	±	0.06
Streptocarpus johannis L.L.Britten	10	▲	3.49	±	0.90	1.08	±	0.35	3.29	±	0.33
Streptocarpus longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	10	△	4.27	±	0.73	3.76	±	0.35	1.13	±	0.13
Streptocarpus modestus L.L.Britten	10	△	5.75	±	0.83	2.53	±	0.40	2.29	±	0.28
Streptocarpus pole-evansii I.Verd.	10	▲	0.70	±	0.06	0.41	±	0.05	1.72	±	0.23
Streptocarpus polyanthus Hook.	10	▲	1.23	±	0.22	0.52	±	0.06	2.35	±	0.26
Streptocarpus rexii (Bowie ex Hook.) Lindl.	10	△	7.53	±	1.02	5.53	±	0.71	1.38	±	0.22
Streptocarpus roseoalbus Weigend & T.J.Edwards	10	△	10.66	±	1.57	3.45	±	0.38	3.08	±	0.27
coop-joha	10	□	4.37	±	1.08	3.03	±	0.77	1.47	±	0.32
coop-long	10	□	5.60	±	1.59	6.00	±	1.10	0.93	±	0.20
coop-mode	10	□	3.70	±	1.08	3.81	±	1.09	0.97	±	0.12
coop-rexi	10	□	8.88	±	1.76	5.72	±	0.58	1.55	±	0.27
coop-rose	10	□	8.44	±	1.42	6.31	±	0.97	1.35	±	0.21
dunn-long	10	□	3.16	±	0.66	3.79	±	0.47	0.83	±	0.14
dunn-rexi	10	□	6.78	±	1.74	5.05	±	0.75	1.34	±	0.29
dunn-rose	10	□	7.79	±	0.61	3.52	±	0.45	2.24	±	0.28
joha-coop	1	■	3.22			2.63			1.22		
joha-dunn	10	■	3.03	±	0.62	2.27	±	0.53	1.35	±	0.19
joha-mode	10	□	3.98	±	1.19	2.60	±	0.55	1.54	±	0.36
joha-poly	10	■	1.38	±	0.29	1.58	±	0.62	0.96	±	0.34
joha-rexi	10	□	6.20	±	0.84	2.81	±	0.54	2.24	±	0.37
joha-rose	10	□	5.28	±	1.17	3.28	±	0.89	1.71	±	0.61
long-coop	10	□	5.51	±	1.15	4.62	±	0.99	1.21	±	0.17
long-dunn	10	□	4.53	±	1.19	5.07	±	0.95	0.89	±	0.17
long-rexi	10	□	3.69	±	1.80	4.36	±	0.89	0.85	±	0.35
long-rose	10	□	4.29	±	0.70	3.81	±	0.40	1.12	±	0.11
mode-coop	1	□	4.37			2.82			1.55		
mode-joha	10	□	2.10	±	0.49	1.93	±	0.51	1.12	±	0.26
mode-long	5	□	5.27	±	2.10	3.71	±	0.43	1.41	±	0.49
mode-poly	10	■	1.85	±	0.48	1.70	±	0.58	1.13	±	0.24
mode-rexi	5	□	5.92	±	1.99	3.89	±	0.65	1.52	±	0.37
mode-rose	10	□	5.74	±	1.37	4.11	±	1.06	1.43	±	0.33
poly-coop	10	■	2.80	±	0.38	3.37	±	0.66	0.85	±	0.15
poly-pole	1	■	0.51			0.60			0.84		
poly-rexi	10	■	3.45	±	0.85	3.08	±	0.96	1.17	±	0.27
poly-rose	9	■	2.94	±	0.72	2.36	±	0.35	1.26	±	0.30
rexi-coop	10	□	8.80	±	3.76	5.93	±	1.04	1.46	±	0.49
rexi-dunn	10	□	5.50	±	1.38	4.08	±	0.62	1.35	±	0.30
rexi-joha	10	□	4.72	±	1.39	2.09	±	0.66	2.29	±	0.46
rexi-long	10	□	7.13	±	1.61	5.53	±	0.65	1.29	±	0.24
rexi-mode	8	□	7.74	±	1.58	4.92	±	1.09	1.60	±	0.27
rexi-rose	10	□	10.64	±	1.15	5.80	±	0.66	1.85	±	0.23
rose-coop	10	□	6.39	±	1.22	5.53	±	1.13	1.19	±	0.30
rose-dunn	10	□	8.85	±	0.91	3.99	±	0.41	2.23	±	0.25
rose-joha	10	□	3.43	±	1.01	2.36	±	0.42	1.49	±	0.51
rose-long	10	□	6.64	±	1.64	4.34	±	1.03	1.54	±	0.31
rose-mode	1	□	3.52			2.58			1.36		
rose-rexi	10	□	9.13	±	1.71	5.42	±	0.64	1.69	±	0.28

TABLE A X.6.2: DATA BANDWIDTH OF FLORAL ARCHITECTURE FOR *STREPTOCARPUS* SPECIES AND HYBRIDS

Presented are the number of samplings (*n*) and arithmetic means for the following variables of floral architecture, in mm: Opening height, opening width, display height, display width, height and width of the unfused part of the upper left petal, height and width of the unfused part of the lower central petal, dorsal and ventral flower length, anther length, filament length and carpel length. Retrieved clusters of floral architecture are indicated (based on data presented here plus display size variables, see table A X.6.1). Cluster 1 (△ parental, □ F1): Larger, funnel shaped flowers with larger flower opening. Cluster 2 (▲ parental, ■ F1): Flowers of moderate size, smaller opening. Cluster 3 (▲parental, ■ F1): Small flowers with complex floral architecture. Abbreviations for hybrids refer to the parental lines involved in the crossing, with the maternal crossing partner named in the first place.

species and hybrids	*n*	cluster	opening height	opening width	display height	display width	petal up. l., height	petal up. l., width	petal l. c., height	petal l. c., width	flower length, dorsal	flower length, ventral	anther length	filament length	carpel length
S. cooperi C.B.Clarke	10	△	12.40	16.10	34.20	31.80	10.25	10.70	11.55	8.20	43.60	63.00	2.45	8.15	41.95
S. dunnii Mast.	10	△	7.40	7.30	13.50	13.50	3.90	5.30	6.80	5.80	37.80	40.10	2.00	12.80	41.25
S. johannis L.L.Britten	10	▲	4.50	2.10	29.10	28.85	10.55	5.50	11.00	7.30	15.85	34.90	1.60	3.40	12.90
S. longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	10	△	10.30	10.90	24.60	28.20	9.50	9.50	11.40	13.10	45.60	58.20	2.40	9.10	40.30
S. modestus L.L.Britten	10	△	12.15	11.60	37.35	40.80	12.60	8.15	15.20	10.00	22.85	43.80	2.85	7.00	16.45
S. pole-evansii I.Verd.	10	▲	2.20	3.10	11.20	11.05	2.80	2.85	4.50	3.15	11.15	18.20	1.40	1.70	5.75
S. polyanthus Hook.	10	▲	3.60	0.95	19.50	17.10	6.70	2.40	7.10	3.65	11.00	25.40	1.55	2.55	6.40
S. rexii (Bowie ex Hook.) Lindl.	10	△	13.80	16.30	41.60	45.70	14.80	10.90	16.40	11.20	49.20	71.00	3.00	9.90	42.30
S. roseoalbus Weigend & T.J.Edwards	10	△	15.85	19.60	42.40	46.20	12.05	12.40	15.90	11.85	29.40	49.90	3.25	8.40	27.00
coop-joha	10	□	5.00	4.90	20.90	31.80	14.00	7.00	13.00	8.90	25.50	50.60	2.00	5.30	22.40
coop-long	10	□	8.57	10.29	22.00	34.20	13.70	8.70	15.80	8.22	45.10	62.30	0	8.40	36.00
coop-mode	10	□	7.80	9.60	20.10	27.90	11.00	7.30	10.50	7.20	27.80	44.70	3.00	7.60	25.30
coop-rexi	10	□	9.20	14.50	31.80	43.40	16.00	11.10	16.10	10.30	46.80	71.80	2.90	9.70	47.10
coop-rose	10	□	10.50	17.00	34.40	46.60	16.60	12.30	18.10	12.20	35.70	63.70	3.10	8.30	37.20
dunn-long	10	□	9.50	10.40	16.70	21.90	6.50	6.80	7.80	6.60	42.20	48.60	3.00	12.10	41.00
dunn-rexi	10	□	10.80	11.40	28.60	32.10	11.30	9.00	12.11	8.22	49.40	61.10	2.70	10.90	45.10
dunn-rose	10	□	11.20	14.80	34.10	37.40	9.90	10.70	13.40	9.80	34.20	49.80	3.10	10.20	36.60
joha-coop	10	■	3.90	2.80	33.20	30.40	11.10	4.50	11.80	6.80	22.70	35.10	2.10	4.90	19.80
joha-dunn	10	■	4.90	3.80	19.40	21.30	8.50	4.60	9.10	5.50	23.60	35.80	1.30	5.40	19.60
joha-mode	10	□	4.40	4.90	21.50	35.50	13.00	6.40	13.80	8.40	22.00	41.20	2.00	4.60	14.40
joha-poly	10	■	3.00	2.30	13.00	21.70	9.40	3.70	9.70	5.50	14.50	32.10	2.00	3.00	9.50
joha-rexi	9	□	3.78	3.67	28.89	34.78	12.22	6.67	12.44	8.78	25.89	46.56	2.11	4.78	23.78
joha-rose	10	□	4.70	4.70	25.20	35.20	11.40	6.80	13.10	8.70	20.50	42.00	2.00	5.70	19.70
long-coop	10	□	8.90	9.80	23.50	31.00	12.70	8.90	13.30	8.30	42.90	62.10	1.83	7.20	41.90

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species and hybrids	*n*	cluster	opening height	opening width	display height	display width	petal up. l., height	petal up. l., width	petal l. c., height	petal l. c., width	flower length, dorsal	flower length, ventral	anther length	filament length	carpel length
long-dunn	10	□	10.60	11.20	21.20	25.20	8.90	7.20	9.90	7.40	46.50	55.70	3.30	12.60	41.80
long-rexi	10	□	7.89	7.00	18.50	25.90	13.80	6.20	16.10	5.20	35.50	53.50	0	0	40.40
long-rose	10	□	8.50	8.70	23.90	29.40	11.00	7.40	12.50	6.40	27.60	42.20	0	7.67	31.20
mode-coop	10	□	6.90	6.20	37.20	36.10	13.00	8.60	14.50	9.30	31.10	49.20	2.70	8.20	27.80
mode-joha	10	□	4.00	3.90	14.00	27.70	10.50	5.20	10.30	6.40	18.90	34.20	1.90	4.60	13.70
mode-long	10	□	8.10	8.20	36.10	37.30	12.00	10.20	13.80	9.70	37.80	49.80	3.30	9.70	34.00
mode-poly	10	■	4.10	4.00	15.60	26.00	9.80	3.70	10.10	4.80	17.30	33.00	2.00	3.50	10.40
mode-rexi	6	□	7.50	8.83	24.83	31.50	12.33	6.67	12.00	7.33	28.83	43.50	2.83	8.00	24.67
mode-rose	10	□	7.30	9.70	27.10	34.90	12.20	8.50	13.90	9.00	24.50	42.30	3.00	7.00	21.30
poly-coop	10	■	4.40	4.80	18.00	25.90	10.80	5.60	11.00	5.80	24.70	44.30	1.70	5.30	17.60
poly-pole	10	■	2.20	2.20	18.10	15.70	5.90	2.50	6.20	4.10	11.00	20.40	0.95	3.50	6.40
poly-rexi	10	■	4.50	4.30	17.40	22.90	11.00	4.60	10.90	5.40	25.20	42.80	2.00	5.70	18.30
poly-rose	10	■	5.90	4.50	19.70	27.70	10.10	5.30	11.40	6.80	19.10	36.70	2.00	4.50	15.30
rexi-coop	10	□	9.50	14.20	30.30	35.60	14.70	10.60	14.13	9.50	45.80	64.90	2.90	9.20	44.80
rexi-dunn	10	□	9.50	11.50	27.00	29.10	9.10	8.30	11.50	6.80	45.70	56.80	2.00	10.50	44.30
rexi-joha	10	□	4.40	3.20	21.60	30.50	11.20	6.30	11.50	7.20	25.00	42.80	5.10	2.60	22.10
rexi-long	10	□	10.80	13.80	26.90	37.30	13.50	10.10	13.60	9.70	46.20	66.80	3.00	10.20	44.60
rexi-mode	10	□	8.20	10.50	30.20	38.80	13.90	8.90	14.50	9.80	33.00	50.50	2.90	8.30	25.80
rexi-rose	10	□	9.10	10.00	36.70	43.20	15.20	11.00	16.40	11.60	35.90	58.40	3.00	8.80	35.30
rose-coop	10	□	11.00	16.10	28.40	42.20	14.90	11.40	15.70	11.60	35.10	60.10	3.00	9.20	34.80
rose-dunn	10	□	11.50	15.20	36.70	38.80	11.30	11.20	14.80	10.40	34.90	51.00	3.00	11.00	35.10
rose-joha	10	□	4.80	5.00	20.00	31.40	11.30	6.50	12.00	8.40	21.10	41.30	3.00	5.10	17.50
rose-long	10	□	9.90	11.50	29.50	40.80	13.10	10.60	14.60	11.30	34.30	55.00	2.89	9.67	34.20
rose-mode	10	□	8.30	8.80	32.60	33.70	10.80	7.70	12.30	9.10	23.30	36.20	2.50	8.60	20.50
rose-rexi	10	□	7.60	10.70	35.20	44.80	14.90	12.00	15.40	11.10	35.50	56.00	2.90	8.40	34.00

TABLE A X.6.3: COEFFICIENTS OF VARIATION FOR FLORAL ARCHITECTURE OF *STREPTOCARPUS* SPECIES AND HYBRIDS

The table shows the number of samplings (*n*) as well as the calculated coefficients of variation (CVs) for every applicable variable and every species and hybrid, in percent. Further, aggregated CVs are presented for variables and clusters. Two clusters were retrieved and are indicated (cluster 1: ▲ parental, ■ F1; cluster 2: △ parental, □ F1). Abbreviations for hybrids refer to the parental lines involved in the crossing, with the maternal crossing partner named in the first place.

species and hybrids	*n*	CV cluster	CV opening height	CV opening width	CV display height	CV display width	CV u. l. petal, height	CV up. l. petal, width	CV l. c. petal, height	CV l. c. petal, width	CV up. flower length	CV und. flower length	CV carpel length
S. cooperi C.B.Clarke	10	▲	9.65	7.44	4.93	6.30	7.71	7.37	8.77	6.55	3.28	2.48	3.66
S. dunnii Mast.	10	△	13.06	13.00	16.84	16.84	8.11	15.53	11.07	10.90	5.26	5.44	6.03
S. johannis L.L.Britten	10	▲	10.48	10.04	7.50	6.33	8.19	17.14	9.58	11.73	5.77	4.21	9.81
S. longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	10	▲	7.99	10.10	6.41	5.49	13.36	12.41	9.43	9.82	3.76	4.42	2.63
S. modestus L.L.Britten	10	▲	8.01	4.45	3.34	5.52	4.51	6.50	4.44	4.08	3.10	2.81	4.17
S. pole-evansii I.Verd.	10	△	19.17	12.72	6.72	9.41	12.49	8.47	5.24	15.06	5.61	5.52	9.39
S. polyanthus Hook.	10	▲	5.86	16.64	6.40	5.65	6.29	8.78	6.47	6.62	5.25	5.15	8.87
S. rexii (Bowie ex Hook.) Lindl.	10	▲	10.69	10.45	3.79	3.10	5.33	5.21	4.26	15.64	3.00	3.87	4.32
S. roseoalbus Weigend & T.J.Edwards	10	△	9.17	5.48	9.58	9.17	12.89	5.31	11.75	7.19	5.60	6.71	2.62
coop-joha	10	□	0.00	6.45	16.34	13.24	11.66	9.52	12.03	12.37	3.81	6.19	2.31
coop-long	10	□	18.88	10.82	26.85	16.12	6.92	7.76	13.93	26.35	13.12	14.61	8.59
coop-mode	10	□	8.11	8.78	24.20	20.17	16.60	19.43	18.10	24.32	10.00	10.39	5.91
coop-rexi	10	■	6.87	4.88	8.49	5.66	6.59	5.11	8.51	9.21	5.31	2.69	2.91
coop-rose	10	■	8.09	2.77	7.15	6.41	5.82	3.93	9.55	3.46	2.31	2.10	1.70
dunn-long	10	□	14.25	9.29	13.55	6.96	13.07	11.60	8.11	7.82	6.49	3.52	5.86
dunn-rexi	10	□	5.86	9.43	16.17	15.43	14.48	7.41	12.00	10.14	3.33	4.66	3.83
dunn-rose	10	■	3.76	6.21	4.68	2.87	5.73	4.51	6.29	6.45	4.09	2.81	2.94
joha-coop	10	□	14.56	15.06	18.56	18.49	25.29	11.71	21.81	23.81	10.80	20.34	5.73
joha-dunn	10	□	15.06	11.10	10.37	18.26	12.71	11.23	13.16	15.45	7.26	6.01	5.48
joha-mode	10	□	11.74	6.45	16.30	14.74	8.88	13.18	15.20	15.06	6.78	7.99	8.78
joha-poly	10	□	0.00	21.00	13.57	15.97	15.21	13.06	16.15	23.08	3.63	11.51	11.37
joha-rexi	9	□	17.65	19.28	13.89	7.30	14.62	10.61	7.09	11.07	7.34	6.45	1.85
joha-rose	10	□	17.52	17.52	8.11	9.06	19.04	13.51	19.86	20.31	5.27	9.19	4.18
long-coop	10	□	8.29	8.05	10.66	10.31	11.17	8.29	12.80	8.13	5.09	5.00	3.07
long-dunn	10	□	6.60	8.20	13.12	17.02	11.17	12.76	10.04	18.24	5.29	7.99	4.48
long-rexi	10	□	14.79	20.20	26.76	28.57	17.68	23.80	28.76	32.43	8.10	10.80	7.40

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species and hybrids	*n*	CV cluster	CV opening height	CV opening width	CV display height	CV display width	CV u. l. petal, height	CV up. l. petal, width	CV l. c. petal, height	CV l. c. petal, width	CV up. flower length	CV und. flower length	CV carpel length
long-rose	10	□	6.20	14.39	9.95	14.80	8.57	9.45	12.07	22.34	5.45	5.78	2.53
mode-coop	10	□	10.69	10.20	8.67	11.93	13.57	6.00	9.89	11.39	7.65	8.77	5.57
mode-joha	10	□	11.79	8.11	13.88	17.19	20.20	12.16	16.53	19.76	6.33	12.16	10.91
mode-long	10	■	9.11	16.06	5.45	5.66	8.78	6.20	4.58	6.96	4.80	4.62	2.77
mode-poly	10	□	7.71	23.57	16.05	19.36	19.12	13.06	18.93	23.65	9.84	11.87	6.72
mode-rexi	6	□	24.94	8.52	14.94	16.89	15.94	12.25	12.91	11.13	6.73	12.32	4.19
mode-rose	10	□	13.00	13.79	16.82	18.54	15.84	11.43	11.97	13.86	5.18	7.73	2.27
poly-coop	10	□	11.74	13.18	11.11	8.23	7.30	9.22	8.57	10.90	1.96	2.62	4.79
poly-pole	10	□	19.17	19.17	11.78	15.62	18.65	21.08	12.72	18.00	14.85	10.13	10.93
poly-rexi	10	□	11.71	11.23	16.07	15.05	16.03	18.33	18.58	21.74	5.55	7.69	5.79
poly-rose	10	□	9.62	11.71	7.95	15.60	13.57	9.11	9.43	6.20	12.20	4.27	5.38
rexi-coop	10	□	25.90	8.66	25.71	18.97	13.24	18.97	15.80	11.25	10.18	9.03	4.91
rexi-dunn	10	□	18.06	14.35	13.64	13.60	10.93	11.43	13.12	21.70	3.27	5.97	6.39
rexi-joha	10	□	11.74	13.18	13.66	17.91	20.96	21.23	16.52	29.13	7.06	17.34	4.98
rexi-long	10	□	8.51	12.22	12.32	9.63	11.18	3.13	9.30	6.96	3.19	3.72	3.21
rexi-mode	10	□	9.62	9.26	12.07	18.22	19.02	18.69	14.26	10.54	6.39	10.53	11.08
rexi-rose	10	□	8.11	14.91	9.09	11.33	7.47	6.06	9.62	6.03	5.79	4.29	2.33
rose-coop	10	□	6.06	9.47	11.28	11.81	10.23	8.47	9.52	9.27	2.49	4.60	2.97
rose-dunn	10	■	4.58	5.19	3.64	5.13	7.29	3.76	6.21	8.11	3.43	3.33	2.49
rose-joha	10	□	8.78	13.33	5.77	18.40	12.55	13.07	12.42	16.07	2.69	4.98	4.04
rose-long	10	□	10.04	10.25	14.04	14.19	17.05	10.14	12.59	11.84	4.57	7.07	2.69
rose-mode	10	□	21.29	17.60	12.20	12.20	14.34	12.32	15.35	15.06	7.31	8.42	4.15
rose-rexi	10	□	12.71	7.69	10.27	9.40	14.31	9.62	12.70	8.96	5.01	5.52	1.96
$\bar{x}$ CV, cluster 1: 6.24 %		▲■	7.74	8.57	5.62	5.28	7.24	7.36	7.10	8.06	4.01	3.50	4.21
$\bar{x}$ CV, cluster 2: 11.63 %		△□	12.16	12.31	13.92	14.63	14.00	12.06	13.42	15.46	6.49	8.08	5.39

TABLE A X.6.4: DATA BANDWIDTH OF NECTAR REWARD FOR *STREPTOCARPUS* SPECIES AND HYBRIDS

The table shows the total number of samplings (*n*), arithmetic means and standard deviations for nectar amount in µl, nectar sugar concentration in percent and derived sugar production in mg per flower for *Streptocarpus* species and hybrids. Four clusters were retrieved and are indicated. Cluster 1 (△ parental, □ F1), in average: 2.72 µl nectar amount, 17.8 % concentration, .47 mg sugar. Cluster 2 (▲ parental, ■ F1): 0.89 µl nectar amount, 36.9 % concentration, .36 mg sugar. Cluster 3 (▲ parental, ■ F1): 1.92 µl nectar amount, 26.8 % concentration, .55 mg sugar. Cluster 4 (▲ parental, ■ F1): 1.79 µl nectar amount, 51.1 % concentration, 1.08 mg sugar. Abbreviations for hybrids refer to the parental lines involved in the crossing, with the maternal crossing partner named in the first place.

species and hybrids	*n*	cluster	nectar amount, µl	concentration, %	sugar production, mg
S. cooperi C.B.Clarke	3 of 25	△	0.86 ± 0.23	16.70 ± 2.90	0.15 ± 0.05
S. dunnii Mast.	25	▲	6.67 ± 4.76	34.78 ± 16.77	2.19 ± 1.21
S. johannis L.L.Britten	25	▲	0.30 ± 0.14	25.28 ± 8.44	0.08 ± 0.06
S. longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	25	△	10.61 ± 6.60	16.40 ± 6.86	1.59 ± 0.86
S. modestus L.L.Britten	25	▲	0.91 ± 0.46	34.08 ± 9.79	0.33 ± 0.14
S. pole-evansii I.Verd.	25	▲	2.51 ± 1.59	57.00 ± 10.34	1.69 ± 0.89
S. polyanthus Hook.	25	△	0.85 ± 0.20	20.16 ± 2.32	0.18 ± 0.04
S. rexii (Bowie ex Hook.) Lindl.	25	△	2.56 ± 1.39	20.60 ± 6.99	0.53 ± 0.29
S. roseoalbus Weigend & T.J.Edwards	25	▲	2.26 ± 1.50	52.08 ± 22.99	1.20 ± 0.49
coop-joha	25	□	0.28 ± 0.17	16.64 ± 5.41	0.05 ± 0.03
coop-long	25	□	3.50 ± 1.25	21.96 ± 5.21	0.79 ± 0.24
coop-mode	25	▲	0.42 ± 0.27	35.36 ± 9.39	0.16 ± 0.10
coop-rexi	25	■	1.34 ± 0.93	23.84 ± 8.35	0.31 ± 0.19
coop-rose	10	▲	0.79 ± 0.39	31.00 ± 7.44	0.27 ± 0.13
dunn-long	25	■	4.79 ± 1.62	28.80 ± 4.11	1.53 ± 0.53
dunn-rexi	25	■	2.37 ± 1.31	27.92 ± 5.37	0.73 ± 0.40
dunn-rose	25	▲	1.69 ± 1.01	40.24 ± 5.34	0.85 ± 0.61
joha-coop	25	□	0.40 ± 0.16	17.24 ± 3.38	0.07 ± 0.03
joha-dunn	25	■	1.54 ± 0.62	29.84 ± 5.10	0.50 ± 0.14
joha-mode	25	▲	0.19 ± 0.11	32.20 ± 9.95	0.08 ± 0.05
joha-poly	25	■	0.61 ± 0.51	27.60 ± 9.23	0.14 ± 0.09
joha-rexi	25	■	0.66 ± 0.45	25.44 ± 5.74	0.17 ± 0.10
joha-rose	25	▲	0.34 ± 0.11	34.88 ± 5.62	0.14 ± 0.05
long-coop	25	□	3.70 ± 1.05	11.72 ± 3.59	0.43 ± 0.14
long-dunn	25	■	6.45 ± 2.48	23.68 ± 2.85	1.62 ± 0.52
long-rexi	25	□	4.24 ± 1.64	19.52 ± 3.96	0.84 ± 0.26
long-rose	25	■	1.99 ± 0.98	25.96 ± 4.68	0.53 ± 0.10
mode-coop	25	■	0.37 ± 0.19	28.88 ± 7.62	0.12 ± 0.06
mode-joha	25	▲	0.22 ± 0.21	36.40 ± 5.44	0.09 ± 0.08
mode-long	25	▲	1.26 ± 0.41	40.88 ± 3.62	0.61 ± 0.20
mode-poly	25	▲	0.15 ± 0.08	39.04 ± 9.82	0.07 ± 0.05
mode-rexi	25	▲	0.30 ± 0.27	35.20 ± 9.97	0.12 ± 0.11
mode-rose	25	▲	0.29 ± 0.11	33.84 ± 11.38	0.12 ± 0.08
poly-coop	25	■	1.03 ± 0.74	24.36 ± 6.97	0.28 ± 0.21
poly-pole	25	▲	0.67 ± 0.22	32.16 ± 10.72	0.24 ± 0.11
poly-rexi	25	▲	0.42 ± 0.15	42.36 ± 8.13	0.21 ± 0.08

species and hybrids	*n*	cluster	nectar amount, µl	concentration, %	sugar production, mg
poly-rose	25		0.55 ± 0.36	38.00 ± 8.78	0.25 ± 0.16
rexi-coop	25		0.70 ± 0.37	39.04 ± 8.40	0.34 ± 0.21
rexi-dunn	25		2.22 ± 0.89	36.60 ± 4.36	0.92 ± 0.33
rexi-joha	3		0.18 ± 0.06	17.33 ± 3.51	0.03 ± 0.01
rexi-long	25		1.67 ± 0.95	30.56 ± 6.14	0.56 ± 0.29
rexi-mode	25		0.38 ± 0.24	40.92 ± 10.19	0.21 ± 0.17
rexi-rose	25		0.35 ± 0.09	42.76 ± 10.40	0.18 ± 0.06
rose-coop	25		0.73 ± 0.22	33.04 ± 5.87	0.27 ± 0.07
rose-dunn	25		0.94 ± 0.49	46.64 ± 10.72	0.56 ± 0.30
rose-joha	25		0.39 ± 0.13	35.12 ± 6.33	0.16 ± 0.07
rose-long	25		1.47 ± 0.39	48.84 ± 4.16	0.87 ± 0.18
rose-mode	25		0.27 ± 0.21	42.72 ± 10.25	0.14 ± 0.08
rose-rexi	25		0.47 ± 0.17	39.08 ± 11.16	0.23 ± 0.11

TABLE A X.6.5: COEFFICIENTS OF VARIATION FOR NECTAR REWARD OF *STREPTOCARPUS* SPECIES AND HYBRIDS
The table shows the number of samplings (*n*) and the calculated coefficients of variation (CVs) for every nectar variable and every species and hybrid, in percent. Aggregated CVs are presented for variables and clusters. Two clusters were retrieved and are indicated (cluster 1: △ parental, □ F1; cluster 2: ▲ parental, ■ F1). Abbreviations for hybrids refer to the parental lines involved in the crossing, with the maternal crossing partner named in the first place.

species and hybrids	*n*	CV cluster	CV nectar amount	CV concentration	CV sugar
S. cooperi C.B.Clarke	3 of 25	△	26.74	17.37	33.33
S. dunnii Mast.	25	▲	71.30	48.21	55.12
S. johannis L.L.Britten	25	▲	47.23	33.40	66.23
S. longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	25	▲	62.21	41.83	53.79
S. modestus L.L.Britten	25	▲	49.92	28.72	42.22
S. pole-evansii I.Verd.	25	▲	63.35	18.15	52.31
S. polyanthus Hook.	25	△	23.92	11.52	22.64
S. rexii (Bowie ex Hook.) Lindl.	25	▲	54.09	33.95	53.97
S. roseoalbus Weigend & T.J.Edwards	25	▲	66.68	44.13	40.46
coop-joha	25	■	60.75	32.50	62.16
coop-long	25	□	35.62	23.72	30.13
coop-mode	25	■	65.15	26.57	58.00
coop-rexi	25	■	69.24	35.03	62.24
coop-rose	10	■	49.04	24.00	47.19
dunn-long	25	□	33.92	14.28	34.87
dunn-rexi	25	■	55.34	19.23	54.88
dunn-rose	25	■	60.11	13.27	71.88
joha-coop	25	□	39.26	19.62	39.93
joha-dunn	25	□	40.17	17.08	28.76
joha-mode	25	■	55.83	30.91	71.63
joha-poly	25	■	83.54	33.44	65.24
joha-rexi	25	■	68.81	22.55	59.61
joha-rose	25	□	31.20	16.12	37.80
long-coop	25	□	28.46	30.62	32.07
long-dunn	25	□	38.42	12.05	32.42
long-rexi	25	□	38.75	20.28	30.57
long-rose	25	□	49.42	18.02	19.48
mode-coop	25	■	52.80	26.40	53.75
mode-joha	25	■	99.11	14.94	88.35
mode-long	25	□	32.67	8.86	33.18
mode-poly	25	■	52.23	25.15	68.08
mode-rexi	25	■	90.30	28.31	86.65
mode-rose	25	■	36.96	33.62	69.54
poly-coop	25	■	72.44	28.61	74.87
poly-pole	25	□	32.41	33.34	44.05
poly-rexi	25	□	34.86	19.19	39.11
poly-rose	25	■	65.62	23.10	64.76
rexi-coop	25	■	52.47	21.53	62.08

species and hybrids	*n*	CV cluster	CV nectar amount	CV concentration	CV sugar
rexi-dunn	25	□	40.17	11.91	35.42
rexi-joha	3	□	32.78	20.26	36.40
rexi-long	25	■	56.98	20.11	51.60
rexi-mode	25	■	63.21	24.90	83.61
rexi-rose	25	□	26.04	24.33	35.71
rose-coop	25	□	30.09	17.77	27.75
rose-dunn	25	■	51.99	22.98	54.56
rose-joha	25	□	32.37	18.01	43.09
rose-long	25	□	26.52	8.52	20.37
rose-mode	25	■	76.45	23.99	61.76
rose-rexi	25	□	36.48	28.55	49.56
$\bar{x}$ CV, cluster 1		△□	33.82	18.64	33.65
$\bar{x}$ CV, cluster 2		▲■	62.61	27.84	62.02

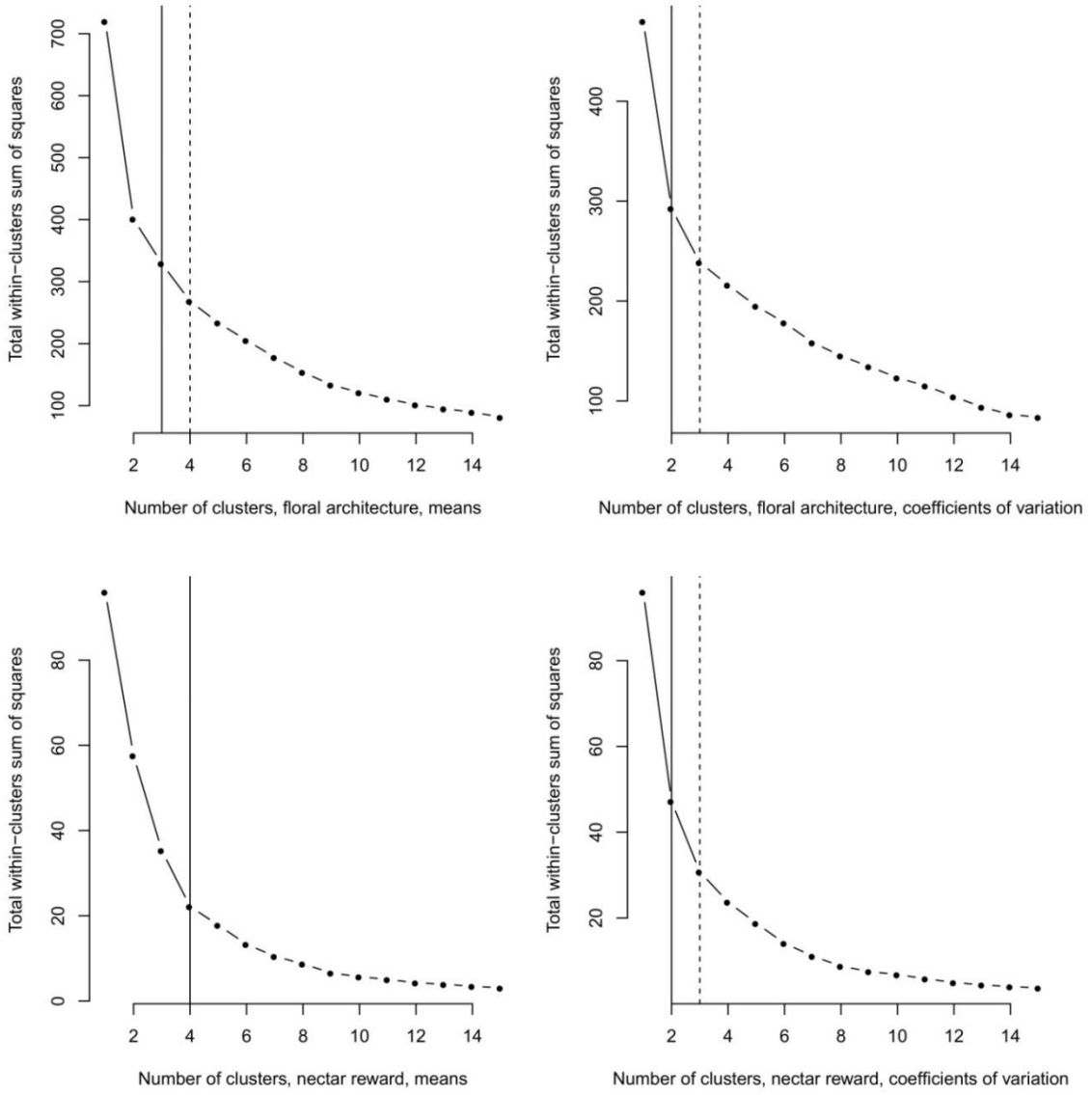

FIGURE A X.6.1: SELECTION OF CLUSTERS (FLORAL ARCHITECTURE, REWARD) FOR *STREPTOCARPUS* SPECIES AND HYBRIDS
Appropriate number of clusters was selected by Elbow method and via R package NBClust, for following metric data sets: a) arithmetic means of floral architecture (top, left), b) CVs for floral architecture (top, right), c) arithmetic means of nectar reward (bottom, left) and d) CVs of nectar reward (bottom, right). The Charts show total intra-cluster variation (y-axis: total within-cluster sum of squares) in dependence to k clusters (x-axis). In regard to Elbow-method, the position of a bend in the plot is considered as indicator for the best number of clusters, with higher cluster numbers not adding substantially to the compactness of the clusters (only monotonous, linear progress). Results of simultaneously tested indices via NBClust (solid lines) were given primacy over decisions based on Elbow-method (dashed lines). a) Arithmetic means of floral architecture, NBClust: 9 out of 24 indices propose 3 as the best number of clusters, only 2 propose 4. Elbow-method: More than 4 clusters would not add substantially to compactness. Decision: 3 clusters selected. b) CVs for floral architecture, NBClust: 12 out of 24 indices propose 2 as the best number of clusters, 10 propose 3. Elbow-method: More than 3 clusters would not add substantially to compactness. Decision: 2 clusters selected. c) Arithmetic means of nectar reward, NBClust: 7 out of 23 indices propose 4 as the best number of clusters, 4 indices propose 3, no index proposes 5. Elbow-method: More than 4 clusters would not add substantially to compactness. Decision: 4 clusters selected. d) CVs of nectar reward, NBClust: 7 out of 23 indices propose 2 as the best number of clusters, 2 propose 3. Elbow-method: More than 3 clusters would not add substantially to compactness. Decision: 2 clusters selected.

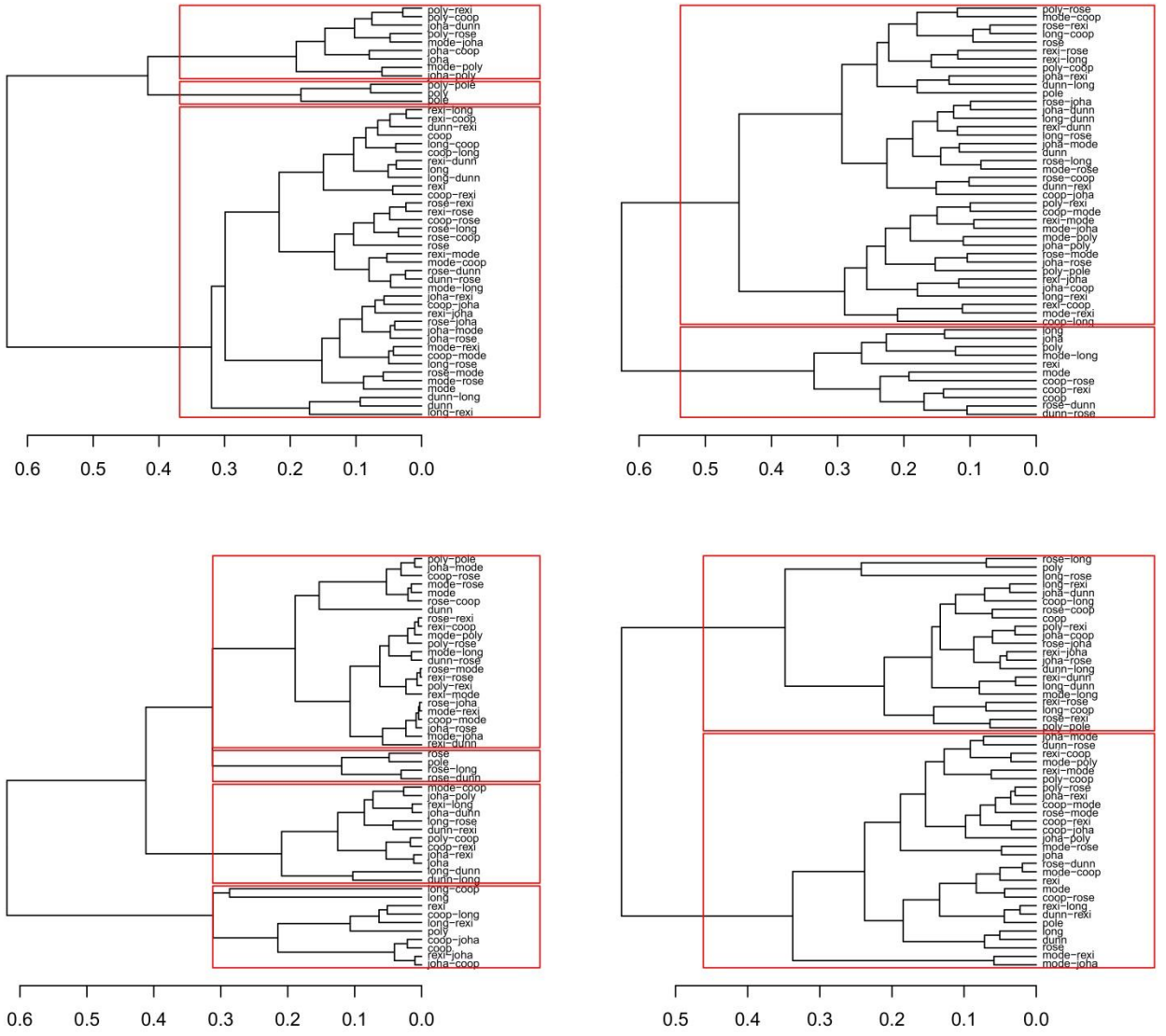

FIGURE A X.6.2: HIERARCHICAL CLUSTERING (FLORAL ARCHITECTURE, REWARD) FOR *STREPTOCARPUS* SPECIES AND HYBRIDS

Hierarchical clustering of four metric data sets. Selected clusters are highlighted in red. a) Arithmetic means of floral architecture (top, left), b) CVs for floral architecture (top, right), c) arithmetic means of nectar reward (bottom, left) and d) CVs of nectar reward (bottom, right). Cluster analyses were conducted with complete linkage, and dissimilarity matrices were produced via Bray Curtis index. Selection of most informative number of clusters was based on visual inspection of retrieved trees, application of Elbow method and simultaneously tested indices via R package NBClust (see figure A X.6.1).

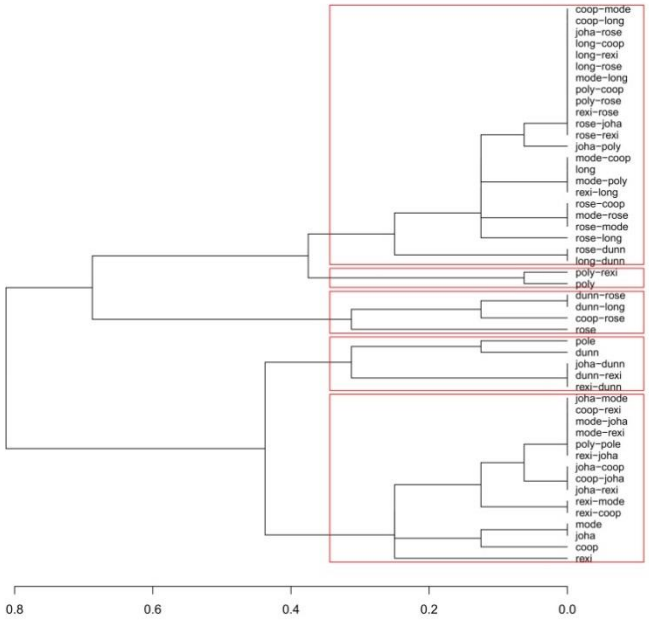

FIGURE A X.6.3: HIERARCHICAL CLUSTERING (OPTICAL FLOWER SIGNAL) FOR *STREPTOCARPUS* SPECIES AND HYBRIDS

Hierarchical clustering of optical flower signal, selected clusters are highlighted in red. Cluster analysis conducted with complete linkage, dissimilarity matrix based on Gower distances. Selection of most informative number of clusters based on visual inspection of the retrieved tree and simultaneously tested indices via R package NBClust: 10 out of 23 indices propose 5 as the best number of clusters, 1 index proposes 6, no index proposes 4. Elbow-Method could not be applied due to emergence of to many cluster centres. Decision: 5 clusters selected.

TABLE A X.6.6: ACCESSION AND VOUCHER NUMBERS FOR *STREPTOCARPUS* SPECIES AND HYBRIDS

Bonn University Botanical Gardens and Herbarium BONN, Nees Institut für Biodiversität der Pflanzen, Germany.

species	accession number	voucher number
Streptocarpus cooperi C.B.Clarke	36665	1313
Streptocarpus dunnii Mast.	33901	-
Streptocarpus johannis L.L.Britten	36678	1297
Streptocarpus longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	36682	1251
Streptocarpus modestus L.L.Britten	36683	1288
Streptocarpus pole-evansii I.Verd.	10393	-
Streptocarpus polyanthus Hook.	36685	-
Streptocarpus rexii (Bowie ex Hook.) Lindl.	36688	1286
Streptocarpus roseoalbus Weigend & T.J.Edwards	36105	1254
hybrids		
S. cooperi x *S. johannis*	38461	2453
S. cooperi x *S. longiflorus*	38462	2540
S. cooperi x *S. modestus*	38463	2451
S. cooperi x *S. rexii*	38464	2427
S. cooperi x *S. roseoalbus*	38465	2424
S. dunnii x *S. longiflorus*	38467	-
S. dunnii x *S. roseoalbus*	38469	2546
S. dunnii x *S. rexii*	38468	2548
S. johannis x *S. cooperi*	38470	2464
S. johannis x *S. dunnii*	38475	2547
S. johannis x *S. modestus*	38471	2549
S. johannis x *S. polyanthus*	38472	2531
S. johannis x *S.rexii*	38473	2721
S. johannis x *S. roseoalbus*	38474	2672
S. longiflorus x *S. cooperi*	38476	2524
S. longiflorus x *S. dunnii*	38477	-
S. longiflorus x *S. rexii*	38478	2649
S. longiflorus x *S. roseoalbus*	38479	2670
S. modestus x *S.cooperi*	38480	-
S. modestus x *S. johannis*	38495	2426
S. modestus x *S. longiflorus*	38496	2423
S. modestus x *S. polyanthus*	38497	2669
S. modestus x *S. rexii*	38498	2534
S. modestus x *S. roseoalbus*	38466	2529
S. polyanthus x *S. cooperi*	38499	-
S. polyanthus x *S. rexii*	38481	2545
S. polyanthus x *S. roseoalbus*	38482	2421
S. polyanthus x *S. pole-evansii*	38483	-
S. rexii x *S. cooperi*	38484	2463
S. rexii x *S. dunnii*	38485	2422
S. rexii x *S. johannis*	38486	2720
S. rexii x *S. longiflorus*	38487	2651
S. rexii x *S. modestus*	38488	2650
S. rexii x *S. roseoalbus*	38489	2454
S. roseoalbus x *S. cooperi*	38491	2455
S. roseoalbus x *S. dunnii*	38490	2573
S. roseoalbus x *S. johannis*	38492	2456
S. roseoalbus x *S. longiflorus*	38500	-
S. roseoalbus x *S. modestus*	38493	2559
S. roseoalbus x *S. rexii*	38494	2457

XI. CURRICULUM VITAE

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