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DIPLOMARBEIT

Titel der Diplomarbeit

Leaf colour patterns, vegetative and sexual reproduction of *Episcia lilacina* (Gesneriaceae)

angestrebter akademischer Grad

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Wissenschaftliche Forschung läuft immer darauf hinaus, dass es plötzlich mehrere Probleme gibt, wo es früher ein einziges gegeben hat.
(Norman Mailer)

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Abstract

Episcia lilacina is a low, stoloniferous, terrestrial herb. Vegetative propagation via stolons is an effective strategy to expand the habitat. On average, the stolons of *Episcia lilacina* grow 6.5 mm per day. Over the time of 80 days a plant can spread over an area of about one square meter.

The colours and patterns of the leaves of *Episcia lilacina* are varied. Almost all conceivable variations between dark red and bright green occur. Anthocyanins are responsible for the red colour and are also present in the bright green leaves, but in a lower concentration. The highest concentration of the anthocyanins is found near the palisade layer.

There is a correlation between the amount of light passing through the leaf and its proportion of red-green. Furthermore, bright green morphs are exposed to higher doses of light than the dark red ones.

Brightening in colour of red leaf areas was observed after transferring to a sunnier location. One possible explanation, and thus the reason for the leaf colouration of *Episcia lilacina* is that chloroplasts of shade plants are not able to deal with enhanced light exposure and therefore were destroyed. On the contrary, chloroplasts of green morphs are adapted to higher sun irradiation from the beginning, thus no additional anthocyanin development was observed.

Episcia lilacina produces single, hypocrateriform flowers of zygomorphic symmetry. The delicate flowers have a lilac-coloured limb, nectar hidden in a spur, with a long distance between the sexual organs and the nectar. The flowers are protandrous. Self-pollination can be excluded. Sexual reproduction is largely replaced by stolon spreading.

The fruit of *Episcia lilacina* is a capsule with globular, reddish or white seeds. Over a time of 25 days the fruit grows averagely 5,5 mm in width and 4 mm in length. The fruit set of *Episcia lilacina* is 19%.

The P/O ratio of *Episcia lilacina* is 36,34 : 1. Due to Cruden (1975) this would indicate obligate autogamy. One possible explanation is pollen agglutination.

The maximum amount of secreted nectar by one flower is 5,4 µl. Further on, the nectar has a sugar concentration of 27%. Both facts indicate butterflies as legitimate pollinators.

However, the only observed flower visitor of *Episcia lilacina* during the investigation period was a species of stingless bees (subfamily Meliponinae). These bees are not the legitimate pollinators, but secondary ones.

Deutsche Zusammenfassung

Diese Diplomarbeit ist in zwei Teile gegliedert. Der klassische Aufbau wurde insofern abgeändert, dass jedes einzelne Kapitel in Einleitung, Material und Methoden, Ergebnisse und Diskussion unterteilt ist. Dies erschien angesichts der vielschichtigen Themengebiete am übersichtlichsten. Die empirischen Daten zu dieser Arbeit wurden im Regenwald der Österreicher (Parque Nacional Piedras Blancas) im Jahre 2006 in der Nähe von La Gamba erhoben.

Die deutsche Zusammenfassung vermittelt einen gerafften Überblick zur Thematik und den wichtigsten Ergebnissen. Auf redundante Wiedergabe von Tabellen, Darstellungen und Literaturliste wurde in der deutschen Zusammenfassung verzichtet, diese sind jedoch im englischen Teil zu finden.

Generelle Beschreibung des Pflanzenhabitus. *Episcia lilacina* ist eine ausläuferbildende, terrestrische, krautige Pflanze. Die Blätter weisen sehr unterschiedliche Farben (von hellgrün bis dunkelrot) und Blattzeichnungen auf. Die Wurzeln von *Episcia lilacina* wachsen nahe der Bodenoberfläche. An den Nodien werden adventive Wurzeln gebildet. Axilläre Ausläufer werden in den Achseln der unteren Blattpaare gebildet.

Ausbreitung über Ausläufer. Vegetative Fortpflanzung spielt eine wichtige Rolle bei *Episcia lilacina*. Sie bildet Ausläufer. Um herauszufinden, ob die Ausbreitung über Stolone eine effektive Alternative zur sexuellen Fortpflanzung darstellt, wurde die Wachstumsgeschwindigkeit der Ausläufer eruiert.

Ausläufer werden bevorzugt in den Blattachseln unterer Nodien gebildet. Der durchschnittliche Längenzuwachs eines Ausläufers pro Tag liegt bei 6,5 mm. Es vergehen einige Wochen bis die Trennung von der Ausgangspflanze erfolgt.

Die Ausläuferbildung ermöglicht ein besseres Ausnutzen eines optimalen Lebensraums. Die Mutterpflanze steckt ihre Energie nicht in die Bildung von Blüten und später Früchten. Sie ist somit unabhängiger gegenüber Bestäubern und der Verbreitung.

Allerdings sind auch Nachteile mit der vegetativen Vermehrung verbunden. Die Möglichkeit entferntere Habitate zu besetzen, ergibt sich nur durch die sexuelle Reproduktion d.h. die

Verbreitung von Samen. Ein weiterer Nachteil ist, dass Ausläufer genetisch idente Kopien sind, somit also keine Rekombination stattfindet.

Beispiel für die Ausbreitung über Ausläufer. Um besser zu veranschaulichen, wie schnell die Ausbreitung über Ausläufer vor sich geht, wurde das Stolonenwachstum einer ausgewählten Pflanze dokumentiert. Dabei ergab sich, dass *Episcia lilacina* unter günstigen Bedingungen im Stande ist, eine Fläche von einem Quadratmeter innerhalb von 80 Tagen mit Ausläufern zu überdecken.

Blattfärbung. *Episcia lilacina* weist eine breite Palette an Blattfarben und -mustern auf. Es wurde eine Einteilung in acht verschiedene Typen vorgenommen. Bei Typ 1 ist das ganze Blatt rot. Der Rotanteil verringert sich von Typ zu Typ immer mehr, bis schließlich bei Typ 8 das gesamte Blatt grün gefärbt ist.

Da eine Daten-DVD einen irreparablen Schaden erlitt, gingen die Daten zur zahlenmäßigen Verteilung der verschiedenen Typen verloren. Auf Beobachtungen basierend konnte jedoch bestimmt werden, dass Pflanzen mit Typ 1 und 2 am häufigsten zu finden waren. Alle anderen Typen kommen eher selten vor.

Anthocyan-Nachweis und Analyse der Farbstoffe durch das UV/VIS Spektrometer. Um sicher zu gehen, dass Anthocyane für die Rotfärbung der Blätter verantwortlich sind, wurde ein pH-Nachweis durchgeführt. Dabei wurde die Tatsache genutzt, dass Anthocyane mit Veränderung des pH-Werts die Farbe wechseln. Die Anthocyane wurden aus den Blättern extrahiert und solange mit Salzsäure (0,01 mol/l) versetzt bis ein pH-Wert von 2 erreicht wurde. Bei niedrigem pH-Wert haben Anthocyane eine deutlich rote Farbe. Anschließend wurde solange eine Ammoniaklösung (50%ig) zugesetzt, bis ein pH-Wert von 9 erreicht wurde. Die Farbe wechselte zu braun. Der Farbumschlag beweist, dass es sich tatsächlich um Anthocyane handelt. Als Vergleich dazu wurde das gleiche Experiment auch mit Blättern von *Saintpaulia ionantha* durchgeführt.

Um den Unterschied im Anthocyangehalt von grünen und roten Blättern zu demonstrieren, wurde das UV/VIS Spektrometer eingesetzt. Aus hellgrünen und dunkelroten Blättern wurden die Anthocyane extrahiert und anschließend im UV/VIS Spektrometer gemessen. Dabei wurde ersichtlich, dass in den hellgrünen Blättern ebenfalls Anthocyane vorhanden sind. Jedoch in so geringer Konzentration, dass diese für das menschliche Auge nicht mehr sichtbar sind.

Lokalisierung der Anthocyane. Durch die Lokalisierung der Anthocyane lassen sich Aussagen über deren Funktion im Blatt treffen. Dazu wurden Querschnitte durch Blätter angefertigt. Dabei wurde sichtbar, dass die Anthocyane überwiegend im Schwammgewebe des Blattes eingelagert sind. Ihre Konzentration ist direkt unter dem Pallisadengewebe am höchsten. Daraus lässt sich schließen, dass die Anthocyane keine Funktion als Schutz der Chloroplasten vor übermäßiger Sonneneinstrahlung haben, denn dazu müssten sie in der Epidermis eingelagert sein.

Blattmimikry. Die verschiedenen Blattfärbungen und –muster lassen den Gedanken an Mimikry aufkommen. Die kontrastreichen Färbungen könnten ein abgefressenes Blatt vortäuschen und somit potentielle Fraßschädlinge in die Irre führen. Beobachtungen diesbezüglich ergaben, dass nur selten Fraßspuren bei *Episcia lilacina* vorkommen. Niemals wurde ein Standort mit großteils abgefressenen Blättern gefunden. Auch konnte kein Blatttyp ausgemacht werden, der bevorzugt angegriffen wurde. Sicherlich spielt auch die dichte Behaarung der Blätter eine Rolle. Womöglich sind in den Blätter Abwehrstoffe als wirksamer Schutz gegen Fressfeinde eingelagert. Es wurde nur eine einzige Raupenart an den Blättern fressend entdeckt. Sie gehört vermutlich zur Familie der Crambidae (den Motten angehörend).

Lichtdurchlässigkeitsmessung. Um zu überprüfen, ob eine Korrelation zwischen Blattmustern und Lichtabsorption der Blätter vorliegt, wurde eine Lichtdurchlässigkeitsmessung vorgenommen. Dazu wurden Blätter verschiedener Typen auf eine Lichtquelle, die konstant 5000 Lux emittierte, gelegt und das durchgelassene Licht mittels eines Luxmeters gemessen. Dunkel gefärbte Blätter lassen durchschnittlich 2-4 Lux, hell gefärbte Blätter bis zu 15 Lux Licht durch. Da die erhobenen Daten keiner Normalverteilung entsprachen, wurden zwei nicht parametrische Korrelationstests durchgeführt (Spearman-Rho Test und Kendall-Tau-b Test). Beide Tests ergaben eine positive Korrelation (0,605 beim Kendall-Tau-b Test; 0,779 beim Spearman-Rho Test). Je grüner die Blätter, desto mehr Licht kann durch das Blatt dringen. Anthocyane absorbieren einen Teil des einfallenden Lichtes, was jedoch nicht bedeutet, dass nur aus diesem Grund Anthocyane in die Blätter eingelagert werden.

Einstrahlungsmessung. Da eine Korrelation zwischen Licht und Anthocyangehalt in den Blättern bewiesen wurde, stellt sich die Frage, ob die verschiedenen Morphen tatsächlich an unterschiedlich hellen bzw. dunklen Standorten vorkommen. Dazu wurde an Standorten, wo Pflanzen mit dem Blatttyp 1, 2, 6 und 8 wuchsen, die einfallende Lichtmenge gemessen. Die

Daten wurden immer zwischen 9 und 12 Uhr vormittags erhoben, um - aufgrund des Wetters - vergleichbare Messergebnisse zu erhalten. Dabei wurde festgestellt, dass am wenigsten Licht auf die Pflanzen des Typ 1 fällt. Die größte Menge an einfallendem Licht wurde bei Pflanzen des Typs 8 gemessen. Je weniger Licht auf die Pflanze fällt, desto mehr Anthocyane werden entwickelt. Die Anthocyane könnten möglicherweise als Schutz vor kurzzeitig intensiver Sonneneinstrahlung dienen (Erklärung siehe nächstes Kapitel). Die Ergebnisse der hierzu durchgeführten Experimente sind jedoch zu wenig aussagekräftig. Weitere Test müssen durchgeführt werden, in denen näher auf die verschiedenen Morphen eingegangen wird.

Farbänderungen der Blätter im Zusammenhang mit einem Standortwechsel. Als logische Konsequenz der oben angeführten Experimente bleibt die Frage offen, ob sich Pflanzen an neue Gegebenheiten die Sonneneinstrahlung betreffend anpassen können. Dazu wurden Pflanzen verschiedener Typen aus dem Regenwald entnommen. Einige wurden an einen dunklen Standort (maximal 6 Lux) gestellt, während andere an einen hellen Standort (maximal 34000 Lux) verpflanzt wurden. Sowohl Pflanzen mit hellem Blatttyp als auch Pflanzen mit dunklem Blatttyp konnten mit dem geringen Lichteinfall an dem dunklen Standort gut umgehen. Es wurden keine Veränderungen in der Blattfärbung festgestellt.

Bei den an einen hellen versetzten Standort Pflanzen kam es zu einem Aufhellen der rot gefärbten Spreitenteile. Bei rein hellgrünen Pflanzen konnte eine leichte Intensivierung des Grüns festgestellt werden. Bei beiden Standorten konnte keine Veränderung der Blatttypen bei den einzelnen Pflanzen beobachtet werden.

Eine eindeutige Erklärung für die Einlagerung der Anthocyane in die Blätter von *Episcia lilacina* wurde nicht gefunden. Einige Autoren haben jedoch eine Erklärung gefunden, die in diesem Fall nahe liegend erscheint: Im Schatten wachsende Pflanzen besitzen eine höhere Konzentration an „Photosystem II“ gegenüber Pflanzen, die sich an sonnigen Standorten entwickeln. Dadurch sind Schattenpflanzen allerdings anfälliger für Photoinhibition und Schäden (wie z.B. reaktive Sauerstoffzwischenprodukte) verursacht durch kurzzeitig intensive Sonneneinstrahlung. Als Schutz der Chloroplasten werden Anthocyane eingelagert, die das übermäßige Licht abfangen. Um diese Hypothese zu überprüfen, sind jedoch noch weitere Experimente nötig.

Beschreibung der Blüten. *Episcia lilacina* bildet Einzelblüten mit zygomorpher Symmetrie. Die Krone hat eine blass-lila bis blass-blaue Farbe und setzt sich aus 5 Petalen zusammen, welche miteinander verwachsen sind und eine lange Röhre mit Stielteller bilden. Der Eingang in die

Blüte ist sehr schmal, somit kann sie als „keyhole"-Blüte bezeichnet werden. Dorsal an der Basis der Corolla sitzt ein Sporn, der dazu dient den gebildeten Nektar aufzufangen.

Die besondere Blütenform von *Episcia lilacina* („keyhole"-Blüte) impliziert, dass der legitime Bestäuber einen langen Rüssel besitzen muss, um an den Nektar im Sporn zu gelangen. Die in Frage kommenden Bestäuber sind also unter den Tagfaltern, Nachtfaltern, Motten, Prachtbienen und Hummeln zu suchen.

Die Blüten von *Episcia lilacina* nehmen leicht Schaden, da sie sehr zart sind. Hummeln bevorzugen jedoch Blüten mit mehr Stabilität. Die helle Farbe der Blüten spricht eher für Schwärmer. Aber auch Schmetterlinge sprechen darauf an. Die Tatsache, dass der Nektar in einem Sporn abgesondert wird bzw. dass ein großer Abstand zwischen Nektar und den Fortpflanzungsorganen liegt, weist ebenfalls auf Lepidoptera als Bestäuber hin. Hingegen liefern der Duft (leicht nach Gurke) der Blüten sowie das UV-Mal am Blüteneingang keine schlüssigen Hinweise.

Morphometrie der Blüten. Um einen Überblick über die Größenrelationen der Blüte von *Episcia lilacina* zu bekommen, wurden verschiedene Blütenteile vermessen: Länge der Kronröhre, Breite und Höhe des Blüteneingangs, Breite und Höhe der weitesten Stelle in der Blüte, Breite und Höhe der engsten Stelle, Tellerdurchmesser, Länge der Staubblätter, Länge des Griffels sowie Höhe und Durchmesser des Fruchtknotens.

Aufgrund der Länge der Kronröhre (durchschnittlich 38 mm) kommen als legitime Bestäuber nur Prachtbienen oder langrüsselige Schmetterlinge in Frage.

Der enge Eingang in die Blüten soll vermutlich verhindern, dass der Bestäuber zu weit in die Blüte vordringt. Der Bestäuber wird auch in die richtige Position gebracht, um mit Pollen beladen zu werden (vermutlich auf Kopf oder Rüssel) bzw. um die Narbe zu bestäuben.

Knospenentwicklung. Die Knospenentwicklung lässt sich in vier Abschnitte einteilen. In Abschnitt 1 (12-14 Tage) ist die Knospe bereits deutlich sichtbar. Während des 2. Abschnitts (3-4 Tage) werden die Petalen sichtbar. Die Petalenlappen formen eine kugelförmige Corollaknospe, aus der sich später der Stielteller entwickelt. In Abschnitt 3 (3-4 Tage) verlängert sich die Kronröhre und nimmt jetzt die typische rosa-lila Farbe an. Der Sporn beginnt sich zu bilden. In Abschnitt 4 (1 Tag) steht die Knospe kurz vor dem Öffnen. Die Blüte hat fast ihre volle Größe erreicht. Der Zeitraum der Knospenentwicklung beträgt somit 19 bis 23 Tagen.

Anthese. Die männliche Phase der Anthese beginnt zwischen vier und fünf Uhr in der Früh. Kurz nach dem Öffnen der Blüte beginnt die Pollenfreisetzung. Die vier Antheren öffnen sich und der Pollen wird frei präsentiert.

Zwischen vier und fünf Uhr am Nachmittag desselben Tages rollen sich die Filamente zurück. Hier endet die männliche Phase. Die Narbe ist zu diesem Zeitpunkt noch nicht rezeptiv. Der Griffel ist noch nicht zu seiner vollen Länge ausgewachsen. Im Laufe der nächsten Stunden wächst der Griffel in die Länge, sodass die Narbe an derselben Stelle, an der vorher die Antheren platziert waren, zu stehen kommt. Währenddessen beginnt die Narbe rezeptiv zu werden. Somit beginnt die weibliche Phase zwischen Mitternacht und vier Uhr früh und dauert ein bis zwei Tage an. Es gibt keine Überlappung zwischen männlicher und weiblicher Phase. Das Ende der Narbenreife ist durch das Eintrocknen der Narbe gekennzeichnet. Bei einer erfolgreichen Befruchtung wird binnen ca. drei Tagen die Fruchtentwicklung sichtbar.

Da sowohl die männliche als auch später die weibliche Phase beide in den frühen Morgenstunden beginnen, muss der Bestäuber (Bienen oder Schmetterlinge) tagaktiv sein. Einen eindeutigen Hinweis auf den legitimen Bestäuber gibt die Anthese von *Episcia lilacina* jedoch nicht.

Fruchtentwicklung. Nach einer erfolgreichen Bestäubung beginnt die Fruchtentwicklung. Die Karpelle bilden eine grünlich bis rötlich gefärbte, kugelige, behaarte Kapsel, die von einem persistierenden Kelch umgeben wird. Die Kapsel öffnet sich in zwei Klappen und ist fleischig.

Die Frucht ist innerhalb von 25 Beobachtungstagen durchschnittlich 5,5 mm in die Breite und 4 mm in die Länge gewachsen. Da der Aufenthalt in Costa Rica zu diesem Zeitpunkt zu Ende war, konnte die Fruchtentwicklung nicht bis zum Schluss verfolgt werden. Somit konnte die Deshiszenz und die Ausbreitungsweise nicht beobachtet werden. Ausgebreitet werden die Samen wahrscheinlich durch Wind oder Regen oder sie fallen einfach nur zu Boden.

Fruchtansatz. Von 141 beobachteten Blüten entwickelten nur 27 eine Frucht. Das entspricht einem Fruchtansatz von 19%. Obwohl kein legitimer Bestäuber gesichtet werden konnte, kommt es doch zur Bestäubung. Der einzige regelmäßige Besuch kommt von einer Meliponinen-Art. Die stachellose Biene könnte auf der Suche nach Pollen an einer Narbe streifen und die Blüte dann (gesetzt den Fall sie war vorher schon bei einer Blüte in der männlichen Phase) bestäuben. Sie ist jedoch zu klein, um der legitime Bestäuber von *Episcia lilacina* zu sein.

Bestäubungsexperimente. Mit sogenannten Bagging-Experimenten wurde untersucht, ob spontane Selbstbestäubung vorkommt bzw. ob manuelle Selbstbestäubung möglich ist. Die Ergebnisse zeigen, dass Selbstbestäubung bei *Episcia lilacina* zwar möglich ist, jedoch durch 2 Mechanismen in der Natur erfolgreich vermieden wird. Die erste der beiden ist das Einrollen der Filamente, sodass die Antheren im hinteren Bereich der Blüten zu liegen kommen. Da während dieses Wegklappens die Antheren nahe der Narbe vorbeibewegt werden, kann es theoretisch zu einer Selbstbestäubung kommen. Doch der zweite Mechanismus verhindert diese: Der Griffel mit der unreifen Narbe wächst erst nach dem Einrollen der Filamente in die Länge. Während dieses Längenwachstums beginnt die Narbe zu reifen. Somit ist gewährleistet, dass der eigene Pollen niemals in Kontakt mit der rezeptiven Narbe kommen kann.

Da das Verhindern der Selbstbestäubung erfolgreich funktioniert, ist unbedingt ein Vektor, der den Pollen auf die Narbe einer anderen Pflanze überträgt, nötig, um eine Fruchtbildung zu ermöglichen.

Pollen/Ovula Verhältnis. Die Erhebung des Verhältnisses von Pollenkörnern zu Samenanlagen lassen Aussagen über das Fortpflanzungssystem zu. Die P/O Rate von *Episcia lilacina* liegt durchschnittlich bei 36,34 : 1. Dies bedeutet, dass ca. 36 Pollenkörner auf 1 Samenanlage kommen. Da Autogamie bei *Episcia lilacina* ausgeschlossen werden kann, erscheint diese P/O Rate sehr niedrig. Sieht man sich allerdings die Anzahl an Pollenkörner und Samenanlagen genauer an, bemerkt man, dass sich sehr viele Samenanlagen in einem Fruchtknoten befinden. Dadurch lässt sich das niedrige P/O Verhältnis besser verstehen.

Auch bei anderen Gesneriaceae-Arten konnten ähnlich niedrige P/O Raten beobachtet werden. Vermutlich spielt das Verkleben der Pollenkörner, das den Pollen klumpiger und somit die Übertragung effizienter macht, ebenfalls eine wichtige Rolle. *Episcia lilacina* wurde daraufhin nicht näher untersucht, aber verklumpter Pollen konnte beobachtet werden.

Nektarsekretion. Die Blüte von *Episcia lilacina* beginnt bereits mehrere Stunden vor dem Öffnen Nektar zu produzieren. Es konnte bereits um 23 Uhr des vorangegangenen Tages Nektar im Sporn der Knospe beobachtet werden. Die durchschnittlich produzierte Nektarmenge in zwei Stunden beträgt 0,5 µl (± 0,2µl Standardabweichung). Die maximale Menge an Nektar, die von einer Blüte produziert worden ist, beträgt 5,4µl. Der durchschnittliche Wert liegt allerdings bei 2µl (±1,37µl Stabw). Es konnte kein signifikanter Unterschied in der Nektarproduktion während der männlichen und weiblichen Phase nachgewiesen werden. Die Nektarmenge deutet auf

Schmetterlinge als legitime Bestäuber, da die angegebenen Mengen für neotropische Bienen höher liegen (9,75µl) als die für Schmetterlinge (0,01 bis 2,0µl).

Der gesammelte Nektar mehrerer Blüten ergab jeweils eine Zuckerkonzentration von 27 %. Dies weist wiederum auf Schmetterlinge als legitime Bestäuber hin, da für Schmetterlinge eine durchschnittliche Zuckerkonzentration von 25 % angegeben wird, wogegen der angegebene Wert für Bienen viel höher liegt (bei 35%).

Blütenbesucher. Die hier besprochenen Beobachtungen deuten eher auf Tagfalter als legitime Bestäuber von *Episcia lilacina* als auf Bienen hin. Leider konnte kein Schmetterling beim Besuch der Blüte beobachtet werden. Die gefangene stachellose Biene kann durchaus ein sekundärer Bestäuber sein. Sie entspricht jedoch auf keinen Fall dem Bestäubungssyndrom von *Episcia lilacina*.

Auf den Blättern wurde noch eine Raupenart gefunden. Oft sind Raupen an den gleichen Pflanzen zu finden, die diese dann später als adulter Schmetterling bestäuben. Dies ist hier jedoch nicht der Fall. Die gefundene Raupenart gehört zu den Motten und ist weiters viel zu klein, um der gesuchte Bestäuber von *Episcia lilacina* zu sein.

Introduction

This diploma thesis deals with the reproductive biology and the leaf colourations and patterns of *Episcia lilacina*. The presented data were raised in 2006 (May to August) in an evergreen lowland rain forest in the south of Costa Rica ("Regenwald der Österreicher") and in the biological station La Gamba. The investigation area lies in the western part of the Piedras Blancas national parks, where *Episcia lilacina* is a widespread, commonly occurring herb. The plant grows along paths and slopes.

Hanstein described *Episcia lilacina* first in 1865. From nine species of *Episcia* only *Episcia lilacina* is native in Costa Rica. The second occurring species is an exotic one, *Episcia cupreata*, which is used as ornamental plant. The geographic range of *Episcia lilacina* reaches from Nicaragua to the North of Columbia (Wiehler 1983).

Regarding the enormous variety of forms and the diversity of tropical forests it is little known about the existing species and their correlation among themselves (Stork 1993, Simon 1995, May 1996). To find an explanation, how the huge diversity in the tropics has developed, many authors (Heithaus 1974, Feinsinger 1987, Gentry 1990, Orians et al 1996) point out the need to study the reproductive biology because it is the basis for all organisms.

Wiehler (1983) estimated that 60% of the Gesneriaceae in the Neotropis are pollinated by hummingbirds. This is a remarkable high percentage of ornithophily. Neotropical male and female Euglossine bees (gynandro-euglossophily) are the second most common pollinators of the Gesnerioideae (about 30%). The remaining 10% of the Gesneriaceae are pollinated by bats, butterflies, moths, flies, bees etc.

Despite its common presence beside paths nobody has investigated the sexual reproduction of *Episcia lilacina* until now. In the first part of this work the anatomy of the flower as well as its morphometric features are described in detail. The bud development, the anthesis and the fruit development are expounded. Pollination experiments clarify if spontaneous self-pollination occurs or if manual self-pollination is possible. Through the determination of the P/O ratio it is possible to compare *Episcia lilacina* with different plant family having a similar reproductive system. Nectar is an important food reward and serves to attract pollinators. The amount of secreted nectar and the sugar concentration gives further indication towards the legimate pollinator. Unfortunately, the legitimate pollinator could not be observed, but other flower visitors, so-called second pollinators, were documented.

Likewise nothing is known about the development (amount of seeds, setting of fruit) and the dispersal of the seeds. Of special interest is the sexual reproduction (development and spreading of seeds) compared to the vegetative one (development of stolons).

Another important issue that will be considered in this work is the natural polymorphism in leaf colours of *Episcia lilacina*. A wide range of leaf-colouration and -drawings from bright green to dark green (dark red) can be observed. These colourings are caused by colouring agents, probably by one or more anthocyanins.

Anthocyanins are a group of water-soluble, pigmented flavonoids spread in many plant parts across the world's flora (Harborne 1967). Anthocyanins can be often found in leaves, even if they cannot be noticed easily because chlorophylls, carotenoids or pubescence can conceal their red to purple colouration (Moore 1965, Sanger 1971, Woodall et al. 1998). There is no unified explanation for the presence and function of anthocyanins in leaves.

Anthocyanins interact with visible wavelengths of the daylight spectrum, which differentiate them from most other flavonoids (Harborne 1967). If anthocyanins can change the light environment within a leaf, then they have the capability to influence photosynthesis and prevent photoinhibition (Gould et al. 1995, Burger and Edwards 1996). The optical properties of anthocyanic leaves seem to diversify within the plant kingdom. An increased adsorption of green-yellow light in anthocyanic leaves was often referred, even though the extent of this increase varies notably among the species (Eller et al. 1981, Gausmann 1982, Burger and Edwards 1996, Woodall et al. 1998). Other observations revealed that anthocyanins increase the red light' reflectance of the leaf surface. On the one hand this fact can result in a reduction of the entire adsorption (Boyer et al. 1988, Ntefidou and Manetas 1996, Barker et al. 1997). On the other hand it conceivably enhances the capture of red light by back-scattering light that would otherwise pass through the leaf (Lee et al. 1979, Lee 1986).

The complex colouration and patterns in the leaves of *Episcia lilacina* occur rather exceptionally in nature. Possibly, there is a connection between the patterns and the environment, in which the plants grow. Does this phenomenon correlate with the incoming light or is it an attempt to camouflage the leaves against herbivores? Exact observations will show the potential presence of any vermins and their amount on the leaves. Are there any leaf morphs, which are preferentially attacked?

The differences in the optical properties of anthocyanic leaves are possibly related to different locations of the colourants within the leaf tissues. Anthocyanins occur most commonly in vacuoles of palisade and spongy mesophyll cells, but they can also be found in the epidermis, hypodermis, and (or) vascular parenchyma (Gould and Quinn 1999). One part of this work is to

determine the tissues where the anthocyanins are located. This gives indications about the possible function.

As *Episcia lilacina* is solely found on the ground it is of interest if there are any differences in the amount of light that is adsorbed from the diverse leaf-morphs. Does less light get to the ground where the plants develop darker leaves? Do the anthocyanins enhance the light adsorption within the leaves? Do the patterns of the leaves change if the plants were transferred to a new habitat? All these questions will be answered in the second part of this work.

The Genus *Episcia* - Systematics and General Characteristics

The family of the Gesneriaceae is spread in the tropics and subtropics of the Old and New World, but it also appears in the warm-temperate regions in Eastern Asia and Europe. Pan-tropically there are 147 genera with 3870 species. In the Neotropics there are approximately 40 genera with more than 900 species: for example *Alloplectus* (75), *Besleria* (over 200), *Codonanthe* (17), *Columnea* (396), *Diastema* (20), *Drymonia* (over 140), *Episcia* (9), *Gasteranthus* (35), *Gesneria* (60), *Kohleria* (17), *Nautilocalyx* (over 70), *Paradrymonia* (over 70), *Sinningia* (60). In Costa Rica there are 29 genera with more than 140 species within the Gesneriaceae.

The representatives of the family Gesneriaceae are perennial (seldom annual) herbs, shrubs or small trees. In the Neotropics lives a huge number of species epiphytically, while in the Palaeotropics most species are terrestrial. The leaves are opposite and often arranged anisophylly, distichally or spirally. They are sometimes fleshy and pilose; the lamina is usually undivided, very rarely lobed or pinnately dissected. The inflorescences are mostly axillary cymes with the flowers placed in pairs. The flowers are mostly zygomorphic, rarely actinomorphic and usually 5-merous. The sepals are free or conate to a variable extent. The petals are sympetalous with a more or less long tube. They own from two to four (rarely five) stamens with often-coherent anthers. The filament is always adnate to the corolla. The ovary is mostly superior or also semi-inferior to inferior. The placentation is parietal; the stigma is capitate, bilabiate or variously bifid. The fruits are fleshy berries or capsules, which open by two or four valves or by a dorsal slit. The seeds are very numerous and very small (Morton 1938, 1971).

The family is divided into four subfamilies:

1. The subfamily of Didymocarpoideae (82 genera/over 2000 species) has representatives in Africa, Madagascar, from Sri Lanka to Malaysia and in the pacific region, isolated also in Europe. Some genera are: *Cyrtandra, Henckelia, Aeschynanthus, Streptocarpus, Agamyla, Paraboea, Didymocarpus*.

2. The subfamily of Epithematoideae (6 genera/75 species) is distributed in India, from Southeast Asia to Malaysia. In West Africa and from Central America to Peru only one representative can be found. The most varied genus is *Monophyllea* with roughly 30 species.
3. The subfamily of Gesnerioideae (53 genera/ 1500 species) is spread in the Neotropics. Some genera are: *Besleria, Episcia, Drymonia, Allopectus, Nautiocalyx, Paradrymonia, Gesneria, Sininngia* and *Columnea*.
4. The subfamily of Coronatheroideae (9 genera/ 20 species) has representatives on the Solomon Islands, the Antilles, in New Caledonia and the south of South America. The most varied genus is *Coronanthera*.

In 1829 the genus *Episcia* was described for the first time by Karl Friedrich Philip Martius in his work "Nova genera et species plantarum". The name stems from the Greek word "episkios" which means shaded. The plants can be found predominantly at shady and damp places, slopes, banks or rocks.

The genus *Episcia* was modified drastically by Wiehler (1978) and reduced from 46 to nine species: *E. andina, E. cupreata, E. elongata, E. fimbriata, E. lilacina, E. prancei, E. reptans, E. sphalera, E. xantha*. Before the new division of Wiehler the definition of the genus was only based on floral and fruit characters. The genus *Episcia* united several species, which today belong to the genera of *Nautilocalyx*, *Paradrymonia* and *Alsobia*. *Episcia* and *Alsobia* differ from *Nauticalyx* and *Paradrymonia* in their stoloniferous habit and their sympodial shoot pattern. Whereas *Episcia* is a terrestrial or saxicolous genus and owns two stolons per node, *Alsobia* lives strictly as an epiphytic and has only one stolon per node (Wiehler 1983). Also the newest investigation of Clark (2006) confirms the monophyly of *Episcia* within the Episcieae.

Episcia consists of stoloniferous low terrestrial or epiphytic herbs, which are rarely lignified at the base. The stems creep or sprawl on the ground but with erect or rising tips. The plants can reach one meter or more in length. At the nodes there can be adventive roots.

The leaves are often crowded or opposite with short petioles. The leaf form varies from ovately or elliptically to lanceolately. Usually the leaf pairs are nearly equal. The upper surface is dark green or has various patterns of variegation. The lower surface is often coloured.

The inflorescences often consist of single or 2-6 axillary flowers on slender pilose peduncles. They are also furnished with two bracts. The showy flowers are zygomorphic. The floral tube is short. The calyx is often irregular and owns five oblong sepals, which are free or shortly connate at the base. The sepals are pilose and green or coloured. The posterior lobe is forced back around a corolla spur. The corolla inserts horizontally in the calyx, has the form of a more or less developed tubus and is conspicuously spurred. Above the spur and at the throat the corolla can

be contracted. The limb is oblique. The corolla has five lobes, which are spreading and can be entire, toothed or fimbriate.

The flower possesses four stamens in two pairs of unequal length with nearly straight filaments, which insert at the base of the corolla. After the anthesis the stamens are depressed or coiled. The anthers are coherent in pairs in a square or arc and dehisce by a longitudinal slit.

Episcia has one large gland, which is located dorsally at the base of the superior ovary. The stigma is stomatomorphic, bilobed and capitate. The placentas wear the ovules on both the outer and the inner surface or only on the inner one. The fruit is a bivalved fleshy capsule. The seeds are ellipsoid, shiny and brown with obliquely striae (Skog 1978).

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PART 1 Investigation of Plants and Leaves of *Episcia lilacina*

General Description of the Plant Habit

The family Gesneriaceae to which *Episcia lilacina* belongs exhibits many types of growth forms. Most species are herbs or shrubs. Gesneriads can be terrestrial, climbing or epiphytic (Weber 2004). *Episcia lilacina* is a widespread forest ground herb in the Piedras Blancas rainforest. The plants can be found predominantly in shady and damp places, slopes, banks or rocks, often forming large patches.

Episcia lilacina is a stoloniferous, terrestrial herb. The stem creeps on the ground, with erect tips only. The plants can reach 15 cm in height. The stem diameter is circular. The opposite leaves have short petioles. The leaf pairs are usually almost equal. The leaf blades are ovate and dentate. The apex of the lamina is blunt, the base rounded. Leaf length averages 6.9 cm, with the largest leaves growing up to 10.8 cm in length. At their widest part the leaves measure 4.1 cm in average, with a maximum of 6.3 cm. The whole leaf is pilose. The hairs are mixed with glandular trichomes. Stipules are not present. The venation of the leaves is net-veined. The roots of *Episcia lilacina* grow shallowly. At the nodes adventive roots are produced. Axillary stolons occur at the lower leaf pairs.

Stolon Spreading

Though sexual reproduction is the primary mode of reproduction in the angiosperms, vegetative propagation also plays an important role. *Episcia lilacina* develops stolons. Stolons are axillary side branches with elongated internodes, creeping over the ground and producing adventitious roots.

The conducted measurements are supposed to reveal the role of stolon development in the process of dispersal of *Episcia lilacina*. The question if vegetative propagation poses an effective alternative to sexual reproduction shall be answered. Therefore, the speed of growth of the stolons has been investigated.

Materials and Methods: The investigations were performed at several locations in the forest. The stolon length was always measured from its beginning at the leaf axil to the first node. After a few weeks the measurements were repeated. The daily increase in length was calculated

and the measurements were compared to each other. The number of leaves as well as the presence of roots and the development of further stolons was documented.

Results: Stolons are preferentially developed in the leaf axils of the lower nodes. Early in the development, the first two leaves of the stolon are visible. The stolon begins to grow in length rapidly. The first pair of leaves are cataphylls. After they have reached a certain size (about 1 cm x 1 cm) a second pair of leaves becomes visible. At that moment the stolon has reached a couple of centimeters in length and continues to grow very fast. The daily increase in length averages 6.5 mm. Before the development of the first roots at the first node begins, another pair of leaves becomes visible. The first roots do not immediately anchor in the ground. In this stage the stem (connection to the original plant) has a length of over 10 cm (15 cm not rarely seen). Further on, roots are developed at the second node. They afterwards anchor in the ground. The growth in length has stopped. The growth is now restricted to the area above the first node. After rooting the shoot apex begins to rise. The connection to the original plant exists for a long time. After weeks, this connection begins to whither and finally drops. At this point the stolon has developed more than four pairs of leaves.

Discussion: Among the gesneriads stolon spreading can be noticed in a few species, for instance, *Henckelia stolonifera, Boeica stolonifera, Chirita stolonifera*. The genus *Alsobia*, which is systemically close to *Episcia*, develops stolons too. The genus *Episcia* differs insofar as two stolons per node regularly develop.

Most herbs inhabiting shaded forest understorey have some ways of clonal growth (Doust and Doust 1988). The development of stolons enables the plants to exploit an optimal environment, meaning to use advantageous living conditions, more effectively. If enough resources are present, these can be quicker made accessible by stolon propagation than by sexual reproduction (Fenner and Thompson 2005). In addition, the survivorship of stolons is relatively higher in comparison with seedlings (Sarukhán and Harper 1973).

The mother plant has no need to put its energy in the development of flowers and, later on, of fruits. It is much more independent from pollinators and dispersal. The energy is invested in a new plant and thus it is not "wasted" by unsuccessful pollination or failed development of fruit (Fenner and Thompson 2005).

Indeed, there are some drawbacks connected with vegetative propagation. No spreading over a wider area is possible (10 to 15 cm at the maximum). The possibility to occupy habitats that are further away is solely opened up by dispersal of seeds. The vector of seeds of *Episcia lilacina* is still unknown at the moment. If dispersal does not take place by vectors but by just opening the seed

capsule and releasing the seeds, the spread is limited too. In addition, seeds can stay dormant and start germination as soon as unfavorable environmental conditions improve.

A further profound drawback of vegetative reproduction is that the stolons are genetically identical to the mother plant and to each other. All members of a clone, though independent and numerous, can all be considered to be part of the same plant. Via sexual reproduction the species can evolve to adapt to any change in selective pressure. Stolons are also liable to inimical mutations and viral infections over the course of time (Fenner and Thompson 2005).

The daily growth in length of the stolons of 6.5 cm is remarkable. Further on, no seedlings could be located during the observation period. Thus the development of stolons seems to be not only an effective strategy to establish itself in a newly occupied habitat, but also a successful alternative to sexual reproduction.

Example for Stolon Spreading

In the last chapter the general development of a stolon was described. In this part an example is used to demonstrate the ability of *Episcia lilacina* to spread in the course of 80 days. This will make clear how fast *Episcia lilacina* can establish itself at a given site.

Materials and Methods: To document how much space the plant of *Episcia lilacina* can cover in two month, a single plant was put in a patch of about 1 m^2. The bed was prepared in the botanical garden of the field station La Gamba, in the vicinity of the main building. All stolons that developed in this period were measured and photographed. Also the temperature and the solar radiation were measured to describe the environmental conditions under which the plant grew. With the use of the program Adobe Illustrator 10 of Adobe Systems a sketch of the plant with its stolons was created.

Results: Figure 1 shows the above-mentioned sketch of *Episcia lilacina*. Since the arrival at the field station, this plant was one of the first that was in flower. To document the anthesis exactly, it was transferred into the station garden. After the documentation was finished, no further attention was paid to the plant. Then by chance, it was discovered, how it had spread over the bed. It had developed four stolons in the course of 80 days that each had developed stolons of their own. The numbers 1 to 4 and the respective letters describe the four developed stolons in figure 1. Stolon 1 and 3 originate from the first node, 2 and 4 from the second. The length specifications of the stolons are filled in.

Figure 2 shows a photo of the mother plant (with two flowers) and its stolons. The schematic diagram in figure 3 helps to identify the stolons belonging to the observed plant. Figure 4 documents the incoming light as well as the temperature. Maxima of 12.000 Lux and 40 °C characterize the environmental influences.

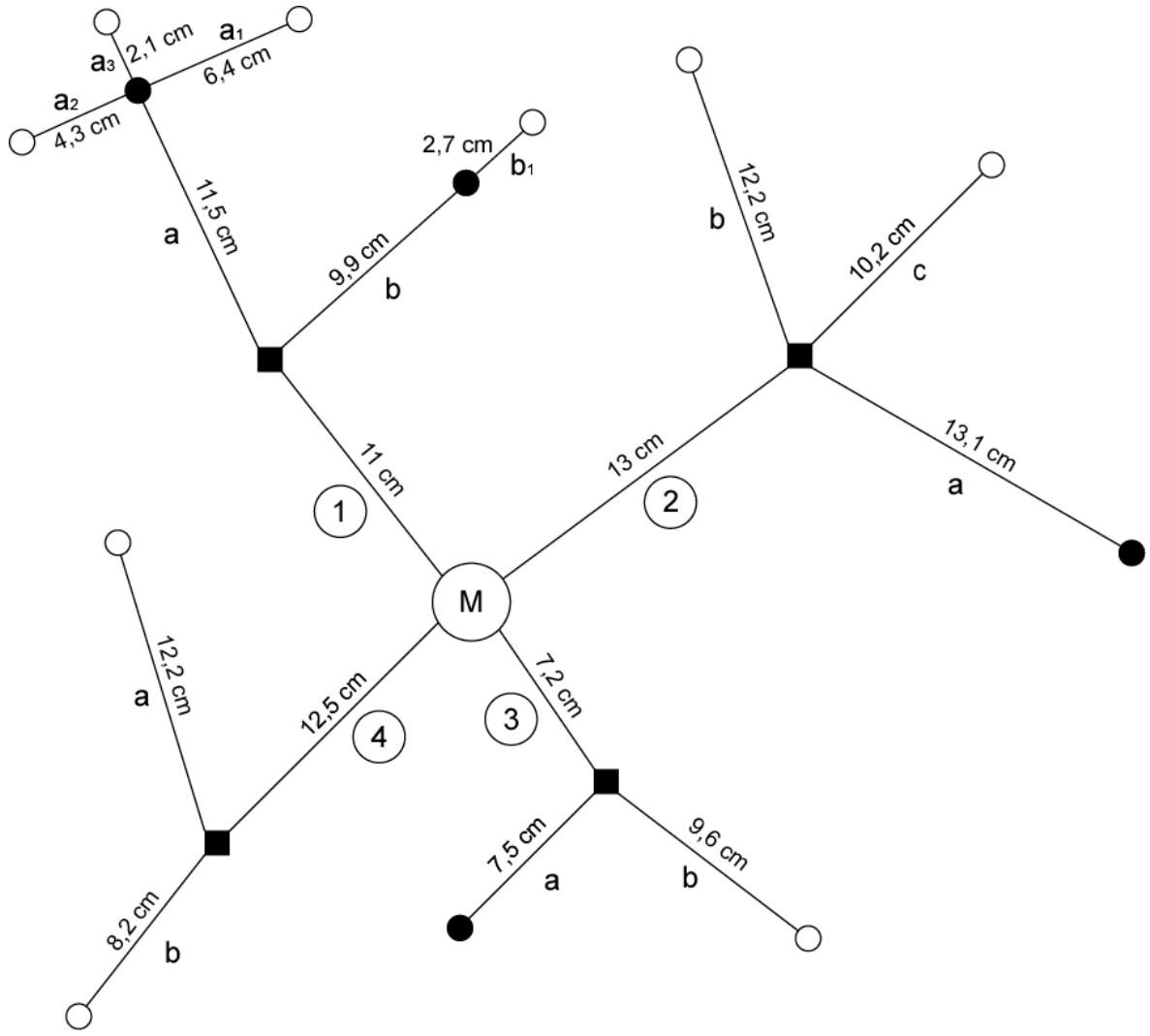

Figure 1 *Episcia lilacina*; a plant spreading over 80 days; ■ = stolon rooted, ● = stolon with roots but rootage not yet having taken place, ○ = stolon without roots; 1-4 designate the stolons of the mother plant in the order of time; a-c respectively a1-b3 designate further developed stolons originated in the stolons of the mother plant (M).

Figure 2 *Episcia lilacina*; the mother plant located in the middle of the photo; all plants of bright green next to it are its stolons; the area in this photo is about 1m².

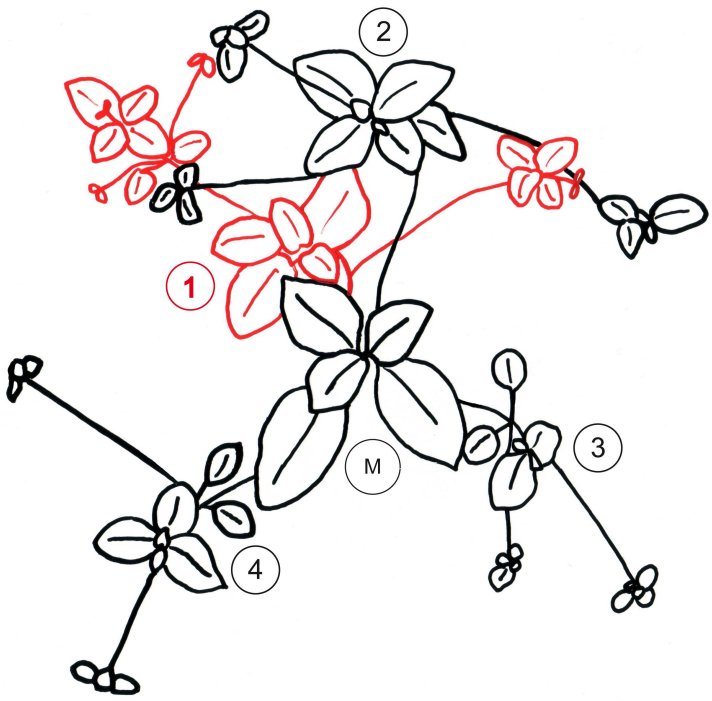

Figure 3 *Episcia lilacina*; schematic diagram of the photo above; the first stolon drawn in red to simplify the determination of the four stolons; 1-4 designate the stolons of the mother plant (M) in the order of time.

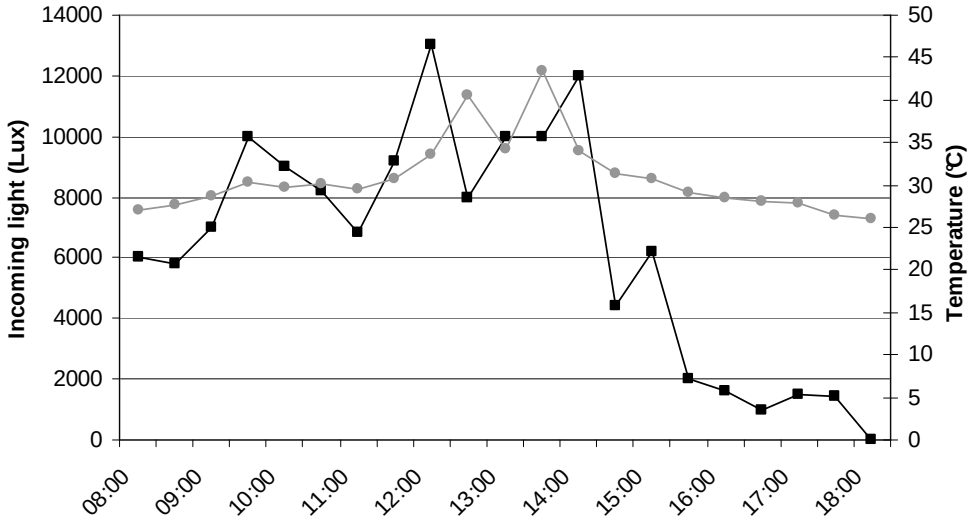

Figure 4 Incoming light (black) and temperature (gray) shown to illustrate the conditions in which the plant did spread; the highest value of incoming light was 13000 Lux; the highest temperature of the day 43,4 °C.

Discussion: The usage of stolons seems to be a good alternative to sexual reproduction. The combination from sexual and vegetative propagation is clearly successful. Via sexual reproduction *Episcia lilacina* can reach a new habitat where it establishes itself quickly via stolon development. Altogether, this is to be considered a very successful strategy of spreading.

Leaf Colouration

Another important issue that will be considered in this work is the natural polymorphism in leaf colours of *Episcia lilacina*. A wide range of leaf colouration and leaf patterns from bright green to dark red can be observed. These patterns are caused probably by one or more anthocyanins. The basic colour of the leaves is bright green. Areas, where larger amounts of anthocyanins are developed, appear dark red. In this chapter a classification of the different types of leaves was created.

Materials and Methods: The applied classification is based on the green to red relation within the leaves. There is always just one type of pattern present in one plant meaning all leaves of a single plant can be assigned to a single type.

At every investigation spot one sample of each kind of leaf was taken and photographed to get a complete collection of all morphs. The leaves are photographed with the underside up in transmitted light, thereby the colouration can be seen clearer.

An attempt to give an overview over the numerical spreading of the different morphs ultimately failed because the DVD containing the required data suffered irreparable damage and thus the data were lost.

Results: Based on the analysis of the different photos a classification in eight types was reached (Fig. 5-12). From type 1 to 8 the red portions decrease until they disappear totally. The intensity of the reddish colour was not taken into consideration in this classification, because brownish colours may also appear in dependence of bleaching through light exposure (see below: „Colour change in correlation with change in habitats").

Type 1: The whole leaf is red. Only a few spots along the main vein can be coloured green.

Type 2: Along the main vein a green band can be seen.

Type 3: Beside the green area along the main vein green spots spread over the whole leaf appear sporadically.

Type 4: The green band in the middle of the leaf broadens along the side veins.

Type 5: The red region withdraws more and more. Green spots near the leaf margin start to merge with the green band. The junctions between red and green are fluent.

Type 6: The boundaries between the red and green areas are definite. The green spots spread over the whole leaf more and more.

Type 7: Green is now the dominant colour. There are only a few red spots left between the leaf margin and the main vein.

Type 8: No more red spots can be detected. The whole leaf is bright green.

The observation of the numerical distribution of the different types revealed that the dark morphs (type 1 and 2) are far more widespread than all the others. All other types occurred rather seldom.

Discussion: The eight different types of morphs show the diversity of the leaf colouration of *Episcia lilacina*. It appears that between dark red and bright green all variations are possible. Therefore, it was difficult to identify as well as to classify the different types.

Taking the numerical appearance into consideration the dark red morphs seem to have an advantage over the others. The reason for that is yet unclear.

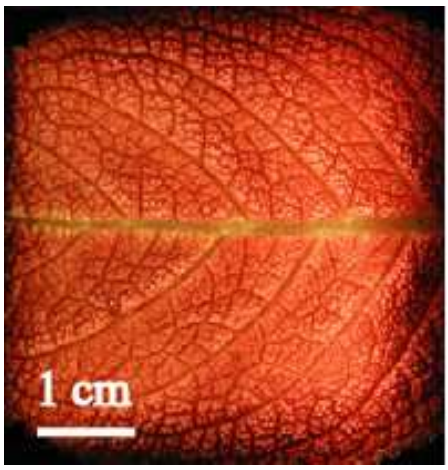

Figure 5: *Episcia lilacina*; leaf type 1.

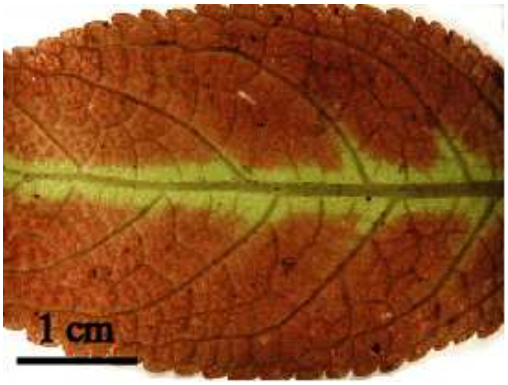

Figure 6: *Episcia lilacina*; leaf type 2**.**

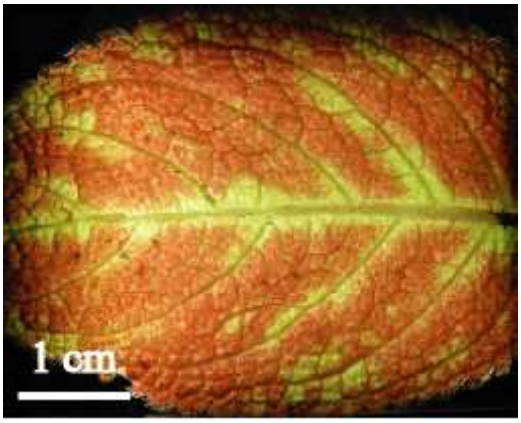

Figure 7: *Episcia lilacina*; leaf type 3.

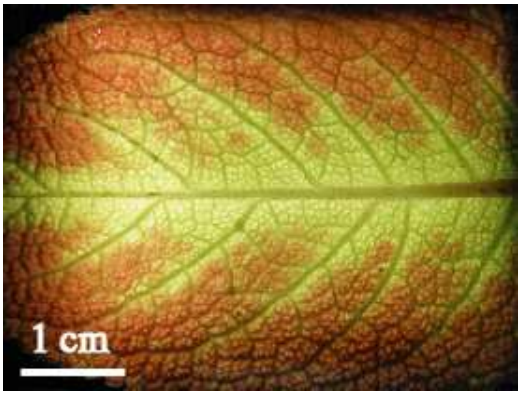

Figure 8: *Episcia lilacina*; leaf type 4.

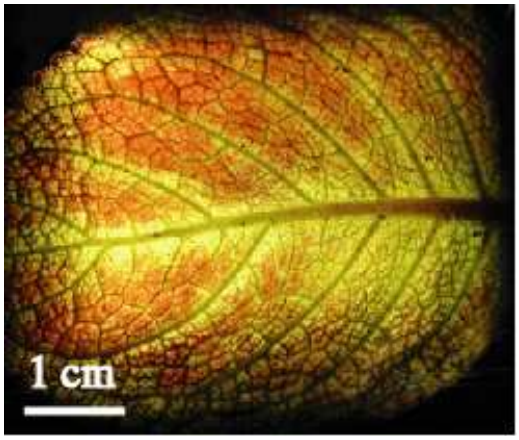

Figure 9: *Episcia lilacina*; leaf type 5.

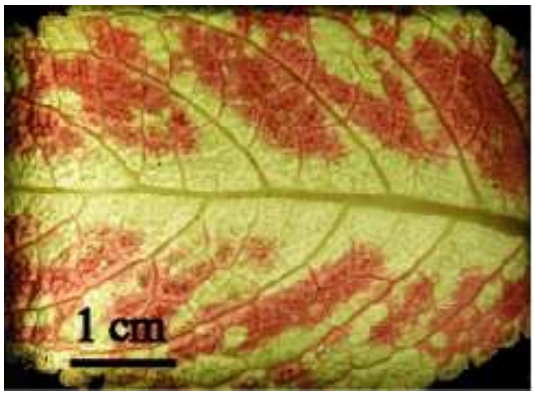

Figure 10: *Episcia lilacina*; leaf type 6**.**

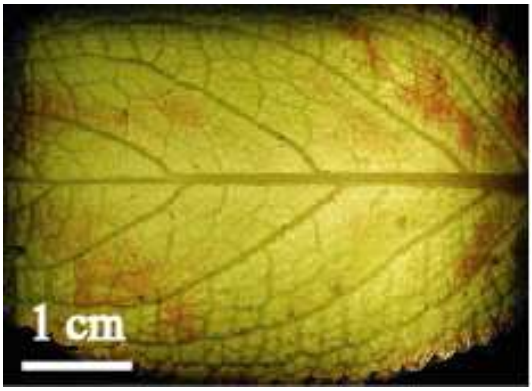

Figure 11: *Episcia lilacina*; leaf type 7.

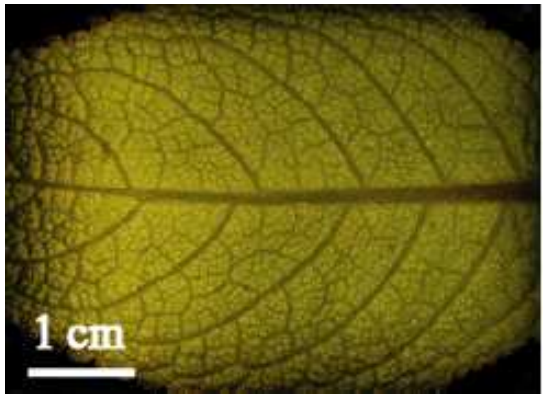

Figure 12: *Episcia lilacina*; leaf type 8.

Proof of Anthocyanins and Analysis of the Colouring Agents with the UV/VIS-Spectrometry

Anthocyanins are secondary metabolites and are part of the large phenolic family collectively known as the flavonoids. The anthocyanin molecule consists of an anthocyanidin (the aglycone chromophore), tied to one or more glycosides (Paech 1950). The maximum absorption of anthocyanins lies between 500 to 550 nm (Harborne 1967).

Anthocyanins have the ability to change their colour in correspondence with changes of the pH-value. The experiments described below were conducted to prove that anthocyanins are responsible for the leaf colouration of *Episcia lilacina* as well as to check if there are anthocyanins even in the bright green leaves.

Materials and Methods: To be sure that anthocyanins are responsible for the red colouration, the pH-value has been increased from low to high. Leaves of the red morphs were cut and anthocyanins were extracted with aqua. Hydrochloric acid (0,01mol/l) was added until the pH-value reached the level of 2. Afterwards, Ammonium hydroxide (50 per cent) was added until a pH-value of 9 was obtained. By comparison, the same experiment was performed with the leaves of African violet (*Saintpaulia ionantha*).

To demonstrate a difference in the content of anthocyanins between the green and the red-morphs of *Episcia lilacina*, the UV/VIS-spectrometry was used. 15 plants were taken from Costa Rica to Austria to accomplish experiments, which were not possible in the laboratory of the Field Station La Gamba. In Austria the plants were kept in a greenhouse of the Botanical Garden of the University of Vienna. The following method was modified after Strigl et al (1995).

To receive the clearest possible results, the two most extreme leaf-morphs were chosen. From the green leaves 2,15 g were weighed, from the red ones 1,14 g. The leaves were cut and grinded in a mortar with about 10 ml of 70% methanol and sea sand. The solid parts were separated through a filter. From the supernatant the methanol was distilled on the rotary evaporator. The solution was shaken out twice with benzine to separate the covalent parts and then with ethyl acetate to extract the flavonoids and other phenolic compounds. To eliminate the ethyl acetate the rotary evaporator was used. One more time the solution was filtered (0,2µm). The measurement of the extinction took place in the UV/VIS spectrometer with a wavelength of 522 nm. The absorption maximum of 522 nm was determined in a previous measurement.

Results: At low pH-values a change in colour to red in the solutions could be observed with *Episcia lilacina* as well as with *Saintpaulia ionantha*. After addition of base, the colour of both solutions changed to brownish.

The measurements in the UV/VIS spectrometer resulted in a value of 1,0266 for the red leaves and a value of 0,4142 for the green ones.

Discussion: The colour change evince that anthocyanins are responsible for the red colouration. The results of the UV/VIS spectrometry indicate that anthocyanins are located in the green leaves as well as in the red ones. In the green morphs the concentration is too low thus the red colour cannot be seen. It is thus not a genetic defect causing a deficiency in anthocyanin production and in further consequence a green colouration. Because the absorption maximum of anthocyanins is between 500 and 550 nm (Harborne 1967) the measured wave length of 522 nm points strongly towards anthocyanins.

Localisation of Anthocyanins

The location of the anthocyanins gives first indication of the function. Do anthocyanins only appear in one tissue or are they distributed in all leaf cells of *Episcia lilacina*? What kinds of possible functions can be excluded by determining the location of anthocyanins?

Materials and Methods: To localize the anthocyanins in the different cell layers of the leaf, several cross-sections were produced. Therefore, leaves were laid between two plates of polystyrene and put in a manual microtome. The cross-sections were made with a razor. The cuts were between 20 and 30 μm thick. They were transferred on top of an object slide with a drop of water and were analysed under the microscope.

Results: Figure 13 shows a photo of a cross-section through a leaf. On the single-layered upper epidermis then follows a palisade layer, in which the chloroplasts are situated. The palisade cells have a cone-shaped form and are noticeably smaller than the epidermis cells. Beneath the spongy layer is situated. The red colourant can be seen clearly. The concentration of the anthocyanins is highest near the palisade layer. But in the lower epidermis also a light red colouration can be seen.

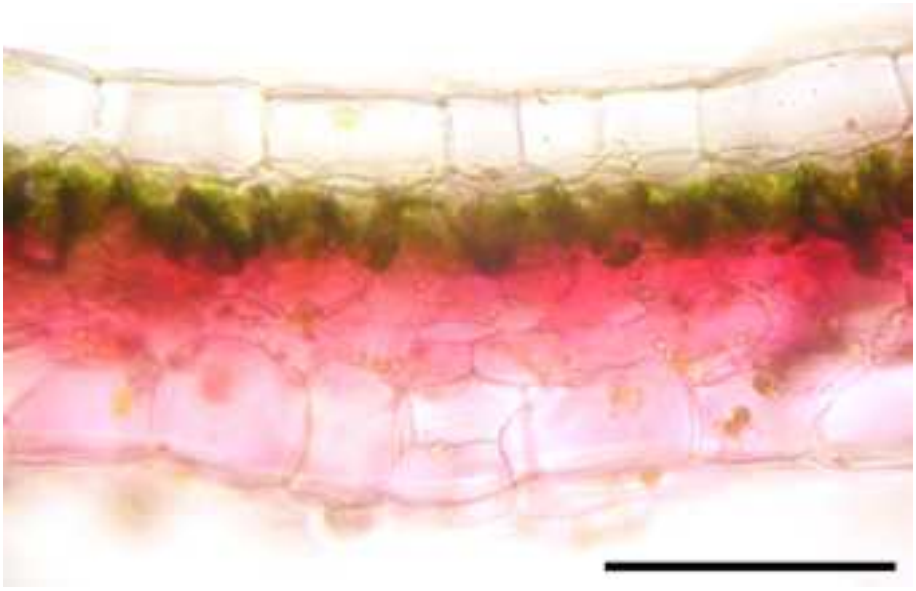

Figure 13 *Episcia lilacina;* cross-section of a leaf, anthocyanins mostly located in the spongy layer; the black bar has a length of 0.1 mm.

Discussion: Even though a lot of experiments deal with the function of anthocyanins, there are surprisingly few scientists who take their distribution within a leaf into consideration (Gould and Quinn 1999). This complicates comparisons of different papers. Due to the location of the anthocyanins at least one conclusion may be drawn: anthocyanins appearing near the upper parts of the leaves can reduce the wavelength of incoming light and thus act as a filter (Timmins et al 2002). This is especially important if chloroplasts are in stages of development and can be damaged by high doses of light. To fulfil this function, the anthocyanins have to be situated above the chloroplast layer. That is, however, not the case with *Episcia lilacina*. As shown in figure 13, the concentration of anthocyanins is highest below the palisade layer. Therefore, a function of anthocyanins as sunscreens seems to be unlikely. Further deductions concerning the function (e.g. protection against photoinhibition or herbivory) can only be made in correlation with other experiments.

Leaf-camouflage

Episcia lilacina grows in the rain forest in the shade casted by giant trees. Effective use of the remaining light reaching the ground is of utmost importance for its process of growth. Reduction of leaf surfaces cause by feeding damage can result in a considerable competitive disadvantage. In that regard, the numerous leaf-types of *Episcia lilacina* bring up thoughts of leaf mimicry.

Materials and Methods: To test this theory the different coloured plants were searched for animals that feed on the leaves. Furthermore, it was documented how many of the leaves was grazed. Additionally, *Episcia lilacina* was tested for other mechanisms to repel such attackers. Insects and larvae, which were sighted on the plants, were caught and conserved in 70 % alcohol.

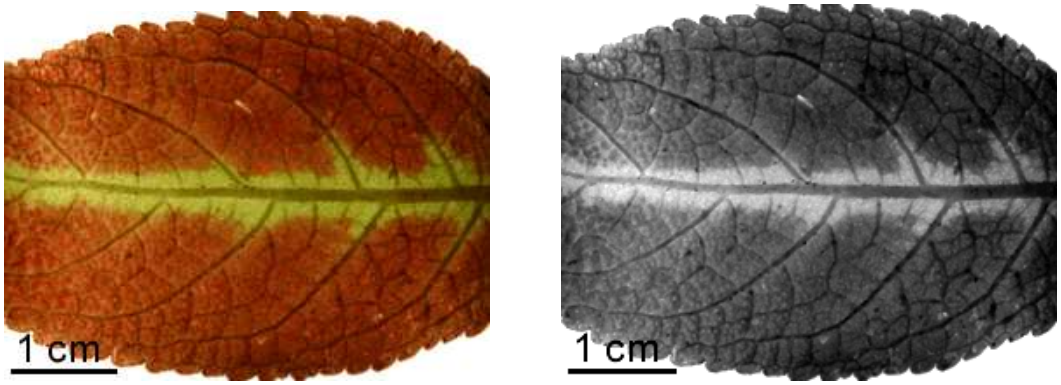

Figure 14 *Episcia lilacina;* left: the contrast between the red and green parts, right: converted to greyscale to elucidate the contrast.

Results: Only minimal traces of feeding damage could be observed on the leaves of *Episcia lilacina*. In addition, marginal areas wither easily because of external influences and look, in further consequences, alike traces of feeding damage.

On the flowers beetles of the family of Chrysomelidae (probably subfamily Alticinae) were found. As shown in figure 15, they can cause considerable damage to the flowers.

Furthermore, a species of caterpillar was detected while feeding on the leaves and cocooning. Probably it pertains to the family of Crambidae (order Lepidoptera). Systematically this family belongs to the moths. An attempt, to keep one of the caterpillars in a container and having it develop into an imago to determine the exact species, failed. Beside the two insects, no further vermin could be observed.

Figure 15 *Episcia lilacina*; Alticinae beetles browse one flower.

Discussion: The evolutionary competition between forage plant and butterfly is not only fought with chemical weapons, but also with optical deception and camouflage (Gilbert 1982). Females of the heliconiine butterfly are geared by the leaf form of their specific forage plant for the placement of their eggs. Thus, with time, a plenty of leaf forms developed that appeared to be grazed or full with eggs (Lunau 2002).

There could be similarities between the strategy of the family of Passifloraceae and *Episcia lilacina*. The different colouration of the leaves could be a simulation of grazed leaves (Fig. 14). The attacking insects are misled by the contrasty colouration. But the results show that the hypothesis of leaf mimicry cannot be proved. The found species of beetles seems to limit its foraging to the flowers. Thus leaves only one species of butterflies to feed on the plant. There are rarely traces of feeding damage on both leaves and flowers. Most notably, no difference between the various leaf-types could be observed. No locations totally grazed by vermin could ever be found. Most likely, there are some kinds of chemical repellents present in the leaves. They are heavily pilose, probably, to avoid being eaten (Schaller 2002). In addition, there are few amongst the types of leaves that look actually grazed.

As a conclusion it can be said that the cause for the diverse leaf patterns is not to appear grazed to attackers. Relating to this, a tendency towards a certain pattern would be recognizable.

Measurement of Light-Translucency

To check, if a connection between the different leaf patterns and the light-adsorption exists, the measurement of light translucency was conducted. If there was any difference in the adsorption between the green and the red morphs, an obvious explanation would be that anthocyanins within the leaves absorb a part of the incoming light.

Materials and Methods: For the experiment a light source emitting a constant amount of light was needed. Therefore a slide projector (Vivitar AutoFocus Slide 5000 AF) was chosen. It has a little illuminated window, which is normally used to preview slides. The window has a size of 4,6 cm x 4,3 cm and constantly emits 5000 Lux. A luxmeter (PANLUX electronic, GOSSEN) was used to measure how much light the source sheds.

Before starting all other sources of light were turned off and the blind value was measured. 300 leaves were sorted by the collecting locations. Then each leaf was laid with the upper surface onto the light source. The amount of light, which was getting through the leaf, was measured three times in a row. Afterwards the leaf was turned upside down and the passed light was measured again. The average was ascertained for the upper and underside. Both, the illuminated upper- and underside, were photographed. During the experiment the blind value was measured several times to ensure that there were not any variations in the emitted light.

To measure, how high the percentage of green areas in the leaves was, an image-editing program (Gimp 2.4.2) was used. Green parts on each photo were marked and the pixels were counted. The same was done with the whole leaf. Next the percentage of the green parts in relation to the whole leaf area was calculated. With the program SPSS 15.0 it was calculated, whether a correlation between the leaf translucency and the quantity of green areas on a leaf existed.

Because the data were not normally distributed, non-parametric correlations (Spearman's and Kendall tau rank correlation coefficient) were used.

Results: The Kendall-Tau-b test resulted in a correlation coefficient of 0.605, the Spearman-Rho test of 0.779 (+ 1 representing the value of a perfectly positive correlation). Both times it was tested unilateral. The correlations are both on a level of 0,01 significant. The graph in figure 16 illustrates the linear correlation of the percentage of green parts to the passed light. The greener the leaf, the more light can pass through it.

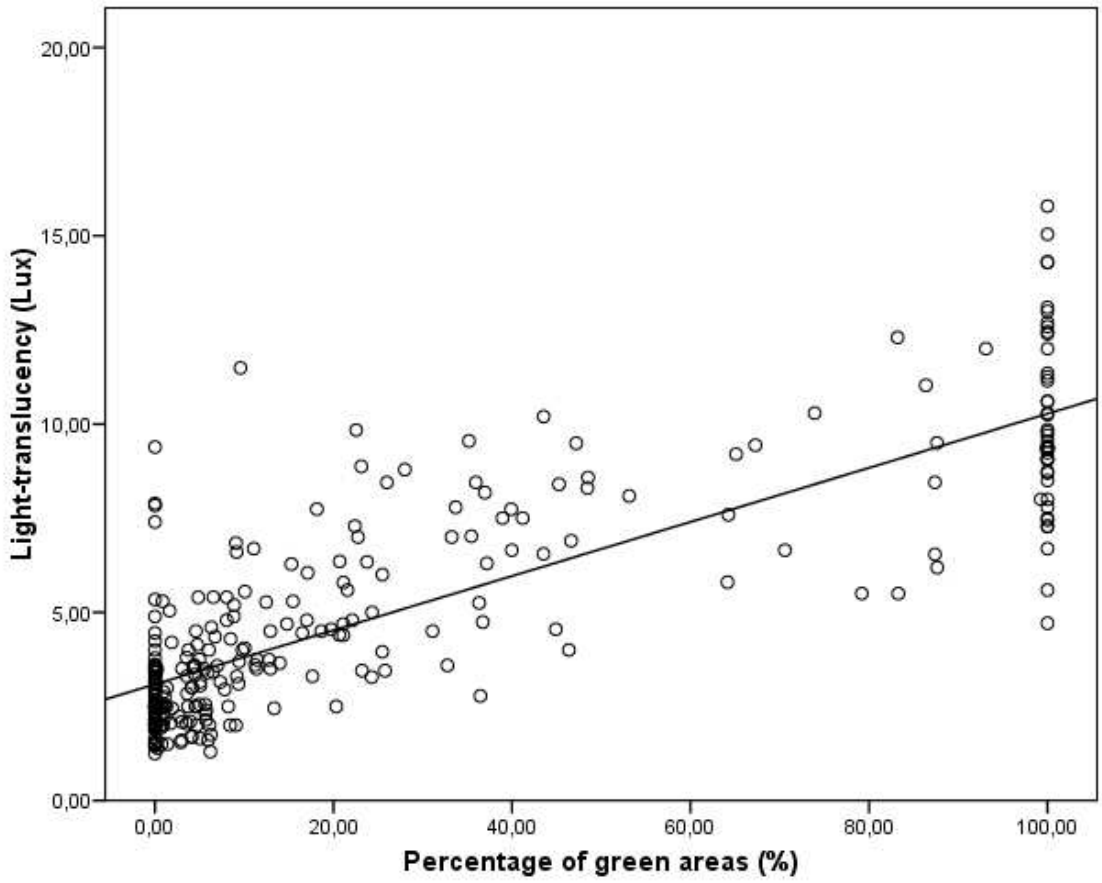

Figure 16: Positive correlation between the percentage of green parts of a leaf and the passed light; with an increase of green parts the amount of passed light grows as well.

Discussion: A correlation coefficient can range from –1 (perfectly negative correlation) to +1 (perfectly positive correlation). Thus the results of both tests point towards a positive correlation. There clearly exists a correlation between the light passing through and the red-green proportion of the leaves. In further consequence the conclusion can be drawn that the anthocyanins within the leaves absorb a part of the incoming light. But that doesn't mean that *Episcia lilacina* develops anthocyanins just because of absorbing light. Thereby the reason for the red colour within the leaves is not yet proven. It could be an ancillary effect of the main function.

Measurement of Radiation

The relation between light and the development of anthocyanins was already often investigated (Gould and Neill 1999, Post and Vesk 1992, Lee and Graham 1986). It has been ruled out earlier that anthocyanins protect the chloroplasts of *Episcia lilacina* from destruction by

UV-light. In the last chapter the correlation between anthocyanin concentration and translucency of the leaves was shown. A connection between leaf colouration and light exposure is most likely.

It is an interesting question if dark red morphs are exposed to higher doses of light than the bright green ones. Do brighter morphs appear more often in sunnier locations than darker ones? These questions will be answered in the following chapter.

Materials and Methods: At first adequate locations with plants of type 1, 2, 6, and 8 were selected (for the different leaf type see above: „leaf colouration"). Using a spectrometer the incoming light at a marked position above the plants was measured in Lux. Every 30 minutes the incoming light was measured three times in a row. This method was repeated on three different days from 9 a.m. to 12 p.m. This period of time was chosen because of the weather conditions. Till midday the weather was mostly sunny, however, in the afternoon clouds often gathered.

Results: With the collected data of the different plants the average for each leaf-type was calculated. With type 1 (dark red, n = 264), the incident light had a mean light intensity of 2428 Lux. With type 2 (dark red with bright main vein, n = 81), the light intensity averaged 1953 Lux. With type 6 (red-green spotted, n = 12), 6624 Lux were averagely measured. With type 8 (bright green, n = 48), the mean light intensity was at 17033 Lux. The varying sample sizes have different reasons: At first the plants with dark red leaves occur more often than all other types. Additionally, at the beginning of the experiment it was not clear if the way of proceeding was proved to be useful and realizable. Thus, the first experiments were conducted mainly at locations, which could be easily reached. At these locations, mainly plants with dark red leaves were found.

Discussion: According to Lee (2002), the underside of leaves belonging to plants of the herb layer of a forest are more likely to have a red colouration. Most of the time it is a shady environment with the exception of sunny spots where plants are exposed to high doses of UV-light. Due to Lee (2002), this and other factors may lead to conditions of stress for the plants, which cause them to produce anthocyanins. The results show that the amount of sun irradiation differs highly from location to location. While the average amount of incoming light energy is similarly low at type 1 and 2, considerably higher results were measured at types 6 and 8. It seems to be the case that the less light reaches the location of a plant, the more anthocyanins are developed within its leaves.

Gould et al (1995) claim that shade plants enriched with photosystem II are more vulnerable to photoinhibition and damage caused by short-term intensive sun irradiation. This could be a possible explanation for the higher rates of anthocyanin development (as a protective function) in

darker morphs. The results can, however, not be interpreted insofar as that the varied morphs are some kind of adaption for different light conditions. Thereto the focus of this investigation was not enough directed towards the differences among the varied morphs. These experiments can only be regarded as preliminary investigation. While gathering the presented data, the way of proceeding seemed adequate. Unfortunately, the intervals of light energy measurements were set too coarse-meshed. The sun irradiation on a plant has to be measured continuously over the course of several days to ascertain the duration and incidence of sun spots. Only by that, the theory of Lee (2002) and Gould et al (1995) concerning the protective function of the anthocyanins against short-term heightened sun irradiation can be assessed. Special attention on the different morphs must be paid in this process.

Colour Change in Correlation with Change in Habitats

The influence of UV-radiation on the leaf colouration is known and was already subject of many investigations (Brandt et al. 1995, Mancinelli 1985, Wellmann et al. 1991). Hoffman (1999) detected changes in leaf and flower colouration of different species caused by UV-radiation. On the contrary, she did not found any shifts in colour, when the UV-wavelengths of the incoming light were blocked.

The enhancement of anthocyanins within a leaf as a protective mechanism was already often observed and investigated (Bormann and Teramura 1993, Teramura 1983, Tevini and Teramura 1989, Wellmann et al. 1991). The following experiment was conducted to clarify how *Episcia lilacina* answers to enhanced solar radiation. For *Episcia lilacina* grows mainly on the shady forest ground, the plant is not used to prolonged sunny periods. What happens if the plants of *Episcia lilacina* were transplanted to a sunnier location? Do they answer to an enhanced sun exposure? Do plants with dark red leaves differ in their reaction from plants with bright green leaves?

Materials and Methods: Plants with bright green leaves and without visible anthocyanin concentration as well as plants with dark red leaves and intermediate stages were selected. A sunny and a shady place were chosen to conduct this experiment. Before starting, all plants were photographed to enable a comparison of the leaf colouration afterwards. For the shady location seven plants were transferred from the rain forest to the main building of the biological station La Gamba. There, a maximum of 6 Lux of light intensity could be measured. The same was done with the plants, which were transferred to a sunny location. The search for a suitable sunny location turned out to be difficult. At first all plants were transplanted in a plastic bag (as a plant pot) and placed near the main building on a lawn, where the sun was shining almost all day long.

But soon, it turned out that the plants could not cope with the intensive sun-irradiation. Because it was becoming too hot, they could not compensate the loss of water. At a second attempt a patch directly besides the main building was chosen. On this location the sun was shining from morning to afternoon. For the moment it seemed again that the plants could not sustain the circumstances. As a consequence a thin net in a height of 50 cm was braced above them (Fig. 17). The net cast enough shadow to prevent them from withering (the measurement results of light energy and temperature can be seen in Fig. 18). After a month the plants were photographed again. Afterwards the photos were compared with the earlier ones. (Fig. 19-22)

Figure 17 *Episcia lilacina*; plants covered to protect them from intense sunlight and as a consequence to prevent them from withering.

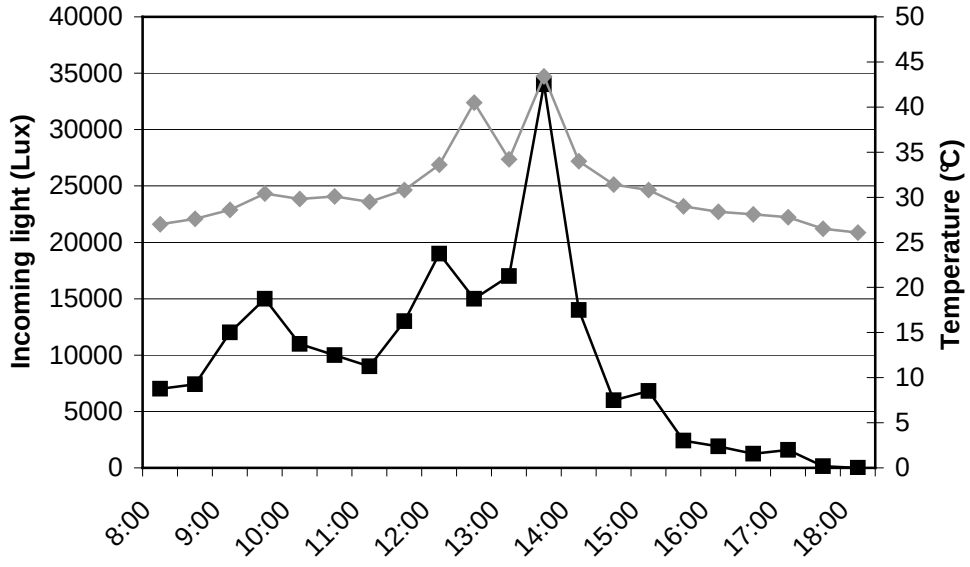

Figure 18: Measurement of incoming light (black) and temperature (grey) reveal the conditions the plants had to deal with; the maximum amount of sunlight was 34000 Lux; the highest temperature 43,4 °C.

Results: After a month a definite change in colour of red leaf areas could be observed in those plants that were planted in sunny locations. They became brighter while in the already bright areas the changes were not so obvious, though it seemed the green had intensified. Due to the fact, that the photos were not taken under controlled lightning conditions it cannot be ascertained that the differences in colour are not caused by the photo camera. With those plants that were kept in dark places in the main building no change in colour could be observed. Even the small amount of light was sufficient for the plants.

Furthermore no difference in leaf colouration was detected in newly developed leaves. Plants with dark leaves developed further dark ones as well as plants with patterned leaves developed further patterned ones et cetera.

Discussion: The fact that the sun exposed plants had to be protected from dying off using a fine net as cover shows that the heightened amount of light irradiation posed a stress factor for the leaves. It is obvious that *Episcia lilacina* is a shade plant, perfectly capable of dealing with low light situations but unable to cope with large amounts of sun irradiation. They cannot compensate the loss of water caused by the increased evaporation and start to wither.

Disturbances in photosynthesis via destruction of the chloroplast structure and in further consequence changes of photosynthetic pigments in leaves caused by UV-B radiation are known

(Bornmann and Teramura 1993, Burger and Edwards 1996, Strid and Porra 1992, Teramura and Sullivan 1994). That can cause a visible change in the colour of the leaves.

A brightening occurs with the darker coloured areas of the leaves of *Episcia lilacina* as well as a colour change to a reddish brown because of a proportional lower availability of chlorophyll; other colourants contained in the plant become visible (Hoffmann 1999). Changes in colour were (to some degree, see above) recognized in the green leaves, too. Due to Hoffmann (1999) a brightening of the leaf colour caused by the destruction of the chloroplasts should have also occurred. But the green intensified. If the reason lies in an increased chloroplast concentration, has yet to be proven in further experiments.

There are still several tests that have to be done to clarify the exact function of the anthocyanins in *Episcia lilacina*. The different leaf colourations are apparently caused genetically. A single plant is thus not able to react directly to changes in light conditions by in- or decreasing the development of anthocyanins in newly formed leaves.

The anthocyanin layer of *Episcia lilacina* has not a protective function against UV-radiation as some scientists proved it to be the case with other plant species (Takahashi et al. 1991, Burger and Edwards 1996). Thus an increased development of anthocyanins should have occurred, respectively, the dark morphs should not have bleached out if the anthocyanins would have prevented the destruction of the chloroplasts. In neither the dark red nor the green plants a visible increase in the anthocyanin concentration was detected under increased sun irradiation. All colour morphs of this test series were able to deal with increased stress. Admittedly, it was not measured if there were different maximum levels of bearable light irradiation at the different colour morphs. There could be variations.

To once again face the results of Gould et al (1995) it has to be said that in his opinion shade plants show a higher concentration of photosystem II and thus are more susceptible to photoinhibition and damage caused by short-term intensive sun irradiation. The anthocyanins would, according to that, not directly protect the chloroplasts in the leaf from destruction (which would require them to be layered above the chloroplasts) but rather diminish consequential damage like photoinhibition as well as reactive oxygen intermediates that are released by chloroplasts under influence of UV-B rays (Yamasaki 1997, Gould et al 2000). This would, in addition, explain why within green leaves even under constantly higher sun exposure no visible increase of the anthocyanin concentration could be observed. Their chloroplasts are adapted to higher sun irradiation from the beginning. These newest results seem to be the most appropriate to explain the phenomenon of leaf colouration of *Episcia lilacina*. But as already mentioned earlier, further investigations are required to verify or falsify this hypothesis.

Figure 19 *Episcia lilacina*; plant of type 1; left: shortly after the transfer to the new location; right: after a month; the dark red colour changed into reddish brown.

Figure 20 *Episcia lilacina*; plant of type 3; left: shortly after the transfer to the new location; right: after a month; change of colour can be seen.

Figure 21 *Episcia lilacina*; plant of type 6; left: shortly after the transfer to the new location; right: after a month; red areas became brighter.

Figure 22 *Episcia lilacina*; plant of type 8; left: shortly after the transfer to the new location; right: after a month; hardly any difference.

Summary

Episcia lilacina is a stoloniferous, low-terrestrial herb. The stem creeps on the ground, with erect tips only. The plant can reach 15 cm in height. The roots of *Episcia lilacina* grow shallowly. At the nodes adventive roots can be produced. Axillary stolons occur at the lower leaf pairs.

On average, the stolons of *Episcia lilacina* grow daily 6.5 mm. The vegetative propagation via stolons is an effective strategy to occupy a habitat as well as an effective option to sexual reproduction.

To show, how fast *Episcia lilacina* can establish itself in a newly occupied habitat the progress in stolon development was documented. Over the course of 80 days one plant can spread over an area of about one square meter.

The leaf colours and patterns of *Episcia lilacina* are varied. After reconsidering all leaf morphs - from dark red to bright green – they were divided into eight types. Between dark red and bright green almost all variations are possible.

Two experiments were conducted to proof that anthocyanins are responsible for the red colour within the leaves of *Episcia lilacina*. Further on, it was revealed that anthocyanins are also present in the bright green leaves, but in a lower concentration.

The highest concentration of the anthocyanins can be found near the palisade layer. This fact excludes one explanation for the anthocyanins development, because in this layer they can not act as sunscreens.

Another explanation for the varied leaf morphs of *Episcia lilacina* could be mimicry. But there are too many leaf types, which do not look like grazed leaves. Additionally, only few plants with feeding damage were found.

Furthermore, it was tested if there exists any relation between the light passing through and the red-green proportion of the leaves. With an increase of green parts the amount of passed light grows as well.

Investigations concerning the leaf colour and the light exposure reveal that bright green morphs are exposed to higher doses of light than the dark red ones. Further analyses are necessary to confirm the presumption that the different leaf morphs are adaptations for different light conditions.

A definite change in colour of red leaf areas was observed after a transplantation to a sunnier location. The leaves became brighter. One possible explanation, and thus the reason for the leaf colouration of *Episcia lilacina* is that chloroplasts of shade plants are not able to deal with enhanced light exposure and therefore were destroyed. On the contrary, chloroplasts of green

morphs are adapted to higher sun irradiation from the beginning, thus no additional anthocyanin development was observed.

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PART 2 Investigations of Flowers of *Episcia lilacina*

General Description of the Flower

Until today, the legitimate pollinator of *Episcia lilacina* could not be observed. Because of the mutual adaptions between flowers and pollinators, the flower shape as well as different morphological characteristics can indicate possible visitors. In this chapter these indications were analysed and therefore some pollination groups were taken into consideration, others were excluded.

Figure 23 *Episcia lilacina;* habit.

Results: *Episcia lilacina* produces single flowers of zygomorphic symmetry (Fig. 23). The pedicel is pilose and of greenish to reddish colour. The calyx consists of five sepals, which are all of equal size, free to the base, erect, pilose, and of greenish to reddish colour. The shape of the sepals is narrow lanceolate, the margin is entire.

Regarding the corolla, five petals are fused together to form a long tube, which apically bears an oblique limb of rounded petal lobes. The limb is divided into two upper and three lower lobes. The entrance into the tube is a narrow slit so that the flower type may be classified as keyhole-

flower. At the base of the corolla tube a spur is present on the dorsal side. It serves for collecting the secreted nectar.

The corolla has a pale lilac to pale blue colour. Sometimes it appears almost white. At the entrance to the corolla tube a yellow blotch is present, which extends a few millimeter into the tube. Sometimes the yellow spot is dotted with red. The outside of the tube is pilose.

The androecium is composed of 4 stamens, which are divided into a filament and a dithecal anther with four pollen sacs. Dehiscence is by longitudinal slits. The stamens are inserted at the base of the corolla tube. The filament bases are broad and adjacent to each other. They form a sheath around the ovary. The four anthers are postgenitally united (Endress 1994). Two are fused laterally and all four are united apically (Fig. 24). During the male phase the anthers are placed at the entrance of the flower. This position is achieved by curving of the filaments.

Figure 24 *Episcia lilacina;* left: androecium, four anthers united postgenitally; right: closer view at two anthers, dehiscence is by longitudinal slits.

The singular nectar gland lies dorsally towards the spur at the base of the ovary.

The ovoid ovary is superior, syncarpous and hirsute. The placentation is parietal with two placentae. The ovules arise from the inner and outer surfaces of the placentae. During the male stage (pollen release) the style has not reached its full length and the stigma is not receptive. At the end of the male stage, the filaments coil and the anthers are drawn back from the corolla entrance. The style starts growing in length so that the stigma reaches the same position the anthers occupied before. The stigma is divided into two lateral lobes. The lobes' surface is covered with an area of papillae.

The flower smells slightly of cucumber.

An analysis of the corolla's capability to reflect UV-light that was conducted using cobalt blue glass showed that the whole limb area except the entry reflects UV-light (Fig. 25).

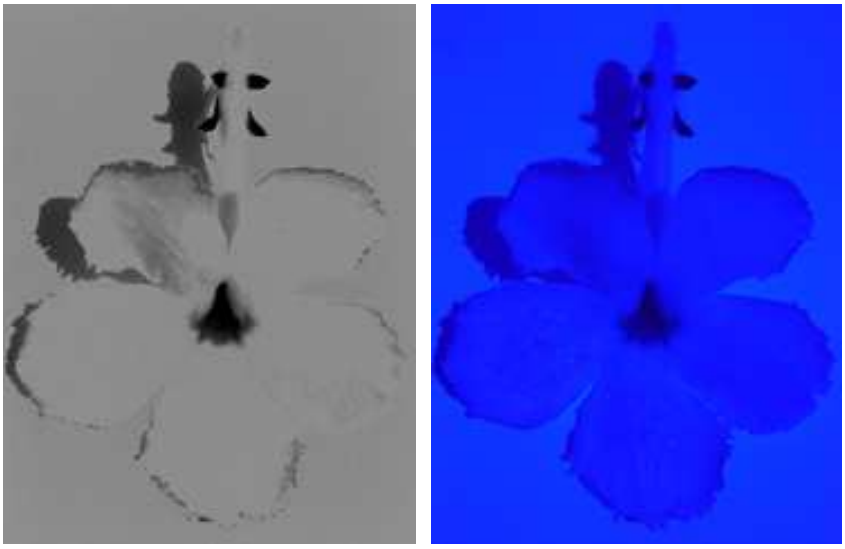

Figure 25. *Episcia lilacina;* photos of one flower taken through cobalt blue glass, the tube entrance absorbs UV.

Discussion: Wiehler (1978) describes the flower of *Episcia lilacina* as the only psychophilous one within the genus of *Episcia*. As previously mentioned the legitimate pollinator could not yet be determined. Nevertheless, the analysis of the *Episcia lilacina's* morphological characteristics allows to draw conclusions concerning this matter.

Because of the long tube and the narrow entrance the general access to the floral centre is impeded in such a way that only certain groups of potential pollinators may reach nectar. The special shape of the keyhole flower of *Episcia lilacina* implies that the pollinators have to be insects featuring long proboscises to get access to the nectar in the spur. Firstly the tube does not proceed straight but is curved. Furthermore, the stamina and style impose an impediment. Thus a pollinator's proboscis has to pass through the curved tube past filaments and style to reach the nectar in the spur.

In addition, the shape of the flower of *Episcia lilacina* offers hints on its legitimate pollinator. Flowers with a narrow tube and flat rim are typical for a psychophilous syndrome (Proctor et al 1996, Endress 1994). The keyhole flower represents an increase of this principle.

A limb formed by the corolla lobes attracts pollinators and serves as a landing area for visitors (Westerkamp 1999, Endress 1994, Scoble 1995). The legitimate pollinator is likely to be a species of insects that uses this landing platform like e.g. Hymnoptera and Lepidoptera species. Hawkmoths (Sphingidae) are able to hover in midair directly in front of the flower (Dreisig 1997)

so they would not need any kind of landing opportunity. Thus they do not match to the pattern of the pollination syndrome of *Episcia lilacina*.

Episcia lilacina develops sensitive flowers that can easily be harmed by, for example, rain. Flowers being pollinated by bumblebees have to be mechanically strong. The deepest flowers can be operated in a non-destructive way by butterflies and birds (and some specialized flies) only (Faegri and Pijl 1971). This circumstance indicates that butterflies (Lepidoptera) belong to the visitors.

Statements concerning the colour of a psychophilous flower vary broadly. Some authors (Endress 1994, Faegri and Pijl 1979, Westerkamp 1999) state that the flowers are brightly coloured, often orange, red or pink, while Proctor et al (1996) think blue to be part of the sample board beside purple, pink or red. Scoble (1995) indicates white as a possibility beside bright colours. It is quite difficult to draw accurate deductions from these statements. Keber (1997) specifies blue-lilac to be around one per cent among the colour preferences of Lepidoptera – examined in Golfo Dulce Rainforest, Costa Rica. That indicates the lilac colour of *Episcia lilacina* to be a rarity. Hawkmoths are rather attracted by pale coloured flowers and are therefore more often present among flowers of sphingophilous syndrome (Faegri and Pijl 1979).

The UV-light absorbing yellow blotch near the entrance seems to be a nectar guide. The limb's UV-light reflecting properties solidify the suspicion of insects serving as legitimate pollinator. A UV-absorbing entrance and a UV-reflecting periphery are commonly found with flowers of psychophilous syndrome (Scoble 1995). Despite these facts, there is no actual proof of what insect it is exactly. Goldsmith and Bernard (1974) describe that bees, bumblebees, butterflies as well as flies are able to percept ultraviolet light.

The bulk of the nectar is collected in the spur that protects the nectar from rain or evaporation. Nectar hidden in tubes or spurs (Faegri and Pijl 1971) and a long distance between sexual organs and nectar occurs in moth and butterfly flowers. This serves not only to exclude bees as pollinators, but also to guide the pollinator's proboscis (Faegri and Pijl 1979).

Flower perfume is produced to serve as an attractant for pollinators (Faegri and Pijl 1979), *Episcia lilacina,* for example, smells slightly like cucumber. The odour is weak with psychophilous flowers but generally agreeable (Vogel 1954), because the olfactory sense of butterflies (Rhopalocera) is not very strong (Faegri and Pijl 1979). The very opposite is applicable for moths (Sphinghidae, Noctuidae): Their olfactory sense is strong with instinctive preferences for heavy-sweet perfume (Faegri and Pijl 1979). The scent that attracts bees can be fresh (Faegri and Pijl 1979), sweet (Proctor et al 1996) and/or agreeable (Vogel 1954). Due to those statements the odour secreted by *Episcia lilacina* cannot be related to a certain pollinator group.

When drawing a conclusion a lot of features of *Episcia lilacina's* flower point towards a psychophilous pollination syndrome – more than those indicating otherwise. Due to Opler

(1983) flowers of a given syndrome often lack one or more of the expected features. But also those flowers that appear to fit in a certain pollinator group could in fact be pollinated by a different group.

Morphometry of the Flower

The dimensions of a flower and its components are an essential part of a description of a plant. This chapter provides a survey of the size relations of the observed flower components. The measurements of the flower and its components should also serve to increase the understanding of the pollination process.

The following parameters were recorded:

- Length of corolla tube; conclusions on the proboscis length of the pollinator can be drawn and thus its size can be predicted to some degree.
- Width and height of the flower entrance; via the size of the entrance a flower can limit the access of unwanted visitors.
- Width and height of the narrowest and widest part of tube; the tube has no consistent diameter throughout its length; the measuring of the narrowest and widest part gives information on the progression of the tube's shape.
- Limb diameter; the limb diameter plays an important role as it is an essential part of the pollination syndrome.
- Lengths of stamina; this suggests the position of the anthers inside the tube; the stamina have to be long enough that the pollen can be easily transferred to the pollinator.
- Length of style; the correct length is important so that the pollen on the pollinator can reach the stigma.
- Diameter and height of ovary.

Figure 26 shows a flower frontal and lateral view, both in its male (upper drawing) and in its female stage (bottom drawing). Most of the measured parameters are given.

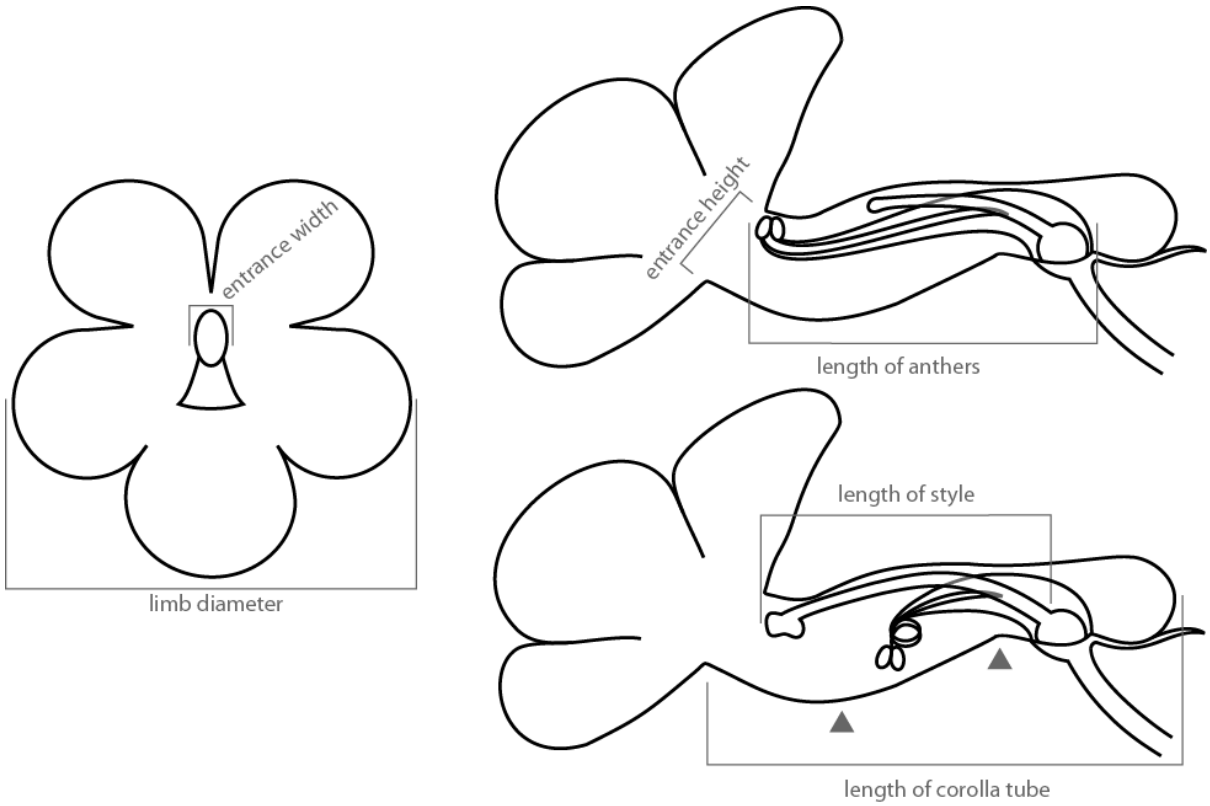

Figure 26 *Episcia lilacina;* schematic drawing of the flower and the parts measured; left: flower in fronted view; upper right: flower in male stage; bottom right: flower in female stage; the arrows indicate the narrowest and widest parts of the tube.

Materials and Methods: For measurements a calliper rule was used. The length of stamina was measured in the male stage, the style length in the female stage. 25 flowers from eight different localities were investigated.

Results: All measured values are listed in table 1. The entrance to the tube is very narrow just like the narrowest part of tube. In fact, the entrance is narrower but higher than the narrowest part. Immediately after the entrance the tube widens noticeably. Here the widest part of the tube is located. Shortly before the ovary and the basis of stamina the tube narrows up once more as shown in figure 26. Using the measurements to calculate the cross sectional area, the entrance has an area of 13,7 mm^2, the narrowest part 13,2 mm^2 and the widest part 26,2 mm^2.

The sum of the length of style and the height of ovary equals the length of stamina. The range in measurements of the length of stamina is considerable, but is equally present with the measurements of length of corolla tube and length of style.

Table 1: Measured floral characteristics of *Episcia lilacina*.

	n (number of flowers measured)	Average value and standard deviation (in mm)	Minimum to Maximum (in mm)
Length of corolla tube	25	38 ± 2,62	29 – 41
Width of the flower entrance	25	3 ± 0,5	2 – 4
Height of the flower entrance	25	5,8 ± 0,64	5 – 7
Width of the widest part of tube	25	4,5 ± 0,91	3 – 6
Height of the widest part of tube	25	7,4 ± 0,65	6 – 8
Width of the narrowest part of tube	25	3,5 ± 0,77	1 – 5
Height of the narrowest part of tube	25	4,8 ± 0,62	4 – 6
Limb diameter	25	42 ± 3,46	35 – 49
Length of stamina	21	17 ± 1,49	13 – 20
Length of style	21	15 ± 1,10	12 – 17
Height of ovary	21	2,9 ± 0,38	2 – 4
Diameter of ovary	21	2,1 ± 0,30	2 – 3

Discussion: The limiting factor considering the legitimate pollinator is the tube of *Episcia lilacina*. The average length of tube of 38 mm implies that the proboscis of the pollinator has to be of about equal length, because there exists an evolutionary correlation between tube length and proboscis length (Nilsson 1988). Bumblebees, for instance, belong to the largest insects acting as legitimate pollinators, but possess proboscises approaching only 10 mm in length (Barth 1991). Insects that are to be considered effective pollinators thus only include butterflies (Lepidoptera) and large neotropical bees e.g. Euglossini.

Euglossini are a tribe within the family Apidae, which are also called orchid bees. The proboscises of orchid bees are up to 41 mm long (Roubik and Hanson 2004). Euglossine bees use the nectar of the flowers of Gesneriaceae as an important food resource (Endress 1994).

Butterflies do not forage on flowers with a corolla depth that exceeds their tongue length (Corbet 2000). From that, it can be concluded that its proboscis has to be of at least equal length as the tube. Because there is a positive relationship between body length and proboscis length in the family of Lepidoptera (Kunte 2007), conclusions considering their size can be drawn. Thus smaller butterfly species can be excluded from the group of possible pollinators.

Length of tube alone delivers no further indication to exclude one of the insects that are considered possible pollinators until now.

The narrow entrance to the flower is supposed to prevent pollinators from penetrating too deep into the inside of the flower. Additionally, the insect is positioned correctly to be brought in contact with the pollen as well as being able to pollinate the style. During this process the pollen is supposedly placed on its head or proboscis. It is quite interesting that the tube broadens right after the narrow entrance significantly to almost double the size of the narrowest part before narrowing again in the rear section. Such an expanse can also be observed with other tubular flowers in the family of Gesneriaceae, e.g. *Nauticalyx* or *Paradrymonia*. The entrance of the flower is alike the narrowest part of the tube because both cross-section areas differ only rarely in size. Because of the narrowest part being located in the rear part of the flower it likely serves as guidance for the proboscis so that it can reach the nectar.

The size of the floral display can matter in attraction of pollinators. Within most plant species, pollinators generally select in favour of large flowers (Pellmyr 2002). The limb diameter of averaged 42 mm forms a spacious landing area for insects. Thus the flower is almost as long as it is wide. The attracting effect of the big limb is quite considerable.

If the sum of the length of style and height of ovary is compared to the length of stamina approximately equal values are reached. This indicates that the stigma indeed comes to a halt on roughly the same position that had held the anthers before. Thus the application of pollen from the body of the pollinator on the stigma is guaranteed.

Bud Development

Before referring to the process of anthesis more exactly, the bud development shall be taken into observation. The morphological changes in bud development and growth are described, rather than absolute changes in bud size or stem growth, because they are more indicative of development than mere increase in size and length.

Materials and Methods: The investigated plants were marked and each of them received a number. At periodical intervals the spots with the marked plants were visited, the progress of the development was written down and photographs were taken. Additionally, injuries or malformations, which influence the normal development, were documented.

Results: The bud development can be roughly divided into four stages. Table 2 shows each stage of bud development including the time period. The records started with stage 1, in which the flower bud as such is clearly recognizable. At this stage the petals are not yet visible. Only the sepals are easy to identify. They are densely white-pilose.

In stage 2 the petals become visible though they are still green. They have about the same length as the sepals. The petal lobes form a globular corolla bud. Out of this part through the unfolding of the petal lobes the corolla limb forms later on. Slight changes of colour to pink-lilac can be observed with the petals.

In stage 3 the tube elongates. The petals have grown longer than the sepals. The corolla bud overtops the sepals. The petal lobes located on the outside can already be discerned. The spur begins to form. The tube takes on the colours of bright pink to lilac. The sepals have nearly reached their full length.

In stage 4 the flower bud is shortly before opening. The corolla is now obviously coloured in lilac and the tube has considerably grown in length. The spur bends upwards to the rear of the flower but has not reached its full size yet. The outer petal lobes can easily be distinguished. The anterior globular section of the flower hat turned upwards resulting in the inclined position of the corolla limb in relation to the tube.

The total duration of the bud development process described above is about 19 to 23 days. Subsequently, the process of anthesis begins.

Table 2: *Episcia lilacina*, overview of the different stages of bud development.

Stage	Photo	Time period
1		12-14 days
2		3-4 days
3		3-4 days
4		1 day

Anthesis

The term "anthesis" describes the whole period in which a flower is active i.e. as long as pollen is presented and/or the stigma is receptive. The flowers of *Episcia lilacina* are proteandrous. Male and female stages proceed separately. This chapter deals with the process of *Episcia lilacina's* anthesis.

Materials and Methods: The process of anthesis was documented using the same plants and methods as in the last chapter. To assure the receptivity of the stigma, potassium permanganate ($KMnO_4$) was dissolved in water. This solution was transferred to the stigma, which in case of receptivity should turn black. Unfortunately the stigma turned black every time as soon as it came in contact with $KMnO_4$. Receptivity was efficiently measured via observation because of the swelling of the stigma lobes.

Results: The male stage (Fig. 27, left) of anthesis starts between four and five o'clock in the morning. Shortly after the opening of the flower the release of pollen begins. The four anthers open and the pollen is presented freely accessible.

Between four and five o'clock in the afternoon the filaments coil. The stigma is still immature. The style has not reached its full length. Thus the male stage comes to an end. In the course of the next hours the style grows in length so that the stigma reaches the location that was held before by the anthers. During this growth the stigma becomes receptive. The female stage (Fig. 27, right) begins in the subsequent night between midnight and four a.m. and lasts one or two days. The end of the receptivity of style is signified by shrivelled stigma lobes. The corolla detaches itself and drops. The style stays prominent for a few days and withers afterwards. If a successful pollination took place a growth of fruit becomes recognizable after around three days.

Figure 27 *Episcia lilacina;* left: flower in male stage; right: flower in female stage.

Discussion: Protandry – like in *Episcia lilacina* – is quite common in the family of Gesneriaceae (Denham 2004). It poses an effective method to avoid self-pollination.

Observations of the longevity of individual flowers are scarce (Wiehler 1983). As noted by Primack (1985), the length of time the anthesis lasts can influence its total number of pollinator visits, which, in turn, can affect the amount and diversity of pollen a flower receives and the amount of pollen it disseminates. A period of 4 days of anthesis is stated for the species of *Drymonia* (Endress 1994). Primack (1985) indicates a longevity of averaged 1.3 days with a range of 1-3 days for tropical rain forests. Due to the fact that *Episcia lilacina*'s flowers can be easily damaged by environmental influences but even though feature a two to three days timeframe for the anthesis the flowers can be considered durable above average.

Over 80% of the flower species tested by Primack (1985) showed a male stage much shorter than the female one. Due to Primack this is caused by the fact that the usefulness of the male flowers to the plant is over as soon as the pollen is shed and that may occur in many species within hours after the flower has opened.

Because the male as well as later on the female stages begin in the early morning the legitimate pollinator has to be day-active. Thus Noctuidae (moth) and Sphingidae (hawkmoth) can be excluded due to their overly night-active nature (Endress 1994). As stated by Faegri and Pijl (1979), the diurnal anthesis without closing at night favours butterflies (Rhopalocera) as pollinators. Opler (1983) claims that most butterflies feed on nectar between 8.00 and 15.00, beginning later and stopping earlier than other diurnal nectarivores. Other authors (Roubik 1989, Armbruster and McCormick 1990) allege that the early morning is a major foraging time for bees. Therefore, many bee flowers open at that time or earlier during the night. This statement concerning psychophilous flowers is in contrast to *Episcia lilacina* because the opening of the bud as well as the beginning of the female stage occurs early in the morning and still in the night,

respectively. Any explicit conclusion cannot be drawn. These statements rather point towards melittophilous syndrome though.

Fruit Development

Fruits are flowers in the state of seed maturity (Leins 2000). In gesneriads two different fruit types occur: dehiscent and indehiscent fruits, whereby the dehiscent dry capsular fruit represents the more primitive type (Weber 2004). The range of dispersal is from autochory, anemochory over ombrohydrochory to zoochory. Skog (1978) describes the fruit of *Episcia lilacina* as fleshy capsule. Residual are questions about the fruit development and the kind of distribution.

Results: After a successful pollination fruit development starts in the course of just a few days. The carpels form a greenish to reddish coloured, globular, pilose capsule that is surrounded by the persistent calyx (Fig. 28). The caspule is bivalved and fleshy.

Such capsules are green when part of green plants without red leaf patterns. The fruit of all colour morphs is reddish and no opening lines are visible. The calyx grows neither in length nor does it change its colour. The inside of the capsule is dark brown to dark red. Seeds are globular, shiny, mostly reddish, rarely white.

Two days after the withering of the flower it can be unmistakeably seen whether a fruit has developed or not. At this point the young fruit is covered completely by the calyx. At the beginning the fruit broadens a little bit faster than it grows in length. After approximately two weeks it is 5 mm (±2,5 mm) in diameter and has reached a length of 6 mm (±3mm). At this point the length starts to increase while the diameter of the fruit further on just slowly widens. After ten more days the diameter is 8 mm (±3mm) and its length 7 mm (±1mm). In summary, it can be said that in the course of 25 days of observation the fruit grows with an average of 5,5 mm in width as well as 4 mm in length.

While the fruit development was not finished yet, it could not be observed until the end because the stay in Costa Rica came to an end at this point. Thus neither dehiscence nor the way the seeds spread could be examined.

Discussion: The ripening of a fruit can take a couple of weeks or even months. Fruits of *Cyrtandra grandiflora* (Gesneriaceae) for e.g. need approximately five month to ripe (Reolofs 1979). Fruit development of *Episcia lilacina* could only be observed for around four weeks. Presumably, it would have taken a few weeks more for the fruit to actually become ripe. Wiehler (1983)

describes the fruit of *Episcia* as more or less a capsule, which dehisces loculicidally. The convex valves form a cup, which holds seeds with prominent funculi.

Supposedly, the seeds are either dispersed by wind or rain or they just drop to the ground.

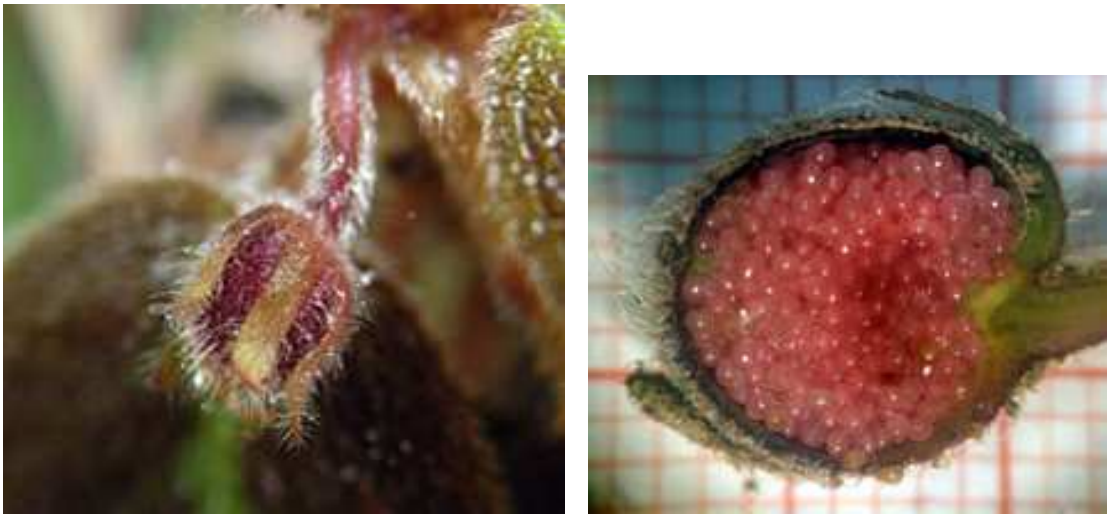

Figure 28 *Episcia lilacina;* left: fruit after 25 days development; right: cross-section of one fruit with reddish seeds.

Fruit Set

The result of a successful pollination is the development of fruits and seeds. The amount of the developed fruits gives information about the "reproduction effectiveness". The fruit set was calculated by the number of developed fruits per 100 flowers.

Materials and Methods: In total, 141 flowers were under observation. Two days after the end of anthesis it could be clearly determined if a successful pollination had taken place and if thus fruit development had begun. In case of a failed pollination the rest of the flower, which initially remained after the dropping of the corolla, decayed in the course of a few days. A premature dropping of fruits was not observed. However, the observation of the fruit development to ripeness could not be completed, because the stay in Costa Rica was too short.

Fruit set was calculated by the following formula:

$$fruit\ \ set = \frac{developed\ \ \ fruits}{100\ \ \ flowers}$$

Results: 27 fruits developed from 141 flowers. That is 19 fruits per 100 flowers, i.e. the fruit set is 19%.

Discussion: A fruit set of 19% seems quite low with regard to the fruit set of *Paraboa rufescens* (Gesneriaceae) of about 40% (Gao et al 2006) and the fruit set of *Cyrtandra grandiflora* (Gesneriaceae) of 32-39% (Roelofs 1979). Even though no legitimate pollinator could be observed, pollination did occur without a doubt. The only regular visitor was a species of Meliponinae. Due to SanMartin-Gajardo & Sazima (2004) pollen collectors can act as pollinators even if they are not the legitimate pollinator. Either the pollinator just could not be encountered during the observation period or it was deterred by the presence of the observer. However, self-pollination can be excluded to explain the fruit set results (see below in „pollination in the field").

The observed fruit set is most likely caused by the visitation of meliponine bees. The stingless bee touched the stigma in search of pollen and thus pollinated the flower in case it had visited a flower in the male stage before. There are a lot of flowers that show a special floral syndrome but are visited by pollinators that do not fit. Waser and Price (1990) quote *Delphinium nelsonii* as an example, whose blue asymmetrical flowers conform to a bee syndrome, but attract humming birds along with hawkmoths, solitary bees, and several species of bumblebees.

Pollination Experiments

The anatomy of the flower as well as proterandry decreases the possibility of self-pollination enormously. Therefore, different pollination experiments were performed to investigate if spontaneous self-pollination occurs or if manual self-pollination is possible.

Materials and Methods: To test spontaneous self-pollination, on the day before the buds opened they were bagged. The bag remained until the anthesis of the flowers had ended. Afterwards the development of fruits was checked and documented.

To test self-compatibility, the anthers were cut out from the flower during the male stage. The anthers were put into a container. On the following day, the pollen was transferred to the stigma of the same flower. From the beginning the flower was covered with a bag to protect it against flower visitors. After the end of the anthesis the bag was removed and the following development was documented and photos were taken.

To ensure that cross-pollination (allogamy) is possible, pollen of a different flower must be used for pollination. After the opening of the flower the anthers were cut out. Afterwards the flowers were covered to prevent visits of pollinators. During the female stage the stigma was pollinated with pollen from a different plant. The flower was covered again. In the following days the fruit set was observed and documented.

Results: None of the covered flowers pollinated itself spontaneously and therefore development of fruits did not take place. However, the pollination of the flowers that were pollinated with their own pollen was successful i.e. self-compatibility is possible. The same is true for the experiments testing on allogamy.

Discussion: The conducted experiments show that self-compatibility in *Episcia lilacina* is indeed possible, but is successfully prevented by two mechanisms in their natural environment. The first method is the curling of filaments so that the anthers are placed in the back area of the flowers. Due to the proximity of the anthers to the stigma during this process there is a possibility of self-pollination, which is prevented by another mechanism: The stigma stays unreceptive till the curling of the filaments has finished. In this way it is assured that the receptive stigma will not ever come in contact with the plants' own pollen.

Self-pollination is not necessary, because *Episcia lilacina* successfully spreads vegetatively via stolons. Additionally, selfing is seen rarely in the family of Gesneriaceae (Weber 2004).

P/O Ratio

According to Cruden (1977), the pollen-ovule ratio reflects the reproductive system. By pollen-ovule ratio Cruden means the proportion of the pollen grain numbers as compared to the number of ovules in a bisexual flower. A high P/O ratio suggests xenogamy, a lower one implies autogamy to cleistogamy. He even considered that P/O ratios better reflect the breeding system than morphological characteristics. To characterize the reproductive system of *Episcia lilacina* the number of the pollen grains and the number of ovule per flower were counted.

Materials and Methods: Altogether, five buds were investigated. To be sure that the whole amount of pollen grains was still in the anthers, the pollen grains were counted with buds shortly before anthesis. The content of each anther was counted per hand using a stereomicroscope and a grid.

For the determination of the number of ovules the same buds as before were used. The gynoecium was dissected out under a stereoscope and the ovules were counted afterwards.

The pollen-ovule ratio per flower was calculated by the following formula:

$$\frac{P}{O} = \frac{number\ of\ \ pollen\ grains\ \ per\ \ flower}{number\ of\ \ ovules\ \ per\ \ flower}$$

Results: The results are listed in table 3. The P/O ratio of *Episcia lilacina* is 36,34 : 1.

Table 3 Results concerning the P/O ratio.

Bud number	Number of pollen grains	Number of ovules	P/O
1	40120	1052	38,14
2	34528	1046	33,01
3	40560	1082	37,49
4	35080	956	36,70
5	35414	974	36,36
		Average value	36,34 (±1,99)

Discussion: Pollen-ovule ratios are correlated with the breeding systems of plants (Cruden 1977). They reflect pollination efficiency, i.e. the likelihood of a pollen grain reaching a stigma (Cruden and Miller-Ward 1981). Cruden (1977) divided the ratios of different plants into five classes of reproductive systems: xenogamy (P/O about 5800:1), facultative xenogamy (about 800), facultative autogamy (about 170), obligate autogamy (about 30) and cleistogamy (about 5). The evolutionary shift from class to class is accompanied by a significant decrease in the mean P/O. The more efficient the transfer of pollen, the lower the P/O. That means for *Episcia lilacina* that about 36 pollen grains come on one ovule. Due to Cruden (1977), this result would indicate obligate autogamy. A much higher P/O ratio would be expected. Mistakes can of course not be excluded entirely. But even if the result of the pollen grain count would be twice as high the next class would be barely reached. The P/O ratio results of this work do not fit in the system of Cruden because obligate autogamy of *Episcia lilacina* can be definitively excluded.

Examining the amount of pollen grains and ovules it is obvious that a lot of ovules (averagely 1022 ovules) were developed in one gynoecium. Comparing this fact with the high amount of pollen grains the low P/O ratio seems to be more comprehensible. The numerical amount of pollen grains and ovules were not further considered by Cruden (1977). This circumstance makes comparisons between the results of *Episcia lilacina* and other plant families difficult.

Nevertheless, many different factors may influence the pollen-ovule ratio (Preston 1986), for example:

- Enlargement of the stigma area; it can receive more pollen grains despite of a constant pollen-transfer area on the pollinator (Cruden and Miller-Ward 1981).

- Pollen-packing theory (Brunet 1992); the amount of pollen grains gets reduced when the presentation period is prolonged.
- Ovule-packing theory (Burd 1995); if more ovules per flower are produced, the reproduction success is enhanced. The success would be higher, even when not all ovules were fertilized. Thus, less pollen grains were needed to enable a successful pollination.
- Pollen-grain junctions; they lead to pollen agglutination, which increases the efficiency of the pollen-transfer (Cruden and Jensen 1979).

Which of these factors is responsible for the low P/O ratio of *Episcia lilacina* was not investigated in detail, but the last mentioned may well apply. Albrecht (1999) made similar observations. She investigated for the first time different species of Gesneriaceae with regard to their P/O ratios. Thereby, species were found (e.g. *Achimenes grandiflora*, *A. misera*, *Nauticalyx adenosiphon*, *Paradrymonia lineata*), which were - like *Episcia lilacina* - despite of a low P/O ratio - not autogamous. Albrecht (1999) claimed that the reason for this phenomenon are pollen agglutinations, which enhance the efficiency of the pollination process. Such agglutinations were observed with *Episcia lilacina,* too. Therefore, they are perhaps the reason for the occurring P/O ratio.

Apparently, each major taxon needs its own standard for evaluating its breeding system, because a lot of factors influence the P/O ratio in such a way that it cannot be meaningfully compared (Vasek and Weng 1988, Erbar and Langlotz 2005). It is necessary to take the family Gesneriaceae more precisely into account to reveal why some genera have reduced their pollen grain to ovule ratio.

Nectar Secretion

The immobility of plants creates an obstacle in the dispersal of their genes. To alleviate this problem, the majority of all plants have developed a mutual partnership with animals, which transport pollen grains and also disperse seeds. In the case of pollination, the animals transport pollen in turn for a food reward. Nectar is one of the rewards. It serves as energy supplier for the pollinators. Its accessibility in relation to floral morphology but also the amount and the concentration of produced nectar allows a conclusion which animal groups use the nectar of *Episcia lilacina* and may be the legitimate pollinator.

Materials and Methods: *Episcia lilacina* has a single dorsal nectar gland. To ensure that the gland is the only tissue, which produces nectar, it was removed. This took place before other experiments were performed.

To make out the beginning of nectar production, the spur of the bud was opened and nectar – if there was any - was absorbed by glass capillaries. At the beginning of the nectar production the amount was too low to measure it.

To find out the maximum amount of secreted nectar the spur was dorsally cut open and the nectar was removed every second hour. The measurement was not possible any longer than several hours per flower because the flowers were very tenuous and got very easily damaged by the glass capillaries. Furthermore the cut spur began to mortify. To remove and measure the nectar, glass capillaries were used (0,5 µl, 1 µl and 3 µl disposable capillaries, Hirschmann Laborgeräte, R≤ 0,5%, CV≤1,0%). In total, nectar was taken on three different days every two hours. The removal was started at different times (11.30 p.m., 5.30 a.m., 7.45 a.m.).

Further on, at different times of the day several flowers were cut open and nectar was removed to find out at which time the maximum was offered. Additionally, flowers in the male and female stage were investigated if there were any differences in the maximum of produced nectar.

Because of the small amount of nectar in a flower, the nectar of several flowers was put together on a refractometer to measure the sugar concentration. After cutting the top of the spur the nectar was transferred on it with glass capillaries and then the range was read. At a temperature of 29°C two measurements on two different days were made. The flowers were taken from two different investigation spots. The first time 16 flowers were collected near the station. The second time 19 flowers were harvested near the "Rio Quebrada".

Results: The flower of *Episcia lilacina* starts its production of nectar a couple of hours before opening. Already at 11 p.m. of the day before nectar could be found in the spur of the flower. The average amount of nectar produced in two hours is 0,5 µl (±0,2µl standard deviation). The longest duration of nectar measuring was 14 hours. After these 14 hours in none of the flowers (male or female) nectar could be measured any longer.

The maximum of nectar produced by one flower is 5,4 µl. The average value is 2 µl (±1,37 µl standard deviation). No significant difference in nectar production during male and female stages could be measured. The collected nectar had a sugar concentration of 27%.

Discussion: The flowers can get easily damaged by glass capillaries. The used method does not seem be the most appropriate. As soon as the spur is cut it starts to wither, a fact that for sure has had its influence on the nectar production.

Research in the tribe *Sinningieae* revealed an average amount of nectar of 15,4 µl (±12,1 µl standard deviation) for bee pollinated flowers (Perret et al. 2001). For *Drymonia serrulata* (Gesneriaceae), a plant that is mostly visited by large neotropical bees, an average of 262,3 µl (±95,9 µl standard deviation) has been published (Steiner 1985). Opler (1983), however, gives values of 9,75 µl for medium to large bee pollinated species and 0,93 µl for butterfly pollinated species. Reddi (1998) investigated among others "tubular flowers with the essential organs inserted" which were pollinated by butterflies. She found a range from 0,01 to 2.0 µl nectar volume. These statements point to butterflies as the legitimate pollinators of *Episcia lilacina* due to the fact that the amount of presented nectar is far below the average of melittophilous flowers.

With higher concentrations of sugar in the nectar the amount of contained energy increases. At the same time its viscosity increases too, resulting in a higher amount of energy requirement for the pollinator during the uptake of the nectar. The „optimal sugar concentration for floral nectar" represents the sugar concentration at which the rate of net energy gain to the pollinator is maximized. It is around 26 % (Heynemann 1983). *Episcia lilacina* is quite close to that value with its 27% of sugar concentration.

Heyneman (1983) published a table containing the average sugar concentrations of flowers in context with their pollinators. Flowers pollinated by butterflies have an average sugar concentration of 25%, those pollinated by bees however a higher concentration of 35%. Similar values are presented by Pyke and Waser (1981), namely 28.8 % for butterflies, 22.1 % for hawkmoths and 41.6 % for bees. In relation to these facts the sugar concentration of *Episcia lilacina* of 27% points again towards butterflies as legitimate pollinators.

Flower Visitors

Field studies on pollination on Gesneriaceae are still scarce (Vogel 1966, San Martin-Gajardo and Sazima 2002a, 2002b). Whieler (1978) described *Episcia lilacina* as the only known case of psychophily in the Episcia complex. Afterwards nobody paid attention to the pollinators of *Episcia lilacina* any more. During the investigation period the legitimate pollinator could not be detected, though the flowers did not remain completely unvisited.

Materials and Methods: To verify the statement of Whieler (1978) numerous observations during the investigation period (May to August 2006) were made. To find the pollinator, observations of the flowers of *Episcia lilacina* took place on different spots during different periods. Especially in the morning hours after the opening of the flowers the arrival of visitors has been observed. Additionally, an observation of twelve hours – continuously from 4 a.m. to 4

p.m. – was made. To avoid scaring off the visitors, a distance of several meters was kept. In this case, binoculars were used for observations.

The visitors were caught with a net. To kill them a piece of felt soaked in ethyl acetate was put with the insects in a plastic container with a screw top. Back in the station the insects were pinned on needles. Afterwards they were frozen to avoid becoming mouldy or getting eaten up by ants.

The determination was done with the help of Professor Vogel of the University of Vienna and Mag. Dominique Zimmermann (curator of the hymenoptera collection) at the Natural History Museum in Vienna.

Results: A stingless bee (subfamily Meliponinae) was caught. It was observed collecting pollen. Unfortunately only stingless bees visited the flowers of *Episcia lilacina* during the monitoring. In the vicinity of the flowers orchid bees as well as bumblebees could be seen, even though none of them visited the flower of *Episcia lilacina*. Additionally, an ant queen of the subfamily Myrmicinae was caught in a net that had been wrapped around a flower.

Discussion: The observations reported in this paper indicate that butterflies (Rhopalocera) – rather than bees- are the legitimate pollinator of *Episcia lilacina*. Sadly, no butterfly visiting the flower could be seen. During the observations Euglossini were spotted in the vicinity that are known to visit flowers that are also pollinated by butterflies (Vogel 1966). One important source of nectar for Euglossini are species of the family of Gesneriaceae (Wiehler 1978). However, none of the observed Apidae species visited the flower of *Episcia lilacina*.

The ant queen that was caught in the net was most likely there by chance. No further observations of similar kinds could be made. Thus it is out of the question to act as pollinator.

On top of the leaves a caterpillar species was found ("Part 1"). Caterpillars are often seen on the very same plants they pollinate later on as adult butterfly (Scoble 1995, Proctor et al 1996). This is not the case here, however. The caterpillar species belongs to the moth and in addition is much too small to be the missing legitimate pollinator. It is definitely possible that the caught stingless bee is a secondary pollinator (see fig. 29). Anyway, it does not fit the pollination syndrome of *Episcia lilacina*.

The importance of particular pollinators to tropical forest plants frequently shifts from year to year (Roubik et al 2003). Thus even though the legitimate pollinator was not seen during the observations this year it could possibly be encountered quite easily the next year. Further observations are absolutely necessary to solve the mystery of the pollinator of *Episcia lilacina* entirely.

Figure 29 *Episcia lilacina*; meliponine bee taking pollen from the flower.

Summary

Episcia lilacina produces single flowers of zygomorphic symmetry in the leaf axils. The five petals are fused, forming a hypocrateriform flower. This suggests butterfly pollination. Further indications are sensitive flowers with a lilac-coloured limb, nectar hidden in a spur, with a long distance between sexual organs and nectar.

Pollinators with shorter proboscis can be excluded as legitimate pollinator because of the length of tube of *Episcia lilacina*. Further on, the narrow entrance prevents that pollinators penetrate too far into the tube and ensures that the insects are positioned correctly to come in contact with the anthers or the style.

From the stage at which the flower bud is clearly recognizable to the stage at which the bud is shortly before opening, about 19-23 days have past. During this period the corolla has grown in length and developed an anterior globular section equivalent to the limb of the later flower.

The flowers of *Episcia lilacina* are proteandrous. The male stage begins shortly after the opening of the flower and ends about 12 hours later with coiling the filaments. Afterwards the style starts to grow in length and after a few couple of hours the female stage begins with the stigma becoming receptive. After two days, when the stigma lobes shrivel, anthesis ends.

The fruit of *Episcia lilacina* is a capsule with globular, reddish or white seeds, surrounded by a persistent calyx. Over the course of 25 days the fruit grows averagely 5,5 mm in width and 4 mm in length.

The fruit set of *Episcia lilacina* is 19%. Self-pollination can be excluded. Manual self-pollination is possible, but spontaneous self-pollination does not occur, for the simple reason of protandry. Sexual reproduction is largely replaced by stolon spreading.

The P/O ratio of *Episcia lilacina* is 36,34/1. Due to Cruden (1975) this would indicate obligate autogamy. But this can be definitively excluded. One possible explanation is pollen agglutination, which enhances the efficiency of pollen transfer and was observed both with *Episcia lilacina* and other species of Gesneriaceae.

The production of nectar starts a couple of hours before the flower of *Episcia lilacina* opens. The maximum amount of secreted nectar by one flower was 5,4 µl. Further on, the nectar has a sugar concentration of 27%. Both facts indicate butterflies as legitimate pollinators.

The only observed flower visitor of *Episcia lilacina* during the investigation period was a species of stingless bees (subfamily Meliponinae). These bees are not the legitimate pollinators, but secondary ones, which do, however, not fit into the pollination syndrome.

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