

THE STAMINAL LEVER MECHANISM AND FLORAL DIVERSITY OF SOME
BEE-POLLINATED *SALVIA* L. (LAMIACEAE) SPECIES

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**THE STAMINAL LEVER MECHANISM AND FLORAL DIVERSITY OF
SOME BEE-POLLINATED *SALVIA* L. (LAMIACEAE) SPECIES**

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ABSTRACT

THE STAMINAL LEVER MECHANISM AND FLORAL DIVERSITY OF SOME BEE-POLLINATED *SALVIA* L. (LAMIACEAE) SPECIES

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The characteristic staminal lever mechanism is defined as a functional unit including the modification of stamens into lever-like structures, their reversible movement and the organisation with the remaining floral structures and they are involved in the process of pollen transfer. The staminal levers play a major role in the process of pollen deposition on the pollinators' body and their proportion and interaction with pollinators may have significant consequences for the pollination system in *Salvia*.

In this study, staminal lever mechanism and floral diversity of bee pollinated species including *Salvia absconditiflora*, *Salvia aethiopis*, *Salvia russellii*, *Salvia tchihatcheffii* which grow sympatrically are investigated in the campus flora of Middle East Technical University.

The functioning of the staminal lever mechanism is tested and pollinators of the *Salvia* species are observed by field investigations. Special attention is given to species-specific patterns of pollen deposition on the pollinators' body. Floral diversity of the *Salvia* species such as corolla shape, size, colour, stamen type are

studied. Floral and pollinator structures are measured morphometrically by laboratory investigations. Moreover, the isolation mechanism among the co-occurring *Salvia* species are investigated by field observations. All field observations are backed by photos and video recordings.

Field and laboratory investigations revealed that co-occurring *Salvia* species are reproductively isolated by several means and the diversity of floral constructions and staminal lever mechanism increases the mechanical isolation by attracting different pollinators and by loading pollen on different parts of their bodies.

Keywords: Labiatae, *Salvia*, staminal lever mechanism, pollination

ÖZ

ARILARLA TOZLAŞAN BAZI *SALVIA* L. (LAMIACEAE) TÜRLERİNİN STAMEN ÇALIŞMA MEKANİZMASI VE ÇİÇEKLERİNİN ÇEŞİTLİLİĞİ

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Salvia türlerinin polen transfer işleminde, stamenlerin kol benzeri yapılara farklılaşması, her iki yönlü hareketi ve diğer floral yapılarla ilişkisi, fonksiyonel bir birim olarak tanımlanır. Stamen kolları, polinatör vücudunda polen depolanması işleminde önemli bir rol oynar. Bu mekanizmanın polinatorlerle olan ilişkisi *Salvia*'nın polinasyon sisteminde önemli sonuçlara sahiptir.

Bu çalışmada, arılar ile tozlaşan ve Orta Doğu Teknik Üniversitesi kampüs florasında bir arada bulunan *Salvia absconditiflora*, *Salvia aethiopsis*, *Salvia russellii* ve *Salvia tchihatcheffii* türlerinin stamen çalışma mekanizması ve çiçeklerinin çeşitliliği incelenmiştir.

Stamen çalışma mekanizmasının fonksiyonelliği test edilmiştir ve *Salvia* türleri ile polinatörleri, arazi çalışmaları ile gözlemlenmiştir. Polinatör vücudu üzerinde türe özgü polen depolanma şekillerine önem gösterilmiştir. *Salvia* türlerinin korolla şekli, büyüklüğü, rengi, stamen tipi çalışılmıştır. Çiçek ve arı organları morfometrik olarak

ölçülmüştür. Ayrıca, bir arada bulunan *Salvia* türlerinin izolasyon mekanizmaları arazi çalışmaları ile incelenmiştir. Tüm arazi çalışmaları fotoğraf ve video kayıtları ile desteklenmiştir.

Arazi ve laboratuvar çalışmaları, bir arada bulunan *Salvia* türlerinin çeşitli üreme izolasyonları ile birbirlerinden izole olduklarını göstermiştir. Çiçeklerin organizasyonu ile stamen çalışma mekanizmasının, farklı polinatörleri cezbederek ve poleni, polinatörlerin farklı vücut bölgelerine bulaştırarak, *Salvia* türleri arasında mekanik izolasyonu arttırdığı gösterilmiştir.

Anahtar Kelimeler: Labiatae, *Salvia*, stamen çalışma mekanizması, polinasyon

To my family

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CHAPTER 1

INTRODUCTION

1.1 The Family Lamiaceae

Lamiaceae Martinov (Labiatae) also known as the mint family, has cosmopolitan distribution. It includes over 250 genera and nearly 7000 species (Thorne, 1992). It is the third largest family in Turkey. Lamiaceae is composed of 45 genera and 574 species, 256 of which are endemic in Turkey and its endemism ratio is about 44.5 % (Davis, 1982).

The flowers of Lamiaceae are unique for the family and petals are composed of an upper and a lower lip. The members of Lamiaceae are used as ornamental, culinary or flavoring herbs. Most of the species which are native to Turkey and Mediterranean area are cultivated throughout the world. (Davis, 1982).

In Labiatae, there are many species from different genera that are economically important, including *Mentha* L. (peppermint), *Nepeta* L. (catnip), *Lavandula* L. (lavender), *Marrubium* L. (horehound), *Origanum* L. (oregano), *Rosmarinus* L. (rosemary), *Thymus* L. (thyme), *Salvia* L. (sage). (Judd *et al.*, 1999). By far the largest genus in the family is *Salvia* comprising more than 900 species in the world and nearly 100 of them grow in Turkey.

1.2 The Genus *Salvia*

It is the largest genus in Lamiaceae and sages are used as herbal tea and for culinary purposes. *Salvia* species possessing antibacterial, antioxidant and antidiabetic properties are used as medicines all over the world (Ulubelen, 2003). Many sage species are grown as garden plants. Some of them have aromatic leaves which can be used as flavoring herbs. They are also grown for their ornamental value. The genus name goes back to ancient times and comes from the Latin word 'salvus' which means 'to heal', regarding the healing properties of *Salvia officinalis* (Turner, 1999).

Etlinger (1777) recognized 48 species in the first monographic treatment of *Salvia*. Bentham (1833, 1848, 1876) recognized four sub-genera characterized mainly by the shape and structure of the calyx, corolla and lower branches of the connectives. This classification was modified by Briquet (1897). Then, many revisions were made (Fernald 1900, Gams 1927, Peter 1936, Epling 1938, 1939a, 1939b, Hedge 1974, 1982a, 1982b, Ramamoorthy 1984a, 1984b, Wood and Harley 1989, Espejo Serna and Ramamoorthy 1993, Santos 1996) and numerous species were described in local floras (Epling and Toledo 1943, Pobedimova 1954, Hedge 1972, 1982a, 1982b, 1985, Standley and Williams 1973, Codd 1985, Li Xi-wen and Hedge 1994, Ilcim *et al.* 2009, Celep *et al.* 2011).

For Turkish *Salvia*, Bentham (1833) recognized 18 species from Turkey. The intrageneric classification of the genus dates back to Bentham (1848) who established 14 sections, nine of them distributed in the Old World and five in the New World. Only sect. *Heterosphace* has species in both hemispheres. Boissier (1875), in his 'Flora Orientalis', recognized 75 species of *Salvia* from Turkey and placed these under seven sections. The last taxonomic revision of *Salvia* in Turkey was made by Hedge (1982), who recognized 86 species, one hybrid and one doubtful species. Since then, six more new species, one new variety, and three new records have been described from Turkey. The species number is now reached to 97 and 55 of which are endemic (Celep *et al.*, 2011) Infrageneric delimitation of the genus was done by Doğan *et al.* (2007). According to this study, *Salvia* species in Turkey were

grouped under seven sections as; *Aethiopsis* Benth, *Drymosphace* Benth, *Hemisphace* Benth, *Horminum* Dumort, *Hymenosphace* Benth, *Plethiosphace* Benth, *Salvia* Hedge.

Up to now, little has been known about *Salvia*'s center of origin and its biogeographical routes of migration. It is assumed that *Salvia* probably originated from an ancient Mediterranean species (Dieringer et al. 1991; Baikova 1999). Some species might have migrated during the Miocene and Pliocene via tropical mountains to South America and South Africa (Baikova 1999). Fossils from Mexico (Graham 1999) and Alaska (Müller 1981) dated from the Lower and Upper Miocene and thus indicate an age of at least 25 million years. It is reasonable that ancestral *Salvia* species migrated from Eurasia to North America via the Bering land bridge. (Claßen-Bockhoff et al. 2004).

1.3 Environmental Selection and Phenotypic Diversity of *Salvia* Flowers

The species of *Salvia* L. contains nearly 1000 species and they display a significant diversity in secondary compounds, floral morphology, and pollination biology. Phenotypic diversity is high in *Salvia* (e.g. Himmelbaur and Stibal 1932–34; Ramamoorthy and Elliott 1998).

Salvia are distributed world-wide (Figure 1). Today, centers of diversity areas are found in Eurasia. In the Mediterranean region there are approximately 210 species, in Central and East Asia 90 species, in East and South Africa 60 species, in North America 40 species, in Central America 350 species, and in South America 210 species (Claßen-Bockhoff et al. 2003). Turkey seems to be a major centre for *Salvia* in South-west Asia, with 97 species (Celep *et al.*, 2011). The distribution in neighbouring countries is as follows: 75 species in the former USSR (Pobedimova, 1954), 70 in *Flora Iranica* (Hedge, 1982a), 36 in Europe (Hedge, 1972), and 21 in *Flora Palaestina* (Zohary, 1966).

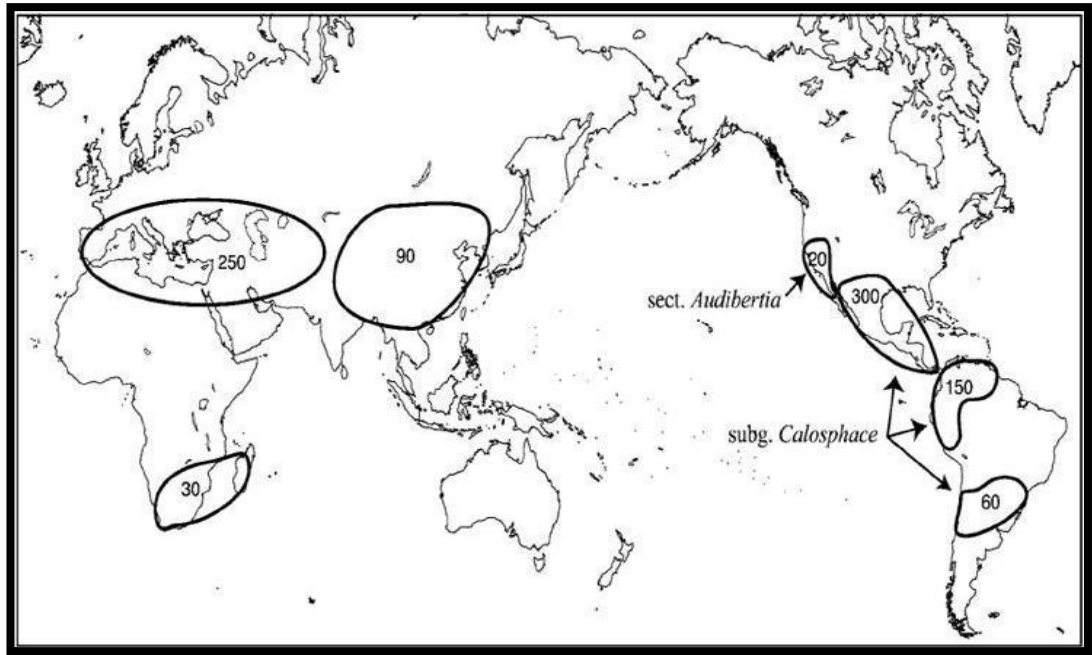


Figure 1. General distribution of the genus *Salvia* (Walker *et al.*, 2004).

Salvia species prefers a wide range of habitats, from sea level to 4000 m. They grow in tropical rainforests, dry bushland, temperate forests, at forest edges, on meadows and degraded sites. Their ecological differentiation is high, including the size and shape of leaves, the presence/absence of simple or glandular hairs, and their secondary metabolite contents. Growth forms vary from perennial herbs and shrubs to annuals (Alziar 1988–1993).

1.4 Floral Characteristics and Stamen Types of *Salvia*

The flowers are mainly bilabiate or tubular. Flowers differ in length for example, flowers of *S. dombeyi* Epl. is 8–9 cm and flowers of *S. tiliifolia* Vahl. is approximately 0.5 cm). The shape and relative proportions of their organs are different (Figure 2). They show colors from yellow and white to red, pink, blue and violet (Alziar 1988–1993). The style and stamens are placed below the upper lip. Size, density and number of flowers vary in the inflorescences (Claßen-Bockhoff *et al.* 2004).

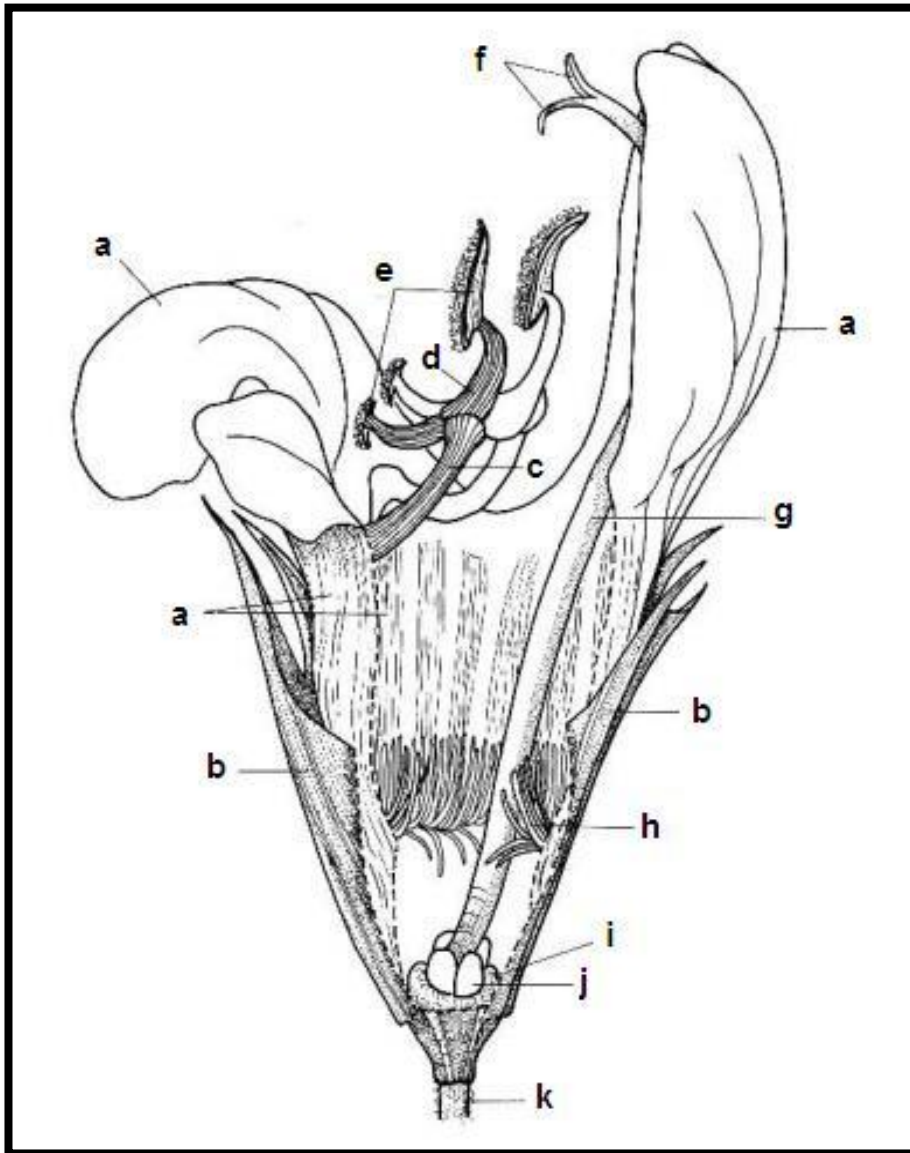


Figure 2. Cross section of a *Salvia* flower showing the floral organs. a; petals, b; sepals, c; filaments, d; connectives, e; anther sacs, f; stigmas, g; style, h; hairs, i; nectar disc, j; ovary, k; pedicel, (Modified from Glimn-Lacy & Kaufman, 2006).

Stamen structures have been used for classification. In addition to other morphological characters they formed the base for the sectional parrangement (Bentham, 1833, 1848). Walker *et al.* (2004) and Walker and Sytsma (2007) tested the phylogenetic significance of stamen morphology. Considering connective

widening, theca reduction, different modes of connective fusion and lever functionality, they illustrated that the lever mechanism has developed at least three times in parallel. The lever-like stamen is the result of a unique developmental processes, such as inhibition and unilateral growth.

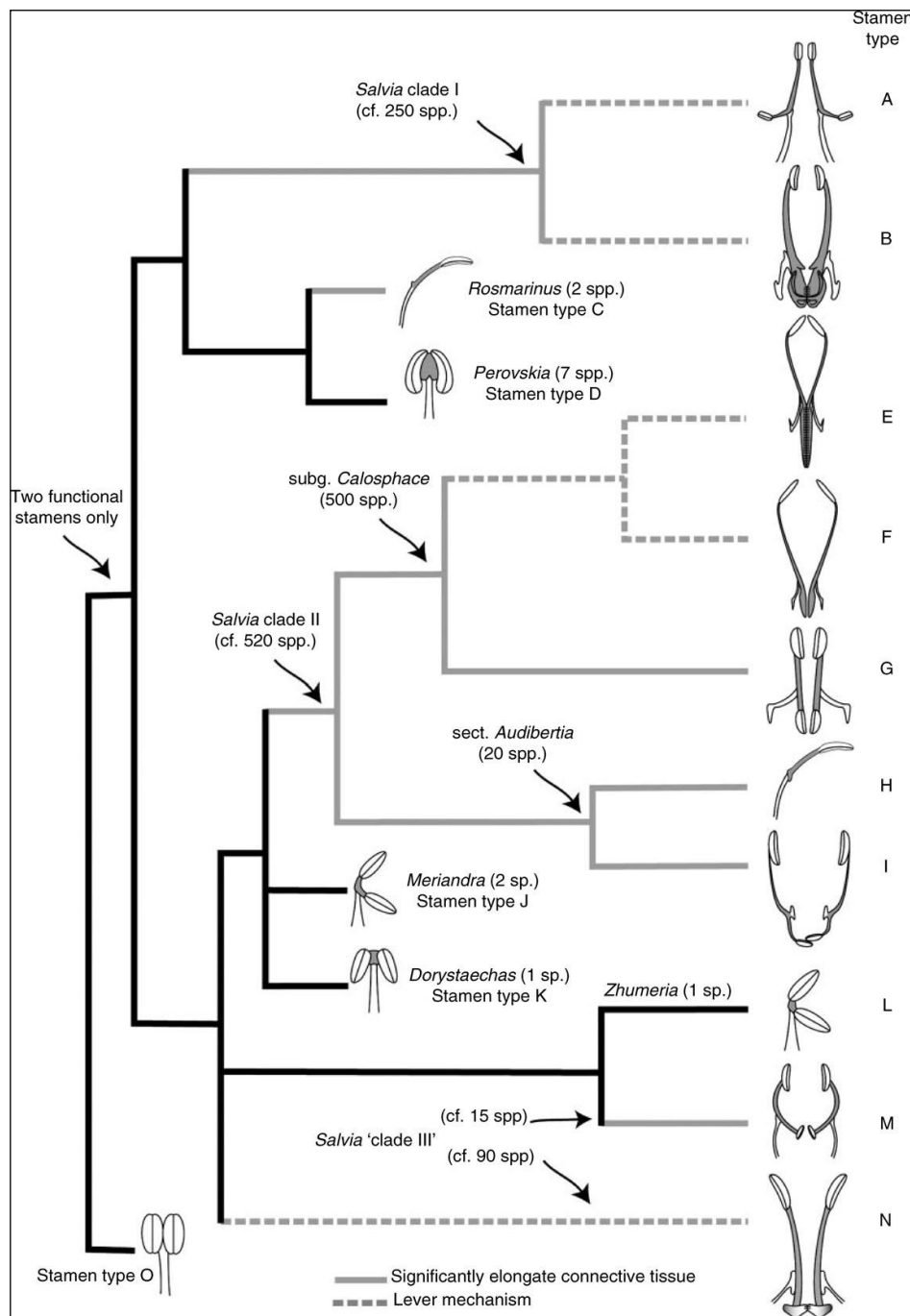


Figure 3. Stamens types (Walker & Sytsma (2007))

The anther in *Salvia* is asymmetric when compared with the basic stamen type in Lamiaceae which has a bithecate anther and a long filament (Troll, 1929). The staminal lever arms results from the extension of the connectives. The adaxial theca forms the monotheccic anther and the short filament results from the inhibited elongation (Claßen-Bockhoff *et al.*, 2004).

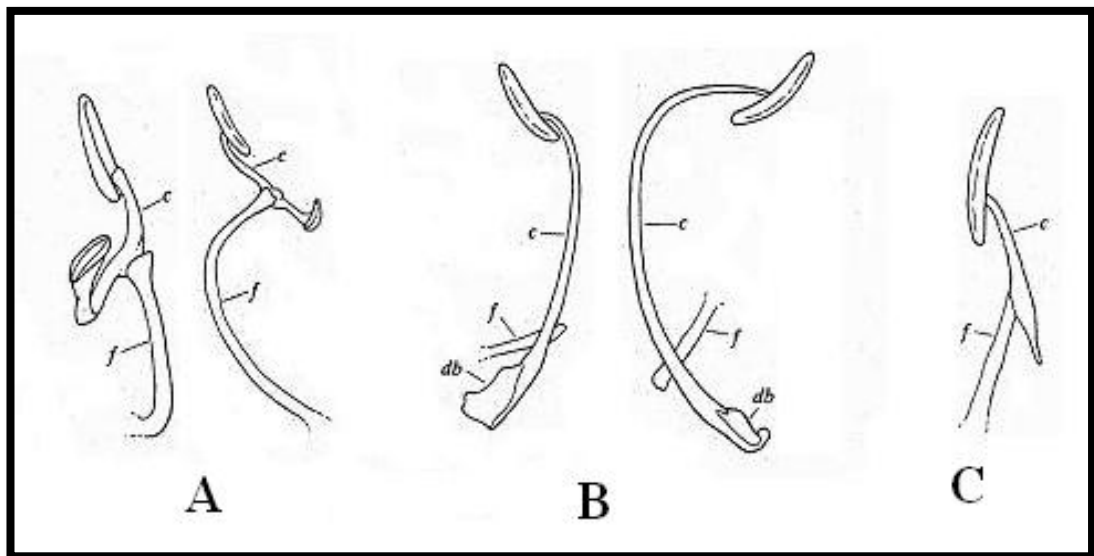


Figure 4. Stamen types of Turkish *Salvia* according to Hedge (1982)

Walker & Sytsma (2007) correlated their major clades of *Salvia* with stamen types from A to O (Figure 3). Hedge (1982) identified three stamen types for Turkish *Salvia* (A to C) in the Flora of Turkey (Figure 4). In stamen type A, both thecas are fertile but the ones in lower lever arms are smaller. The length of connective is almost equal to the length of filament. In stamen type B, the staminal connectives are longer than filaments. The lower thecas are reduced. In stamen type C, the staminal connectives are longer than filaments. The lower thecas are sterile. Stamen types M and N of Walker & Sytsma (2007) are represented in Turkey via *S. aristata* (recently found in Turkey), and *S. glutinosa*, respectively.

1.5 Flower Pollination by Bees

The transfer of pollen from an anther to the stigma of a pistil is called pollination.

In wind-pollinated ancestors of bee-pollinated plants, the main food source offered for the pollinators is pollen. Most of the wind-pollinated flowers are unisexual and female flowers are not normally visited by the pollen feeding insects. Bee-pollinated plants either have changed to produce organs of two sexes in a single floral structure or they have enabled female flowers to produce a food source for attracting the pollinators, such as nectar (Proctor & Yeo, 1973). Bees collect pollen and nectar as a food source. All the pollen is not collected and much of it is brushed off on the flowers which ensures pollination (Glimn-Lacy & Kaufman, 2006).

Pollinators often limits their visits to one type of flower while bypassing other equally rewarding ones which is known as flower constancy. Pollinators specialize on different flower types and individuals. This case leads to an effective pollination process for the flowers ensuring out-crossing by flower constancy (Grant 1950).

Cross-pollination which provides genetic diversity takes place when pollen is transferred to another plant. The pollen and stigma may not mature at the same time thus pollen must be transferred from one individual to the other to enable fertilization (Glimn-Lacy & Kaufman, 2006). In most common bee-pollinated flowers, there is a landing platform as in the two-lipped (bilabiate) flowers of Lamiaceae. The pollination system is rather unique for the genus *Salvia* (Proctor & Yeo, 1973).

1.6 The Pollination System in *Salvia*

Flowers are functional units and they are involved in sexual reproduction. There are several conflicts which have to be balanced by flowers. Flowers ensure the production of offspring by self-pollination but they also need to increase genetic diversity by outcrossing. While offering pollen as a floral attractant, flowers also have to save pollen for effective reproduction. Thus, floral construction is the combination of adaptive characters which are needed for pollen transfer. Lamiaceae,

which is characterized by ‘bilabiate blossom’, is a construction for dorsal pollen deposition (Classen-Bockhoff *et al.*, 2007).

Sprengel (1793) described the dorsal (nototribic) pollination mechanism in *Salvia*. The morphology of the staminal lever was recognized by Hildebrand (1865) for the first time. According to the latter author, the lever is formed by the connective that separates the two thecae from each other. The upper connective arm always has two pollen sacs and is placed below the upper lip, while the lower connective arm is often sterile and limits access to nectar. An insect searching for nectar pushes the sterile lower connective arm back. Then the insect gets loaded with pollen on its head, bill or back (Figure 5). During a subsequent visit to a flower of the same species, pollen on the insect can be transferred to the stigma. Regarding the precision of pollen transfer by the lever mechanism and the morphometric fit of floral structures and pollinators, each *Salvia* species deposits its pollen on specific domain of the pollinator’s body. (Classen-Bochoff *et al.*, 2004).

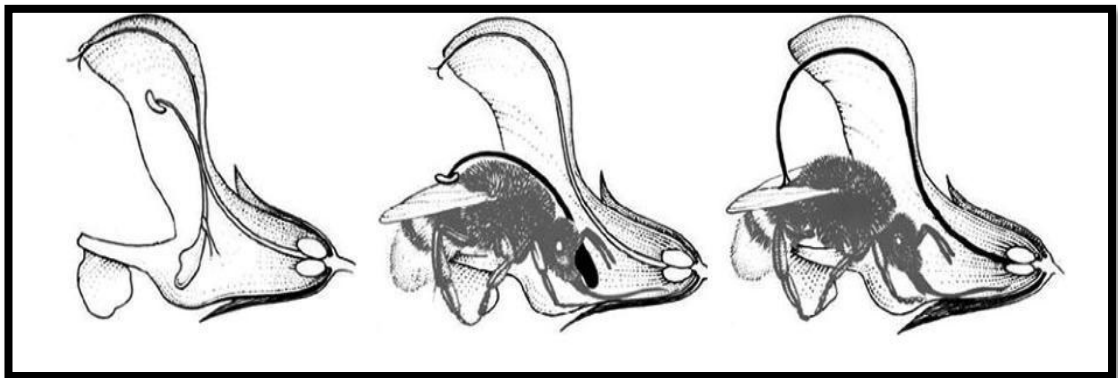


Figure 5. Longitudinal section of a *Salvia* flower showing the lever-like stamens and an insect looking for nectar (Classen-Bockhoff, 2004).

The staminal lever mechanism in *Salvia* is the best example for nototribic pollination mechanisms (Epling 1947; Grant and Grant 1964; Haque and Ghoshal 1981; Palomino *et al.* 1986; Huck 1992; Owens and Uberta-Jimenez 1992; Ramamoorthy

and Elliott 1998). The genus *Salvia* represents a taxon that possibly has undergone adaptive radiation to its pollinator spectrum. Ultimately, several sympatric species that flower at overlapping times share some of their pollinator species without mixing pollen (Classen-Bockhoff *et al*, 2004).

According to the literatures, In Europe, Asia and Africa, the dominant pollinators are honey bees, carpenter bees and bumble bees (Sprengel, 1793; Hildebrand, 1865; Müller, 1881; Knuth, 1898; Himmelbaur and Stibal, 1933-1935; Faegri and Van Der Pijl, 1979) and a lot of flowers which are pollinated by hummingbirds are found in America (Trelease, 1881; McGregor, 1899; Sazima et al., 1995; Ramamoorthy and Elliott, 1998).

1.7 The Functioning of the Staminal Lever Mechanism

Flower visit of a bee on *Salvia pratensis* flower was described by Proctor and Yeo (1973). According to them, when a bee pushes its head into the flower tube, the lower connective arm is pushed upwards and backwards. Then the upper connective arm bearing the fertile anther lobes swing downwards and their pollen sacs contact with the abdomen of the bee (Figure 6).

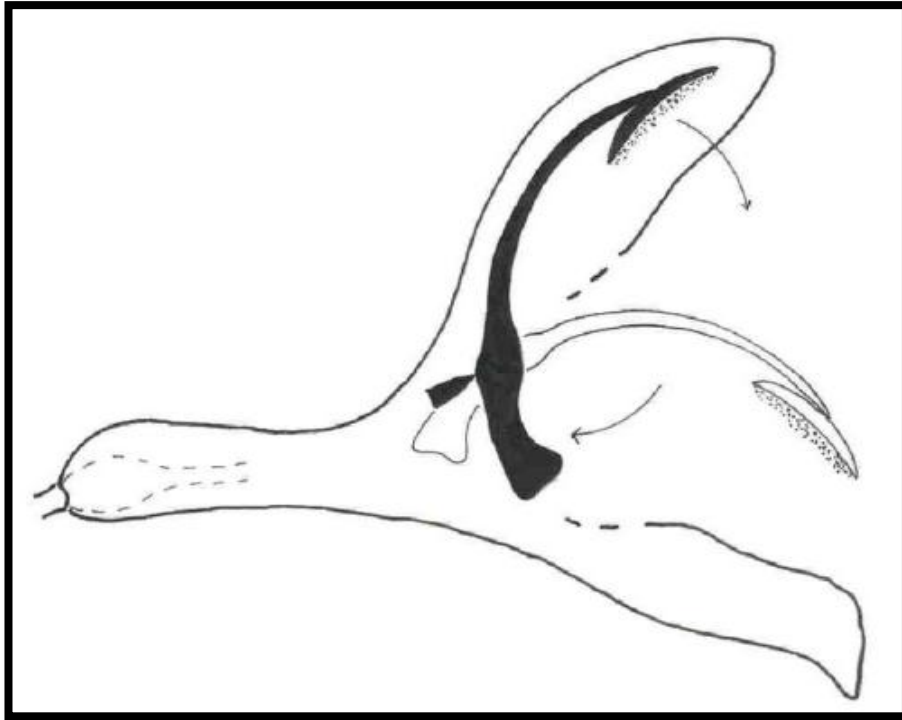


Figure 6. The reversible movement of the staminal levers (Classsen-Bochoff, 2004).

There are three phases in the visit of a bee on a *Salvia* flower. In the first phase the bee pushes its head into the flower and triggers the lever mechanism and then the lever moves. The second phase is almost static. The bee absorbs nectar and the lever is still triggered. During the third phase the bee leaves the flower. Then the lever swings back into position. Since the force that the bee applies on the lever is more than the forces that resets the lever mechanism, the lever swings around its joint in the first phase. In the third phase, the force that resets the lever mechanism becomes greater than the forces applied by the bee. Therefore the lever mechanism swings back to its original position (Classsen-Bockhoff *et al*, 2004).



Figure 7. Longitudinal section of a *S. abconditiflora* flower showing the staminal levers.

1.8 Location of the Study Area

The study area is located in Middle East Technical University campus area and lies approximately at 39° 53' 58".17 N, 32° 46' 47".45 E and 39° 52' 14".91 N, 32° 46' 54".42 E. The study area falls in the B4 square according to Davis's grid system (1965). The altitude is approximately 850 meters.

Turkey is divided into three phytogeographical regions which are Euro-Siberian (Euxine province), Mediterranean (East Mediterranean provinces of West Anatolia, Taurus and Amanos mountains) and Irano-Turanian (Central and Eastern Anatolia) phytogeographical region (Figure 8). Each phytogeographical region covers different floristic composition (Davis, 1965). The study area is in the Irano-Turanian phytogeographical region of Turkey.

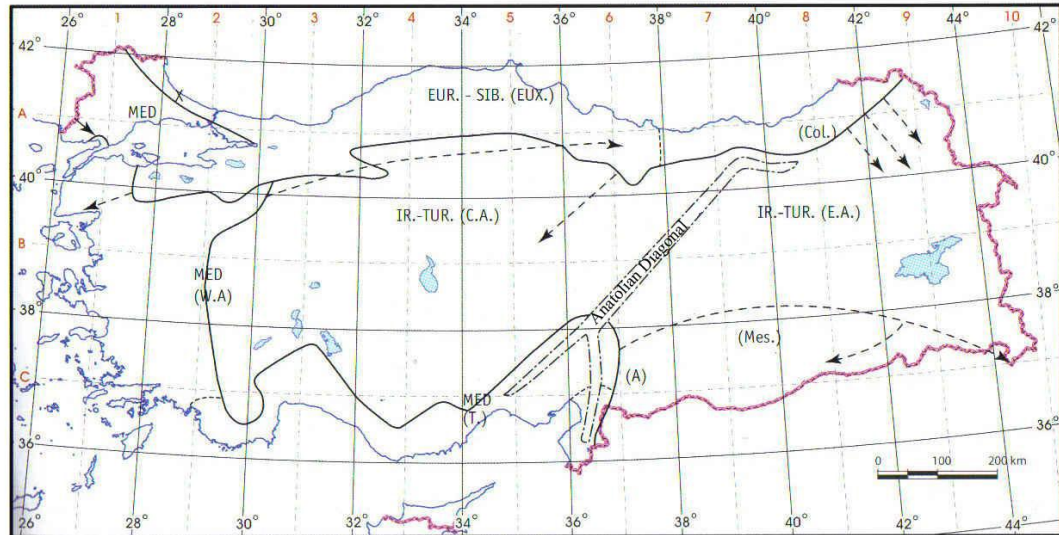


Figure 8. Phytogeographical regions in Turkey (Davis, 1971). Eur. Sib. (Eux): Euro-Siberian, Ir.-Tur.: Irano-Turanian, Med.: Mediterranean Phytogeographical Region. C.A: Central Anatolia, E.A.: East Anatolia.

According to Kaya *et al.* (1999), over 500 plant species grow in the campus area. All studied stations have great species diversity due to the METU campus being relatively a protected area over the years. Fourteen *Salvia* species (*Salvia absconditiflora* Greuter & Burdet, *S. aethiopis* L., *S. bracteata* Banks & Sol., *S. hypargeia* Fisch & Mey., *S. candidissima* Vahl, *S. cyanescens* Boiss. & Bal., *S. russellii* Benth, *S. sclarea* L., *S. suffruticosa* Montbret & Aucher, *S. syriaca* L., *S. tchihatcheffii* Boiss., *S. verbenaca* L., *S. virgata* Jacq. and *S. viridis* L.) grow in the campus area, however nine of them grow sympatrically. *Salvia absconditiflora*, *S. cyanescens*, *S. hypargeia*, *S. tchihatcheffii* are endemic. *Salvia absconditiflora*, *S. aethiopis*, *S. russellii* and *S. tchihatcheffii* are the target species of this study (Figure 9).



Figure 9. Target *Salvia* species, a; *Salvia absconditiflora*, b; *S. aethiopis*, c; *S. russellii* and d; *S. tchihatcheffii*.

1.9 Climate of the Study Area

Since the study area is in the Irano-Turanian phytogeographical region of Turkey, a typical steppe climate occurs and it is characterized by cold winters and hot dry summers (Davis, 1965). The northern part of central Anatolia receives lower amount of precipitation than the southern part. The critical period for this area is during May and September due to the more amount of evaporation than precipitation, resulting in water deficiencies (Doğan 2003). The climate of central Anatolia resembles the

climate of West and south Anatolia but the precipitation is less and much of it falls as snow (Davis, 1965).

The average rainfall is 40 cm per year while the driest part which is the semiarid region of Central Anatolia receives 30 cm precipitation per year. Average temperature is -0.7°C in January which is the coldest month. Average temperature is 22°C in July which is the hottest month (Davis, 1965).

1.10 Topography of the Study Area

Central Anatolia is a part of the Irano-Turanian phytogeographical region and it contains more than 50% of the endemic taxa of *Salvia* in Turkey. Altitude varies from 300 to 3900 meters. There are two major vegetational areas in the region with deciduous scrubs and the basin occupied by Salt Lake (Tuz Gölü). Formations of saline and gypsum creates the alluvial deposits of Central Anatolia which is the main geological formation. Central Anatolian basin is composed of saline lakes, halophytic vegetation, salt steppe, rivers and marshes (Davis, 1965).

1.11 Objectives of the Study

The staminal lever mechanism is defined as a functional unit. Its organization with the remaining floral structures are involved in the process of pollen transfer. The existence of a large number of species and almost a unique structure which is essential for reproduction raise the question whether if the lever mechanism might drive speciation in the genus. The four *Salvia* species have different stamen types and the species are growing sympatrically. Regarding these phenotypic differences of co-occurring *Salvia* species;

The main objectives of this study are;

- To understand the functioning and significance of the staminal lever mechanism in the pollination of the genus *Salvia*.

- To identify the isolating mechanisms among co-occurring *Salvia* species.
- To investigate the morphometric fitting between floral and insect characters and species-specific patterns of pollen deposition on the pollinator's body.
- To understand whether the staminal lever mechanism forces speciation by increasing mechanical isolation among sympatric *Salvia* species.

CHAPTER 2

2. MATERIAL AND METHODS

2.1 *Salvia* Species

Four co-occurring *Salvia* species (*S. absconditiflora* Greuter & Burdet, *S. aethiopsis* L., *S. russellii* Benth., *S. tchihatcheffii* Boiss.) and their visitors were observed in three stations in their natural habitats during field studies from May to August 2010. Investigated co-occurring *Salvia* species have a great diversity in terms of their habit, population size, inflorescence type, flowering sequence, flowering time, the number of flowers per verticillasters, flower size and morphology, stamen type and morphology, flower colour and pollinator ranges.

2.2 Stations

Field investigations are conducted in three stations in Middle East Technical University campus area where *Salvia* species grow sympatrically (Figure 10). Station 1 is in central Anatolian steppe and open *Pinus nigra* Arnold forest, around Department of Biological Sciences, Middle East Technical University (B4 sensu Davis 1965, Ankara,), GPS: 39° 53' 48".60 N 32° 46' 47".94 E (Figure 11). Station 2 is in steppe and open *Pinus nigra* forest around Yalıncak village in the Middle East Technical University Campus area as station 2, GPS: 39° 52' 54".39 N 32° 47' 09".88 E (Figure 12). Station 3 is in steppe, between Department of Aerospace Engineering and DSI Lake, GPS: 39° 52' 40".04 N 32° 46' 31".11 E, in Middle East Technical University (Figure 13).



Figure 10. Google Earth Image of the study area showing 3 stations.



Figure 11. A general view from station 1.



Figure 12. A general view from station 2.



Figure 13. A general view from station 3.

All the plots were located in a protected area where animal grazing is not allowed, therefore the studied area has great species diversity. Four of the studied species in the stations have large populations (Table 1).

Table 1. Population density of the species in the studied stations ; number of crosses indicate density from high (*****) to low (*)

	Station 1	Station 2	Station 3
<i>S. absconditiflora</i> *	****	***	***
<i>S. aethiopsis</i>	*	*	*
<i>S. russellii</i>	***	**	**
<i>S. tchihatcheffii</i> *	*****	*****	***

2.3 Field Investigations

According to Reith *et al.* (2007), generally, a visit of an insect on a flower can be divided into two functional groups. First is illegitimate flower visits, for example nectar robbing or pollen theft in which the lever mechanism is usually not triggered. Second, legitimate flower visits in which the lever mechanism is triggered. This study concentrates on legitimate flower visits (Claßen-Bockhoff *et al.*, 2004; Reith *et al.*, 2005).

From early May to early August 2010, stations were visited at least four times in a week. The observations were random in the morning, noon and afternoon. Field observations were backed by photographs and video recordings. Over 1000 photos and 34 video records were taken and they provided a close observation on the flower-insect interactions. Canon G10 compact camera, Sanyo TH1 video camera, Canon 50 D with 100 mm macro objective were used throughout the field investigations. During field investigations, over 150 bees were captured from the

field. Their specific pollen deposition areas were observed both in the field and in the laboratory using photos and video records.

2.3.1 Pollination observation

We recorded the frequency of each species of pollinators while observing the visit of pollinators on the flowers in a defined area of populations in the stations. We measured the handling time of bees per flower visit, i.e. the duration from landing on the lower lip to leaving the flower after absorbing nectar. Probing time was calculated for the dominant pollinators of the studied species. Close-up photographs and video recordings provided information about the pollinator visits whether they are legitimate or not. The functioning of the staminal lever mechanism is analysed with video records and photographs (Figure 14). For each *Salvia* species the flowering season, the range of pollinators (touching pollen and stigma) and the pollinator's body loaded with pollen were recorded (Figure 15).



Figure 14. A general view from the pollination observation.



Figure 15. Flower-bee interaction on *Salvia tchihatcheffii*.

2.4 Laboratory Investigations

The internal structure and morphology of the flowers were studied in a number of longitudinal sections and cross-sections. Morphological measurements were made using Leica DM1000 binocular light microscope with a Leica DFC280 camera. Large number of *Salvia* specimens were collected and dried as herbarium specimens. Vouchers of all the investigated species are deposited at Department of Biological Sciences, Middle East Technical University. Moreover, fresh materials were fixed in 70% ethanol for further investigations.

The *Salvia* specimens were identified by Dr. Ferhat Celep using the Flora of Turkey (Hedge, 1982). Bee species were identified by Fatih Dikmen. Field photographs were taken by Zeynep Atalay.

2.4.1 Floral Measurements

Floral structures were measured morphometrically. Following characters were measured from: (a) pedicel length, (b) flower length, (c) length of corolla tube, (d) flower entrance (d_h , horizontal, d_v , vertical), (e) distance flower entrance to nectar cover, (f) distance thecae to nectar cover, (g) length of total connective, (h) length of upper connective arm, (i) length of filament, (j) style length in natural position, (k) length of the lower lever arm, (l) proportion of upper to lower lever arm, (m) theca length and (n) calyx length (Figure 16). All measurements were taken from fresh specimens and at least 30 measurements were made for each species. Their minimum-maximum range, mean and standard deviations were also calculated.

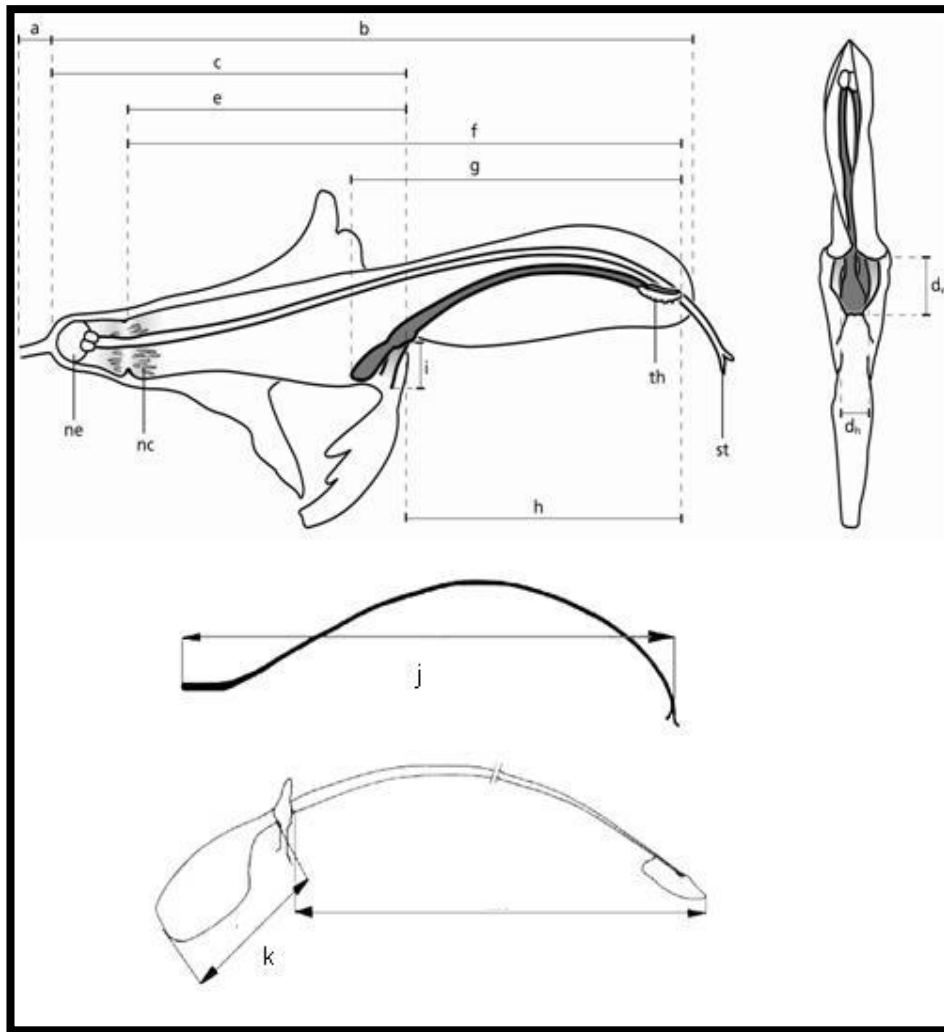


Figure 16. Morphometric data of flowers (Classen-Bockhoff, 2004).

Inflorescence architecture and flower number of the investigated species were observed in the field and in the laboratory. We try to determine the total flower numbers on each inflorescence by counting the number of pedicels which remain on the inflorescence after flower withering. Moreover, we try to calculate the expected minimum and maximum number of fruits and seeds per inflorescence and individual.

2.4.2 Insect Measurements

Insect structures were morphologically investigated and measured morphometrically from following characters: (a) head height, (b) length of maxillae, (c) glossa length, (b+c) length of proboscis, (d) max. head width, (e) head width below eyes, (f) head length, (g) thorax, (h) abdomen and (f+g+h) total insect size (Figure 17). All measurements were taken from proper specimens and at least 1-8 measurements were made. Their minimum-maximum range, mean and standard deviations were also calculated (Table 3).

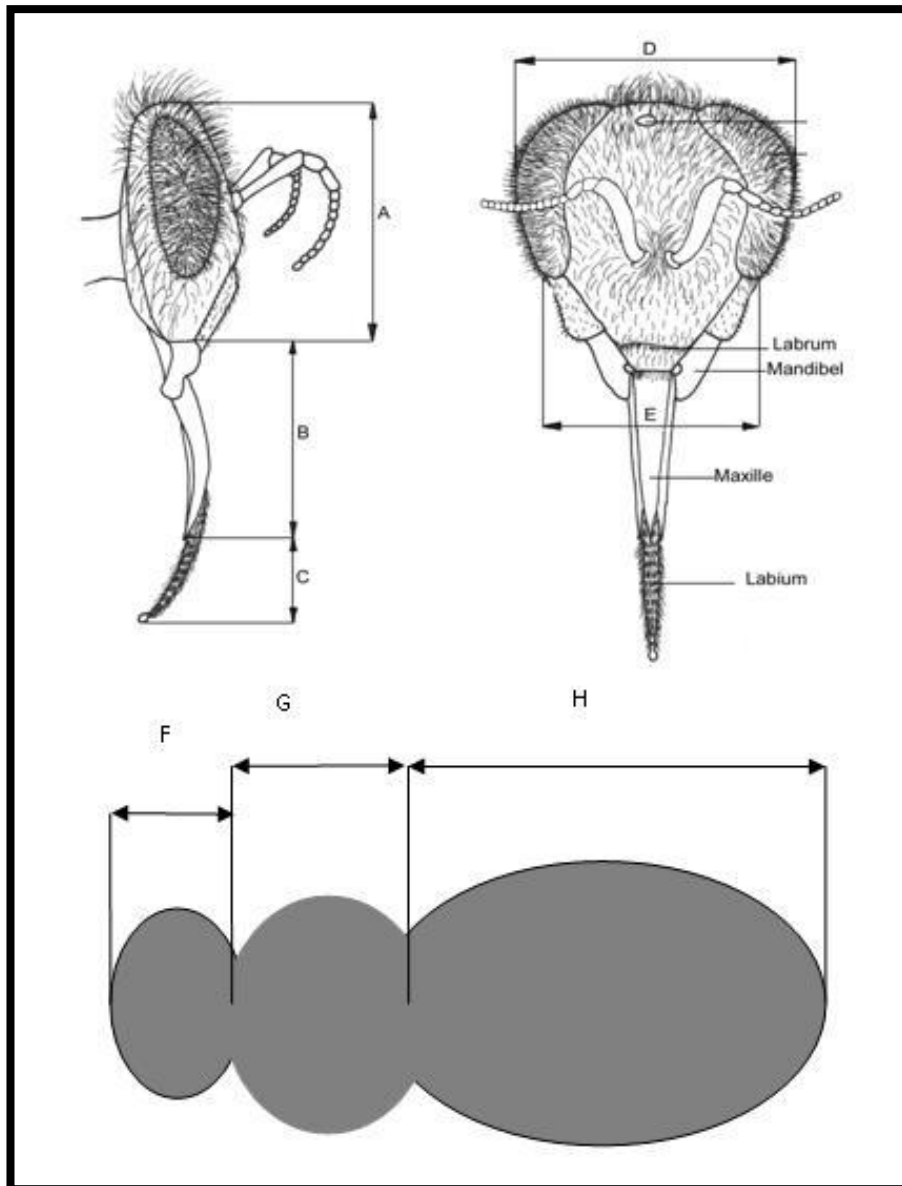


Figure 17. Morphometric data of insects, a, head hight, b, length of maxillae, c, glossa length, b+c, length of proboscis, d, max. head width, e, head width below eyes, f, head length, g, thorax, h, abdomen. All measurments mm, (Classen-Bockhoff, 2004).

CHAPTER 3

3. RESULTS

3.1 Floral Morphology

During laboratory investigations, the species were observed morphologically and their floral characters were measured morphometrically on the basis of 15 characters given in (Table 2). The measurements indicated that floral organs differed significantly in terms of their sizes. Minimum and maximum range for the measurements of each character is given.

These characters and their minimum and maximum values are as follows; the pedicel length (a) ranged from 1.1 mm (*S. aethiopsis*, sect. *Aethiopsis*) to 4.2 mm (*S. absconditiflora*, sect. *Hymenosphace*), the flower length (b) ranged from 8.1 mm (*S. russellii*, sect. *Hemisphace*) to 27.3 mm (*S. absconditiflora*), the length of corolla tube (c) ranged from 5.2 mm (*S. russellii*) to 17.6 mm (*S. absconditiflora*), the horizontal flower entrance (dH) ranged from 1.0 mm (*S. russellii*) to 3.3 mm (*S. absconditiflora*), the vertical flower entrance (dV) ranged from 0.8 mm (*S. russellii*) and to 3.6 mm (*S. tchihatcheffii*, sect. *Salvia*), the distance flower entrance to nectar cover (e) ranged from 3.2 mm (*S. aethiopsis*) to 13.4 mm (*S. absconditiflora*), the distance thecae to nectar cover (f) ranged from 5.7 mm (*S. russellii*) to 22.2 mm (*S. absconditiflora*), the length of total connective (g) ranged from 2.4 mm (*S. russellii*) to 14.6 mm (*S. aethiopsis*), the length of upper connective arm (h) ranged from 1.4 mm (*S. russellii*) to 12.1 mm (*S. aethiopsis*), the length of filament (i) ranged from 0.6 mm (*S. russellii*) to 5.3 mm (*S. absconditiflora*), the style length in natural position (j) ranged from 6.3 mm (*S. russellii*) to 26.1 mm (*S. absconditiflora*), the length of

lower lever arm (k) ranged from 0.4 mm (*S. russellii*) to 3.6 mm (*S. aethiopsis*), the proportion of upper to lower lever arm (l) ranged from 1.6:1 (*S. tchihatcheffii*) to 3.27:1 (*S. aethiopsis*), the thecae length (m) ranged from 1.0 mm (*S. russellii*) to 2.7 mm (*S. tchihatcheffii*) and the calyx length (n) ranged from 5.1 mm (*S. russellii*) to 18.1 mm (*S. absconditiflora*). According to the results, *Salvia russellii* has the smallest floral characters while *S. absconditiflora* has the largest floral characters in terms of their sizes (Figure 18).

Table 2. Morphometric Data of Flowers; (a) pedicel length, (b) flower length, (c) length of corolla tube, (d) flower entrance (d_h , horizontal, d_v , vertical), (e) distance flower entrance to nectar cover, (f) distance thecae to nectar cover, (g) length of total connective, (h) length of upper connective arm, (i) length of filament, (j) style length in natural position, (k) length of the lower lever arm, (l) proportion of upper to lower lever arm, (m) theca length, (n) calyx length, all measurements in mm, (n: 30)

	a	b	c	dH	dV	e	f	g
<i>S. absconditiflora</i>	3.0±0.5 (2.1-4.2)	20.6±2.4 (16.2-27.3)	13.4±1.3 (11.1-17.6)	2.7±0.3 (2.3-3.3)	2.9±0.3 (2.1-3.4)	9.3±1.5 (6.6-13.4)	18.1±2.1 (14.3-22.2)	3.6±0.5 (2.8-4.5)
<i>S. aethiopsis</i>	2.1±0.4 (1.1-2.9)	18±1.9 (13.5-21.2)	7.5±0.5 (5.5-8.3)	1.5±0.2 (1.2-1.9)	1.2±0.2 (0.9-1.8)	5.9±0.6 (3.2-6.6)	14.2±1.6 (10-17.8)	10.4±2.1 (4.8-14.6)
<i>S. russellii</i>	2.6±0.5 (1.7-3.5)	9.3±0.7 (8.1-10.7)	6.5±0.5 (5.2-7.7)	1.2±0.1 (1-1.4)	1±0.1 (0.8-1.3)	5.3±0.5 (3.9-5.9)	7.6±0.7 (5.7-8.5)	3.2±0.3 (2.4-3.7)
<i>S.tchihatcheffii</i>	2.8±0.4 (2-3.4)	15±1.7 (10.4-17.3)	8.9±0.9 (6.5-10.6)	2.3±0.2 (2-2.8)	2.5±0.5 (1.4-3.6)	7.3±1 (5.0-9.3)	13±1.7 (8.1-15.5)	3.6±0.4 (2.5-4.3)
	h	i	j	k	l	m	n	
<i>S. absconditiflora</i>	2.4±0.3 (1.8-2.7)	3.8±0.7 (2.3-5.3)	21.5±2.8 (17-26.1)	1.4±0.1 (1.2-1.7)	1.71:1	2.1±0.2 (1.8-2.6)	14.7±2.3 (11.8-18.1)	
<i>S. aethiopsis</i>	8.5±1.9 (3.5-12.1)	2.8±0.5 (1.5-3.7)	18.6±2.5 (12.7-22.7)	2.6±0.4 (1.8-3.6)	3.27:1	1.8±0.2 (1.3-2.2)	9.8±0.6 (8.3-11.5)	
<i>S. russellii</i>	2.5±0.4 (1.4-3.3)	1.2±0.3 (0.6-1.7)	8.3±1 (6.3-10)	0.8±0.1 (0.4-1)	3.13:1	1.3±0.1 (1-1.5)	5.4±0.2 (5.1-5.9)	
<i>S.tchihatcheffii</i>	2.4±0.3 (1.7-3.1)	3.4±0.4 (2.8-4.3)	16±1.7 (11.2-18)	1.5±0.2 (1.1-2)	1.6:1	2.2±0.2 (1.7-2.7)	9.8±1.2 (6.4-11)	

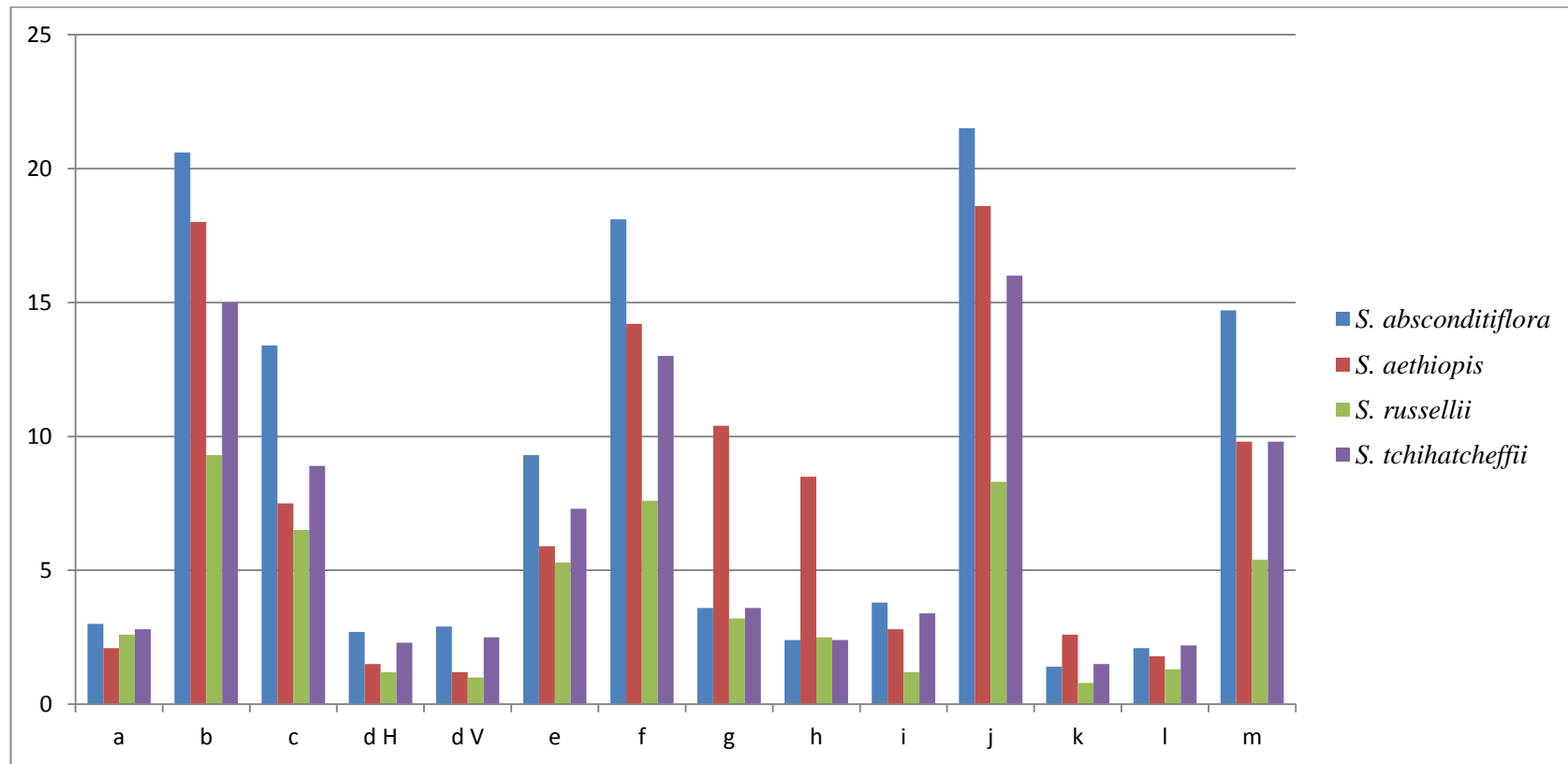


Figure 18. Schematic view of the morphometric data of flowers. (a) pedicel length, (b) flower length, (c) length of corolla tube, (d) flower entrance (d_h , horizontal, d_v , vertical), (e) distance flower entrance to nectar cover, (f) distance thecae to nectar cover, (g) length of total connective, (h) length of upper connective arm, (i) length of filament, (j) style length in natural position, (k) length of the lower lever arm, (l) theca length (m) calyx length, all measurements in mm, (n: 30)

3.2 Insect Morphology

Similarly, the pollinators were investigated morphologically and measured morphometrically on the basis of 10 characters given in (Table 3). Measurements indicated that insect structures differed in terms of their sizes. Minimum and maximum range of each character is given.

These characters, minimum and maximum values are as follows ; (a) the head height ranged from 1.2 mm (*Hylaeus sp.*) to 3.35 mm (*Xylocopa cf. iris*), (b) the length of maxillae ranged from 0.6 mm (*Hylaeus sp.*) to 6.7 mm (*Anthophora robusta*), (c) the glossa length ranged from 0 (majority of the species) to 3.5 mm (*Xylocopa cf. iris*), (b+c) the length of proboscis ranged from 0.6 mm (*Hylaeus sp.*) to 6.7 mm (*Anthophora robusta*), (d) the max. head width ranged from 1.8 mm (*Evylaeus laticeps*) to 6.0 mm (*Anthophora robusta*), (e) the head width below eyes ranged from 1.0 mm (*Evylaeus laticeps*) to 3.9 mm (*Anthophora robusta*), (f) the head length ranged from 0.7 mm (*Evylaeus laticeps*) to 2.6 mm (*Anthophora robusta*), (g) the thorax ranged from 1.9 mm (*Osmia versicolor*) to 5.9 mm (*Anthophora robusta*), (h) the abdomen ranged from 2.8 mm (*Osmia versicolor*) to 9.2 mm (*Anthophora robusta*) and (f+g+h) the total insect size ranged from 5.4 mm (*Evylaeus laticeps*) to 18.36 mm (*Anthophora robusta*, Female).

Table 3. Morphometric Data of Insects; (a) head height, (b) length of maxillae, (c) glossa length, (b+c) length of proboscis, (d) max. head width, (e) head width below eyes, (f) head length, (g) thorax, (h) abdomen and (f+g+h) total insect size, all measurements in mm.

	Gender	A	B	C	B+C	D	E	F	G	H	F+G+H	
	<i>Anthophora aestivalis</i>	F	2.78±0.18	5.42±0.37	0	5.42±0.37	4.58±0.22	28,08±1,88	21,6±1,51	42,58±2,78	70,3±3,87	13.7±0.70
	<i>Anthophora robusta</i>	F	3.72±0.63	6.46±0.08	0	6.46±0.08	5.78±0.14	3.7±0.15	2.61±0.11	5.93±0.4	8.94±0.4	18.36±0.64
		M	3.65±0.21	6.7±0.42	0	6.7±0.42	5.45±0.63	3.25±0.21	2.4±0.14	5.7±0.84	9.2±0.71	18.1±1.27
	<i>Apis mellifera</i>	F	2.8±0.23	2.93±0.33	1.01±0.5	3.81±0.84	3.61±0.2	2.4±0.22	1.4±0.13	3.77±0.28	5.33±0.63	11.15±0.44
	<i>Eucera cf. pollinosa</i>	F	2.83±0.11	3.6±0.08	0	3.6±0.08	4.54±0.08	2.84±0.05	1.84±0.08	4.85±0.23	7.7±0.46	15.65±0.66
	<i>Chalicodoma ericetorum</i>	M	2.8	3	0	3	3.5	1.9	1.7	3.4	4.9	11.5
	<i>Coelioxys rufescens</i>	F	2.1	?	?	?	3.2	1.8	1.2	2.9	6.3	10.2
	<i>Eucera sp.2</i>	F	1.93±0.09	2.27±0.15		2.27±0.15	3.37±0.12	2.12±0.12	1.25±0.06	3.4±0.25	5.45±0.25	10.07±0.59
	<i>Eucera sp.3</i>	M	2.15±0.07	3.5±0.14		3.5±0.14	3.7±0.14	2.25±0.35	1.65±0.35	3.8±0	7.5±7,07	14.25±1.77
32	<i>Eucera sp.4</i>	M	2.4	3	0	3	3.6	2.2	1.6	4	5	11
	<i>Eucera sp.5</i>	F	2.7	6.4	0	6.4	4.2	2.8	1.5	4.8	9.1	15.7
	<i>Eucera sp.7</i>	M	2.2	4.2	0	4.2	3.6	2.3	1.4	3.6	4.3	10
	<i>Evylaeus laticeps</i>	F	1.25±0.07	1±0		1±0	1.8±0.14	1±0	0.75±0.07	2.1±0.56	3.25±0.354	5.95±0.78
	<i>Evylaeus sp.2</i>	F	?	?	?	?	?	?	?	?	?	6.7
	<i>Halictus tetrazonianellus</i>	F	1.9	0	0	0	3	1.9	1.3	2.8	4.9	9.3
	<i>Hylaeus sp.</i>	F	1.2	0.6	0	0.6	2	1.1	0.9	2.7	3.3	6.7
	<i>Megachile pilicrus</i>	M	2.6±0	3.9±0	0±0	3.9±0	3.8±0	2.5±0	2.2±0	3.3±0	6.5±0	12.5±0
	<i>Nomada sp.</i>		2.1	2.4	0	2.4	3.5	2.1	1.5	3.5	5	10.4
	<i>Osmia mustelina</i>	F	2.77±0.23	2.86±0.11	0	2.86±0.11	3.67±0.15	2.23±0.11	1.7±0	3.23±0.11	4.7±0.36	9.3±0.1
	<i>Osmia versicolor</i>	F	1.9	1.7	0	1.7	2.2	1.5	1.1	1.9	2.8	6.5
	<i>Xylocopa cf. iris</i>	F	3.35±0.07	2±2.83	1.75±2.57	3.75±0.35	4.7±0.28	3.05±0.49	2.6±0.14	4.6±0.14	9.25±0.35	17.65±0.21

According to the results, *Salvia russellii* and *Salvia tchihatcheffii* have the smallest floral characters, while *S. absconditiflora* has the largest floral characters in terms of their sizes (Figure 18). On the other hand, the bee pollinators, *Evyllaes laticeps* has the smallest morphological characters, while *Anthophora robusta*, *Xylocopa cf. iris* have the largest morphological characters in terms of their sizes (Table 3).

3.3 Floral characteristics of the *Salvia* species

Floral characteristics including corolla colour, stamen types, sections, life forms, chromosome number are investigated for *Salvia* species (Table 4). Detailed information about the floral characteristics of each species are given.

Table 4. Species, corolla colour, stamen types, sections, life and chromosome numbers of investigated species. The chromosome number of *Salvia russellii* is unpublished data (in review). * endemic.

	Corolla colour	Stamen Type (Hedge, 1982)	Stamen Type (Walker et al., 2007)	Section	Life	Chromosome Number (2n)
<i>S. absconditiflora</i> *	White	A	A	<i>Hymenosphace</i>	Perennial	16, 32
<i>S. aethiopsis</i>	White	B	B	<i>Aethiopsis</i>	Perennial	24
<i>S. russellii</i>	Violet	C	C or H	<i>Hemisphace</i>	Perennial	?
<i>S. tchihatcheffii</i> *	White	A	A	<i>Salvia</i>	Perennial	18

Salvia absconditiflora is a perennial endemic species. It is mainly distributed in central Anatolia, eastern Anatolia and partly in other regions of Turkey except for the European portion of Turkey. Flowers of *S. absconditiflora* are white to pinkish, but sometimes purplish morphs can be found. Upper lip of the corolla is more or less straight and corolla tube is non-squamulate (Figure 19). There is a hairy ring “annulus” at the base of the corolla tube. Flowers have long filaments. All connective lever arms are exerted out of the flower. Lower lever arms present pollen from lateral sides.



Figure 19. Flower of *Salvia absconditiflora*.

Salvia aethiopsis is a biennial or perennial, widely distributed species. Flowers of the *S. aethiopsis* are usually white. Upper lip of the corolla is falcate and corolla tube is squamulate. In addition, there is no hairy ring “annulus” at the base of the corolla tube. It has spoon-shaped stamens (Figure 20).



Figure 20. Flower of *Salvia aethiopsis*.

Salvia russellii is a perennial, widely distributed species. Flowers of the *S. russellii* are usually violet-blue. Upper lip of the corolla is straight and narrow at base. The corolla tube is non-squamulate (Figure 21). There is hairy ring “annulus” at the base of the corolla tube. It has stamens similar to *Salvia verticillata* (Stamen type C according to Hedge 1982, Stamen type C or H according to Walker and Systema, 2007; Stamen type III according to Himmelbaur and Stibal 1934). The stamens in *S. russellii* shows a highly reduced lower connective arm, thus representing an extreme form of stamen type (Figure 27). The species lack any lever mechanism, due to the reduction of the lower lever arm. In *S. russellii*, the bee has to push back the movable upper lip to gain access to nectar (Hildebrand 1865; Correns 1891). As a result it is loaded with pollen on its forehead. When visiting a second flower, the pollen-loaded forehead of the bee first touches the style protruding from the flower.

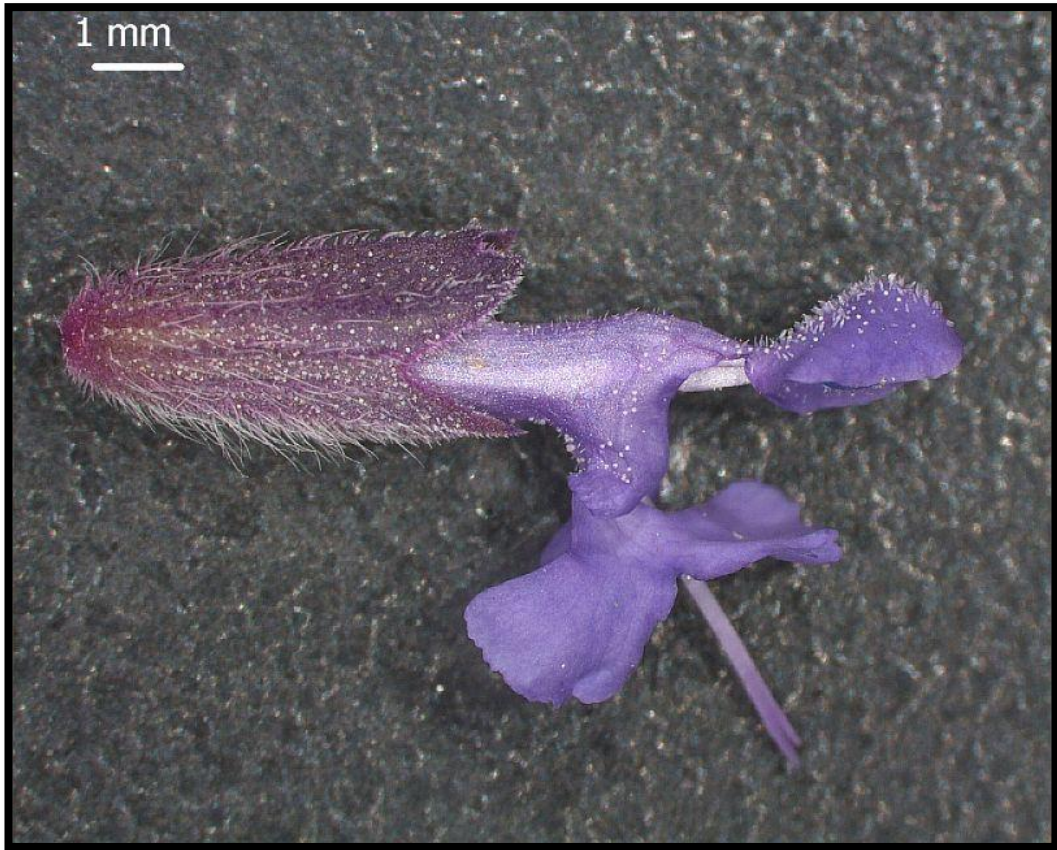


Figure 21. Flower of *Salvia russellii*.

Salvia tchihatcheffii is a perennial endemic species which is mainly restricted in Ankara, Kütahya and Eskişehir (central Anatolia and partly Aegean region of Turkey). Flowers of the *S. tchihatcheffii* are usually white, but rarely light violet morphs can be found. Upper lip of the corolla is more or less straight and corolla tube is non-squamulate (Figure 22). In addition, there is a hairy ring “annulus” at the base of the corolla tube. The flowers have long filaments, and the entire connective levers are protruded from the adult flower. While the upper pollen sacs contribute to dorsal pollination, the lower theca is not turned and thus presents pollen from the lateral side. In the species, lower lever arm of the stamen does not fully close the corolla tube entrance.



Figure 22. Flower of *Salvia tchihatcheffii*.

3.4 Stamen Types of the *Salvia* Species

Stamen types of the four *Salvia* species are investigated in the laboratory. According to the research findings, there are three types of stamens in these species according to Himmelbaur and Stibal (1932-1934). *S. absconditiflora* and *S. tchihatcheffii* have the stamen type A (Figure 24 and Figure 25) while *S. aethiopsis* (Figure 26) has the stamen type B and *S. russelli* has the stamen type C, (Figure 27). (Figure 23) shows the stamen types of the studied species according to Himmelbaur and Stibal (1932–1934).

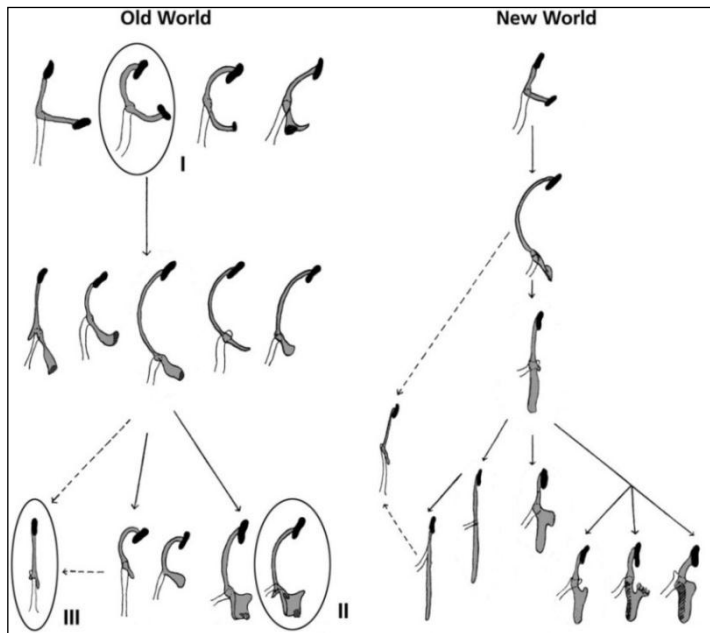


Figure 23. Stamen diversity according to Himmelbauer and Stibal (1932–1934, slightly changed). The encircled structures correspond to the stamens of the investigated species: I; *Salvia tchihatcheffii* and *S. absconditiflora*, II; *S. aethiopsis*, III; *S. russellii*.



Figure 24. Stamen type A, *S. absconditiflora*



Figure 25. Stamen type A, *S. tchihatcheffii*



Figure 26. Stamen type B, *S. aethiopsis*



Figure 27. Stamen type C, *S. russellii*

3.5 Isolation Types Among Co-occurring *Salvia* species

The *Salvia* species growing sympatrically are observed in three stations. For each of the *Salvia* species, flowering season, range of pollinators (touching pollen and stigma) and pollinator's body loaded with pollen were recorded. The results show that the studied *Salvia* species have overlapping flowering season and also share some of the pollinators. The question arises how reproductive isolation is maintained and hybridization precluded among these *Salvia* species.

3.5.1 Phenologic Isolation

The *Salvia* species occurred sympatrically with largely overlapping flowering times. The field observations showed that the duration of the anthesis of each plant is about four weeks between early May to early June. The anthesis of *S. absconditiflora* and *S. tchihatcheffii* are approximately 4 weeks but they have an effective pollination period at the end of May and the beginning of June. *S. russellii* stays in flower for

approximately 3 weeks and individuals in the populations flower fully at the beginning of June. *S. aethiopsis* stays in flower for only one week and flowering of each individual is in a sequence. (Figure 28) shows the flowering times of the species in three stations.

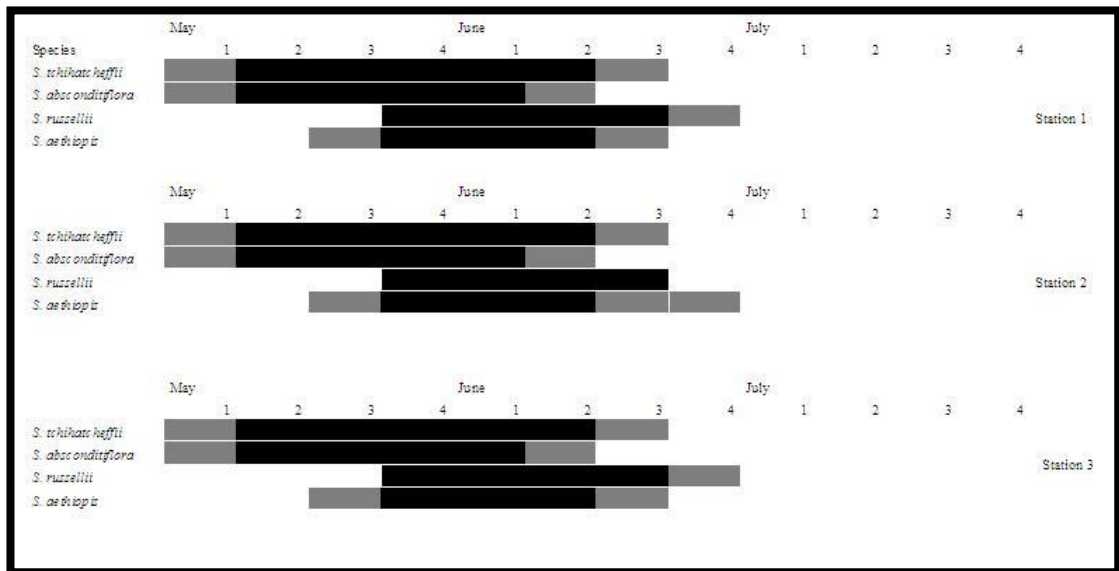


Figure 28. Flowering season of the *Salvia* species in three stations. Blacks indicate the main flowering seasons.

3.5.2 Mechanical Isolation

Since the studied species have overlapping flowering times and occur sympatrically, there must be an isolating mechanism precluding hybridization. Our results indicated that the *Salvia* species are reproductively isolated by mechanical means and it was governed by both relatively different sizes of the flowers and the bees and the species-specific pollen deposition on the body of the pollinators.

3.5.3 Phenotypic Isolation

Our field observations and laboratory studies showed that the flower morphology and pollinator morphology are consistent in terms of their sizes and preferences. For example, the smallest bee species, *Evyllaeus laticeps*, only visits *S. russelli* and *S. tchihatcheffii*, which have the smallest floral characters. Similarly, one of the largest bee species, *Anthophora robusta* visits *S. absconditiflora* which has the largest floral characters. These results imply that the *Salvia* species and their pollinators construct mutualistic relationship. Moreover, the size of the flowers and relatively different sizes of staminal lever arms provide limitation to pollinators and phenotypic isolation is achieved among the *Salvia* species. Our observations and measurements pointed out that the staminal lever mechanism (stamen type, length of upper and lower connective arm and filament length) and the length of corolla tube in *Salvia* flowers and head height, the length of proboscis in bee species are the most important characters for the individual fitness between the flowers and their pollinators (Figure 29, Figure 30, Figure 31).

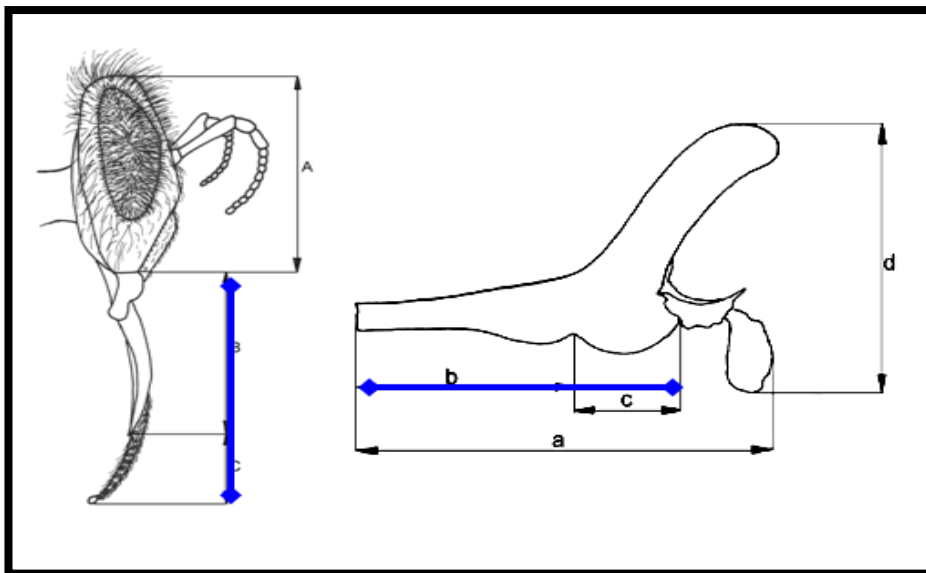


Figure 29. The consistency in the length of proboscis and the length of corolla tube (Classen-Bockhoff, 2004).

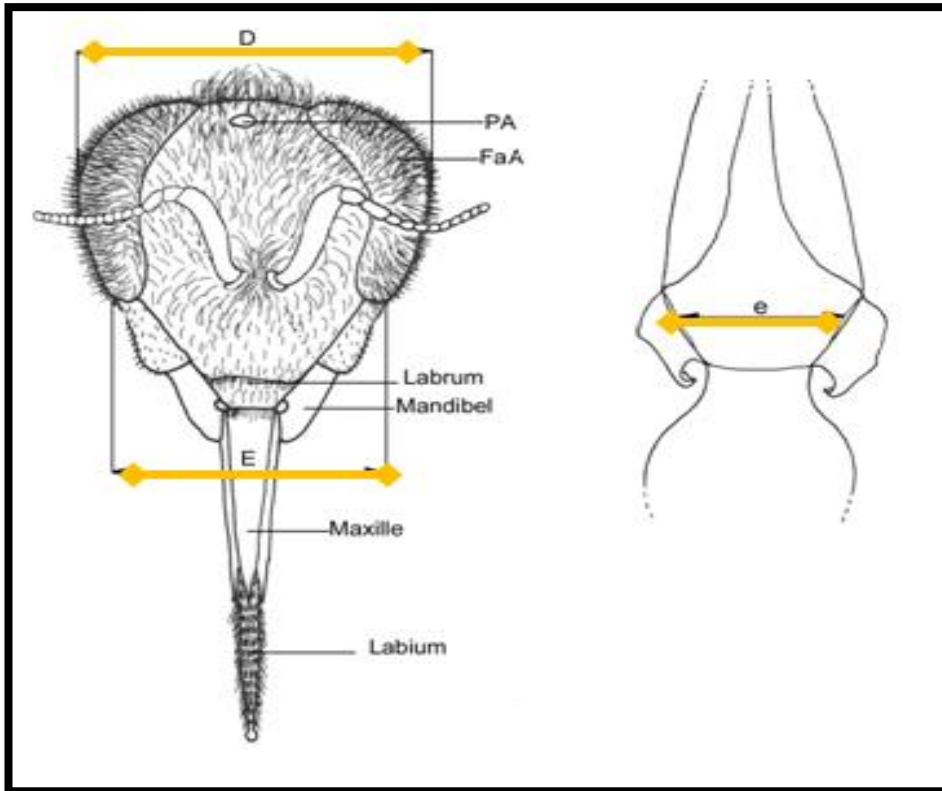


Figure 30. The consistency in the width of bee's head and the width of flower entrance (Classen-Bockhoff, 2004).

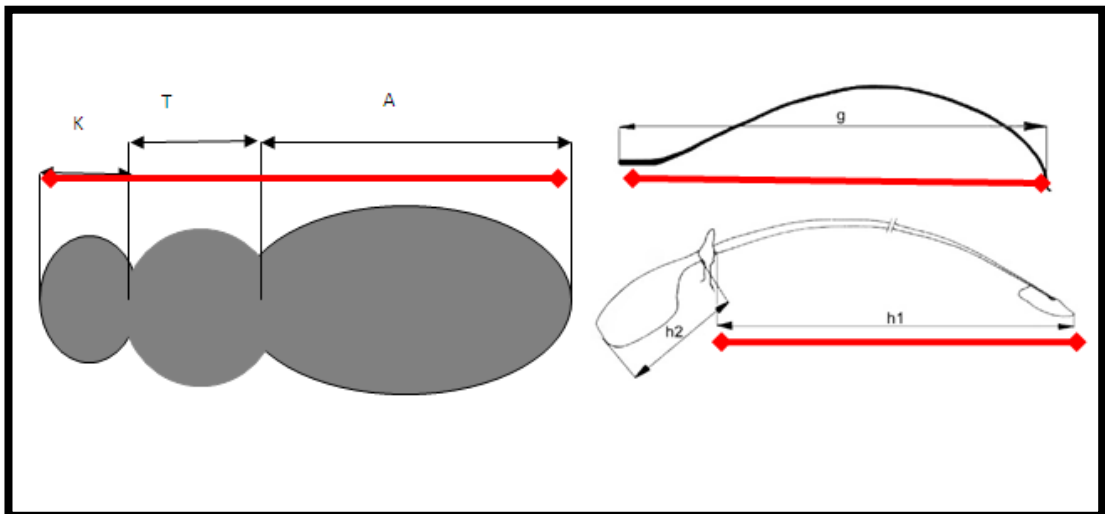


Figure 31. The consistency in the body length of bees and the length of upper connective arm and style (Classen-Bockhoff, 2004).

3.5.4 Species-Specific Pollen Deposition

The flower-pollinator interactions and pollinators' body domain loaded with pollen were investigated to understand the precise pollen placement of the staminal lever mechanism. Letters from a to g are given for the pollen accumulating places on bees' body (Figure 32). Species-specific patterns of pollen deposition places are given (Table 5).

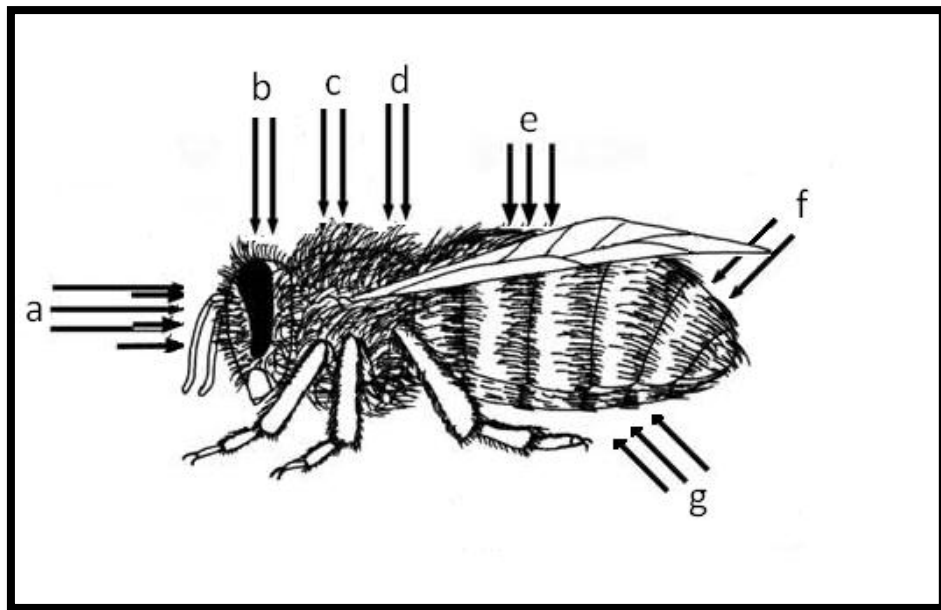


Figure 32. Pollen accumulating places on the pollinator's body. Small letters indicate the pollinator's body domain loaded with pollen (Figure modified from Classen-Bockhoff)

Regarding temporal, ethological and mechanical isolation, *S. tchihatcheffii* (endemic, sect. *Salvia*) and *S. absconditiflora* (endemic, sect. *Hymenosphace*) have overlapping flowering times with *S. russelli* and *S. aethiopsis* in all investigated stations. Moreover, *S. tchihatcheffii* and *S. absconditiflora* have different flower and stamen morphology from the remaining species. Therefore, *S. tchihatcheffii* and *S. absconditiflora* are clearly isolated from *S. russelli* and *S. aethiopsis*. Although *Salvia*

tchihatcheffii and *S. absconditiflora* were placed in two different sections (Table 4) they have the same stamen type (A) which still bears two fertile thecae per stamen. While the upper pollen sacs contribute to dorsal pollination, the lower theca is not reduced and thus presents pollens from the lateral or front side. The species have the shortest upper/lower lever arm ratio and have the shortest total connective length in the other studied species. Moreover, the flower entrances of both of the species are wider than the remaining species.

S. tchihatcheffii and *S. absconditiflora* share *Apis mellifera* and *Osmia mustelina* as the pollinators, but their flowers are different in size, such as the flower length, the length of the corolla tube, the distance from flower entrance to nectar cover and the distance thecae to nectar cover that pollen is deposited on different domains of the bee's body and cannot therefore be exchanged between the two species. A small number of hybrids could be expected between large flowers of *S. tchihatcheffii* and small flowers of *S. absconditiflora* due to the lack of an effective mechanical isolation.

However, no hybrid populations were found at the localities investigated. There have been no literature record for a hybrid species between *S. tchihatcheffii* and *S. absconditiflora*. This could be explained that the specific and highly accurate pattern of pollen placement seems to be an effective mean of maintaining genetic isolation. Prezygotic, postzygotic isolations and flower constancy seem to play an important role for the isolation between the species. On the other hand, *S. tchihatcheffii* and *S. absconditiflora* have different chromosome numbers. While *S. tchihatcheffii* has $2n: 18$ as the chromosome number, *S. absconditiflora* has $2n: 16$ & 32 as the chromosome number (Table 4).

Salvia russellii differs from the remaining species by its smaller size and its unique pollen transfer mechanism sticking the pollen to the bee's forehead. It shares pollinators with each of the remaining species, but is completely mechanically isolated. *S. tchihatcheffii*, *S. absconditiflora* and *S. russellii* share *Apis mellifera* as a pollinator, but the flowers are so different in size that pollen is deposited on different

domains of the bee's body and therefore cannot be exchanged between the species (Figure 34 and Figure 35).



Figure 33. *Anthophora robusta* on *S. absconditiflora*



Figure 34. *Apis mellifera* on *S. tchihatcheffii*



Figure 35. *Apis mellifera* on *S. russellii*

The anthesis time of *Salvia aethiopsis* is rather shorter than the remaining species. Each individual stays in flower for one week. In three stations, regarding to the different habits of *S. aethiopsis*, the species are widely spread from each other and presents many more flowers per individual. Our detailed field observations and video recordings showed that, the pollinators of *S. aethiopsis* never switched from *S. aethiopsis* to another *Salvia* species (Figure 36). Also, *Salvia aethiopsis* does not share any pollinator with the remaining species. In addition to these findings, *S. aethiopsis* (sect. *Aethiopsis*) has $2n: 24$ as chromosome number while *S. tchihatcheffii* (sect. *Salvia*) has $2n: 18$ and *S. absconditiflora* (sect. *Hymenosphace*) has $2n: 16$ & 32 as chromosome number (Table 4).



Figure 36. *Xylocopa cf. iris* on *S. aethiopsis*



Figure 37. *Anthophora aestivalis* on *S. aethiopsis*

Table 5. Species-specific pattern of pollen deposition on pollinators body.

Species (*,endemic)	* <i>S. absconditiflora</i>	<i>S. aethiopsis</i>	<i>S. russellii</i>	* <i>S. tchihatcheffii</i>	Total
<i>Anthophora aestivalis</i>		b+c			1
<i>Anthophora robusta</i>	b				1
<i>Apis mellifera</i>	b+c+d		a+b	a+b+c	3
<i>Eucera cf. pollinosa</i>			a+b		1
<i>Chalicodoma ericetorum</i>				d+e	1
<i>Coelioxys rufescens</i>			a+b		1
<i>Eucera sp.2</i>			a+b+c		1
<i>Eucera sp.3</i>			a+b		1
<i>Eucera sp.4</i>			a+b		1
<i>Eucera sp.5</i>			a+b		1
<i>Eucera sp.7</i>				x	1
<i>Evylaeus laticeps</i>			visitor	visitor	2
<i>Evylaeus sp.2</i>		visitor			1
<i>Halictus tetraonianellus</i>			b+c		1
<i>Halictus resurgens</i>	visitor				1
<i>Hylaeus sp.</i>			visitor		1
<i>Megachile pilicrus</i>		b+c+d			1
<i>Nomada sp.</i>			c+d		1
<i>Osmia mustelina</i>	b+c+d			b+c+d	2
<i>Osmia versicolor</i>				c+d+e	1
<i>Xylocopa cf. iris</i>		c			1
** <i>Zygaene minos</i>			visitor		1
Total	3	3	9	5	20

3.6 Reproductive Success of the *Salvia* Species

All *Salvia* species have an inflorescence of oppositely reduced cymes which form false whorls usually known as verticils or verticillasters. Some, such as *S. aethiopsis*, have widely branched paniculate inflorescences but most species have much less spreading inflorescences and may be unbranched even “spike” like inflorescence (Hedge 1973). In addition, raceme type of inflorescence was observed in *S. absconditiflora*. The number of flowers in a verticel is occasionally diagnostic as in *S. russellii* and *S. verticillata*, where they are always 20-40-flowered.

The investigated species exhibit different inflorescences, as *S. tchihatcheffii* “spike”, *S. absconditiflora* “raceme”, *S. russellii*, “cyme”, *S. aethiopsis* “widely branched panicle” inflorescence. The number of flowers in the inflorescence and verticillasters and number of seeds produced among the investigated species are shown in (Table 6). We observed and counted flowers in a verticillaster and an inflorescence. However, seed numbers of the species just indicate the expected and approximate minimum, maximum numbers. They were not counted from the individuals. This is also not possible for many of the species, because seeds of species become mature in a sequence in *Salvia* both in the inflorescence and verticillaster. For example, lower flowers open earlier and their seeds also become mature earlier.

According to our field studies and observations, expected seed numbers show positive correlation with the population size of the species. Moreover, according to the field observations and video records (slow motion), approximate probing time per flower of the species were calculated (Table 6).

S. absconditiflora

S. absconditiflora is a perennial species. It is branched below and has several stems about 3 to 20. Inflorescence is not branched. In each stem, there are 4-10 verticillasters and each verticillaster has about 3-10 flowers. Therefore, each individual has about 12 to 200 flowers. The characteristic feature of section *Hymenospatha* is membranous calyces which greatly enlarge after anthesis. Usually, there are four seeds, but only one or two reach maturity (Hedge 1973). Therefore, each individual may produce (if fertilization occur on all flowers and they produce fully fertile seeds) about 12 to 400 seeds.

S. aethiopsis

S. aethiopsis is a perennial species. The species is unbranched at the base, but widely branched above “widely paniculate”. In the inflorescence, there are many branches. In each branch, there are many verticillasters and each verticillaster has 4-10 flowers.

According to the field observation and laboratory studies, each individual has about 100 to 1500 flowers. Each flower has 4 seeds, therefore each individual produces (if fertilization occurs on all flowers and they produce fully fertile seeds, this is an assumption) about 400 to 6000 seeds.

S. russellii

S. russellii is a perennial species. The species is branched both below and above (inflorescence cyme). It has several to many stems between 4 to 20. In each stem, there are about 6 to 12 verticillasters, and each verticillaster has about 12 to 40 flowers. Therefore, each individual has about 288 to 9600 flowers. Each flower has 4 seeds, therefore each individual could produce (if fertilization occur on all flowers and they produce fully fertile seeds) about 1152 to 38400 seeds. The great number of seed production does not provide any advantage for the species. This case could be explained as the less fertilization ratio, low ecologic tolerance and difficult seedling process of *S. russellii*.

S. tchihatcheffii

S. tchihatcheffii is a caespitose perennial species. The species is much branched at the base. However, stems are not branched above and have spike like inflorescence. Verticillasters are very closely arranged. In each stem, there are 3-7 verticillasters and each verticillaster has 5-10 flowers. Therefore, each stem has at least 15 to 70 flowers. According to the field observation and laboratuvarly studies, each individual has about 150 to 2500 flowers. Each flower has 4 seeds, therefore (if fertilization occurs on all flowers and they produce fully fertile seeds, this is an assumption) each individual could be produce about 600 to 10000 seeds.

3.7 Pollinator Foraging Behavior

- *Salvia absconditiflora*

In *S. absconditiflora*, honeybees land on the flower and use the lobes of the lower lip as a foothold. While the bee introduces its proboscis through the constriction into the flower tube, the lever starts to swing. The bee also introduces its mandibles into the flower tube. Most of the head is introduced between upper and lower lip of the flower, because *S. absconditiflora* has wider flower entrance than the other species (Table 2). The head of the honey bees are not visible clearly. Morphometric measurements show that the average length of proboscis of the honey bee and the length of the corolla tube and the distance from flower entrance to nectar cover are clearly consistent when the honey bee introduce its head to corolla tube. However, honey bees stay quite shorter on *S. absconditiflora*. This can be resulted from the small number of flowers in both verticillasters and individuals.

On the other hand, we observed that honey bees first touch the anther and then the stigma, because the stigma of *S. absconditiflora* is shortly exerted from the upper lip. This could cause autogamy and geitonogamy. In this respect, the degree of protandry and male sterility of the individual flowers are important.

-*Salvia aethiopsis*

According to the field observations with photos and video records, the main pollinators are *Anthopora aestivalis* and *Xylocopa cf. iris* for the *S. aethiopsis*. The pollinators visit flowers randomly. In *S. aethiopsis*, pollinators land on the flower and use the lobes of the lower lip as a foothold. While the bee introduces its proboscis through the constriction into the flower tube, the lever starts to swing. The bee also introduces its mandibles into the flower tube. Most part of its head does not pass the filaments, which is due to the narrow the flower tube and the head remains clearly visible outside the flower tube. The bee inserts its proboscis through the hole of the

spoon-shaped part of the lever when introducing its proboscis into the flower like *S. pratensis*.

Pollinators take nectar from both the flowers of the same verticillaster and flowers of the same individual to the other individuals. Although *S. aethiopsis* has the a large number of flowers, pollinators stay for a very short time in each flower and plant. During daytime, the activity of the pollinators is quite low. On the other hand, we observed that pollinators first touch the anther and then stigma (herkogamy), because the stigma of *S. aethiopsis* is shortly exerted from the upper lip. This could cause autogamy and geitonogamy. In this respect, the degree of protandry and male sterility of the individual flowers are important.

-*Salvia russellii*

According to the field observations based on photos and video records, the main pollinator is *Apis mellifera* for the *S. russellii*, although 11 the other bees and moths visit it. *Apis mellifera* visits flowers randomly. In *S. russellii*, honeybees both land on the flower and use the lower lip and the other flowers as a foothold. In *S. russellii* the bee has to push back the movable upper lip to gain access to nectar. As a result it becomes loaded with pollen on its forehead. When visiting a second flower, the pollen-loaded forehead first touches the style protruding from the flower like in the case of *S. verticillata* (Claßen-Bockhoff et al., 2004). *S. russellii* lack the movable staminal lever mechanism and has a very narrow corolla tube. The bee introduces its mandibles into the flower tube. The head of the honey bees remain clearly visible. Morphometric measurements show that the average length of proboscis of the honey bee and the length of the corolla tube and the distance flower entrance to nectar cover are clearly consistent when the honey bees touch the anthers. On the oher hand, when the honey bees and the other pollinators are taking nectar, their ventral side touch the flower anther and stigma, it may also provide pollination in low degrees.

The pollinators take nectar from both the flowers of the same verticillaster, flowers of the same individual and other individuals. Because of the a large number of

flowers, honey bees and the other pollinators stay for a very longer time than the other *Salvia* species. During daytime, the activity of the honey bees is quite much.

-S. tchihatcheffii

According to the field observations with photos and video records, the main polinator is *Apis mellifera* for the *S. tchihatcheffii*. *Apis mellifera* visits flowers, randomly. In *S. tchihatcheffii*, honeybees land on the flower and use the lobes of the lower lip as a foothold. While the bee is introducing its proboscis through the constriction into the flower tube, the lever starts to swing. The bee also introduces its mandibles into the flower tube. Most of the head introduce between upper and lower lip of the flower, because *S. tchihatcheffii* has a lit bit wider flower entrance (like *S. absconditiflora*) than the other species. The head of the honey bees does not remain clearly visible.

Honey bees take nectar from both the flowers of the same verticillaster, flowers of the same individual and the other individuals. Because of the many numbers of flowers which offer large amount of nectar, honey bees stay for a very long time on each plant which increases the chance of pollination. During daytime, the activity of the honey bees is quite much. On the other hand, we observed that honey bees first touch the anther then touch the stigma, because the stigma of *S. tchihatcheffii* is shortly exserted from the upper lip. This could cause autogamy and geitonogamy. In this respect, the degree of protandry and male sterility of the individual flowers are important.

Table 6. Inflorescence type, flower number in each verticillaster, approximate flower and seed number in each individual and probing time of pollinators for the studied species.

	Inflorescence type	Flower number in each verticillaster	Approximate flower number in each individual	Approximate seed number in each individual (if fertilization occur on all flowers and produce fully fertile seeds)	Approximate probing time according to field observations and video records on each flower
57	<i>S. absconditiflora</i> *	Raceme	3-8 (-10)	12-200	12 (or less) - 400 (max.) <i>Apis mellifera</i> , app. 1.8 sc.
	<i>S. aethiopsis</i>	Widely paniculate	4-8 (-10)	100-1500	400 (or less) - 6000 (max.) <i>Xylocopa iris</i> , app. 2.5 sc. <i>Anthophora aestivalis</i> , app. 2.4 sc.
	<i>S. russellii</i>	Cyme	12-40	288-9600	1152 (or less) - 38400 (max.) <i>Apis mellifera</i> , app. 1.4 sc.
	<i>S. tchihatcheffii</i> *	Spike	5-8 (-10)	150-2500	600 (or less) - 10000 (max.) <i>Apis mellifera</i> , app. 5.4 sc.

3.8 Flower Colour- Pollinator Interaction and Pollinator Probing Time

In Turkey, there are white, yellow, pink, blue, violet and reddish (only *Salvia viscosa*) flowered *Salvia* species and they may have differently shaped nectary signals (Claßen-Bockhoff et al. 2004). However, in the studied species, there are only white and light violet flowers. Field observations illustrated that there are significant visiting sequence between the white and violet flowered species. For example, one of the white flowered species, *S. aethiopsis* was visited by 4 (one of them is visitor), *S. absconditiflora* was visited by 4 (one of them is visitor) and *S. tchihatcheffii* was visited by 6 (one or two of them is visitors) insect species including bees, and moths. On the other hand, (light) violet flowered species, *S. russellii* was visited by 12 (two of them is visitor) species including bees and moths.

The other important observation that probing time of the bees on *S. aethiopsis* (white), is very short. On the other hand, the same one in *S. russellii* and *S. tchihatcheffii* are quite longer than the other investigated species (Table 6). There is no correlation for the reason of it in terms of colour and stamen type. It could be directly related with flower numbers in one individual and the population size.

3.9 Preference of the Pollinators

Due to the phenotypic diversity of the flowers, *Salvia* species are specialised to certain sub-groups of bees, so there should be isolation mechanisms preventing pollen mixture and pollen loss. Among captured insects, we identified 22 species and 21 of them are bees, 1 is a moth species however 16 of the bee species are pollinators while 5 of them are visitors (Table 7). They either preferred a single *Salvia* species or more than one species. Among them, *Apis mellifera* visited three *Salvia* species which are *S. absconditiflora*, *S. russellii* and *S. tchihatcheffii*. However 18 bee species preferred only a single *Salvia* species.

Table 7. Pollinator sharing among sympatric *Salvia* species in three stations, number of crosses indicates pollinator frequency from high (XXX) to low (X), (**,moth), (v); visitor.

Species (*,endemic)	* <i>S. absconditiflora</i>	<i>S. aethiopsis</i>	<i>S. russellii</i>	* <i>S. tchihatcheffii</i>	Total
<i>Anthophora aestivalis</i>		x			1
<i>Anthophora robusta</i>	x				1
<i>Apis mellifera</i>	xxx		xxx	xxx	3
<i>Eucera cf. pollinosa</i>			x		1
<i>Chalicodoma ericetorum</i>				x	1
<i>Coelioxys rufescens</i>			x		1
<i>Eucera sp.2</i>			xx		1
<i>Eucera sp.3</i>			x		1
<i>Eucera sp.4</i>			x		1
<i>Eucera sp.5</i>			x		1
<i>Eucera sp.7</i>				x	1
<i>Evylaeus laticeps</i>			x(v)	x(v)	2
<i>Evylaeus sp.2</i>		x(v)			1
<i>Halictus tetraonianellus</i>			x		1
<i>Halictus resurgens</i>	x(v)				1
<i>Hylaeus sp.</i>			x(v)		1
<i>Megachile pilicrus</i>		x			1
<i>Nomada sp.</i>			x		1
<i>Osmia mustelina</i>	x			x	2
<i>Osmia versicolor</i>				x	1
<i>Xylocopa cf. iris</i>		xx			1
** <i>Zygaene minos</i>			x(v)		1
Total	3	3	9	5	20

CHAPTER 4

4. DISCUSSION

4.1 Reproductive Isolation

To test the functionality of the staminal lever mechanism and the morphometric fitting between flowers and bees, pollinator observations in the field were conducted to understand the pollen transfer process. The pollination systems of actually co-occurring species in their natural habitats are observed by field and laboratory investigations in order to evaluate the adaptive value of the staminal lever mechanism. Determining the isolation types among co-occurring species and testing the functioning of the lever mechanism will help to understand the evolutionary significance of the staminal lever mechanism.

The functioning of the staminal lever mechanism has been observed by many scientists since Sprengel (1793). Classen-Bockhoff *et al.* (2004) explained possible scenarios what happens if co-occurring *Salvia* species that may potentially hybridize share their pollinators. First scenario assumes that pollen is mixed and hybrids may occur and second scenario assumes that pollen is not mixed due to the precise manner of pollen deposition on pollinators' body. In second scenario, the *Salvia* species remain reproductively isolated and this leads to the use of entire range of pollinators. So the *Salvia* species do not have to specialize on certain pollinators.

There are many studies on sympatrically flowering species of *Salvia* from California (Grant and Grant, 1964), Mexico (Dieringer *et al.*, 1991), Austria (Tweraser and Weiniger-Höllrigl, 2001; Claßen-Bockhoff *et al.* 2004). The morphological differences in floral organs and their correlation with the pollinators caused

mechanical isolation due to the precise pollen deposition on the pollinator's body. Their studies indicate that sympatric species share pollinators without mixing pollen and reproductive isolation is achieved by temporal, ecological, morphological and mechanical factors (Claßen-Bockhoff *et al.*, 2004).

In this study on the four co-occurring *Salvia* species, the precise pollen deposition due to the effective functioning of the lever mechanism and several isolating factors enabled the *Salvia* species to share their pollinators. The *Salvia* species have different floral characteristics and stamen types. It is evident that the diversity of floral constructions increases mechanical isolation by attracting different pollinators and by loading pollen on different parts of their bodies.

The four *Salvia* species have overlapping flowering times. However, their flowering sequences show differences and this provided phenological isolation among species. Morphometric fitting between floral and insect characters are consistent in terms of their sizes. These results imply that the *Salvia* species and their pollinators construct mutualistic relationship. Moreover, the size of the flowers and relatively different sizes of staminal lever arms provide limitation to pollinators and phenotypic isolation is achieved among the *Salvia* species.

Regarding the reproductive isolation between the four *Salvia* species,

- *S. absconditiflora* has overlapping flowering time and shares its pollinators with the remaining *Salvia* species (Figure 27). However its relatively different size of floral organs provide mechanical isolation by depositing pollen on different domains of pollinators' body (Table 5). *S. absconditiflora* and *S. tchihatcheffii* are the most abundant species in 3 stations (Figure 1) and they grow side-by-side however no hybrid populations are observed in 3 stations and it is completely mechanically isolated from each other and they have different chromosome numbers (Table 4). Also, the pollinators first touch the anther and then the stigma because the style is not protruded from the flower which results in geitonogamy.

- *S. tchihatcheffii* shares its pollinators with the remaining species and have overlapping flowering time (Figure 27). *S. tchihatcheffii* is the second most attractive species for the pollinators but its relatively different sized flowers and proportions of staminal lever arms, its pollen is deposited on different domains of bees' body (Table 5). Also, the pollinators first touch the anther and then the stigma because the style is not protruded from the flower which results in geitonogamy.

- *S. aethiopsis* have a rather distinguishing habit than other species. Individuals of *S. aethiopsis* are widely spread from each other and they offer many more flowers per individual. Moreover, each individual has a sequence of flowering such that flowering of each individual is followed by the other individual of *S. aethiopsis*. The anthesis time is rather shorter and *S. aethiopsis* do not share any of its pollinators with the remaining species (Figure 5). Thus *S. aethiopsis* is phenologically isolated from the remaining *Salvia* species. Also flower constancy plays an important role for the reproductive isolation of *S. aethiopsis*.

- *S. russellii* is the most attractive species in the stations for the pollinators. It has light violet flowers and offers the maximum number of flowers per individual than the remaining species. Besides, it differs from the remaining species by its smaller size and its unique pollen transfer mechanism, sticking the pollen to the bee's forehead. The staminal lever arms are not moveable in *S. russellii*. It shares pollinators with each of the remaining species, but it has the smallest floral characters and it is completely mechanically isolated by its phenotypic differences.

4.2 Reproductive Success of *Salvia* species

In principle, autogamy and geitonogamy are possible in *Salvia*. The size and density of inflorescences, the sequence of flowering, the anthesis of individual flowers, length of probing time of a pollinator affect the degree of autogamy and geitonogamy (Barrett 2002, Claßen-Bockhoff *et al.*, 2004).

In this study, the size and density of inflorescences of the four *Salvia* species show differences. Flowers in a verticillaster and inflorescences are observed and counted. However, seed numbers of the species just indicate the minimum and maximum expected and approximate numbers, it was not counted from the individuals. This is not possible for many of the species, because the seeds of species mature in a sequence both in inflorescences and verticillasters. The synchronicity in the order of flowering is different for each *Salvia* species. For instance, the flowers on the lower parts of the inflorescence open earlier and their seeds also mature earlier. At the same time, the flowers on the upper parts of the inflorescence mature later than the flowers on the lower parts of the inflorescence. According to our field studies and observations, expected seed numbers show positive correlation with the population size of the *Salvia* species.

Zhang *et al.* (2011) found that total seed number per plant is positively correlated with the flower number per inflorescence. Our results and observations, on the four co-occurring *Salvia* species in three stations, are also supported by their findings. However, seedling success and population size show differences among the species.

4.3 Flower colour-Pollinator Interactions

A flower reflecting all light wavelengths except ultra-violet appears coloured to a bee but a flower reflecting all light wavelengths with ultra-violet appears white to a bee. Many flowers have patterns caused by the absence of ultra-violet from certain regions and these regions are invisible to man however they act as a guide to bees for the available sources. (Proctor & Yeo, 1973).

In Turkey, there are white, yellow, pink, blue, violet and reddish (only *Salvia viscosa*) flowered *Salvia* species. However, only white and (light) violet flowered *Salvia* species are studied in this research. Field observations illustrated that there are significant visiting sequence between the white and violet flowered species. For example, *S. aethiopsis* which has white flowers was visited by 3, *S. absconditiflora* was visited by 3 and *S. tchihatcheffii* was visited by 5 bee species. On the other hand, (light) violet flowered species, *S. russellii* was visited by 11 bee species.

Similar results were found by Claßen-Bockhoff *et al.*, 2004. In their study, violet or blue flowered species such as *S. pratensis*, *S. nemorosa* and *S. verticillata* were visited by large number of species than the white and yellow flowered species. In our study, *S. russellii* (sect. *Hemisphace*) is the most attractive species with 11 visiting species like *S. pratensis* (sect. *Plethiosphace*) with 13 species and *S. nemorosa* (sect. *Plethiosphace*) with 8 species and *S. verticillata* (sect. *Hemisphace*) with 7 visiting species.

Both studies indicated that violet flowers are more attractive than other colours for the pollinator species and other visitor insects. As well as, the most attractive species are placed in the section *Plethiosphace* and the *Hemisphace*. These could be resulted from ultraviolet reflectance patterns of violet colour, shape and attractiveness of the staminal lever mechanism, inflorescence architecture, flower number in the inflorescence and/or in verticillasters, or the amount of the nectar produced at the base of the corolla tube.

4.4 Flower Constancy

Pollinators often limits their visits to one type of flower while bypassing other equally rewarding ones which is known as flower constancy. Pollinators specialize on different flower types and individuals (Grant 1950). Reproductive benefits are provided to out-crossed plants by flower constancy as the interaction between

foreign pollen and stigma is prevented. However the reason behind flower constancy has some different aspects which have to be understood (Waser 1983).

Since the cross-fertilisation among distinct individuals of the same species is of great importance for the plant, the insect behaving in this manner is not for the good of the plant. The insects probably learned the best position to work on the flower and how to insert their proboscides and it enabled the insects to work quicker on flowers (Darwin 1876). There may be morphological differences among flowers such as colour, odor, type of floral reward which are involved in flower recognition (Waser 1983).

Zhang *et al.* (2011) tested the effect of flower constancy through changing the floral construction of *Salvia* flowers. The foraging pattern, flower constancy and reproductive success of *Salvia* species are affected by the variation in floral structures. Particular floral designs are related with flower constancy and pollinator cognition (Zhang *et al.* 2011). The staminal lever arms may be an attractive structure for pollinators (Classen-Bockhoff *et al.*, 2004).

In this study the probing time and foraging behavior of the pollinators are observed in the field and the investigations indicated that flower constancy may be an effective way for precise pollination but it may increase the degree of geitonogamy. *Salvia* species offering many more flowers per verticillaster may be more rewarding to bees.

CHAPTER 5

5. CONCLUSION

The staminal lever mechanism is considered as a floral syndrome which plays a major role in the process of pollen transfer. To understand the evolutionary significance of the lever mechanism, different methodological approaches are needed. There are several methods for this and they range from functional morphology, ecological field investigations, molecular systematics and experimental biomechanics.

In this study, field observations on the co-occurring *Salvia* species and their pollinators in their natural habitats provided valuable information for understanding the flower-pollinator interactions and the pollination system in genus *Salvia*. Species-specific patterns of pollen deposition on the pollinators' body and floral diversity of the *Salvia* species such as corolla shape, size, colour, stamen type are investigated.

The size and density of inflorescences, the synchronicity in the order of flowering, number of flowers in verticillasters and total number of flowers in one individual and anthesis period affect pollinators foraging behavior and probing time. Differences on flower colours and ultra-violet patterns may have impact on the behavior of pollinator species. More field observations and data on pollinator behavior are needed. Different floral designs are directly related with the flower constancy and the reproductive success of the *Salvia* species.

Regarding the different sizes of bee species as pollinators, the four *Salvia* species entirely use different sized bees as pollinators but the precise pollen deposition on

pollinators body is a way for maintaining genetic isolation. Hybridization may occur between morphologically similar species such as *S. absconditiflora* and *S. tchihatcheffii*. The degree of hybridization depends on the size of parental populations, the size of the flowers, the overlapping flowering times and sharing pollinators. Although the *Salvia* species have overlapping flowering periods and share their pollinator species, no hybrid populations of *Salvia* were observed. Thus, *S. absconditiflora* and *S. tchihatcheffii* are completely mechanically isolated from each other. *S. aethiopsis* does not share any of its pollinators with the remaining species and the sequence of flowering and anthesis of *S. aethiopsis* enable the species to be phenologically isolated from the other *Salvia* species. *S. russelli*, which has a rather unique staminal lever mechanism, is completely mechanically isolated from the remaining species. These cases result from effective means of mechanical, phenotypical and ethological isolation factors.

Consequently, the four sympatric *Salvia* species are reproductively isolated from each other by the precise pollen deposition of the staminal lever mechanism and by other isolating factors such as; phenotypical, phenological an ethological isolation.

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