The staminal lever mechanism in *Salvia* L. (Lamiaceae): a key innovation for adaptive radiation?

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Abstract

Floral key innovations play a significant role in the discussion of adaptive radiation in plants. The paper brings together a brief review of morphological key innovations in plants, elucidating their evolutionary significance in flower–pollinator interactions, and new data on *Salvia*, a genus being examined as an example for presumed adaptive radiation. We hypothesize that the characteristic staminal lever mechanism functions as a key innovation. It is defined as a functional unit including the modification of stamens to lever-like structures, their reversible movement, and the organization of the remaining floral structures involved in the process of pollen transfer. We follow the assumption that structure and functioning of the staminal levers play a major role in the process of pollen deposition on the pollinator's body, and that minute changes of both their proportions and their interactions with pollinators may have significant consequences for the pollination system. The functioning of the staminal lever mechanism is tested by field investigations, biomechanical experiments and pollination simulations. First results are presented, and possible modes of allopatric and sympatric speciation are discussed, based on morphometry of *Salvia* flowers and pollinators as well as on the operating mode of the staminal lever mechanism. Special attention is given to species-specific patterns of pollen deposition on the pollinator's body. We assume that, depending on the precision of the lever movement, sympatric *Salvia* species flowering during overlapping periods and sharing the same pollinating species may be either mechanically isolated from each other or able to hybridize. The latter may result in speciation, as may spontaneous mutations influencing the flower-pollinator interaction, e.g. by significant changes in morphometry of the staminal lever system and/or other flower structures. As a consequence, *Salvia* individuals may deposit pollen on a different part of the pollinator's body, or even adapt to a new pollinator species, both resulting in reproductive isolation from the parental population.
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Introduction

Due to the increase in molecular-systematic studies, which provide phylogenetic information independent of phenotypic characters, the study of adaptive radiation has undergone a renaissance over the past 20 years (Givnish and Sytsma 1997; Schluter 2000). Among flowering plants, adaptive radiation has been studied in Hawaiian *Schiedea* (Caryophyllaceae; Sakai et al. 1997) and the silversword alliance (Asteraceae: *Argyroxiphium, Dubautia, Wilkesia*; Carr et al. 1989; Baldwin

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1997), the Asteraceae *Encelia* (Ehleringer and Clark 1988), and *Argyranthemum* (Francisco-Ortega et al. 1997), *Dalechampia* (Euphorbiaceae; Armbruster 1993), *Aquilegia* (Ranunculaceae; Hodges and Arnold 1995; Hodges 1997) and the monocotyledonous taxa *Brocchinia* (Bromeliaceae; Givnish et al. 1997), *Platanthera* (Orchidaceae; Nilsson 1988; Hapeman and Inoue 1997), Orchidaceae-Oncidiinae (Chase and Palmer 1997), Pontederiaceae (Barrett and Graham 1997), and Rapateaceae (Givnish et al. 2000).

The genus *Salvia* represents a further taxon that possibly has undergone adaptive radiation as to its pollinator spectrum, eventually allowing the co-existence of several sympatric species that flower at overlapping times and share some of their pollinator species. *Salvia* includes more than 900 species (Alziar 1988–1993) and is by far the largest genus in the family Lamiaceae. It is characterized by modified lever-like stamens playing a central role in the process of pollen transfer. The existence of a large number of species and an almost unique structure essential for reproduction raises the question as to whether the lever mechanism might have driven speciation in the genus. Considering the huge structural and functional diversity of *Salvia* flowers, we hypothesize that the modification of the stamens into levers that ensure pollen deposition, and their 'coordination' with the remaining floral structures might represent a key innovation promoting adaptive radiation in *Salvia*.

Background

Adaptive radiation

Since the times of Darwin the concept of adaptive radiation has been related to the Galapagos finches which diversified by occupying diverse niches on the individual islands. However, the corresponding terms 'adaptive', 'radiation', 'niche', and 'key innovation' are under continuous discussion and have only recently been summarized by Givnish (1997), Bateman (1999a, b) and Schluter (2000), reevaluating ideas formulated, e.g. by Stebbins (1950, 1974), Simpson (1953) and Mayr (1970).

In the present paper we define 'adaptive radiation' as the evolution of ecological and phenotypic diversity within a lineage in adaptation to the specific biotic and abiotic environment. We agree with Givnish (1997) and Barrett and Graham (1997) in that adaptive radiation is usually linked with speciation but that it should not be defined by the process of 'rapid speciation' as Schluter (2000) proposed. A 'radiation' does not merely result from the passage of time. Rather, it results from an increase in phenotypic diversity formed through adapta-

tion to specific environments. It differs from 'speciation' which points to the increase of species numbers by any speciation mechanism. Speciation can occur by any interruption of gene flow; and is not necessarily coupled with phenotypic diversification. The term 'adaptive' is restricted to cases in which ecological divergence is accompanied by changes that increase the capacity for utilizing those environments. It excludes speciation without phenotypic divergence (Gittenberger 1991); and species proliferation in which differentiation is not triggered by the environment (Kambysellis and Craddock 1997). Although from the theoretical point of view 'non-adaptiveness' is not verifiable, adaptive radiation cannot be assumed 'a priori' (Schluter 2000).

'Adaptive radiation' is the process that can be interpreted as a link between ecology and phylogeny and thus poses several fundamental questions of evolutionary biology (Givnish 1997; Rowe and Speck 2004). It may be triggered through extrinsic causes due to new environmental conditions, and through intrinsic characters of organisms due to the evolution of a 'key innovation'. According to Simpson (1953), a 'key innovation' is defined as the acquisition of evolutionary novelties causing large-scale adaptive radiation by enabling a taxon to utilize existing niche space in a novel manner (Hodges 1997; Hunter 1998; but see Bateman 1999a, b). It acts by (1) allowing escape from competition by utilizing new adaptive zones, (2) increasing individual fitness, and (3) favoring reproductive and ecological specialization (Heard and Hauser 1995).

For the term 'niche' we follow the broad definition given by Grinnell (1917), including any biotic or abiotic environmental feature that may be utilizable by a single species. It thus differs from the more general term 'environment' which includes all features of a given habitat irrespective of whether or not they potential influence population dynamics and natural selection (Schluter 2000).

The *Salvia* case

Sprengel (1793) described and illustrated the dorsal (nototribic) pollination mechanism in *Salvia*, but the morphology of the staminal lever was first recognized by Hildebrand (1865). The latter author found that the lever is formed by the connective that widens and separates the two thecae from each other. The upper connective arm always bears two pollen sacs and is usually placed below the upper lip, whereas the lower connective arm is often sterile and restricts access to nectar. An insect or bird searching for nectar pushes the barrier back and is thus loaded with pollen on its head, bill or back. During a subsequent visit to a flower of the same species, pollen can be transferred to the stigma,

which at a more advanced flowering stage often assumes the position earlier occupied by the pollen sacs (Fig. 1; for a detailed description see Claßen-Bockhoff et al. 2003). In the present paper, we define the staminal lever mechanism as a functional syndrome including the modification of stamens to lever-like structures, their reversible movement, and their 'coordination' with the remaining floral structures involved in the process of pollen transfer.

Although the staminal lever mechanism in *Salvia* is cited as one of the best examples of nototribic pollination mechanisms, we know little about its evolutionary significance. Starting from the findings that many *Salvia* species occur sympatrically and that hybridization may occur (Epling 1947; Grant and Grant 1964; Haque and Ghoshal 1981; Palomino et al. 1986; Huck 1992; Owens and Ubera-Jiménez 1992; Ramamoorthy and Elliott 1998), we test if the lever-like stamens may be involved in prezygotic isolation mechanisms (Levin 1971). One approach for this purpose are field investigations which should include sister groups. However, as the phylogeny of *Salvia* is still unclear this is not yet possible. Although we cannot reconstruct the processes of speciation, we can study the pollination systems of actually co-occurring species in order to evaluate the adaptive value of the floral structures involved.
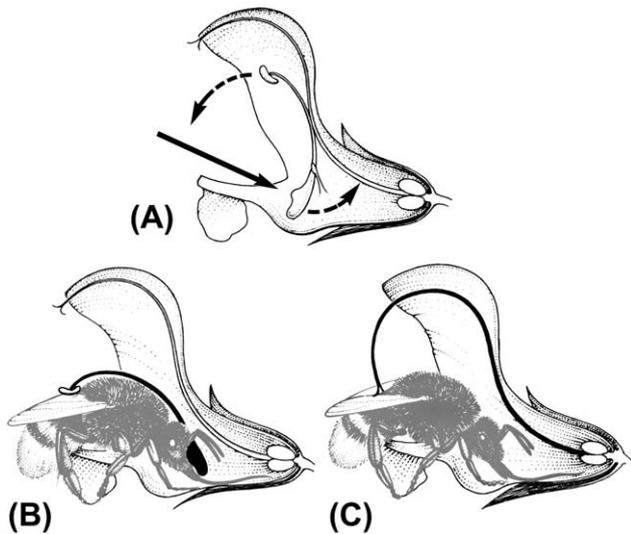

Fig. 1. Pollen transfer in *S. pratensis* L. (schematic, after Meeuse and Morris 1984). (A) Longitudinal section through the flower, showing one of the two modified, lever-like stamens, and the position of the style at the beginning of anthesis; arrow indicates contact of pollinator with the sterile connective plate, dotted lines illustrate movement of lever arms. (B) An insect looking for nectar in a young flower pushes the platform back, thus triggering pollen loading on its back. (C) In an older flower, in which the style arrests the position of the anthers (the latter not drawn), the insect deposits the pollen on the stigmas.

With respect to the lever mechanism in *Salvia*, we focus on mechanical and ethological isolation (Grant 1994a), which may be caused by the physical force or energies needed to release the lever mechanism. In principle, two directions of selection are conceivable: (a) the evolution of levers that are difficult to trigger, or levers with mechanical locks that mechanically restrict the number of potential pollinator species; and (b) the evolution of levers that are easy to trigger, which may allow a large number of pollinator species to gain access to nectar. The latter may convey reproductive isolation by other means, such as the precision of the reversible lever movement and the corresponding precision of the pollen placement on the pollinator's body. Both possibilities mean that mechanical isolation likewise may be caused by the selective use of the pollinators as part of the 'environment' a given *Salvia* species is interacting with.

Dependent on the precision of pollen transfer by the lever mechanism and on the morphometric fit of floral structures and pollinators, each *Salvia* species deposits its pollen on a more or less specific domain of the pollinator's body. Grant (1994a) already listed as one example for mechanical isolation assemblages in which, due to different proportions, pollinator behavior, and sexual dimorphism, pollen of sympatric species is transported by the same pollinator without being mixed. Among them are bee-pollinated species in *Pedicularis* (Macior 1982; Grant 1994b), *Rhinanthus* (Kwak 1978), *Polygala* (Brantjes 1982), *Stylidium* (Armbruster et al. 1994), and orchids (Dressler 1968, 1981), as well as bird-pollinated species in *Heliconia* (Stiles 1975), and heterostylous species (Barrett 2002).

If co-occurring *Salvia* species that potentially may hybridize share the same pollinator, two scenarios are possible: (a) pollen is mixed and hybrids may occur, and (b) pollen is not mixed because it is deposited on distinctly separated areas of the pollinator's body in a manner precluding contact with any heterospecific *Salvia* stigma. In the latter case the species remain reproductively isolated from each other. In consequence, they do not have to specialize in different pollinators, but instead can use the entire range of pollinators each of which is loaded with pollen in a specific way (see Waser et al. 1996 for a recent discussion of specialization and generalization).

As an alternative hypothesis to adaptive radiation (not mutually exclusive with it) we have to consider selective pressures affecting pollination success and breeding systems in *Salvia*, that only indirectly contribute to phenotypic diversification and speciation. According to the current state of knowledge, the genus *Salvia* lacks any genetic incompatibility system, hence autogamy and geitonogamy are possible in principle (Read 1983; Owens and Ubera-Jiménez 1992; Navarro 1997; Miyajima 2001). Their extent depends on the

degree of protandry and male sterility of the individual flowers (gynomonoecy) and individuals (gynodioecy), the size and density of inflorescences, the synchronicity in the order of flowering, the duration of the anthesis of individual flowers, and the length of stay of a pollinator with an individual plant (Barrett 2002). The staminal lever mechanism may have evolved to promote male fertility by reducing gamete loss. Male fitness increases with the complete emptying of the pollen sacs, the extent of pollen partitioning, the number of pollen grains getting to receptive conspecific stigmas, and of course the accuracy of pollen disposal and reception. All these features may more or less depend on the staminal lever mechanism.

Only recently, Thomson et al. (2000) reviewed the pollen presentation theory (PPT) formulated by Percival (1955). According to this theory, selection for successful pollination is also reflected by the scheduling and control of pollen release to visitors. Bees are classified as HRLD-pollinators (high removal, low deposition), whereas birds appear to be LRHD-pollinators (low removal, high deposition). Selection may favor pollen partitioning in bee-pollinated flowers and pollen packaging in bird-pollinated flowers. As Salvia includes both bee flowers and bird flowers this theory should be tested as describing a fact possibly contributing to pollinator shifts found in the genus.

Systematic background

Salvia belongs to the subfamily Nepetoideae within the Lamiaceae, a monophyletic group well supported by both molecular and morphological data (Cantino and Sanders 1986; Cantino 1992; Wagstaff et al. 1995, 1998). The genus itself was assumed to constitute a natural group mainly because of the highly derived staminal structures. However, recent molecular findings have generated doubt (Kaufmann 1994; Walker et al. 2002). The first molecular data, based on rbcL and trnL-F sequences of 54 Lamiaceae, including 36 Salvia species, show three different Salvia clades and thus do not favor a monophyletic origin of the genus (Sytsma and Walker 2003). In addition, staminal levers highly similar to those of Salvia are also found within the subfamily Lamiaceae–Prostanthereae (Tweraser and Claßen-Bockhoff 2002). It thus might be possible that the staminal lever mechanism has evolved several times independently within the Lamiaceae or even in Nepetoideae.

A basic assumption of the adaptive-radiation concept is common ancestry of the group under consideration. However, this does not necessarily involve monophyly of the adaptively radiating group, which would demand all descendant taxa to be included in the process of adaptive radiation (Schluter 2000). Irrespective of the mono-, para- or polyphyletic origin of the genus Salvia, all species descend from the common ancestral species of the Nepetoideae, and thus fulfill the criterion of common ancestry on this level. If adaptive radiation can be demonstrated in each of the independent Salvia clades, the Nepetoideae would be an impressive example of parallel adaptive radiation. If Salvia will indeed prove to be polyphyletic, most of the new clades will probably include more species than their newly assigned sister groups, and therefore each new 'Salvia' clade will still give a picture characteristic of adaptive radiation (Table 1).

Up to now we know little about Salvia's center of origin or biogeographical routes of migration. Keeping in mind the above discussion about para- or polyphyly we also have to consider several independent origins and several phases of migration. At present it is assumed that Salvia originated in the Old World, probably from an ancient Mediterranean species (Dieringer et al. 1991; Baikova 1999), and that member species 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) date from the Lower and Upper Miocene and thus indicate an age of at least 25 million years. In accordance with Panero et al. (1999), it is conceivable that ancestral Salvia species belonged to the Tertiary boreotropical flora and migrated from Eurasia to North America via the Bering land bridge.

Table 1. Number and size of genera within the monophyletic Nepetoideae (after Cantino et al. 1992; Mabberley 1997); it is assumed that even in case of a polyphyletic origin of Salvia the individual Salvia clades have many more species than their sister groups

Tribes	Genera	Number of species
Mentheae	Salvia	> 900
	Thymus	350
	Nepeta	250
	68 further genera, including:	< 100
	Perovskia[a]	7
	Rosmarinus[a]	2
	Dorystoechas[a]	1
Ocimeae	*Hyptis*	300
	Plectranthus	200
	Ocimum	150
	Acrocephalus	130
	47 further genera	< 100
Elsholtzieae	6 genera	< 10
Lavanduleae	1 genus	30
not classified	3 genera	1–2

[a] = preliminary sister groups according to Sytsma and Walker (2003).

However, these currently open questions can only be answered by phylogenetic studies combining molecular and morphological data. Further molecular analyses are essential for testing the hypothesis of adaptive radiation in *Salvia*. Phylogenetic trees will not only provide insights into the inter- and intrageneric relationships of *Salvia*, the time(s) of origin and probable speciation rates, but also into the biogeographical migration routes, the sequence of ecological changes and the development of phenotypic traits throughout the evolution of *Salvia*. Further morphological and ecological studies are essential to interpret the molecular findings and to reconstruct the constraints underlying phenotypic diversification.

Phenotypic diversity and specific environment of *Salvia* flowers

Phenotypic diversity is high in *Salvia* (e.g. Himmelbaur and Stibal 1932–34; Ramamoorthy and Elliott 1998). Members of *Salvia* are distributed world-wide. Present centers of diversity are Eurasia including the Mediterranean (approx. 210 species), Central and East Asia (approx. 90 species), East and South Africa (approx. 60 species), North America (approx. 40 species), Central America (approx. 350 species), and South America (approx. 210 species) (Claßen-Bockhoff et al. 2003). The group occurs across a wide range of habitats, from the sea level up to more than 4000 m. *Salvia* plants grow in tropical rainforests, dry bushland, semi-deserts, temperate forests, at forest edges, on meadows and degraded sites. Their ecological differentiation is correspondingly 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 — more rarely — annuals, vines and trees (Alziar 1988–1993).

The flowers are mainly bilabiate or tubular, but differ in length (*S. dombeyi* Epl.: 8–9 cm, *S. tiliifolia* Vahl.: approx. 0.5 cm), shape and proportions. They show all floral colors from white and yellow to red, pink, blue and violet (Alziar 1988–1993), and may have differently shaped nectary signals. The style and stamens are hidden below the upper lip, or are exposed in various ways (Fig. 2; for color photographs see the Electronic Supplement 04-04, Pt 1). The staminal levers show a wide range of forms, relative proportions, and additional structures of functional importance (Fig. 3; Claßen-Bockhoff et al. in press). Inflorescences vary in flower number, size and density, present flowers in unilateral, horizontal and vertical directions, and may have showy bracts or leaves.

Despite the large number of species and great structural diversity of the flowers, only melittophilous and ornithophilous flowers appear to have evolved (Huck 1992; Owens and Ubera-Jiménez 1992). While bee-pollinated species occur world-wide and are associated with a wide array of pollinators from small to large solitary bees, honey-bees, bumble-bees, and carpenter bees (e.g. Hildebrand 1865; Knuth 1898; Faegri and van der Pijl 1979), bird-pollinated plants predominantly evolved in response to neotropical hummingbirds (Sazima et al. 1996; Ramamoorthy and Elliott 1998). The only known exception in the Old World is *S. africana-lutea* L., a species from South Africa visited by sunbirds and white-eyes (Scott Elliott 1890; Brieschke 1991). Although we cannot rule out the existence of further bird-pollinated species in the Old World, the shift from bee- to bird-pollinated species appears to be an almost exclusively neotropical phenomenon. According to the pollen presentation theory (PPT) mentioned above (Thomson et al. 2000), birds can be more effective pollinators than bees, because they achieve higher pollen deposition rates. In *Salvia*, the hypotheses have to be tested that (a) many shifts from bee- to bird-pollinated flowers occurred in parallel, and (b) that, therefore, also less specialized flowers appear being pollinated by both bees and birds.

As the floral structures make direct use of features of their pollinators, it is expected that they diverged accordingly. However, considering the 'environment' of *Salvia* plants we have to distinguish at least three different levels. (1) The individual plant has to survive in its actual habitat to which it is predominantly adapted by physiological and vegetative characters such as growth form, leaf construction and metabolic substances. (2) The flowers have to attract and reward their pollinators. Floral traits might have evolved in relation to the structural, physiological and behavioral properties of the insects and birds involved. (3) Referring to the staminal lever mechanism, the 'environment' can be understood to represent the pollinator's body offering different areas for pollen deposition. Analyses of phenotype-environment correlations should thus focus on the relative proportions and interactions of flowers and pollinators and their significance for the process of pollen transfer.

In terms of the diversity of the pollen transfer mechanisms, it is obvious that the classical case of dorsal (nototribic) pollination is true for only some of the *Salvia* species, e.g. for the most frequently cited *S. pratensis* L. (Figs. 2A and B; Electr. Suppl. 04-04, Pt 1). Other species, such as *S. scabra* L. (Fig. 2E; Electr. Suppl. 04-04, Pt 1), still bear two fertile thecae per anther and transfer the pollen in both a nototribic and lateral (plagiotribic) way. It was already discussed by Correns (1891), using the example of *S. officinalis* L. which has a similar flower structure, that the pollen of the lower lever arm did not contact the stigma and thus was reduced in most of the *Salvia* species. *Salvia*

austriaca Jacq. (Fig. 2D; Electr. Suppl. 04-04, Pt 1) is an example of plagiotribic pollination, with the widely exposed thecae striking the nectar-seeking insect from the sides. Ventral (sternotribic) pollination can be realized by resupinate flowers (*S. jurisicii* Košanin, see Fig. 2C and Electr. Suppl. 04-04, Pt 1; Hildebrand 1865; Schmucker 1929), or by hanging inflorescences (*S. nutans* L.; Hildebrand 1865; Correns 1891); in either
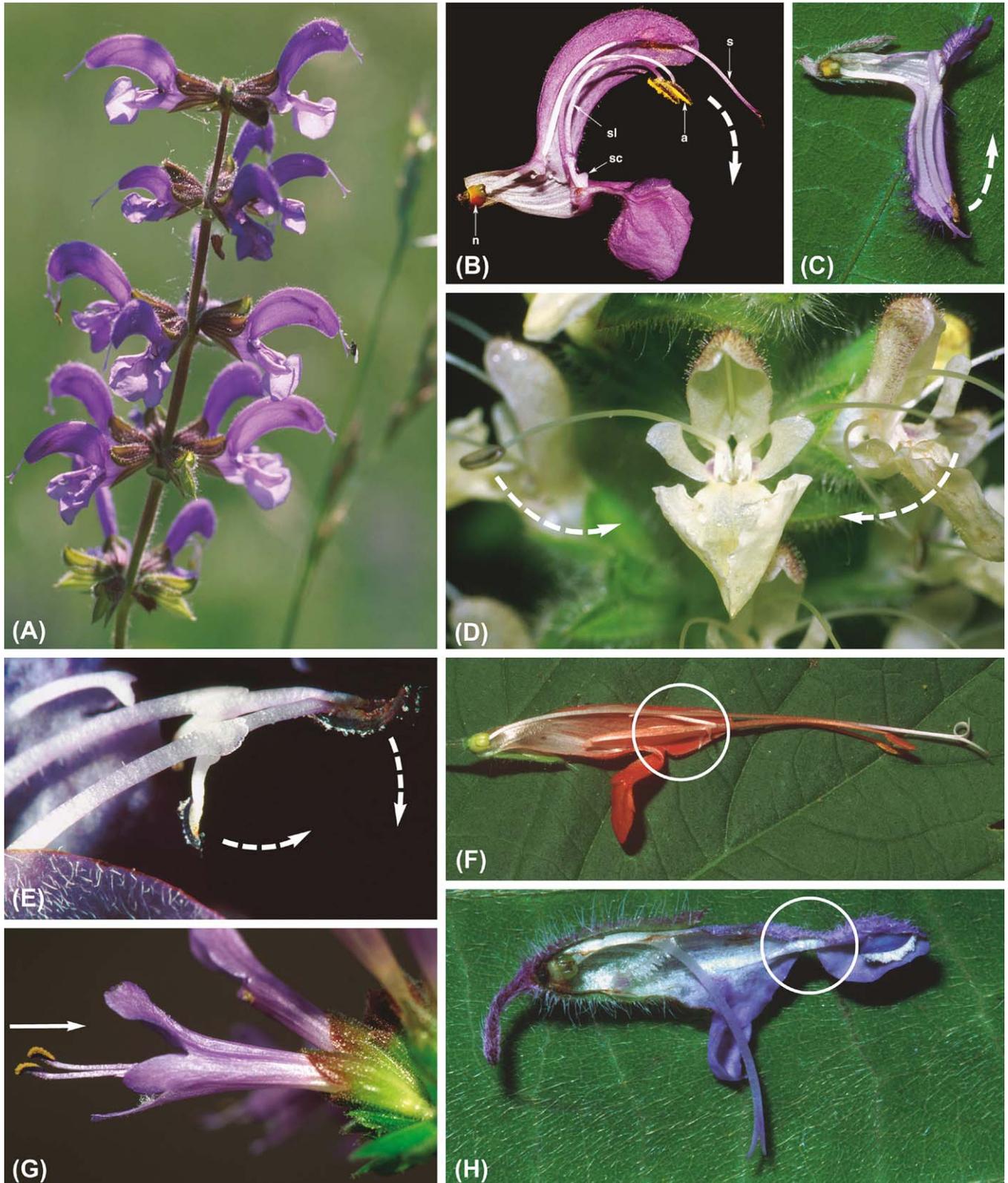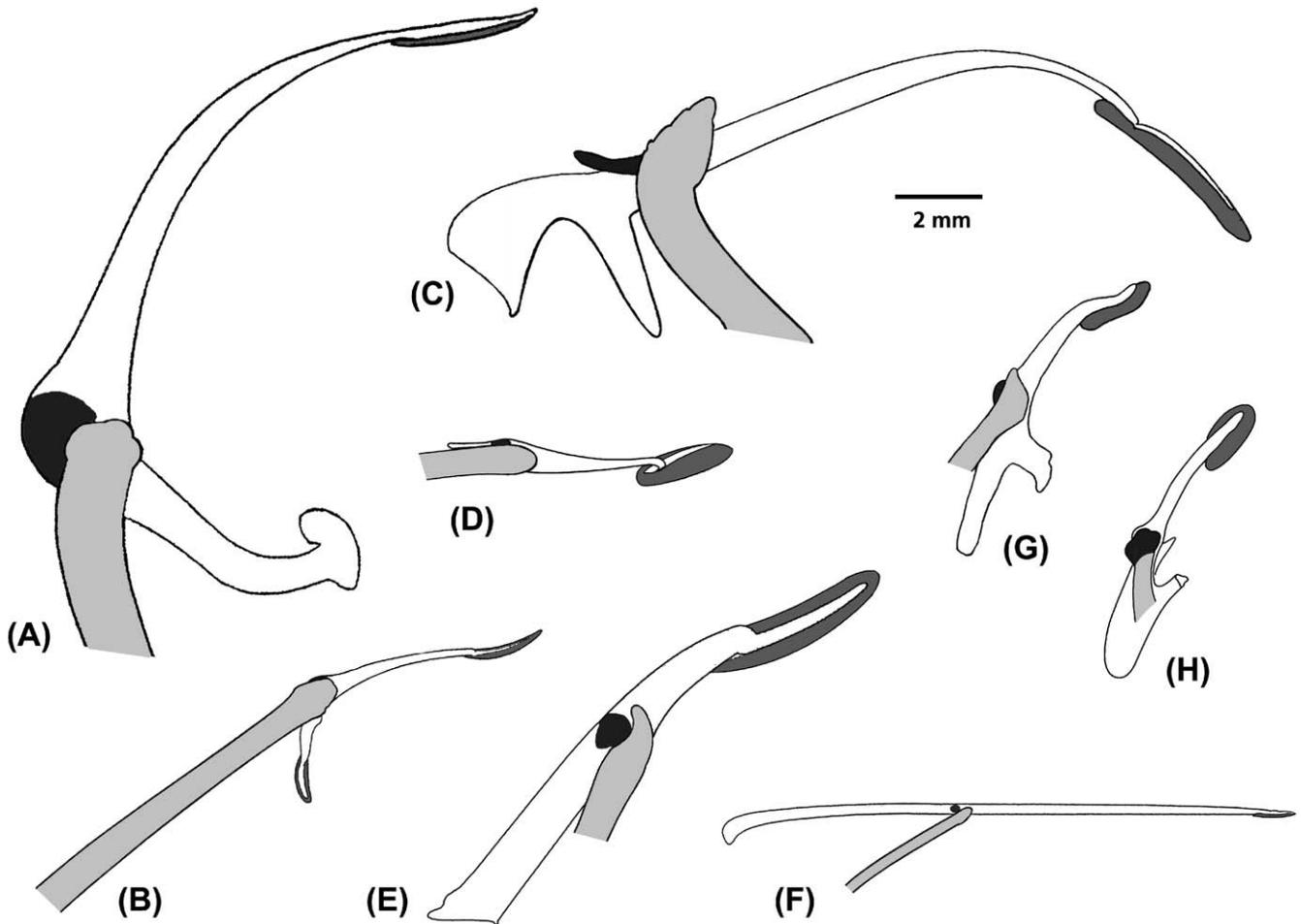
Fig. 3. Diversity of staminal levers in *Salvia*; variation includes size and shape of both the lever and its substructures. At the joint area, secondary formations of the filament (light gray) and connective (black) flank the thin ligament and stabilize the lever movement. (A) *S. glutinosa* L.; (B) *S. scabra* L.; (C) *S. argentea* L.; (D) *S. verticillata* L.; (E) *S. leucantha* Cav.; (F) *S. coccinea* Juss. ex Murr.; (G) *S. rypara* Briq.; and (H) *S. uliginosa* Benth. All stamens proportional to their natural size (after Claßen-Bockhoff et al., in press). dark grey: pollen sacs.

case, the insects land on the upper lip and are loaded with pollen on their ventral side.

Not only the site of pollen deposition on the pollinator's body varies between the individual flower–pollinator interactions, but so does the functionality of the lever mechanism itself. In *S. praeclara* Epl. (Fig. 2F; Electr. Suppl. 04-04, Pt 1), the joint is rather stiff and only the long and elastic filament enables the lever movement (authors' unpublished data). *Salvia clevelandii* (Gray) Greene and *S. verticillata* L. lack any lever

Fig. 2. Diversity of pollination mechanisms in *Salvia*. (A) *S. pratensis* L., inflorescence structure and flowering sequence enable geitonogamy among zygomorphic bumble-bee pollinated flowers. (B) *S. pratensis* L., flower in longitudinal section; a = anthers, n = nectary, s = style, sc = sterile connective plates, sl = staminal levers. (C) *S. jurisicii* Košanin, resupinate flower offering upper lip as a landing for bees; as anthers are enclosed by the upper lip, an insect only receives pollen after having released the lever. (D) *S. austriaca* Jacq.; the laterally exposed anthers strike against the bee's side when the pincer-like lever movement is released. (E) *S. scabra* L.; due to the bithecate anther, pollen is transferred in a nototribic and in a plagiotribic way; however, pollen from the lower lever arm does not contribute to pollination. (F) *S. praeclara* Epl.; the rather stiff joint is functionally substituted by a long elastic filament enabling the lever movement. (G) *S. clevelandii* (Gray) Greene; there is no lever mechanism, pollen is instead unprecisely smeared onto the underside of the pollinator. (H) *S. verticillata* L.; the reduced lever mechanism is compensated for by a unique pollen-transfer mechanism including a fold of the mobile upper lip. Dotted arrows in B–E indicate the dorsal (B,E), ventral (C), and lateral (D, E) pollen transfer mechanisms; arrow in G illustrates the smear effect caused by the unprecise pollen transfer; circles in F and H locate the elastic filament (F) and mobile upper lip (H) involved in the respective pollen-transfer process. (Photographs B and E by Michael Crone.)

mechanism, due to the reduction of the lower lever arm. In *S. clevelandii* (Fig. 2G; Electr. Suppl. 04-04, Pt 1) the thecae are exposed out of the flower and present pollen for ventral (sternotribic) pollination (see Cox 1981). As the pollinator passes over the pollen sacs while searching for nectar, pollen is not as precisely transferred as in the species with active levers. In *S. verticillata* (Fig. 2H; Electr. Suppl. 04-04, Pt 1) 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 first touches the style protruding from the flower.

These few examples already illustrate part of the phenotype-environment correlation in *Salvia*. 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.

Results

Adaptive significance of the staminal lever mechanism

To test the significance of the association between the floral traits in *Salvia* and the exploitation of the specific environment in more detail, field investigations are necessary. First observations already date from the late 19th century (e.g. Hildebrand 1865; Trelease 1881). They illustrate the flower–pollinator interactions in several European bee-pollinated *Salvia* species and neotropical hummingbird flowers. Among the many papers on pollination biology in *Salvia*, we find several experimental studies testing the breeding system (Visco and Capon 1970; Read 1983; Ouborg and Van Treuren 1995) and the ability and degree of hybridization among sympatric species (Haque and Ghoshal 1981; Ouborg and Van Treuren 1994). However, there are only a few studies dealing with the lever mechanism and its possible evolutionary significance (Ramamoorthy and Elliott 1998).

To get a deeper insight into the process of pollen transfer two studies are presently in progress analyzing the flower–pollinator interactions in bee-pollinated species and in bird-pollinated species.

Here, we first summarize some results from two localities investigated in eastern Austria where several *Salvia* species occur sympatrically. A detailed account of the methodological approach and quantitative results will be the subject of a separate paper (Tweraser et al., in prep.). For each *Salvia* species the flowering season, the range of pollinators (touching pollen and stigma) and the pollinator's body domain loaded with pollen were

recorded. The results show that the *Salvia* species have overlapping flowering seasons and also share some of the pollinators (Fig. 4; Table 2).

The question arises how reproductive isolation is maintained and hybridization precluded among these species. Regarding temporal, ethological and mechanical isolation, *S. glutinosa* L. is separated from the remaining species by its late flowering time overlapping only with *S. verticillata* L. *Salvia glutinosa* and *S. verticillata* share *Bombus pascuorum* Scop. as a pollinator, but the flowers are so different in size that pollen is deposited on different domains of the bee's body and cannot therefore be exchanged between the two species (Fig. 4: details 5, 6). *Salvia verticillata* differs from the other species by its small 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. *Salvia austriaca* Jacq. (Fig. 4: 2), the only species depositing its pollen on the lateral sides of the pollinator, also seems to be mechanically isolated due to its pollination mechanism. *Salvia aethiopis* L. (Fig. 4: 4) shares one pollinator with *S. nemorosa* L. and two with *S. pratensis*. However, as this species occurs in large populations and presents many more flowers per individual than the two other species, it has never been observed that pollinators switch from *S. aethiopis* to another *Salvia* species. Our detailed field observations suggest that *S. aethiopis* may be ethologically isolated from the remaining species. *Salvia pratensis* and *S. nemorosa* often occur sympatrically (Fig. 4: 1, 3). They have overlapping flowering periods, share their five main pollinators (Table 2), and have similar pollen transfer mechanisms, but differ in flower size. However, both species include small and large flower morphs, and the small, mainly female flowers in *S. pratensis* have the same size as the large flowers in *S. nemorosa*.

To elucidate how far the latter two species tend to hybridize, the respective flower and insect proboscis lengths as well as flower entrance and insect head sizes were measured and statistically evaluated. It could be shown that hybridization is only possible between small flowers of *S. pratensis* and large flowers of *S. nemorosa*. Accordingly, a small number of hybrids between these species should be expected due to the lack of an effective mechanical isolation. Indeed, Kerner von Marilaun (1891) previously mentioned *S. × sylvestris* L. as a natural hybrid between *S. nemorosa* and *S. pratensis*, producing up to 60% fertile seeds. However, no hybrid populations were found at the localities we investigated.

The field investigations illustrate that the species are less isolated by flowering time than by the mechanical isolation due to flower size, different pollination mechanisms and different relative morphometric proportions of staminal levers and pollinators. The species-specific and highly accurate pattern of pollen placement
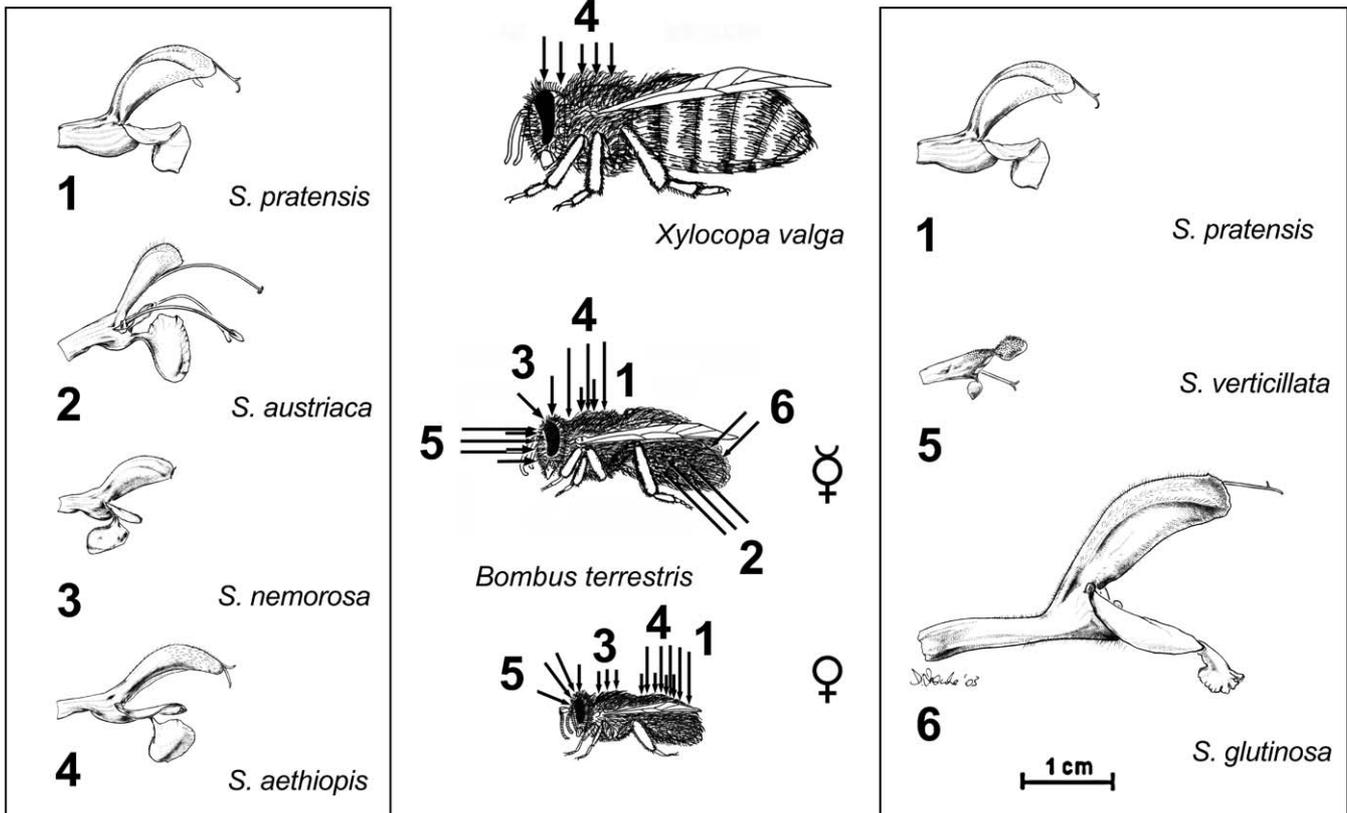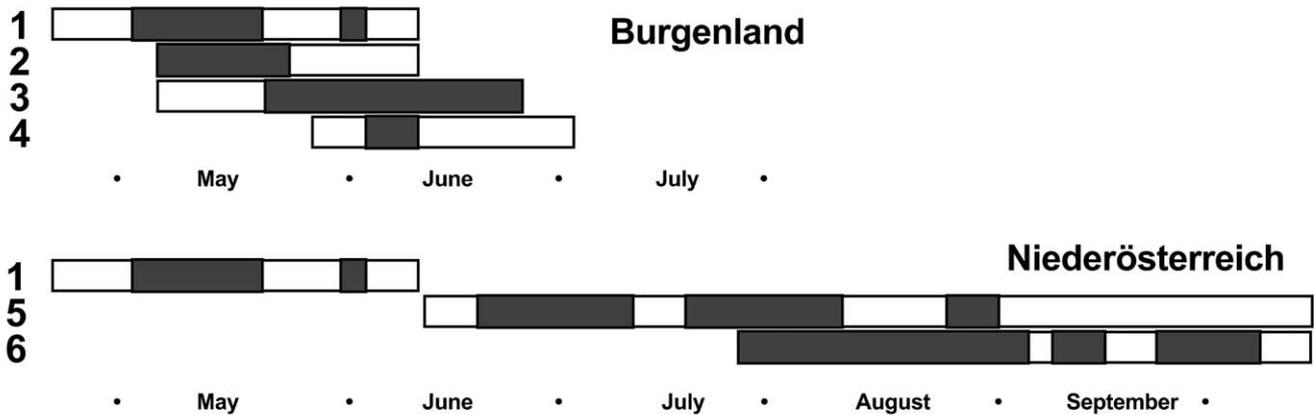
Fig. 4. Results from field studies in eastern Austria (see Table 2; modified from Tweraser and Weiniger-Höllrigl 2001). At the two localities in Burgenland and Niederösterreich (Lower Austria), four and three *Salvia* species, respectively, occurred sympatrically with largely overlapping flowering times (dark gray: main flowering seasons). Mechanical isolation was caused by both the relative sizes of flowers and bees and the species-specific pollen deposition on the body of shared pollinators. Examples are given for a *Bombus terrestris* queen, a *B. terrestris* worker, and for *Xylocopa valga*. (1) *S. pratensis* L.; (2) *S. austriaca* Jacq.; (3) *S. nemorosa* L.; (4) *S. aethiopis* L.; (5) *S. verticillata* L.; (6) *S. glutinosa* L. All flowers and bees shown proportional to their natural size (see text).

seems to be an effective means of maintaining genetic isolation. However, if the lever does not function in such a precise manner, hybridization may occur and possibly result in speciation.

Additional field investigations were carried out in the Bolivian Andes, concerning the significance of the staminal lever mechanism in hummingbird-pollinated species (Wester and Claßen-Bockhoff 2002). Special attention was given to *S. orbignaei* Benth. and *Salvia haenkei* Benth. Both species occur sympatrically and flower nearly simultaneously. According to Epling (1939) they belong to two different sections within the

Table 2. Pollinator-sharing among sympatric *Salvia* species in eastern Austria (see Fig. 4); number of crosses indicates pollinator frequency from high (xxx) to low (x)

Pollinator	S. aethiopis	S. austriaca	S. glutinosa	S. nemorosa	S. pratensis	S. verticillata
Bombus hortorum L.		x	xx		xxx	
B. humilis Illiger						xx
B. hypnorum L.					x	
B. lapidarius L.	xxx	x		xx	xx	xx
B. pascuorum Scopoli		x	xx	x	xxx	xx
B. pratorum L.					x	
B. ruderarius Müller				xx	xx	
B. soroeensis Fabr.						xx
B. sylvarum L.		xx		xxx	xx	x
B. terrestris L.	xxx				xx	xx
Apis mellifera L.				xxx	xxx	xxx
Andrena dorsata Kirby		x			x	
A. hattorfiana Fabr.					x	
Eucera clypeata Erichson				x		
E. chrysopyga Pérez				x		
E. longicornis L.				x		
E. nigrescens Pérez					x	
Osmia caerulescens L.					x	
O. rufa L.		x				
Anthophora aestivalis Panzer		x				
Megachile willughbiella Kirby	x					
Xylocopa valga Gerstaecker	xx					

subgenus *Calosphace* (sect. Chariantha, sect. Cylindriflorae), and thus probably do not constitute sister taxa.

The two species differ in the size, shape and color of the flowers, as well as in the presentation of the anthers which are hidden below the upper lip in *S. orbignaei* (Fig. 5B), but jutting out from the flower in *S. haenkei* (Fig. 5A). A particular difference concerns the lever mechanism which is active in the former species, inactive in the latter. While pollen is assumed to be precisely transferred in *S. orbignaei*, it is smeared on the pollinator's beak or head in *S. haenkei*. Hybrids were found in the field, each showing a different combination of the parental characters (Figs. 5 and 6; for a color version of the latter see Electr. Suppl. 04-04, Pt 2). It is assumed that hybridization is caused by (a) the imprecise pollen transfer in *S. haenkei*, resulting in the styles of *S. orbignaei* picking up *S. haenkei* pollen; and (b) by the protruding style in *S. haenkei* picking up *S. orbignaei* pollen (Wester and Claßen-Bockhoff 2002).

As in the Austrian bee-pollinated species, morphometric data were taken from both the flowers and the birds. In addition, we have started to reconstruct the process of pollen transfer by using beaks and heads of museum specimens of the hummingbirds. However, more field investigations are urgently needed to get more detailed insight into the interactions between hummingbirds and *Salvia* flowers.

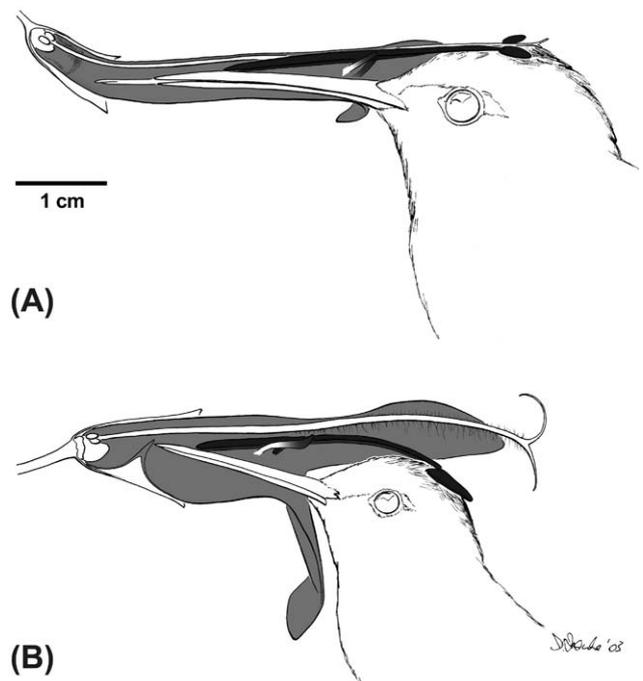These first results illustrate what can be learned from field investigations and why they are inevitably

Fig. 5. Hummingbird-pollinated *Salvia* species from Bolivia. (A) *S. haenkei* Benth. being visited by *Sappho sparganura* Shaw. (B) *S. orbignaei* Benth. being visited by *Chlorostilbon aureoventris* Orbigny and Lafresnaye. In *S. orbignaei* (B), pollen is transferred via the lever mechanism which in *S. haenkei* (A) cannot be moved due to lack of space in the flower; instead pollen is smeared onto the bird's head (from Wester and Claßen-Bockhoff 2002).

Fig. 6. Side-views of individual flowers of *S. haenkei* Benth. (top), *S. orbignaei* Benth. (bottom), and selected hybrids *S. haenkei × S. orbignaei*. Flowers of the parent species differ in several characters which show a continuum within the hybrid swarm (from Wester and Claßen-Bockhoff 2002).

necessary to elucidate the evolutionary significance of the lever mechanism in the genus *Salvia*. Even if the examples for mechanical isolation and hybridization presented above may not be causally responsible for the speciation processes in the species involved, they illustrate how isolation between co-occurring species may be effected or prevented. Our preliminary interpretation is that *Salvia*, despite its obviously highly derived lever mechanism and its specialization in bee or bird pollinators, exists in a balance between reproductive isolation and hybridization among species. It appears to be a reasonable hypothesis that minute changes in the proportions of floral and in particular staminal structures may have far-reaching consequences for the pollination mechanism and for adaptive radiation and speciation.

Trait utility

To test possible fitness advantages in *Salvia* due to the staminal lever mechanism, experimental approaches are currently in progress (Thimm et al. 2003; Speck et al. 2004). They include force measurements determining both the forces and energies needed to release the lever mechanism and those exerted by the pollinators, as well as simulations of pollen transfer processes.

To evaluate the evolutionary significance of staminal levers, we have to learn more about their intrinsic properties. A set of preliminary biomechanical measurements (Speck and Claßen-Bockhoff 2000; Speck et al. 2003) showed that forces ranging from 1.5 mN in *S. glutinosa* to 32 mN in *S. sclarea* are necessary to trigger the staminal levers and gain access to the nectary (Table 3). More detailed investigations in *S. pratensis*, including 55 flowers and more than 1000 individual measurements, revealed an average value of the maximum force of 2.98 ± 2.43 mN (Thimm et al. 2003). The high standard deviation found in this species results

Table 3. Results of the first force measurements in *Salvia* flowers

Species	Numbers of flowers (left) and measurements		Average maximum force per flower (mN) ± standard deviation	Proportion of upper to lower lever arm	Pollinators	Flower size (cm)
S. sclarea L.	1	2	31.83	4.3:1	Bees	2.5
S. africana-lutea L.	4	18	16.86 ± 4.11	5.4:1	Birds	4.0
S. dorsiana Stand.	1	3	15.88 ± 11.02	1.4:1	Birds	5.5
S. splendens	5	25	4.60 ± 0.50	0.7:1	Birds	4.5
S. patens Cav.	1	7	4.10 ± 0.20	4.7:1	Birds	5.0
S. pratensis L.	**55**	**1140**	**2.98 ± 2.43**	**2.8:1**	**Bees**	**2.0**
S. divinorum Epl. & Jativa	4	23	4.68 ± 1.23	2.0:1	Birds	3.0
S. glutinosa L.	**39**	**780**	**1.47 ± 1.05**	**1.6:1**	**Bees**	**3.0**

Only for two species (boldface) the number of measurements is high enough to present statistically confirmed data; the remaining data illustrate preliminarily the range of force values and possible correlations.

from both inter- and infra-individual variation, the within-species range is 2–12 mN. First records of the forces and energies that bumble-bees are able to produce when being guided past a barrier to an artificial food source show values of up to 90 mN for queens and 50 mN for workers (Speck et al. 2004). It is thus evident that the levers in *S. pratensis* do not represent strong barriers to bumble-bees. Field studies showing that the levers of *Salvia* species can be triggered by several different-sized bee species (see Table 2) also do not favor a narrow selection of species-specific pollinators by the force or energy required for triggering the lever. It seems more likely that the levers are designed for ease of triggering. This would allow a large range of pollinators while the species-specific transfer of pollen is achieved through the exact placement of the pollen on the animal's body. A quantitative test of this hypothesis is the subject of ongoing research.

To determine the fatigue properties of the lever and the precision of its movement during multiple releases, each flower was measured at least twenty times in succession (Fig. 7). The infra-individual values in *S. pratensis* varied by up to 2.8 mN, illustrating that the staminal tissue indeed may become fatigued and slack with age. However, the similarity between the repeated force–distance curves was remarkable in which the maximum required force gradually decreased and the initial force recorded got slightly delayed. Both of these result from the fact that the lever does not return to its exact initial position after being triggered. The repeatability of the lever action indicates that the lever mechanism may also act as a means for portioning pollen. In *S. pratensis*, 12–17 separate portions of pollen could be recorded (unpublished data), thus it is evident that the repeatability of the lever movement ensures the complete emptying of the pollen sacs. The nearly identical shapes of the force–distance curves may be interpreted as the result of high selection pressure on the precision of the lever movement.

An additional question relating to the mechanical measurements concerns the structural basis of the precise lever movement. As up to now no clear correlations have been found between forces, lever proportions, pollinators and flower sizes (Table 3), it is assumed that the differences in shapes of the force–distance curves between individual *Salvia* species are correlated with the functional morphology of the flowers and lever arms, an hypothesis that will be tested in
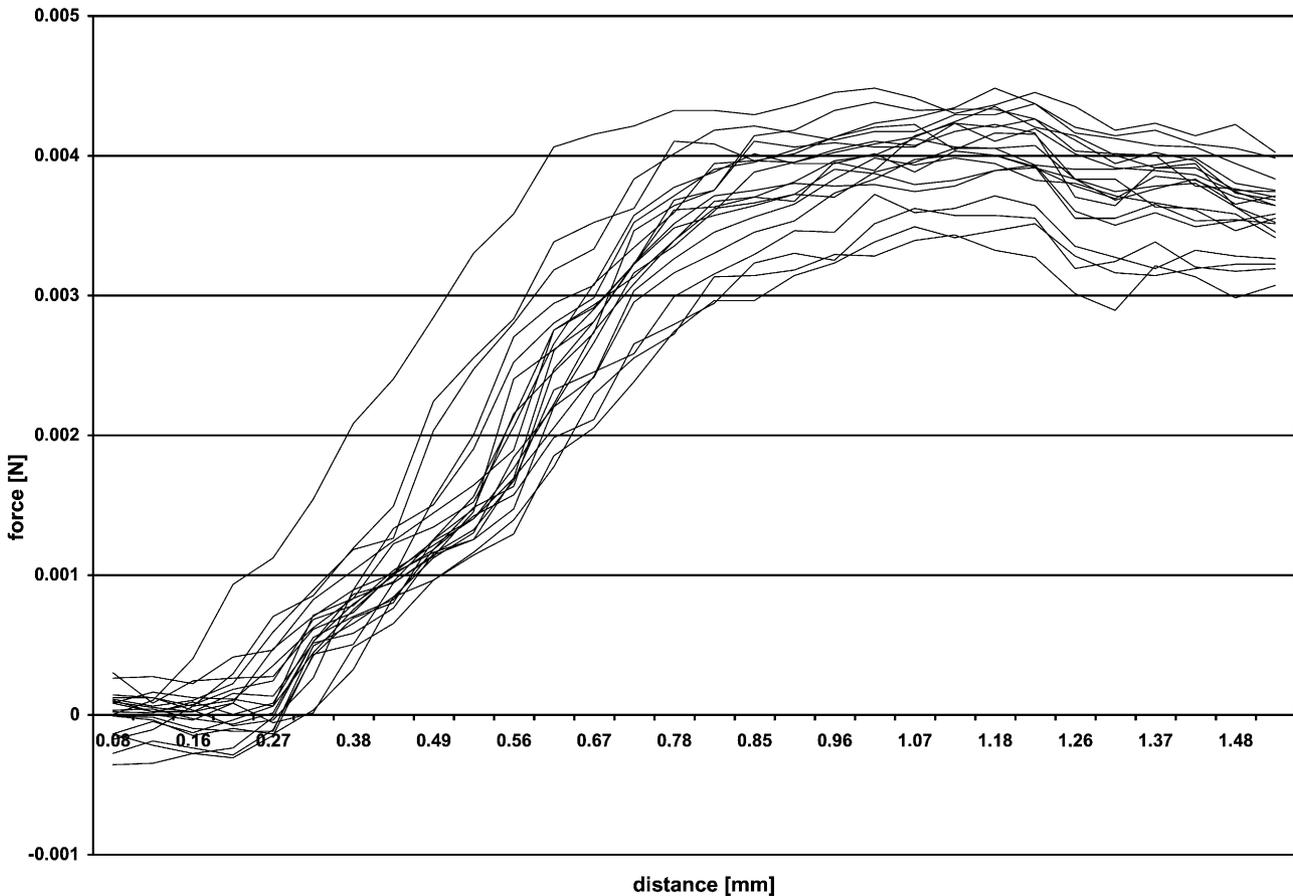

Fig. 7. Force–distance diagram for 20 artificially induced releases of the staminal lever in *S. pratensis* L. Note the congruence of the curves, indicating a high precision of pollen transfer via the reversible staminal lever movement (from Thimm et al. 2003).

ongoing research. Stamens vary considerably in size, shape and proportions (Fig. 3), but usually agree in having a thin, ribbon-like joint flanked by secondary formations of both the filament and the connective (Claßen-Bockhoff et al. in press). We assume that the lateral structures stabilize and guide the lever movement and thus increase its precision. Based on the early studies of Correns (1891) it is supposed that elastic cell walls and turgor pressure contribute to the lever movement.

Conclusions

Understanding the evolutionary significance of the staminal lever mechanism in *Salvia* still requires many more efforts in molecular phylogeny, functional morphology, biomechanics, ecology, observations and experiments regarding flower–pollinator interactions, and model-based theories. Based on our present knowledge we consider it reasonable to assume that the staminal lever mechanism and floral structures involved in the process of pollen transfer might constitute a key innovation for adaptive radiation and speciation within the group.

As for the hypotheses posed in the beginning: (a) it appears to be obvious that some visiting insects are excluded from access to nectar and the process of pollen transfer mainly by the size and shape of the flower and not by the physical force necessary to release the staminal lever. Our preliminary data indicate that in bumble-bee pollinated *Salvia* species it is rather easy for the pollinators to push back the sterile lever arm, and that the staminal levers themselves are constructed to be easily triggered.

(b) The staminal lever mechanism is likely to cause reproductive isolation between sympatric species because variation in lever arm length, shape and orientation can facilitate pollen deposition on certain domains of the pollinators' bodies while excluding others. As a consequence it is evident that minute changes of these proportions might have significant effects on the flower–pollinator interaction.

Regarding mechanical isolation, Grant (1994a) distinguished two types, characterizing the "*Salvia*-type" as sympatric species adapted to different pollinators, and the "*Pedicularis*-type" as sympatric species sharing the same pollinator(s). The first type is represented, for instance, by the nectar and oil spurs in *Aquilegia* (Hodges 1997), *Platanthera* (Nilsson 1988) and *Diascia* (Steiner and Whitehead 1990), which have been tested as representing key innovations. Examples for the second type are mentioned above. Co-occurring species of *Salvia* may belong to both types, by being adapted to bees and birds, respectively, as well as able to deposit

pollen species-specifically on the pollinator's head or body.

For *Salvia*, it may be speculated that the lever mechanism has a different meaning in bee- and bird-pollinated species. In Bolivia we always found bee- and bird-pollinated species in the same area, avoiding hybridization by being adapted to these different pollinator groups. In co-occurring bee-pollinated species, on the other hand, the need to occupy specific sectors of the 'pollination niches' (Armbruster et al. 1994, see below) might be much higher.

To test the hypothesis that reproductive interactions may generate assemblage structure and character displacement in plants, Armbruster et al. (1994) studied the assemblage structure of 31 *Stylidium* species at 25 sites in western Australia. In *Stylidium*, pollen is specifically transferred by an explosive mechanism caused by the touch-sensitive, fast-moving column of fused staminate and pistillate tissues. The authors defined 'pollination niches' by characterizing the length of the nectar tube, the position of the column, and the reach of the column in each *Stylidium* species and floral morph. Comparing their observations with the results of null models they indeed found evidence that assemblage structure has been generated, at least in part, by the evolution of morphologically distinct populations in areas where two species occur sympatrically. In *Salvia* it should be possible likewise to define 'pollination niches' by measuring the length and width of the flower tube (accessibility of nectar), the position of the staminal levers (dorsal, ventral, lateral pollination), and the length of the lever arms (site of pollen deposition). Comparable field investigations have to be conducted in species-rich areas as Central America or the Andes, and cannot be done in Central Europe, for instance.

Armbruster et al. (1994) also illustrated that the separation of mean pollen placement of two sympatric species by as little as 2 mm would lead to effectively segregated pollen flow. This observation encourages our efforts to analyze quantitatively the sites of pollen deposition on a pollinator's body as precisely as possible. We are aware that already very little gene flow may be sufficient to erase species boundaries, and that therefore more experimental studies in artificial systems and especially in the field will be necessary to test this hypothesis.

(c) Besides the significance of the staminal lever mechanism for reproductive isolation, it also contributes to the increase in male fitness and pollination success in general. The repeatability of the lever movement may ensure complete emptying of the pollen sacs and increase the rate of out-crossing due to pollen partitioning. The easy way of triggering allows many pollinators access to the nectar. In *S. pratensis* 13 bee species have been identified as effective pollinators, which shows that this species can use a large range of different-sized bees

as potential pollinators. If the staminal lever mechanism is a key innovation in *Salvia*, it obviously forces diversification and speciation less by specialization to different pollinators than by increasing the precision of pollen deposition on any potential pollinator. This is in accordance with the view of Waser et al. (1996) that pollination systems are more generalized and dynamic than previously suggested. Selection and gene flow vary in time and space, and the contribution of pollinators to reproductive isolation of plant species may be overstated. Despite the highly derived staminal lever mechanism and the general specialization to bees and birds, *Salvia* populations appear not to depend on specific pollinators. Instead, they are 'flexible' enough to change pollinators within the framework of their pollination niche according to the temporal and spatial variance in pollinator quality.

According to our first results the staminal proportions seem to enable species-specific pollen transfer with a minimum of pollen loss. Thus, both female and male reproductive functions are influenced by the staminal lever mechanism, with the rate of self-pollination and inbreeding depression being reduced by pollen partitioning, and the out-crossing rate being promoted through effective pollen dispersal.

Considering the staminal lever mechanism to be a key innovation in *Salvia*, the following scenario is conceivable showing the possible influence of the staminal lever mechanism on adaptive radiation and allopatric and sympatric speciation. Allopatric populations of a *Salvia* species become specialized for pollen deposition on different parts of the pollinator's body. If these populations come into secondary contact, the morphometric differences may provide a prezygotic reproductive isolation mechanism. However, as *Salvia* species are largely interfertile it may depend on the precision of the lever movement whether sympatric *Salvia* species are mechanically isolated from each other or able to hybridize. If the new hybrids are fertile and mechanically and/or ethologically isolated from their parental species (Grant 1994a; Ellis and Johnson 1999), sympatric speciation may result from hybridization. Hybrids occurring in nature are, however, rarely competitive if the new genotype competes for the same habitat as the parental plants. But if the conditions of the habitat change, for example, through the colonization of a new niche, then stable species may develop from the hybrid plant. Sympatric speciation may also follow spontaneous mutations influencing the flower–pollinator interaction. As a consequence, *Salvia* individuals may deposit pollen on a different part of the previous pollinator's body or even adapt to a new pollinator species, both resulting in reproductive isolation from the parental population.

As a preliminary view we conclude that the staminal lever mechanism may support adaptive radiation in *Salvia*. It may act as a key innovation (see Heard and Hauser 1995) increasing both individual fitness and ecological specialization. Future ecological and evolutionary studies should integrate studies of phylogeny, floral morphology, and pollination ecology. Particularly valuable will be experimental studies to estimate the demographic and selective consequences of interspecific pollen flow among naturally or artificially co-occurring species, and morphometric studies of inter- and infraspecific variation in floral characters. While studies in contemporary assemblages more likely reflect adaptation to the contemporary biotic environment rather than speciation, it would be of special interest to test recently diverged sister groups as to their speciation processes. Detailed molecular systematic studies are therefore desperately needed to evaluate the phylogenetic position of the different *Salvia* species.

Compared to the remaining Nepetoideae lacking the staminal lever mechanism, species of *Salvia* are able to adapt to their environment 'pollinator' in a more multifarious and precise way, resulting in the present high phenotypic diversity and species number. If *Salvia* is polyphyletic, many new interesting questions arise. In particular, patterns of parallel evolution should favor the hypothesis of the staminal lever mechanism being a key innovation for adaptive radiation.

Acknowledgements

References

Alziar, G., 1988–1993. Catalogue synonymique des *Salvia* L. du monde (Lamiaceae). I–VI. Biocosme Mesogéen 5, 87–136; 6, 79–115 + 163–204; 7, 59–109; 9, 413–497; 10, 33–117.

Armbruster, W.S., 1993. Evolution of plant pollination systems: hypotheses and tests with the neotropical vine *Dalechampia*. Evolution 47, 1480–1505.

Armbruster, W.S., Edwards, M.E., Debevec, E.M., 1994. Floral character displacement generates assemblage structure of western Australian triggerplants (*Stylidium*). Ecology 75, 315–329.

Baikova, E.V., 1999. Some trends of morphological evolution in the genus *Salvia* L. (Lamiaceae). 14th Symposium 'Biodiversität und Evolutionsbiologie', Jena (poster abstract).

Baldwin, B.G., 1997. Adaptive radiation of the Hawaiian silversword alliance: congruence and conflict of phylogenetic evidence from molecular and non-molecular investigations. In: Givnish, T.J., Sytsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, pp. 104–128.

Barrett, S.C.H., 2002. The evolution of plant sexual diversity. Nat. Rev. Genet. 3, 274–284.

Barrett, S.C.H., Graham, S.W., 1997. Adaptive radiation in the aquatic plant family Pontederiaceae: insights from phylogenetic analysis. In: Givnish, T.J., Sytsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, pp. 225–258.

Bateman, R.M., 1999a. Architectural radiations cannot be optimally interpreted without morphological and molecular phylogenies. In: Kurmann, M.H., Hemsley, A.R. (Eds.), The Evolution of Plant Architecture. Royal Botanic Gardens, Kew.

Bateman R, M., 1999b. Integrating molecular and morphological evidence of evolutionary radiations. In: Hollingsworth, P.M., Bateman, R.M., Gornall, R.J. (Eds.), Molecular Systematics and Plant Evolution. Taylor & Francis, London, pp. 432–471.

Brantjes, N.B.M., 1982. Pollen placement and reproductive isolation between two Brazilian *Polygala* species (Polygalaceae). Plant Syst. Evol. 141, 41–52.

Brieschke, H., 1991. Brutbiologische und nahrungsökologische Untersuchungen am kleinen Halsbandnektarvogel (*Panaeola chalybea*) und am Großen Halsbandnektarvogel (*Panaeola afra*) im Freiland (Südafrika) und im Labor. Unpubl. doctoral dissertation, University of Frankfurt, Germany.

Cantino, P.D., 1992. Evidence for a polyphyletic origin of the Labiatae. Ann. Missouri Bot. Gard. 79, 361–379.

Cantino, P.D., Sanders, W., 1986. Subfamilial classification of the Labiatae. Syst. Bot. 11, 163–185.

Cantino, P.D., Harley, R.M., Wagstaff, S.J., 1992. Genera of Labiatae: status and classification. In: Harley, R.M., Reynolds, T. (Eds.), Advances in Labiatae Science. Royal Botanic Gardens, Kew, pp. 511–522.

Carr, G.D., Robichaux, R.H., Witter, M.S., Kyhos, D.W., 1989. Adaptive radiation of the Hawaiian silversword alliance (Compositae–Madiinae): a comparison with Hawaiian picture-winged *Drosophila*. In: Giddings, L.V., Kaneshiro, K.Y., Anderson, W.W. (Eds.), Genetics, Speciation, and the Founder Principle. Oxford University Press, New York, pp. 79–97.

Chase, M.W., Palmer, J.D., 1997. Leapfrog radiation in floral and vegetative traits among twig epiphytes in the orchid subtribe Oncidiinae. In: Givnish, T.J., Sytsma, K. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, pp. 331–352.

Claßen-Bockhoff, R., Wester, P., Tweraser, E., 2003. The staminal lever mechanism in *Salvia* — a review. Plant Biol. 5, 33–41.

Claßen-Bockhoff, R., Crone, M., Baikova, E., 2004. Stamen ontogeny in *Salvia* L. — homology reinvestigated. Int. J. Plant Sci., in press.

Correns, C., 1891. Zur Biologie und Anatomie der Salvienblüthe. Pringsheims Jhb. Wiss. Bot. 22, 190–240.

Cox, G.W., 1981. Pollination ecology of the Cleveland Sage. Environ. Southw. 494, 15–19.

Dieringer, G., Ramamoorthy, T.P., Tenorio Lezama, P., 1991. Floral visitors and their behavior to sympatric *Salvia* species (Lamiaceae) in Mexico. Acta Bot. Mex. 13, 75–83.

Dressler, R.L., 1968. Pollination by euglossine bees. Evolution 22, 202–210.

Dressler, R.L., 1981. The Orchids. Natural History and Classification. Harvard University Press, Cambridge (Massachusetts) and London. (German edition: 1996. Die Orchideen. Bechtermünz, Augsburg.).

Ehleringer, J.R., Clark, C., 1988. Evolution and adaptation in *Encelia* (Asteraceae). In: Gottlieb, L.D., Jain, S.K. (Eds.), Plant Evolutionary Biology. Chapman & Hall, New York, pp. 221–248.

Ellis, A.G., Johnson, S.D., 1999. Do pollinators determine hybridization patterns in sympatric *Satyrium* (Orchidaceae) species? Plant Syst. Evol. 219, 137–150.

Epling, C., 1939. A revision of *Salvia*, subgenus *Calosphace*. Repert. Spec. Nov. Regni Veg. Beih. 110, 1–383.

Epling, C., 1947. Natural hybridization of *Salvia apiana* and *S. mellifera*. Evolution 1, 69–78.

Faegri, K., van der Pijl, L., 1979. The Principles of Pollination Ecology 3rd Edition. Pergamon Press, Oxford.

Francisco-Ortega, J., Crawford D, J., Santos-Guerra, A., Jansen, R.K., 1997. Origin and evolution of *Argyranthemum* (Asteraceae: Anthemideae) in Macaronesia. In: Givnish, T.J., Sytsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, pp. 406–431.

Gittenberger, E., 1991. What about non-adaptive radiation? Biol. J. Linn. Soc. 43, 263–272.

Givnish, T.J., 1997. Adaptive radiation and molecular systematics: issues and approaches. In: Givnish, T.J., Sytsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, pp. 1–54.

Givnish, T.J., Sytsma, K.J., 1997. Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge.

Givnish, T.J., Sytsma, K.J., Smith, J.F., Hahn, W.J., Benzing, D.H., Burkhardt, E.M., 1997. Molecular evolution and adaptive radiation in *Brocchinia* (Bromeliaceae:

Pitcairnioideae) atop tepuis of the Guayana Shield. In: Givnish, T.J., Sytsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, pp. 259–311.

Givnish, T.J., Evans, T.M., Zihra, M.L., Patterson, T.B., Berry, P.E., Sytsma, K.J., 2000. Molecular evolution, adaptive radiation, and geographic diversification in the amphiatlantic family Rapateaceae: evidence from *ndh*f sequences and morphology. Evolution 54, 1915–1937.

Graham, A., 1999. Studies in neotropical paleobotany. XIII. An Oligo-Miocene palynoflora from Simojovel (Chiapas, Mexico). Am. J. Bot. 86, 17–31.

Grant, K.A., Grant, V., 1964. Mechanical isolation of *Salvia apiana* and *Salvia mellifera* (Labiatae). Evolution 18, 196–212.

Grant, V., 1994a. Modes and origin of mechanical and ethological isolation in angiosperms. Proc. Natl. Acad. Sci. USA 91, 3–10.

Grant, V., 1994b. Mechanical and ethological isolation between *Pedicularis groenlandica* and *P. attollens* (Scrophulariaceae). Biol. Zentralbl. 113, 43–51.

Grinnell, J., 1917. The niche-relationships of the California Trasher. Auk 34, 427–433.

Hapeman, J.R., Inoue, K., 1997. Plant–pollinator interactions and floral radiation in *Platanthera* (Orchidaceae). In: Givnish, T.J., Sytsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, pp. 433–454.

Haque, M.S., Ghoshal, K.K., 1981. Floral biology and breeding system in the genus *Salvia* L. Proc. Ind. Natl. Sci. Acad. B 47, 716–724.

Heard, S.B., Hauser, D.L., 1995. Key evolutionary innovations and their ecological mechanisms. Histor. Biol. 10, 151–173.

Hildebrand, F., 1865. Über die Befruchtung der *Salvia*arten mit Hilfe von Insekten. Jb. Wiss. Bot. 4, 451–476.

Himmelbaur, W., Stibal, E.1934., 1932. Entwicklungsrichtungen in der Blütenregion der Gattung *Salvia* L.—eine phylogenetische Studie. Biol. Generalis 8, 449–474 tables 1 + 2; 9, 129–150, tables 8 + 9; 10, 17–48, tables 1 + 2.

Hodges, S.A., 1997. Rapid radiation due to a key innovation in columbines (Ranunculaceae: *Aquilegia*). In: Givnish, T.J., Sytsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, pp. 391–406.

Hodges, S.A., Arnold, M.L., 1995. Spurring plant diversification: are floral nectar spurs a key innovation? Proc. R. Soc. London, Ser. B, Biol. Sci. 262, 343–348.

Huck, R.B., 1992. Overview of pollination biology in the Lamiaceae. In: Harley, R.M., Reynolds, T. (Eds.), Advances in Labiatae Science. Royal Botanic Gardens, Kew, pp. 167–181.

Hunter, J.P., 1998. Key innovations and the ecology of macroevolution. Trends Ecol. Evol. 13, 31–36.

Kambysellis, M.P., Craddock, E.M., 1997. Ecological and reproductive shifts in the diversification of the endemic Hawaiian *Drosophila*. In: Givnish, T.J., Sytsma K, J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, pp. 475–509.

Kaufmann, M., 1994. Molekulare Systematik der Lippenblütler (Familie Labiatae). Unpubl. doctoral dissertation, University of Heidelberg, Germany.

Kerner von Marilaun, A., 1891. Pflanzenleben. Bd. 2. Geschichte der Pflanzen. Bibliographisches Institut, Leipzig and Wien.

Knuth, P.1905., 1898. Handbuch der Blütenbiologie. Engelmann, Leipzig.

Kwak, M.M., 1978. Pollination, hybridisation and ethological isolation of *Rhinanthus minor* and *R. serotinus* (Rhinanthoideae: Scrophulariaceae) by bumblebees (*Bombus* Latr.). Taxon 27, 145–158.

Levin, D.A., 1971. The origin of reproductive isolating mechanisms in flowering plants. Taxon 20, 91–113.

Mabberley, D.J., 1997. The Plant-Book 2nd Edition. Cambridge University Press, Cambridge.

Macior, L.W., 1982. Plant community and pollinator dynamics in the evolution of pollination mechanisms in *Pedicularis* (Scrophulariceae). In: Armstrong, J.A., Powell, J.M., Richards, A.J. (Eds.), Pollination and Evolution. Royal Botanic Gardens, Sydney, pp. 29–45.

Mayr, E., 1970. Populations, Species, and Evolution. Belknap Press, Cambridge, MA.

Meeuse, B., Morris, S., 1984. Blumen-Liebe. Sexualität und Entwicklung der Pflanzen. DuMont, Köln.

Miyajima, D., 2001. Floral variation and its effect on self-pollination in *Salvia splendens*. J. Hortic. Sci. Biotech. 76, 187–194.

Müller, J., 1981. Fossil pollen records of extant angiosperms. Bot. Rev. 47, 1–142.

Navarro, L., 1997. Is the dichogamy of *Salvia verbenaca* (Lamiaceae) an effective barrier to self-fertilization? Plant Syst. Evol. 207, 111–117.

Nilsson, L.A., 1988. The evolution of flowers with deep corolla tubes. Nature 334, 147–149.

Ouborg, N.J., Van Treuren, R., 1994. The significance of genetic erosion in the process of extinction. IV. Inbreeding load and heterosis in relation to population size in the mint *Salvia pratensis*. Evolution 48, 996–1008.

Ouborg, N.J., Van Treuren, R., 1995. Variation in fitness-related characters among small and large populations of *Salvia pratensis*. J. Ecol. 83, 369–380.

Owens, S.J., Ubera Jiménez, J.L., 1992. Breeding systems i n Labiatae. In: Harley, R.M., Reynolds, T. (Eds.), Advances in Labiate Science. Royal Botanic Gardens, Kew, pp. 257–280.

Palomino, G., Mercado, P., Ramamoorthy, T.P., 1986. Chromosomes of *Salvia* subgenus *Calosphace*, a preliminary report. Cytologia 51, 381–386.

Panero, J.L., Francisco-Ortega, J., Jansen, R.K., Santos-Guerra, A., 1999. Molecular evidence for multiple origins of woodiness and a New World biogeographic connection of the Macaronesian Island endemic *Pericallis* (Asteraceae: Senecionreae). Proc. Natl. Acad. Sci. USA 96, 13886–13891.

Percival, M.S., 1955. The presentation of pollen in certain angiosperms and its collection by *Apis mellifera*. New Phytol. 54, 353–368.

Ramamoorthy, T.P., Elliott, M., 1998. Lamiaceae de México: diversidad, distribución, endemismo y evolución. In:

Ramamoorthy, T.P., Bye, R., Lot, A., Fa, J. (Eds.), Diversidad Biológica de México: Orígenes y Distribución. Instituto de Biología, UNAM, México, pp. 501–525.

Read, E., 1983. Pollen dispersal and the pollination ecology of *Salvia carduacea* Benth. (Lamiaceae). Crossosoma 9, 1–13.

Rowe, N.P., Speck, T., 2004. Hydraulics and mechanics of plants: novelty, innovation and evolution. In: Hemsley, A.R., Poole, I. (Eds.), The Evolution of Plant Physiology. Academic Press, UK, pp. 297–326.

Sakai, A.K., Weller, S.G., Wagner, W.L., Soltis, P.S., Soltis, D.E., 1997. Phylogenetic perspective on the evolution of dioecy: adaptive radiation in the endemic Hawaiian genera *Schiedea* and *Alsinidendron* (Caryophyllaceae: Alsinoideae). In: Givnish, T.J., Sytsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, pp. 455–473.

Sazima, I., Buzato, S., Sazima, M., 1996. An assemblage of hummingbird-pollinated flowers in a montane forest in southeastern Brazil. Bot. Acta 109, 149–160.

Schluter, D., 2000. The Ecology of Adaptive Radiation. Oxford University Press, Oxford.

Schmucker, T., 1929. Blütenbiologische und morphologische Beobachtungen. Planta 9, 727–735.

Scott Elliott, G.F., 1890. Ornithophilous plants in South Africa. Ann. Bot. 4, 265–280.

Simpson, G.G., 1953. The Major Features of Evolution. Columbia University Press, New York.

Speck, T., Claßen-Bockhoff, R., 2000. Biomechanik und Funktionsmorphologie der Staubblätter von insektenbl-ütigen und vogelblütigen *Salvia*-Arten. Biona-Rep. 14, 153–157.

Speck, T., Claßen-Bockhoff, R., Reith, M., 2004. Biomechanics, functional morphology of the interaction between pollinators and the staminal levers: key characters for the adaptive radiation in the genus *Salvia*? Proceedings of the 4th International Plant Biomechanics Congress, Michigan State University, East Lansing, in press.

Speck, T., Rowe, N., Civeyrel, L., Claßen-Bockhoff, R., Neinhuis, C., Spatz, H.-C., 2003. The potential of plant biomechanics in functional biology and systematics. In: Stuessy, T.F., Mayer, V., Hörandl, E. (Eds.), Deep Morphology. Toward a Renaissance of Morphology in Plant Systematics. Koeltz Scientific Books, Königstein, Germany, pp. 241–271.

Sprengel, C.K., 1793. Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen. Vieweg, Berlin.

Stebbins, G.L., 1950. Variation and Evolution in Plants. Columbia Press, New York.

Stebbins, G.L., 1974. Flowering Plants: Evolution above the Species Level. Belknap Press, Cambridge, MA.

Steiner, K.E., Whitehead, V.B., 1990. Pollinator adaptation to oil-secreting flowers — *Rediviva* and *Diascia*. Evolution 44, 1701–1707.

Stiles, F.G., 1975. Ecology, flowering phenology, and hummingbird pollination of some Costa Rican *Heliconia* species. Ecology 56, 285–301.

Sytsma, K., Walker, J.B., 2003. *Salvia* Research Network. http://www.botany.wisc.edu/Salvia.

Thimm, S., Tweraser, E., Speck, T., Claßen-Bockhoff, R., 2003. Pollinators of *Salvia pratensis* L. are not excluded by physical force — evidence from biomechanical measurements and field investigations. Fourth Plant Biomechanics Conference, East Landing, Michigan State University, July 2003 (poster abstract).

Thomson, J.D., Wilson, P., Valenzuela, M., Malzone, M., 2000. Pollen presentation and pollination syndromes, with special reference to *Penstemon*. Plant Species Biol. 15, 11–29.

Trelease, W., 1881. The fertilisation of *Salvia splendens* by birds. Am. Nat. 15, 265–269.

Tweraser, E., Claßen-Bockhoff, R., 2002. *Hemigenia eutax-ioides* C.R.P. Andr. — the inverse *Salvia* flower from Australia. Botanikertagung 2002, Freiburg (poster abstract).

Tweraser, E., Weiniger-Höllrigl, S., 2001. Isolating mechanisms among six sympatric *Salvia* species in eastern Austria. 15th Symposium Biodiversität und Evolutionsbiologie, Bochum (poster abstract).

Visco, F.J., Capon, B., 1970. Pollination mechanisms in three species of *Salvia* native to southern California. Aliso 7, 231–242.

Wagstaff, S.J., Olmstead, R.G., Cantino, P.D., 1995. Parsimony analysis of cpDNA restriction site varition in subfamily Nepetoideae (Labiatae). Am. J. Bot. 82, 886–892.

Wagstaff, S.J., Hickerson, L., Spangler, R., Reeves, P.A., Olmstead, R.G., 1998. Phylogeny in Labiatae sl, inferred from cpDNA sequences. Plant Syst. Evol. 209, 265–274.

Walker, J.B., Sytsma, K.J., Wink, M., 2002. *Salvia* (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and subf. Nepetoideae. Botany 2002 symposium, Madison, Wisconsin (poster abstract).

Waser, N.M., Chittka, L., Price, M.V., Williams, N.M., Ollerton, J., 1996. Generalization in pollination systems, and why it matters. Ecology 777, 1043–1060.

Wester, P., Claßen-Bockhoff, R., 2002. *Salvia haenkei* Benth. and *S. orbignaei* Benth. — two ornithophilous species from Bolivia and their hybrids. Botanikertagung 2002, Freiburg (poster abstract; publication in prep.).