



Evolution of anther connective teeth in sages (*Salvia*, Lamiaceae) under bee and hummingbird pollination

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ABSTRACT

Salvia (Lamiaceae) is a sub-cosmopolitan genus of about 1000 species that often employ a “staminal lever mechanism” that is thought to have spurred species diversification within the genus. The function and evolution of ventral outgrowths or connective teeth, associated with the lever mechanism in some species of *Salvia*, is often unclear despite the wealth of pollination observations across the genus and the major role that pollinators play in driving diversification within the genus. We document the role of these teeth in pollination studies of bee-pollinated *Salvia farinacea*, examine connective teeth across other bee- and hummingbird-pollinated species of New World subg. *Calospatha*, and provide an evolutionary scenario for the connective teeth in context of the staminal lever. Our observations show that the larger teeth function as pressure points at the floral entrance and, when pressed by a pollinator, facilitate movement of the lever. Multiple shifts in pollinator within subg. *Calospatha* may have resulted in further modifications of the connective and possible further losses and independent origins of connective teeth in *Calospatha*. Other distantly related subgenera (e.g., *Sclarea*, *Glutinaria*) display morphologically and spatially different protuberances on the connective, suggesting that connective teeth only evolved near the base of subg. *Calospatha*.

1. Introduction

The sage genus (*Salvia* L. s. l., Drew et al., 2017) exhibits one of the most dramatic species radiations in plants, evolving ca. 32 mya in southwestern Asia and subsequently spreading to and diversifying within most continental regions of the world (Will and Claßen-Bockhoff, 2017; Kriebel et al., 2019). During this diversification, *Salvia* has radiated into different biomes (Kriebel et al., 2019), adapted to both bee (Ott et al., 2016; Celep et al., 2014, 2020; Ajani et al., 2022; Xiao et al., 2022) and bird pollinators (Wester and Claßen-Bockhoff, 2006, 2007, 2011; Cairampoma et al., 2020), and evolved a suite of correlated floral morphologies in response to pollinators (Kriebel et al., 2019, 2020, 2021, 2022). Perhaps the most unique of these traits is the staminal lever mechanism present in most of ~1000 species of the genus (Fig. 1). The lever is formed by two stamens and facilitated by elongated connective tissue that can be either free from the connective of the adjacent second stamen or adhered to it. The lever-like stamens vary in the degree of postgenital fusion between the two connectives, as well as in their shape,

from strongly curved to straight (Kriebel et al., 2020, 2021). In addition, each stamen can have two fertile thecae or show modifications of the posterior thecae into a completely sterile, enlarged paddle or spoon shaped structure that blocks access to the nectar reward: in extreme cases the lower lever arm is almost completely reduced. The pioneering studies of Himmelbauer and Stibal (1932–1934) defined different types of stamens and postulated possible paths of the staminal lever evolution in *Salvia* (Fig. 2).

The staminal lever mechanism ostensibly has evolved independently several times within *Salvia* (Walker et al., 2004; Walker and Sytsma, 2007; Will and Claßen-Bockhoff, 2014; Ajani et al., 2022) based on morphological differences in levers across the genus (Claßen-Bockhoff, 2017) and the phylogenetic inclusion (Drew et al., 2017; Kriebel et al., 2019; Rose et al., 2021) of five small subgenera (former genera *Dorystaechas* Boiss. & Heldr., *Meriandra* Benth., *Perovskia* Kar., *Rosmarinus* L., and *Zhumeria* Rech.f. & Wendelbo) with slightly swollen (Fig. 2n, o, p, r) or elongated connectives (Fig. 2q), but no lever mechanism. Pollination biology studies demonstrated that the lever mechanism is

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lost, at least functionally, in many hummingbird- pollinated species of New World (NW) subg. *Calosphace* (Wester and Claßen-Bockhoff, 2006, 2007, 2011). The aforementioned loss of the levering mechanism occurs by modifications of the joint or corolla shape whereas the basic lever construction is usually maintained, retaining the lever construction (see Wester and Claßen-Bockhoff, 2007).

These results prompted in depth morphological studies of floral development related to the staminal levers and the reinterpretation of older studies considering the new molecular phylogenetic framework for *Salvia* (Claßen-Bockhoff et al., 2004). Key to these studies was the determination of which components of the different levers are homologous and what are their functions. Perhaps most perplexing are the

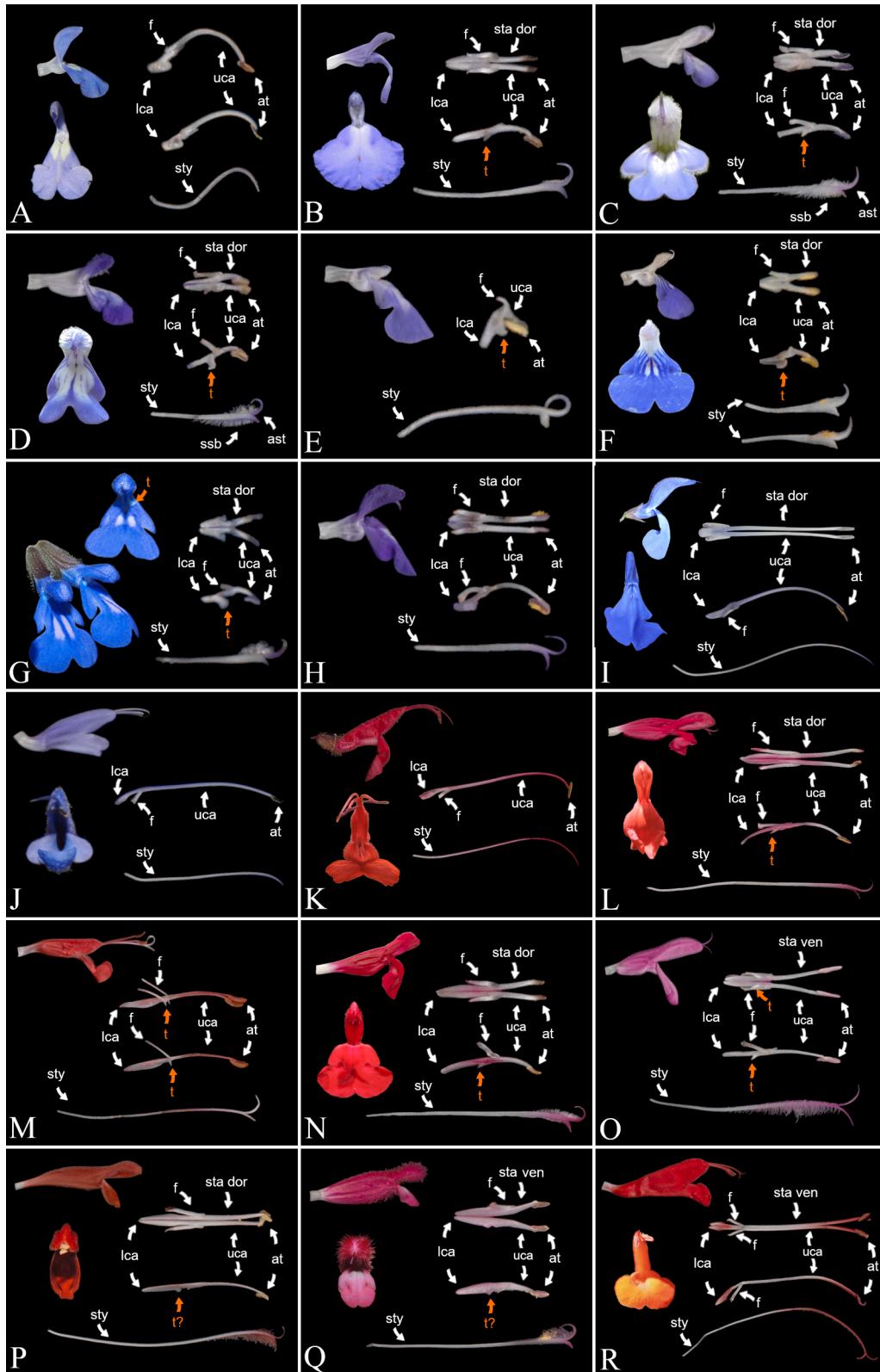


Fig. 1. Flowers and floral structures in *Salvia* subg. *Calosphace*. (A-I) Bee-pollinated species. Hastatae clade. (A) Lacking evident teeth: *S. costaricensis*. Core *Calosphace* clade. (B-C) Acute tooth: *S. chamaedryoides* and *S. xanthophylla*. (D-E) Square tooth: *S. rypara* and *S. compositachys*, the latter species with abruptly curved upper connective arm. (F-G). Geniculate projection: *S. procurrens* and *S. sinaloensis*. (H) Lacking evident teeth: *S. cuspidata*. (I-R) Bird-pollinated species. Hastatae clade. (I-K) Lacking evident teeth: *S. patens*, *S. cacaliifolia*, and *S. rubriflora*. Core *Calosphace* clade. (L-O) Acute tooth: *S. darcyi*, *S. exserta*, *S. microphylla*, and *S. orbignaei*. (P-Q) Small tooth: *S. lineata* and *S. oxyphora*. (R) Without tooth: *S. pauciserrata*. In all figures, the corollas are not to scale, but the stamens are to scale with respect to the styles of their own species. Acronyms: ast=asymmetric stigma with longer upper lobe; at=thecae; f=filament; lca=lower connective arm; ssb=sub stigmatic brush; sta=dorsal view; sta dor=stamen, dorsal view; sty=style; t=connective tooth; uca=upper connective arm.

toothlike structures near the abaxial side of the joint in staminal connectives (Figs. 1, 2) seen in many bee and hummingbird-pollinated members of the NW subg. *Calosphace* (Hildebrand, 1865; Claßen-Bockhoff et al., 2004; Wester and Claßen-Bockhoff et al., 2011). Claßen-Bockhoff et al. (2004) confirmed the view of Himmelbauer and Stibál (1932-1934), that these toothlike structures were of analogous origin, representing either ventral outgrowths of the lower connective arms, referred to as teeth in this study, or the reduced thecae of geniculate connectives (Fig. 2: 3***, 3°, 3°°). Hildebrand (1865) hypothesized in the context of bee-pollinated members of subg. *Calosphace* that these structures may function in pollen transfer precision by guiding an insect's proboscis to the correct position. Teeth (and other connective structures such as furrows or outgrowths) have also been hypothesized to increase the mechanical stabilization of the lever in bee-pollinated species of subg. *Glutinaria* and *Sclarea* (Claßen-Bockhoff et al., 2004), but no experimental tests of these or other hypotheses have been performed. In bird-pollinated species of subg. *Calosphace*, the connective teeth are either arranged laterally or in a distal position not forming a barrier in the flower entrance (Wester and Claßen-Bockhoff, 2011). The function of these teeth has left researchers puzzled and demand experimental tests.

Most studies examining bee pollination in *Salvia* come from species in Old World (OW) subgenera (Ohashi et al., 2002; Claßen-Bockhoff et al., 2003; Zhang et al., 2011; Celep et al., 2014, 2020; Huang et al., 2015; Claßen-Bockhoff, 2017; Reith et al., 2006; Senol et al., 2017; Xiao et al., 2022). Only a few come from the largest subgenus with ca. 580

species - NW subg. *Calosphace* (Hedström 1985; González et al., 2006; Strelin et al., 2017; Cairampoma et al., 2020, 2022; Barrionuevo et al., 2021). Thus, it remains unclear within subg. *Calosphace* how the various components of the flower and its specialized staminal lever, including the connective teeth, interact with the visiting bee's morphology. Here, we first contribute novel observations of bee visitation to *Salvia farinacea* Benth. (subg. *Calosphace*) to discuss the relationship between the lever mechanism with its 'seesaw' function mentioned in some species (Correns, 1891; Ohashi, 2002; Reith et al., 2007; Thimm, 2008), as well as the possible role of the connective teeth in pollination. Second, we survey the morphology of connective teeth across Mexican clades of subg. *Calosphace* that possess both bee and hummingbird pollination. Third, we propose why the distinctive connective teeth are similar between bee-pollinated species of subg. *Calosphace* and their hummingbird-pollinated relatives.

2. Material and methods

2.1. Observations of the function of anther teeth in bee-pollinated *Salvia farinacea*

Salvia farinacea (mealy blue sage) is a commonly cultivated species native to the south-central United States and northern Mexico (Peterson, 1978). It has small purple flowers with a short galea and a long lower lip that serves as a landing pad for bees. It is similar to many other, but not all, bee-pollinated species of subg. *Calosphace* in its overall

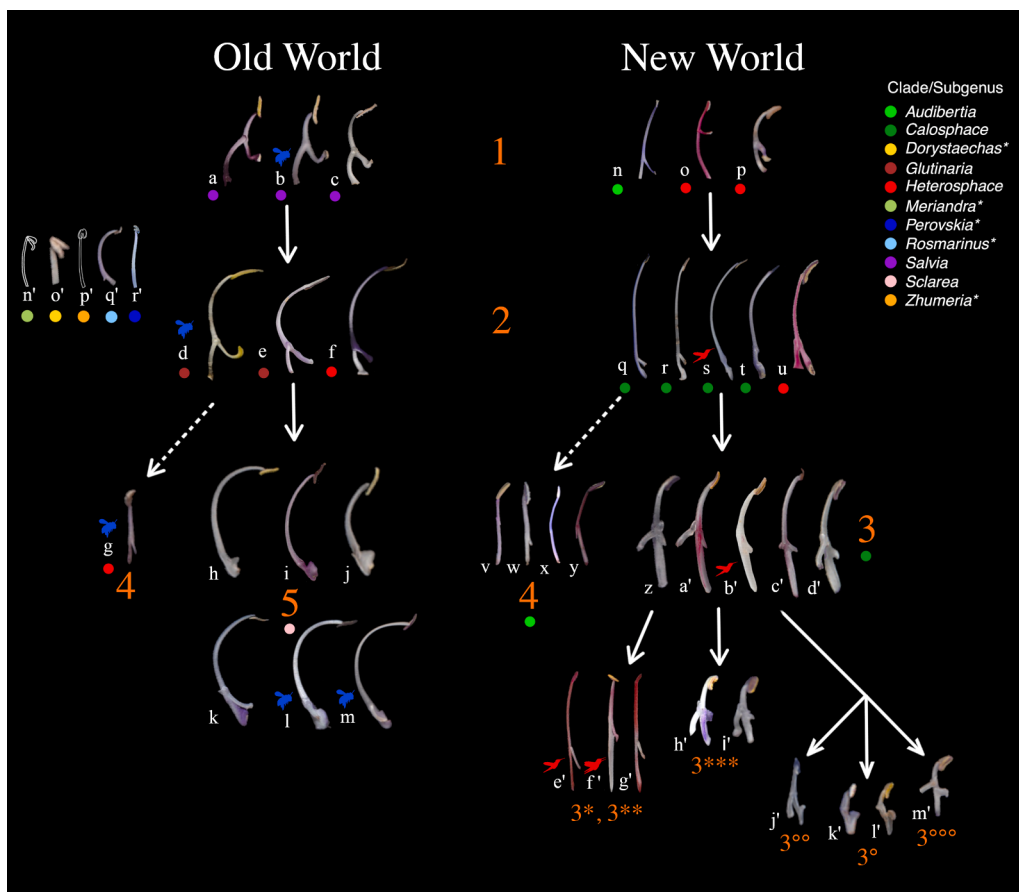


Fig. 2. Diagram of hypothetical pathways of morphological stamen evolution within geographical areas (modified from Himmelbauer and Stibál, 1932-1934). Stamens used as examples come from scans of live material and species were chosen based on those listed in the stamen types assigned by the original authors. Clade names are based on Drew et al. (2017) and Kriebel et al. (2019). Monotypic or small genera found to be nested and now considered in *Salvia* s.l. are from the Old World and added to the left of the diagram to illustrate the total variation in the genus. The bee and bird symbols are a few of the species cited by Himmelbauer and Stibál (1932-1934) for which pollinator information was available at the time. 3*–3*** and 3°–3°° indicate stamen types distinguished by the authors. Stamens not at same scale. Example species: (a) *Salvia hydrangea*, (b) *S. officinalis*, (c) *S. potentillifolia*, (d) *S. glutinosa*, (e) *S. miltiorrhiza*, (f) *S. forskohlei*, (g) *S. verticillata*, (h) *S. aethiopis*, (i) *S. canariensis*, (j) *S. frigida*, (k) *S. phlomoides*, (l) *S. pratensis*, (m) *S. sclarea*, (n) *S. californica*, (o) *S. roemeriana*, (p) *S. texana*, (q) *S. cacialifolia*, (r) *S. macrophylla*, (s) *S. patens*, (t) *S. scutellarioides*, (u) *S. pentstemonoides*, (v) *S. leucophylla*, (w) *S. mellifera*, (x) *S. sonomensis*, (y) *S. spathacea*, (z) *S. coahuilensis*, (a') *S. greggii*, (b') *S. microphylla* (instead of *S. pulchella* of sect. *Fulgentes* which was lacking), (c') *S. purpurea*, (d') *S. roscida*, (e') *S. coccinea*, (f') *S. elegans*, (g') *S. haenkei*, (h') *S. roscida*, (h') *S. farinacea*, (i') *S. ovalifolia*, (j') *S. subincisa*, (k') *S. glechomaefolia*, (l') *S. procurrens*, (m') *S. rypara*, (n') *S. bengalensis*, (o') *S. dorystaechas*, (p') *S. majdae*, (q') *S. rosmarinus*, (r') *S. yangii*. All stamens from scans of live flowers except (n') and (p') which were illustrated after Bokhari and Hedge (1971, 1976).

corolla morphology, with a straight style that is hairy below the stigma, a stigma with a longer upper lobe, and lower connective arms that are postgenitally fused for most of their length and possessing a conspicuous tooth near the middle of the connective (Fig. 3A–B). These peculiar connectives, in which the lower arm is about as long and straight as the upper arm and where the two lower arms of the adjacent stamens are united into a paddle, are frequently called “rudders” (Epling, 1939; Peterson, 1978; Wood and Harley, 1989; Wood, 2007) in reference to the overall shape of the fused extensions of the anther connective.

Previous studies have shown that bumble bees are the main pollinators of *Salvia farinacea* and its close relatives in sect. *Farinaceae*

(Peterson, 1978). Peterson (1978) described such a bee visit: “Bumblebees grasp the lower lip of the corolla with their feet, sometimes even bending the abdomen and rear legs underneath a flower, where the legs grasp the underside of the lower lip. The head and mouthparts are inserted under the galea, and only the mouthparts are inserted into the corolla tube. The mouthparts push the stamen rudder upward and cause the upper, fertile two anther thecae to be depressed on the bee’s frons”. However, the role of the connective teeth has not been discussed.

During July 2021 in Madison, Wisconsin, USA, we observed females of the common eastern bumble bee (*Bombus impatiens*), the two spotted long-horned bee (*Melissodes bimaculatus*), the European wool carder bee

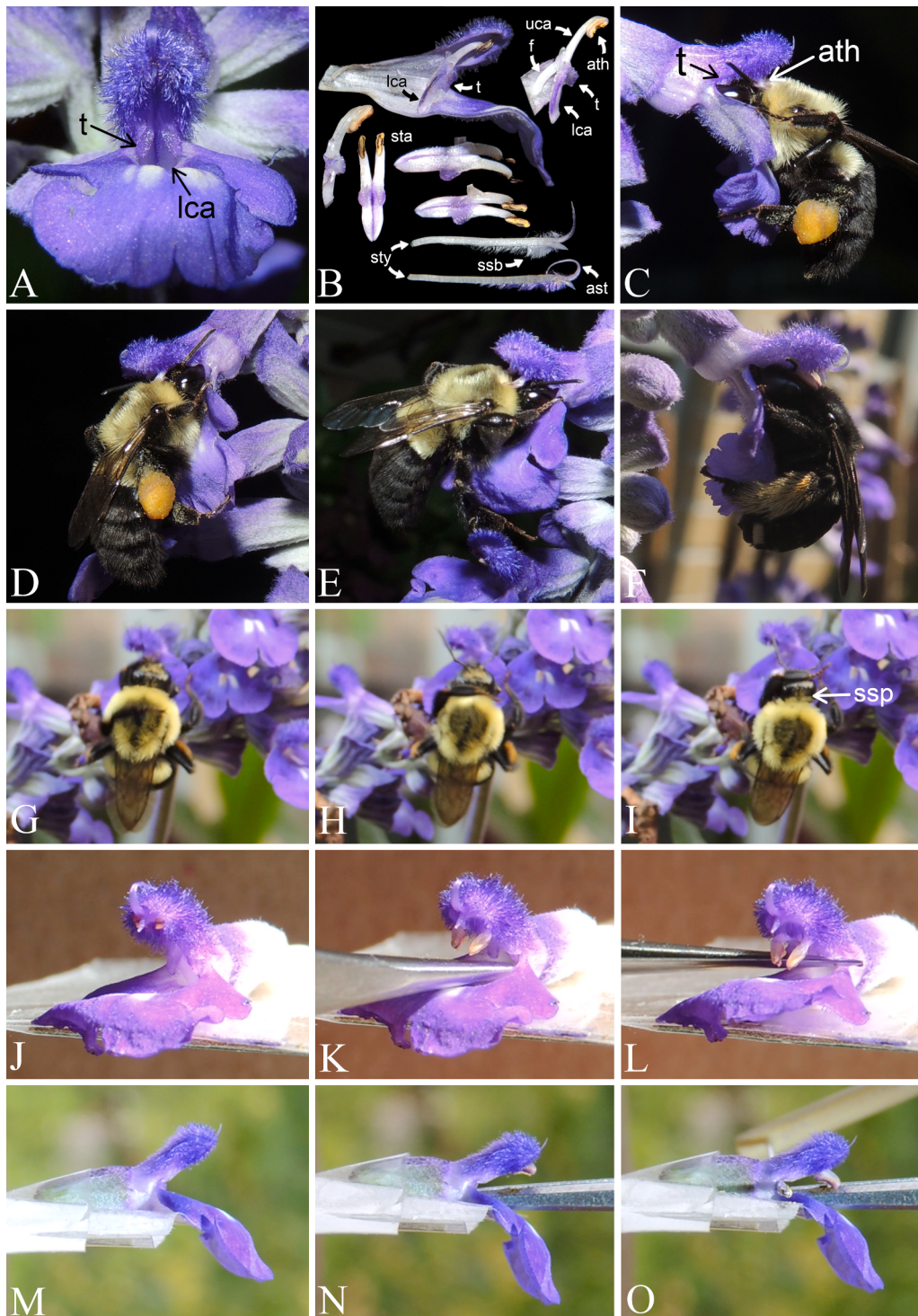


Fig. 3. Floral morphology and bee visitation to *Salvia farinacea*. (A) Front view of the corolla the entrance being almost completely closed by the connective teeth. (B) Dissection of the corolla and various views of the stamens and style. (C–E) *Bombus impatiens* and (F) *Melissodes bimaculatus* visiting flowers. (G–I) *Bombus impatiens* grooming pollen from its head. (J) Flower placed on adhesive tape in diagonal view with the anthers, lever, connective teeth and stigma visible. (K) Forceps pressing the lower connective arms resulting in their movement downwards. (L) Forceps pressing the connective teeth resulting in the lever moving downwards. (M) Flower placed on adhesive tape in side-view. (N) Forceps pressing the lower connective arms, resulting in their movement downwards. (O) Forceps kept pressing the lever with the addition of a needle pressing the teeth making them curve slightly downwards. Acronyms: ast=asymmetric stigma with longer upper lobe; ath= thecae; f=filament; lca=lower connective arm; ssp=hypothetical pollen safe spot; sta=stamens in different views; sty=style; ssb=sub stigmatic brush; t=connective tooth; uca=upper connective arm.

(*Anthidium manicatum*), and the European honeybee (*Apis mellifera*) visiting flowers of cultivated specimens of *Salvia farinacea* (Fig. 3C–F). To document interactions between the bees and the flower and test the role of the connective teeth during bee visitations, we filmed and photographed them. Flowers were also brought into the lab and placed on adhesive tape to film the movement of the lever and test the effects of pressuring the lever and the teeth with forceps. To investigate what would happen when the staminal lever and teeth were activated in concert, we applied pressure to both the lever and teeth simultaneously. A pair of forceps was taped together and affixed to a hard drive which was used as a base. The taped forceps on the base were then moved straight into the corolla, activating the staminal lever without touching the teeth. Then, these forceps were left in place (holding the staminal lever in a lowered position) and the teeth were manually pressed backwards with a pin applied horizontally at the entrance of the corolla (Fig. 3O; Video 1–5).

To compare the morphology of the connective teeth of *Salvia farinacea* to other species from subg. *Calosphace*, we used scans of stamens and styles from live flowers (e.g., Kriebel et al., 2021, 2022) as well as dissections available in the literature.

2.2. Survey of connective teeth in subg. *Calosphace* from western Mexico

Although connective teeth are ostensibly common in subg. *Calosphace* (and lacking apparently in other subgenera), their frequency and morphological variability remain unclear (Claßen-Bockhoff et al., 2004). To help fill this void, we surveyed taxonomic descriptions of 105 species distributed in 37 sections (with six species not assigned to section) included in the treatment of the mints of western Mexico (González-Gallegos et al., 2016), these species being representative of a wide diversity of clades within subg. *Calosphace* (González-Gallegos et al., 2020). As these descriptions distinguish between teeth and the presence of a geniculation (sharply bent structure) in the connective, we surveyed the species for both features. These two modifications of the connective can be achieved independently (Baikova, 2002; Claßen-Bockhoff et al., 2004; Claßen-Bockhoff, 2017). Species were also scored for the known or presumed pollinator (when available) from an exhaustive list in the literature (Wester and Claßen-Bockhoff, 2011; further references in Kriebel et al., 2020) to determine if there was a relationship between the presence of a tooth and pollination processes.

3. Results

3.1. Pollination of *Salvia farinacea* and the role of the connective teeth

3.1.1. Visitors of *Salvia farinacea* and activation of the staminal lever

The two non-native bee species, the European wool carder bee and the European honeybee, were rare visitors to flowers of *S. farinacea*. The honeybee specifically was unable to activate the lever and access the nectar due to its body proportions relative to the floral construction. Two native bee species, the eastern bumble bee and the long-horned bee, also visited *S. farinacea*. The most common visitor each day was the common eastern bumble bee. The bumble bees flew from one inflorescence to the next, landed on lower (proximal) flowers and moved upwards before flying to the next inflorescence. When landing on the broad lower lip, the bees held on with one or more pairs of legs, causing the pedicel to bend downwards ~45°. They then inserted their heads in between the galea (upper lip) and the lower lip, passed by the stigma, which is held below the tip of the galea, potentially deposited foreign pollen on the stigma and entered the tube with their mouthparts to about where their eyes start (Fig. 3C–F; Videos 6–10). It is in this region of the floral tube, where the mouthparts enter, that the two connective teeth are positioned. The teeth contacted the lower part of the bee's face and at about the same time the lever was observed pressed against the back of the bee's head (Fig. 3C–F). Before leaving the flowers, bumble bees were frequently seen grooming pollen from their heads (Fig. 3G–I; Videos

11–13). The long-horned bee visited the flowers in a similar way but only one individual was seen on the days of observation. Although bumble bees and the long-horned bee accessed the nectar reward and are likely pollinators, the larger number of bumble bee individuals and visits and their perfect body fit to the floral parts support the view that bumble bees are the main pollinators of this species.

3.1.2. Experimental lever activation of flowers of *Salvia farinacea* using forceps

In the lab, forceps were inserted into flowers and pressed against connectives and/or connective teeth to record staminal lever activation. Pressing only the lower connective arms on the lever with forceps activated the lever, causing the upper connective arm with the fertile thecae to lower; this lever activation also occurred while pressing both teeth at the level of the mouth while not touching the lever proper (Fig. 3J–L; Videos 1–2). Pressing both the lever and teeth resulted in an initial lowering of the lever followed by additional lowering and additional curvature when pressing the connective teeth (Fig. 3M–O; Videos 3–5).

3.2. A survey of connective teeth in subg. *Calosphace* from Western Mexico

The survey of 105 species (and 4 varieties which are not included in the numbers below) of *Salvia* from western Mexico revealed that the vast majority had a tooth (80 spp., ~76%), often acute but also of different shapes, seven species (~7%) had just an anther geniculation, and an additional three species (~3%) had both a geniculation plus a tooth. Nine species (~9%) were variable in terms of presence or absence of teeth/geniculations, and six species (~6%) lacked teeth and/or geniculations altogether (Appendix 1). Eighty of the 105 species have been clearly assigned to a pollinator syndrome, whereas six species were not clearly assignable; no pollinator data exist about the remaining species (Wester and Claßen-Bockhoff 2011). Of the 80 species with assigned pollinator information, 61 (76%) were bee-pollinated, and 19 (24%) bird-pollinated. Most bee-pollinated species showed the presence of a tooth on the connective (50/61). Of the remaining 11 bee-pollinated species, six had just a geniculation, two were variable for the presence of teeth geniculations, and three lacked teeth and geniculations altogether. Two species had both teeth and geniculations. The same pattern was seen in the bird-pollinated species, with the majority having teeth (12/19). Of the remaining seven bird-pollinated species, six were variable for the presence of teeth and one lacked teeth/geniculations altogether. A geniculation in the connective was only observed in bee-pollinated species, never in bird-pollinated ones.

4. Discussion

4.1. Connective teeth function to assist the staminal lever mechanism

The evolution of connective teeth, their function, and morphological variation have puzzled sage researchers who nevertheless provided detailed analyses of the unusual teeth in *Salvia* subg. *Calosphace* (Hildebrand, 1865; Himmelbaur and Stibal, 1932–1934; Claßen-Bockhoff et al., 2004; Claßen-Bockhoff, 2017). Diversity in teeth morphology and spatial arrangement is quite broad, ranging from minute ventral teeth, laterally arranged teeth, teeth in proximal or distal position, teeth forming a barrier in the flower entrance. Further barriers result from analogously formed connective geniculations (Fig. 1). We suggest that the connective teeth forming a barrier at the corolla tube entrance, like those described here in *Salvia farinacea*, function as additional pressure points for the efficient movement of the staminal lever and its “seesaw” movement. The lever mechanism has been less commonly called a seesaw mechanism, recognizing the mobility afforded by the joint formed between the anther connective and the filament (Correns, 1891; Ohashi, 2002; Reith et al., 2007; Thimm, 2008). In particular, the

seesaw analogy has been mentioned in the OW species *Salvia nipponica* Miq. of subg. *Glutinaria* and *S. pratensis* L. of subg. *Sclarea*. Notably, levers are common within the species rich subg. *Calosphace*, but the seesaw analogy has not been used previously.

We hypothesize that most connective teeth in subg. *Calosphace* are involved with facilitating lever movement. Our observations of bumble bee visitation on *Salvia farinacea* suggest that the corollas, levers, and their connective teeth may function similarly in *S. rypara* and many other species of subg. *Calosphace* by providing an additional pair of pressure points against the bee's face to ensure effective levering. Measurements provided by Peterson (1978) and summarized here (Fig. 4) demonstrate that the corolla tube of *S. farinacea* and close relatives (*S. azurea* Michx. ex Lam. and *S. reptans* Jacq.) are longer than their bee pollinators tongues, which would force bees to enter the tube briefly with their face to reach the nectar, pressing along the way the connective teeth as we report here in *S. farinacea*. In addition, studies of the nectary in *S. farinacea* showed that nectar never moves from the base of the corolla to the gullet area in the middle of the tube (Zhang et al., 2014), emphasizing the importance of the length of the tongue plus the additional distance needed with the face to reach the nectar. In analogous fashion, the same function is provided by geniculated stamens in subg. *Calosphace* and the curved stamens in OW *Salvia* species in which the lower connective itself forms the barrier (Claßen-Bockhoff 2017). In *Salvia pratensis* of Old World subg. *Sclarea*, detailed functional morphological studies demonstrate from a biomechanical standpoint the different forces applied by a bees' head when touching different areas of the posterior arms of the lever (Reith et al., 2006, 2007). It is evident that barriers closing the flower entrance evolved several times independently in different subgenera of OW and NW *Salvia*, assisting bees to lower the pollen-sacs.

These observations are compatible with the hypotheses that the connective teeth may contribute to higher precision and stabilization of the lever, and thus facilitate pollination. These hypotheses are also compatible with the observation that some hummingbird-pollinated species which have lost the lever function also lack connective teeth (e.g., *Salvia longistyla* in our sample and others such as *S. haenkei*; Wester and Claßen-Bockhoff, 2006). Lastly, in *S. exserta*, a hummingbird-pollinated species with a stiff joint and without connective teeth, the seesaw mechanism operates with the filaments elastically moving the connective alone (Wester and Claßen-Bockhoff, 2007).

Our observations of bumblebees as the likely pollinators of *Salvia farinacea* and their great fit to the corolla of this species are consistent with a study on *S. assurgens* Kunth, also of subg. *Calosphace*, which found that the most likely pollinators among several visiting bees were two

species of *Bombus* (Cultid-Medina et al., 2021 and videos therein). That study also found that pollen was placed on the back of the bees' head, an area they could not easily groom, as we have shown with the pollinators of *S. farinacea*. These areas on the pollinators have been called "safe sites" and are important in pollination because they protect pollen from being groomed and are available for fertilizing conspecific ovules (Koch et al., 2017). Thus, selection for different functions of the connective teeth, such as assistance in allowing the anthers to reach farther around the bees' head, may improve placing pollen in such safe spots.

4.2. Staminal lever and connective teeth diversity and evolution in subg. *Calosphace*

Subg. *Calosphace* displays great diversity in staminal lever and connective traits across its ca. 580 recognized species (Figs. 1, 2). The evolution of these traits is becoming clearer as the phylogenetic relationships within subg. *Calosphace* are crystallizing (Jenks et al., 2013; Drew et al., 2017; Fragoso-Martínez et al., 2018; Kriebel et al., 2019; Rose et al., 2021). The rarest form is the presence of two fertile thecae in each anther and is only found in *S. axillaris* Moc. & Sessé ex Benth. (stamen Type I of Himmelbaur and Stibal, 1932-1934). This species is sister to a small clade of 20 species, the Hastatae clade, based on nuclear genes (Drew et al., 2017; Kriebel et al., 2019; Lara-Cabrera et al., 2021; Rose et al., 2021). Although plastome sequences place *S. axillaris* sister to all 580 species of subg. *Calosphace* (e.g., Jenks et al., 2013; Fragoso-Martínez et al., 2018; Lara-Cabrera et al., 2021; Rose et al., 2021), this relationship is likely due to cpDNA capture (Rose et al., 2021). The Hastatae clade (with *S. axillaris*) then is sister to the remainder of the subgenus referred to as the core-*Calosphace* clade (sensu Kriebel et al., 2019, 2020, 2021, 2022; Rose et al., 2021). The Hastatae have Type II stamens (Fig. 2q-t) which consist of a small, sterile lower portion of the lever and a long upper portion bearing the fertile anther. These tend to be briefly united in Hastatae which also have a glabrous, curved style ending in a stigma with a longer lower lobe (Fig. 1A-B). Connective teeth are absent or inconspicuous in species of the Hastatae clade (including *S. axillaris*), most of which are hummingbird-pollinated (Fig. 1J-K).

In contrast, most of the core *Calosphace*, comprised of both bee and hummingbird species, have NW endemic Type III anther connectives (Fig. 2z-m') with more evenly lengthened and straighter lower and upper arms (Himmelbaur and Stibal, 1932-1934), the former postgenitally fused and frequently difficult to separate (Claßen-Bockhoff et al., 2004). The core *Calosphace* group is also distinct because of its straight style that is usually pubescent below the stigma and a stigma with a longer upper lobe that wraps around the galea (Kriebel et al., 2021). These features might be acquired due to hummingbird pollination which is postulated to be ancestral in the subgenus (Kriebel et al., 2019, 2020, 2021, 2022; but see Sazatornil et al., 2022). Most of the core *Calosphace* possess obvious connective teeth (Fig. 1C-H) which are located about a quarter to halfway up the connective. These Type III stamens have +/- equal length upper and lower connective arms compared to the Type II stamen, which have a much longer upper connective arm. This similarity in lengths between connective arms together with the postgenital fusion of the lower ones likely resulted in increasing the weight of the posterior arms, which in turn selected for the development of teeth to provide support to the lever in the form of a seesaw. Connective teeth in hummingbird-pollinated species vary in shape, with triangular to subulate teeth being common (Fig. 1L-O). Other species have protuberances resulting in the appearance of teeth (Fig. 1P-Q). These protuberances on the connective are positioned near the mouth of the corolla in some taxa (Fig. 1Q), so it is possible that the staminal lever seesaws against this area of the corolla.

The innovation of connective teeth might have evolved together with the type III stamens in that they may aid with balancing the lever arms and thus make lever movement more efficient. Given the dominance of hummingbird-pollinated species in core *Calosphace* (Fragoso-Martínez

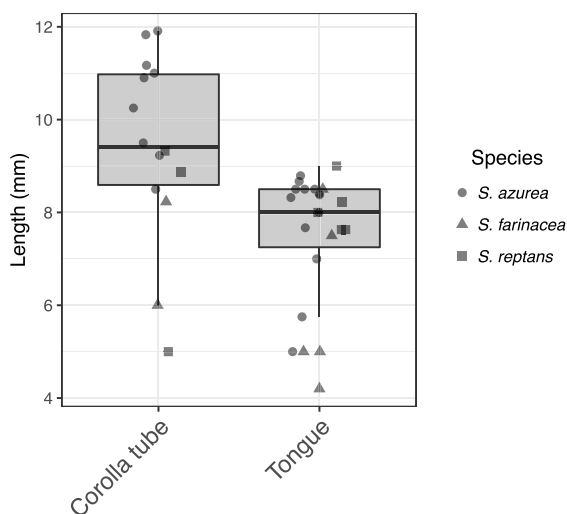


Fig. 4. Boxplots of measurements from corolla tubes of three species of *Salvia* and tongue length of their respective flower visitors (data from Peterson, 1978).

et al., 2018; Kriebel et al., 2019), it is likely that the teeth might have evolved first in this group and act in a similar fashion as with bees by contacting the birds' beak (the function of teeth in hummingbird pollination has not been examined, however). If so, the connective teeth present in many derived bee-pollinated species of subg. *Calosphace* could be a modification of the teeth in the levers of a presumed hummingbird-pollinated ancestor (Fig. 2h', i, -m'). Secondary bee-pollinated species then coopted the seesaw to work by hitting the bees' face. In contrast, other bee-pollinated species, predominantly within the first diverging lineages of core *Calosphace*, have geniculated stamens. Considering that the sterile thecae in geniculate connectives are placed on the ventral side, it is evident that the stamen is originally curved and not straight (Claßen-Bockhoff et al., 2004). Thus, the barrier in these flowers is analogous to the teeth described above and comparable to the curved levers in the bee-pollinated sages from the (Himmelbauer and Stibál, 1932-1934; Claßen-Bockhoff, 2017). The lower connective arm itself acts as a barrier rendering additional teeth unnecessary. Nevertheless, the sterile lower levers of some species in subg. *Sclarea* are modified to cup-, finger- or spoon-like structures guiding the bee's proboscis to nectar (Reith et al., 2007). Considering the genus as a whole, lower connective structures evolved multiple times being morphologically analogous (convergent evolution) or homologous (parallel evolution) to each other. Whereas they usually function as barriers in bee-pollinated species, their function in bird-pollinated species is not yet fully understood.

A function not previously discussed may be related to a secondary pollen presentation mechanism that promotes species diversity. A sub-stigmatic brush, restricted to subg. *Calosphace*, arising at the crown of the core-*Calosphace*, and prevalent in both hummingbird and bee-pollinated species (Kriebel et al., 2021), appears to interact with connective features including the teeth. In most species of subg. *Calosphace*, when the pollinator presses against the lower lever arms, the upper arms lever downward and contact the pollinator. In the process, the pollen sacs also can contact the sub-stigmatic brush and deposit pollen in the brush. This secondary pollen deposition appears to be common in subg. *Calosphace* as dissected flowers of species have brushes that are frequently full of pollen (R. Kriebel, pers. observation). Although the function of the brush in this clade is not well understood (Kriebel et al., 2021), it is likely that the brush stores some pollen and releases it through time with successive triggering of the lever. In this way, the phase of pollen presentation could be prolonged and early damage or fading of the anthers compensated for.

Since there is variation between species with respect to the density of the brush and its position on the lower and/or upper surface of the style, an additional function may be to brush off competing pollen from incoming bees. In species such as *S. farinacea*, where the brush can be restricted almost entirely to the bottom of the style, bees visiting a flower touch the stigma which is positioned first in their path (approach herkogamy). Subsequently, and before pressing the lever, they move forward passing through the brush exposed below the galea allowing the brush to potentially remove pollen that is now in competition with that which is about to be levered on the bee.

Despite this great diversity in staminal levers and connective teeth in subg. *Calosphace*, the levers of most species are similar with their lower arms straight and united (Fig. 1C-I, L-R). This fusion may constrain where and how the anther connective teeth can evolve in subg. *Calosphace* and may help explain their distinct differences to those occurring in other bee-pollinated subgenera. Reversals from Type III to Type II stamens may have occurred occasionally in this clade (e.g., *S. cuspidata* Ruiz & Pav.; Fig. 1D), but this hypothesis needs further examination. In these species, the upper lever arm is longer than the lower arm and curved, a characteristic of Type II stamens common in OW subgenera and in the early diverging *Hastatae* clade of subg. *Calosphace*. Selection for mechanical reproductive isolation through differential pollen placement on bees may have prompted the re-evolution of this Type II staminal morphology.

4.3. One more trait uniting bee and hummingbird-pollinated species in *Salvia* subg. *Calosphace*

Morphological similarities between species of subg. *Calosphace* that distinguish them from the rest of *Salvia*, regardless of pollinators, have long been documented. A phenetic analysis of 100 species in the genus concluded that "The main subdivision represents a distinction between Central and South American *Salvias* and the rest and reflects variation in stylar morphology" (El-Gazzar et al., 1968). This distinction now includes three gynoecial traits based on morphometric analyses: lower lobe of style shorter than upper, straight shape of the style, and the sub-stigmatic brush (Kriebel et al., 2020, 2021). These traits appear to unite all species of NW subg. *Calosphace* excepting those of the *Hastatae* clade (and *S. axillaris*).

The morphology of the androecium also unites this clade of core subg. *Calosphace* and distinguishes it from the rest of the genus. In particular, their generally linear connectives and postgenital fusion for much of their length (Bentham, 1834; Fernald, 1900) was cited as supporting this clade morphologically (Will and Claßen-Bockhoff, 2017). A morphometric analysis confirmed their distinctive straight shape (Kriebel et al., 2020). The distinctiveness of the staminal lever in core *Calosphace*, regardless of pollinating vector, prompted Epling (1939) to name them "rudders." Wood (2007) commented on the confusion this term introduced: "Following Epling, the sterile, fused extension to the anther connectives is referred to as a 'rudder' in the following descriptions, even though this term conceals its function as part of the lever mechanism used in pollination." Here we add another trait to the three gynoecial (straight style, stigma with longer upper lobe, and sub stigmatic brush) and two androecial (straight lower lever arms that are mostly fused) features that distinguish core subg. *Calosphace* regardless of pollinator - the frequent presence of connective teeth near the middle of the connectives.

5. Conclusion

We present observations of bees visiting *Salvia farinacea* that suggest the connective teeth function as additional pressure points or pivots for the staminal lever mechanism. Because similar teeth are common throughout subg. *Calosphace* as shown in a survey of *Salvia* species from western Mexico, a similar function of these teeth across this clade appears likely. Since both bee and bird-pollinated species of the core *Calosphace* group have connective teeth, the evolution of these teeth may involve functional support for the seesaw-like, straight connectives of core *Calosphace* in which the lower and upper arms are of similar length. Particularly in bee-pollinated flowers which need a pressure point for the release of the lever mechanism, the teeth appear to have the same function as the curved lower lever arms in OW *Salvia*. The use of different morphological structures to close or narrow the flower entrance, i.e., connective teeth, geniculated and curved lower connective arms, and their repeated appearance indicate convergent and parallel evolution at the same time. Obviously, a strong selection pressure promotes the formation of connective structures. The function of teeth in bird-pollinated species is not as obvious as in bee-pollinated species. They might play a role in balancing the lever arms thereby improving the lever movement. Moreover, the connective teeth interact with a stylar brush only present in NW core *Calosphace*. The original Himmelbauer and Stibál (1932-1934) hypothesis of transitions from Type I to Type II and type III stamens in the NW followed by the radiation of Type III stamens is supported.

Type III stamens are the ancestral condition in core *Calosphace* and connective teeth evolved in bee and bird-pollinated species. Some species might have lost the teeth during evolution or had them modified in shape and size. The core *Calosphace* is distinctive from the rest of *Salvia* by a suite of characters involving corolla, anther, and style shape, stigma lobing, stylar brush, and now anther connective teeth associated with a functional modification of the staminal lever.

Author's contribution

R.K, B.T.D., R.C.-B., and K.J.S. conceptualized the project; R.K. performed the the formal analysis; R.K. led the writing with contributions from all authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data used in this study is provided as Supplemental Information with the article.

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Supplementary materials

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