

THE BOTANY OF *SALVIA DIVINORUM* (LABIATAE)

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ABSTRACT

Salvia divinorum, ceremoniously employed by the Mazatec Indians of Oaxaca, is endemic to the sierra inhabited by the Mazatec, its distribution anthropogenic. Plants spread vegetatively, flourishing in shaded, humid sites, flowering sporadically from October until June. Flower nectar and corolla dimensions suggest ornithophily, and the only pollination event observed involved a single hummingbird, but other factors suggest that visits by birds to the flowers in their present range are opportunistic, and not a product of plant-pollinator coevolution. The species is diploid with $n=11$, pollen fertility is reduced, there is no active pollen tube inhibition within the style, but some event or process after the pollen tube reaches the ovary is aberrant, as no fully developed nutlet has ever been collected from a Mexican plant, and greenhouse cross-pollinations led to only 3% seed set. Hybridity is suggested, although intermediacy between two known species has not been recognized.

RESUMEN

Salvia divinorum, que fue usada en las ceremonias por los Indios Mazatecas de Oaxaca, es una planta endémica de la sierra habitada por los Mazatecas y su distribución antropogénica. Se reproduce vegetativamente, prosperando en lugares húmedos y sombríos, y ocasionalmente florece de octubre a junio. Las dimensiones de la corola y la presencia de néctar sugieren ornitofilia y la única polinización observada fue realizada por un colibrí, pero hay otros factores que sugieren que las visitas de los pájaros a las flores son oportunistas y no el producto de una coevolución plant-polinizador. La especie es diploide, $n=11$, la fertilidad del polen reducida, no hay inhibición estilar activa del tubo polínico, pero algunos procesos posteriores a la llegada del tubo polínico al ovario son aberrantes, por lo que nunca se ha recolectado ninguna nuclua perfectamente desarrollada en plantas mexicanas, y las polinizaciones cruzadas realizadas en invernadero producen sólo un 3% de semillas. Se ha sugerido hibridación, aunque no se ha reconocido que sea intermedia entre dos especies conocidas.

INTRODUCTION

Of the almost 1000 species of *Salvia* in the world, none has fired the imagination as much as *Salvia divinorum* Epling & Játiva-M, the enigmatic species ceremoniously employed by the Mazatec Indians of Oaxaca, Mexico. The western world first learned of this salvia, or sage, in 1962, when Epling and Játiva-M described the entity from specimens given to them by Albert Hofmann and Gordon Wasson (Wasson 1962; Hofmann 1980), naming it *S. divinorum* after its reported use in divination and curing by the Mazatec. Hofmann, the chemist famous for discovering LSD and isolating psilocybin and lysergic acid amides from the mushrooms and morning glories used by the Mazatecs, had explored the

Sierra Mazateca that year with Wasson, the self-styled ethnomycologist who pioneered the investigations into the Mazatec rituals. The two criss-crossed the rugged highlands on horseback searching for *S. divinorum* in the wild, but never were able to locate it. The flowering branches that eventually reached Epling were brought to Hofmann and Wasson by Indians in the village of San Jose Tenango, though no one was willing to take them to a living plant. Wasson (1962), therefore, concluded that *S. divinorum* is a cultigen that may not exist in the wild state.

Because of the aura of secrecy surrounding *S. divinorum*, the scientific community has not known of this species until recently. A botanist making general collections is not likely to collect *S. divinorum* because its distribution is highly restricted and its flowering infrequent, thus the few existing collections of this species have all been made in conjunction with ethnological investigations.

Several aspects concerning this species beg inspection: it has been found growing only in the region inhabited by the Mazatec Indians; no plants have ever been observed to set seed in the wild; and though plants may be found flowering at any time from October until May, they apparently rarely do so. The corolla conformation suggests no clear pollination syndrome and the flowers do not point to any obvious taxonomic affinities within *Salvia*. These and other questions regarding the biological status of *S. divinorum* cannot be fully understood without consideration of the magico-divinatory aspects of the species. Although information regarding the Mazatecs is limited, recent interest in these people and their medico-religious approach to healing has shed light on certain facts that may be relevant to the natural history of the species, as the pertinent writings of Wasson and Valdés have elucidated (Wasson 1962, 1963, 1980; Wasson et al. 1974; Wasson & Wasson 1957; Valdés 1983; Valdés et al. 1983; Valdés et al. 1987).

THE MAZATEC LANDSCAPE

The Mazatec Indians have lived in a relatively isolated area in northernmost Oaxaca, wedged in between the states of Puebla and Veracruz, since well before the arrival of the Spanish in the Sixteenth Century (Fig. 1). The topography of the region is diverse, characterized by rugged highland areas virtually without level ground. The dissection of the terrain is reflected by the many dialects of the Mazatecan language spoken, and the unique "language silbado," or whistle language, by which the Mazatecs can carry on a conversation with whistle sounds only. Like the yodeling of the Alpine shepherds, communication from one peak to another is made easier using a vocabulary of sounds especially tailored for that purpose. The temperate highlands of the Sierra Mazateca, roughly 1200–2500 m in elevation, grade into tropical lowlands, which comprise somewhere near 40 percent of the total area inhabited by the Mazatec (McMahon 1973; Vásquez 1981). South and east of the highland region, these areas are part of the extensive

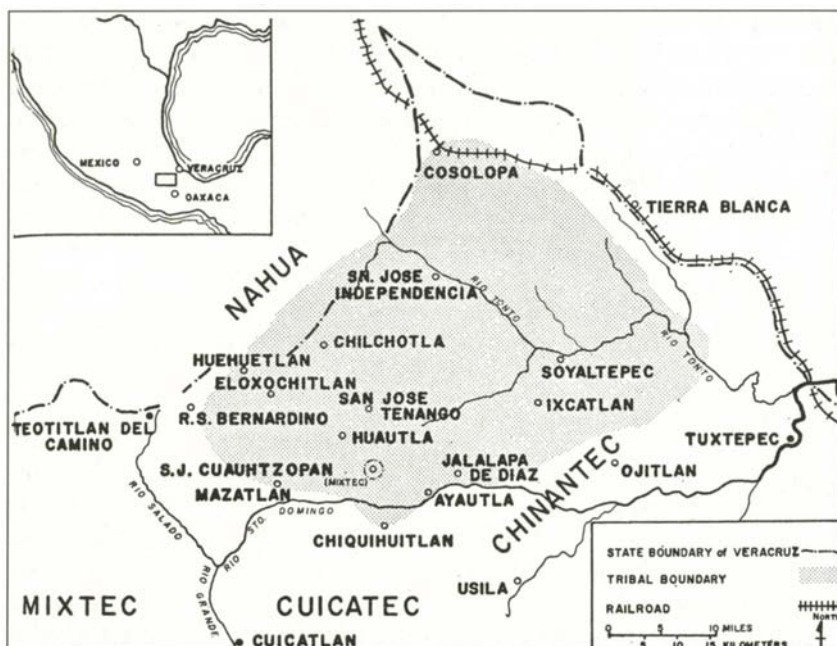


FIG. 1: Stippled region represents the area inhabited by the Mazatec. Note that the Aleman Dam, created with waters from the Tonto River, is not shown. (Map taken from Weitlaner & Hoppe 1969.)

Papaloapan river basin. About half of the roughly 1000 km² of lowland terrain once inhabited by the Mazatec was inundated with water in 1955 to form the Miguel Aleman Dam. This huge hydro-electric project forced over 20,000 Mazatecs to abandon their homes, and many of those displaced now live on the banks of the dam, including its eastern shores (where *S. divinorum* does not grow, see below).

The climates of highland and lowland Mazatec country are quite unlike each other. At an elevation of 1700 m, the large and central community of Huautla de Jimenez is cool and wet. Relief from the unrelenting fog comes only during the short dry season between late March and early May, though the fog is often replaced by torrential downpours in the summer. The annual precipitation ranges from 256 cm to over 400 cm in very wet years, and the average annual temperature is 61°F. To the east, at an elevation of 733 m, the village of Ayautla receives a similar amount of precipitation annually, but is warmer, with an average temperature of 68°. Still farther east is San Pedro Ixcatlan, on a peninsula surrounded by the waters of Aleman Dam, and the adjacent low-lying "tierra caliente" on the eastern side of the lake. These areas also receive a similar yearly

amount of rainfall, but they are still warmer, with annual average temperatures of over 78° (García 1973; Vásquez 1981). The low-lying areas which surround the dam are much more uniform in temperature and moisture regime than the dissected highlands, and therefore are more easily characterized. The rainy seasons (June-August; September-October) and dry seasons (August, in part; March-May) are less variable than in the highlands, where different faces of a mountain may have widely different microclimates.

BOTANICAL INVESTIGATIONS

Previous Research

In 1963, Carl Epling placed cuttings of Hofmann and Wasson's original type collection of *S. divinorum* in the University of California, Los Angeles (UCLA) Botanical Gardens. Epling never saw these plants flower in the gardens at UCLA, and mistakenly described them as having blue corollas. The source of this error is made apparent by Hofmann (1980), who recalls the time he and Wasson received the plant material:

"From an old Curandera, a venerable woman in a strikingly magnificent Mazatec garment, with the lovely name Natividad Rosa, we received a whole bundle of flowering specimens of the sought-after plant, ... [she would not] tell us where she had gathered the leaves. They grew in a very, very distant forest valley. Wherever she dug up the plant, she put a coffee bean in the earth as thanks to the gods.... We now possessed ample plants with flowers and roots, which were suitable for botanical identification.... The plants had blue flowers crowned with a white dome..."

In fact, the white dome referred to by Hofmann was the corolla, which, in the specimen described, had apparently not yet opened. Likewise, the illustration of *S. divinorum* in Schultes and Hofmann (1980) includes only flowers in bud, and the artist's rendition of the individual flower parts emphasizes the mistake: the stamen, style, and corolla are each drawn as they appear before the flower opens. Hofmann and Wasson, neither of whom had any idea what the flowers of this *Salvia* look like, did not realize what they described as "blue flowers, crowned with a white dome" were actually blue calyces with unopened white corollas. The mistake survived in Epling and Játiva's (1962) original description of the species because they never themselves saw living flowers, and the white corollas turn brown upon drying. Díaz (1975), Emboden (1979), Valdés (1983), and Valdés et al. (1987) have all correctly reported that the corollas of *S. divinorum* are pure white, while the calyx and flowering stem are violet blue.

In the course of his pharmacological research, L.J. Valdés (1983) and Valdés et al. (1987) performed several experiments designed to help answer questions regarding the reproductive biology of *S. divinorum*. Of the 14 flowers he cross-pollinated by hand, four set seed, though the number of nutlets that reached maturity is unclear (the ovary of each flower consists of four mericarps). Valdés

concluded that the species is self-sterile, though apparently no attempt was made to self-pollinate any flowers. Daylength experiments, carried out in order to explain the blooming requirements of *S. divinorum*, suggested that it is an obligate short-day plant, with plant height a minor factor in flower initiation. Still, the sporadic flowering of wild populations, the conditions that promote flower initiation, and the failure of the flowers to lead to fruit formation are aspects which remained unclear. These and other questions regarding coevolved pollinators and biological status are addressed below. Investigations of the author (Reisfield 1987) described here have involved visiting and collecting material from many populations in the field, a "stakeout" at a flowering population to observe pollinators, chromosome number determination, greenhouse flower induction experiments, artificial self- and cross-pollinations, pollen stainability studies, fluorescence microscopy of pollen tube growth through styles, and nectar analyses.

MATERIALS AND METHODS

Fieldwork carried out during the winter of 1983-84 consisted mainly of searching the Sierra Mazateca for populations of *S. divinorum*. Flowering populations near Cerro Quemado, a village on the western side of the Aleman Dam, were visited in October, 1985, and watched for two days and one night in order to note any visitors to the flowers. Plants were observed during the night by periodical inspection using a red-filtered incandescent lamp. After being visited, flowers were inspected to see whether pollen had been deposited on the stigma, and whether the store of nectar had been depleted.

Plants of *S. divinorum* from several sources were propagated at the University of Wisconsin-Madison Botany Department (UW) Greenhouses. Valdés generously provided potted plants derived from three sources: a collection from Cerro Rabon, near the Mazatec village of Ayautla, a collection from near the village of Cerro Quemado, and clonotypic material obtained from Berkeley. Later, plants I collected near Cerro Quemado (Reisfield & Solheim 1102) and Ayautla (Reisfield & Solheim 1111) were added to the living collections.

Valdés learned from Robert Ornduff (pers. com.) that plants grown in the gardens at both UC Berkeley and UCLA formed flower buds that subsequently reverted to vegetative growth. (This, in fact, also occurred on plants growing in the UW greenhouses during the winter of 1986-87). Apparently, a limited exposure to light during the night will upset the hormonal mechanism by which the plant perceives a decrease in daylength. Thus, beginning in late October, 1984, a subset of plants at the UW greenhouses were subjected to artificially shortened days of 8-10 hours by covering them with a black cloth each afternoon.

Flowers were hand-pollinated by removing the stamens and immediately

brushing the dehiscing anthers against the inner surfaces of both stigma branches until pollen grains adhered to the stigma. Self-pollinations were performed within individual flowers, between flowers of the same plant, and between plants derived from a common source, while cross-pollinations were performed between plants derived from different sources. Pollinations were performed at different times during the day (and night), and between flowers of different ages.

To study pollen germination and pollen tube growth, styles were collected from flowers that had been self- or cross-pollinated between 4 and 18 hrs earlier. The styles were fixed in FAA and stored in distilled water at approximately 5°C. They were cleared with 8N sodium hydroxide for 24 hrs, then taken through several washes with distilled water, and stained with aniline blue at a concentration of .01 percent for 4 hrs. Fluorescence microscopy was performed with a Zeiss microscope equipped with a Zeiss UG1 excitation filter and 47,-65 barrier filters. The UV source was an Osram HBO 200W mercury vapor lamp. Staining and microscopy techniques mostly followed Martin (1959) as modified by Stettler and Guries (1976). Styles were slightly crushed beneath a coverslip and observed whole in a darkened room. The callosic lining of the pollen tubes fluoresces a bright yellow-green, but the amount and distribution of callose varies between taxa (Martin 1959). Scanning several unrelated species of *Salvia* showed that pollen tubes come in and out of visibility over the length of the style, and can easily be distinguished from the two vascular bundles which fluoresce a uniform, much less brilliant yellow. Since fluorescence was most visible at the stigmatic and ovary ends of the style, an inability of the tubes to reach the ovary should have been readily detectable.

Pollen grains from FAA-preserved flowers on wild and greenhouse-grown plants were analyzed for cytoplasm stainability. Sterile or aborted pollen grains did not take up the cotton blue-lactophenol stain, and were also conspicuous by their shrunken size and shriveled form.

Nectar studies included an analysis of constituents, a study of daily secretion patterns, and also total volumes produced per flower. Calibrated micropipettes were inserted into the pool of nectar that accumulates at the base of the corolla tube, with nectar extracted by capillary action. Sugar concentrations were measured with an Extech model 2132 pocket refractometer, with several nectar samples also analyzed for sugar constituents (ratio) by Irene Baker at UC Berkeley. The nectar was spotted on Whatman #2 filter paper, the diameter of the spot outlined with four pencil marks, and the volume and percent sugar for each spot provided to Dr. Baker.

Young anther sacs were dissected and meiocytes squashed according to the technique of Beeks (1955). These were viewed with a Zeiss phase-contrast microscope.

RESULTS AND DISCUSSION

Distribution, Ecology, and Flower Initiation

During the winter of 1984-85, approximately 15 populations of *S. divinorum* were located, though for the purpose of mapping their distribution, several populations are merged because of their proximity (Fig. 2). *Salvia divinorum* was first located (Reisfield & Solheim 1977) about 2 km north of the village of Huautla de Jimenez in a very wet, somewhat disturbed, shaded ravine at the edge of a coffee plantation. The coffee plantation apparently replaced a cloud forest, the remnants of which included *Liquidambar macrophylla* Oerst. and *Hedyosmum mexicanum* Cordem. Plants of *S. divinorum* were found growing along a stream bank, with some stems trailing near or in water, rooting copiously at the nodes and sometimes along internodes. Broken, trailing, and drooping stems were noticed to resume erect growth at the stem apex or by axillary branching, with new, vigorous shoots often arising from the axils of old, senescent stems. All living stems were observed to arise from stems that had since died, the dead stems lying on or in the ground, sometimes appearing as woody caudices. Many stems were cut, apparently by people who collected the leaves for medico-religious use. Several old, dried, inflorescence branches (rachises) were present but no fruit were found. Later, similar populations (Reisfield & Solheim 1990, 1992) were found in other ravines near Huautla.

The road from Huautla (1700 m) to Ayautla (760 m) is copiously criss-crossed by streams and wet ravines that were searched for populations of *S. divinorum*. A few such populations (Reisfield & Solheim 1991-92) were finally found near Ayautla, and these again showed signs of past flowering, but all the old floral stems were entirely naked (in *Salvia*, the developing fruit are enclosed by a persistent calyx, but failure of the nutlets to develop normally leads to calyx abscission). The plants were spreading vigorously along the rocky stream banks, and erect shoots emerged mostly from a thick litter composed of older decaying stems. One branch was completely severed, lying in shallow water and rooting along the internodes. An additional population in this area, chosen by a local shaman, Maria de la Oz Unda, to supply the leaves for the divinatory ceremony we had requested, grew among the trees of a coffee plantation. Although there was no running water at this site, the many epiphytic ferns and orchids suggested that this wooded mountain slope is regularly blanketed in fog. Similar stands of *S. divinorum* in "cafetals" were found near Huautla (Reisfield & Solheim 1990) and Cerro Quemado (Reisfield & Solheim 1993).

Flowering of *S. divinorum*, as in many forest understory species, is promoted by sunlight, and the extent of flowering of a given population is dictated by the amount of sunlight that penetrates the canopy. We saw our first flowering population of *S. divinorum* (Reisfield & Solheim 1993) near the village of Chilchotla

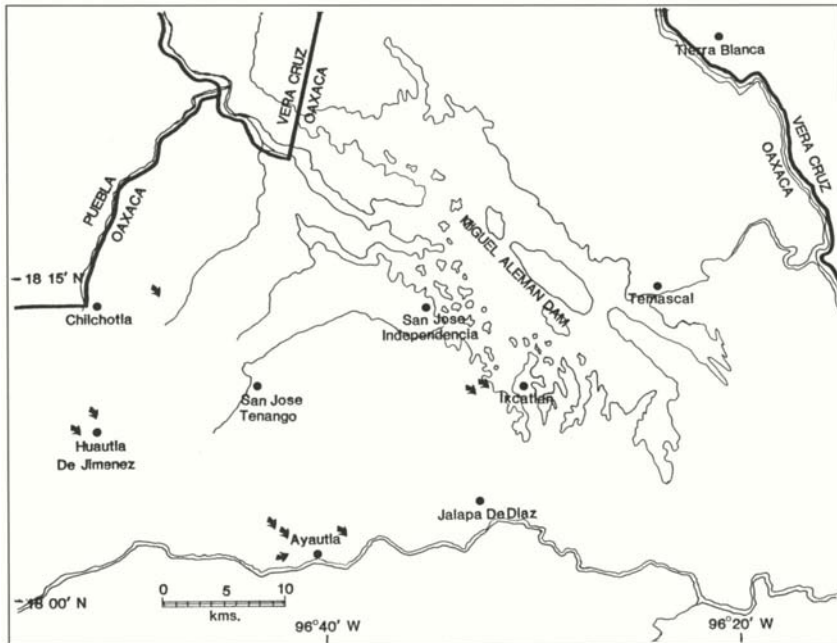


FIG. 2: Distribution of *Salvia divinorum* Epl. & Jat. Arrows point to populations found during fieldwork in the winter of 1984–85. In addition to the locations shown, the type specimen was collected in (near?) San Jose Tenango. Miguel Aleman Dam refers to the lake. (Adapted from McMahon 1973.)

(1200 m). The plants colonized the banks of a broad, shaded ravine with several pools of standing water. The channel of the ravine was mostly without vegetation and almost certainly flooded during wetter periods. The vegetation suggested a transition between cloud forest (e.g., *Hedyosmum*, *Liquidambar*) and tropical evergreen forest (e.g., *Syngonium* and other Araceae), and climatic data (Garcia 1973) indicates that this area is extremely humid, with an annual average of 472 cm of rain. This flourishing population of plants appeared to be clonal, spreading vegetatively in the same fashion as populations observed previously. Inflorescence rachises past the flowering stage were again entirely naked, and not a single mature nutlet was found. The beautiful white and violet flowering stems, found only in patches where sunlight penetrated the canopy, were very conspicuous. This observation suggests the main distinction between this and previous sites where populations of *S. divinorum* were found. The breadth of this ravine allows a greater penetration of sunlight, while the extreme humidity of the region prevents even sunny microhabitats from drying out. It is this interplay between sunlight and humidity that apparently dictates the success and the extent of flowering of a given local population of *S. divinorum*.

Another flowering population (Reisfield & Solheim 1109) was found on a steep face of Cerro Alto, the mountain adjacent to the village of Ayautla. This trailside population formed a thick, shaded stand, with crowded stems over 2 m tall. Flowering branches, up to 3 m tall, rose above the rest and received filtered or direct sunlight. Valdés (1983) reported he collected plants from Cerro Rabon, a somewhat more distant mountain, but our local guide, Pedro Díaz, insisted that he knows "La Maria [*S. divinorum*]" very well, and in 40 years of walking Cerro Rabon, [he] never saw it up there."

Several populations of *S. divinorum* (Reisfield & Solheim 1102-03, 1106-08) were found on the east-facing bluffs above the village of Cerro Quemado, on the western bank of the Aleman Dam. At roughly 300-400 m elevation, these stands were scattered along a steep trail that winds between the peaks of Cerro Quemado and Cerro Camaron. Some plants were found in a slash-and-burn cornfield, where most of the associated vegetation was disturbed, replacing a tropical evergreen forest with *Brosimum*, *Dendropanax*, and *Urera*. Many flowering stems were found, these always in partial to full sunlight, sometimes dried out to the degree that the leaves were badly wilted. Climatic data for the nearby village of Ixcatlan (Garcia 1973) indicates that this area is considerably warmer than the highland regions of the Sierra Mazateca, the wilting plants perhaps demonstrating that *S. divinorum* is here at its limit of evapo-transpiration tolerance. On the other hand, these same populations included more flowering stems than any other, again indicating that flowering of *S. divinorum* is promoted by sunlight, and perhaps the stress of drying out to a certain degree. This is reasonable in light of the heliotrophic nature of *Salvia* in general, with mostly species of open ground and with brightly colored flowers. *Salvia divinorum*, though, with its crisp, watery, easily broken, hollow stems, is clearly a hydrophyte, and most aggressively colonizes sites that are dark and humid. Vigorous, flowering populations are found in conditions of marginal light, and in very humid areas (e.g., Chilchotla), the plants can "venture out of the shade" into the sunlight where they will flower.

Conditions that promote vegetative growth of *S. divinorum* are different than those that promote flowering, and this is reflected in the character and distribution of populations. The Mazatecs displaced by the Aleman Dam, who now live in the low-lying "tierra caliente" on the eastern side of the dam, have tried, unsuccessfully, to cultivate *S. divinorum* (Díaz, pers. com.). This region receives similar amounts of rainfall as the highland areas of the Sierra Mazateca, but is warmer, and consequently drier. Thus, in order to gather fresh leaves, some of these Indians travel by boat across the lake to the hillsides near Cerro Quemado, where the populations are at their limit of evapo-transpiration. Throughout the higher, cooler regions inhabited by the Mazatec, flourishing populations may be found in shaded ravines near water, or on mountainsides continuously bathed in fog. In the latter type of site, often a hillside planted to coffee, *S. divinorum* is almost certainly introduced, and the Mazatecs do this by simply sticking a severed

branch into the soil. Though the more remote, aggressive populations along watercourses seem not to have been planted, they may in fact have been introduced long ago. The Mazatecs do not distinguish between wild and cultivated populations, nor do they attach any significance to the flowers.

While Emboden (1979) reported that *S. divinorum* flowers only when the "branches" [stems] are seven feet or more in length, Valdés et al. (1987) concluded that plant height is a minor factor in flower initiation. In the Sierra Mazateca, most flowering stems are, in fact, very long, since the stems that elongate the most are most likely to receive direct sunlight. Plants grown in the University of Wisconsin Greenhouses received unfiltered sunlight, and those which were subjected to the short-day treatment flowered profusely on branches of varying lengths.

Flower buds on greenhouse-grown plants were first noted roughly 2 months after the beginning of the short day treatment, and the first flowers did not open until almost one month later. The nearly 3 month lag between the time the plants first perceived the stimulus to flower and the onset of flowering correlates with the results of Valdés et al. (1987), but raises the question of whether any critical threshold period is really perceived by the plants. The type specimen was collected in flower by Hofmann and Wasson on October 8 (I am unaware of the collection flowering in August, referred to by Valdés), which suggests that these plants perceived the stimulus to flower more than 3 months earlier, that is, in late June, during the period with the longest days of the year. The mechanism responsible for flower induction is apparently not as simple as our greenhouse and growth chamber experiments would suggest, and the actual induction of flower primordia probably involves several factors, including temperature and water regimes. Perhaps the critical stimulus perceived by the plant is an increase in the length of the night *per se*, an hypothesis that could easily be tested. Flowering plants have been collected from near Cerro Quemado in March, and local villagers insisted *S. divinorum* flowers most abundantly in March, April, and May, when it is the driest. In light of the conditions that promote flowering during the cool and wet winter, these assertions seem more than reasonable.

Co-Evolved Pollinators

Although several flowering populations were finally found during the winter of 1983-84, at no time was a legitimate pollinator observed visiting flowers. While poor flight conditions for Hymenoptera often prevailed, pollinators were also conspicuously absent during an entire, sunny, hot afternoon while flowers were collected near Cerro Quemado. Bumblebees were active in the area, but they ignored the white and violet inflorescences of *S. divinorum* as they do scarlet flowers of many ornithophilous *Salvia*. The explanation for this behavior is suggested by the dimensions of the *S. divinorum* corolla, which more resembles those of ornithophilous salvias than melittophilous ones (Fig. 3). The sigmoid corolla

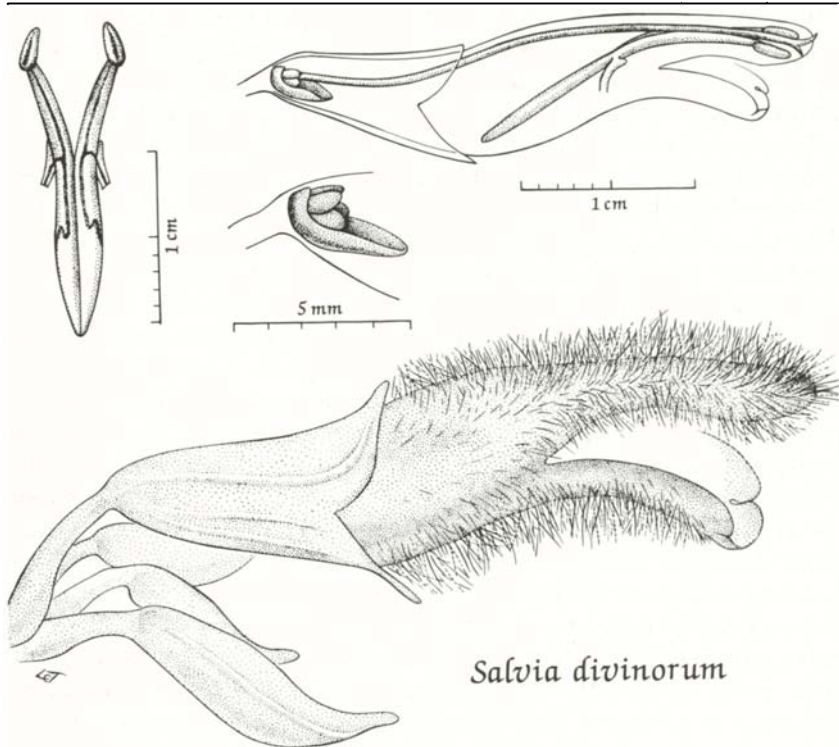


Fig. 3: a. Flower of *Salvia divinorum*; b. Diagrammatic illustration of flower with contained androecium and pistil; c. Stamen connectives; d. Ovary and gynobase. (Illustration by Lucy Taylor.)

tube is 19–22 mm long, and measures only 2 by 1.5 mm at its narrowest point, near the throat. The lower lip, which is horizontally expanded to form a landing platform on bee flowers, is instead vertically oriented, with the middle lobe somewhat cupped, like those of classical hummingbird pollinated salvias. The sigmoid curvature of the corolla tube is uncommon in bee flowers, but characterizes several bird pollinated species of *Salvia*, such as those in sect. *Flexuosae* Epling.

Data obtained from analyzing nectar constituents are consistent with ornithophily. As might be expected, the nectar sugar is sucrose dominant, as are most nectars from flowers pollinated by long-tongued bees, hawkmoths, or birds. Flowers from clonotypic plants yielded a nectar sugar composed of 86% sucrose, 10% fructose, and 4% glucose. This evidence is even less meaningful in light of the fact that the Labiatae, as a group, are characterized by sucrose-rich or sucrose-dominant nectar sugars (Baker & Baker 1983).

While the corolla tube of *S. divinorum* suggests a co-evolved pollinator with

long mouthparts, information concerning nectar sugar concentration and the volume of nectar secreted can help resolve further the identity of this long-tongued pollinator. Nectars sampled by Cruden et al. (1981) from a spectrum of Mexican plants below 2400 m showed that bee flowers produce more concentrated nectars (34.7%) than hawkmoth- (22.6%) and bird-pollinated (23.8%) flowers. The concentration of nectar sugar produced by greenhouse-grown plants of *S. divinorum* was mostly between 21% and 23%. In general, hummingbird flowers have low nectar-sugar concentrations, but produce larger total quantities of nectar than bee flowers. Because of the interplay between pollinator adaptation, on the one hand, and the constraints of phylogenetic relationship, on the other (Baker & Baker 1983), data presented by Cruden et al. (1981) from several species of *Salvia* are especially useful for comparison with data obtained from *S. divinorum*. Ornithophilous species of *Salvia* sampled by Cruden et al. included *S. cardinalis* (17.71 μ l per flower), *S. elegans* (7.52 μ l), *S. greggi* (3.49 μ l), and *S. pubescens* (20.72 μ l). Melittophilous species sampled included *S. tiliaefolia* (0.015 μ l per flower), *S. reflexa* (0.14 μ l), and *S. cobuilensis* (0.53 μ l). Despite the fact that nectar accumulation data obtained by intermittently sampling flowers of *S. divinorum* grown in the greenhouse are plagued with inconsistencies, there is no question that the quantity of nectar produced suggests ornithophily, with measurements ranging from 8–16 μ l, most flowers producing a total quantity of nectar near 9 μ l. Still, several questions emerged, such as the possible negative effect of the intrusive sampling technique, the effect of nectar removal on secretion rates, and the effect of the time of secretion on the quantity of nectar produced, since flowering and nectar production were asynchronous.

No nocturnal visitors were observed during the overnight stakeout near flowering populations of *S. divinorum* at Cerro Quemado. Flowers opened asynchronously in the evening or during the night, and nectar production was also initiated asynchronously, mostly during the night. Virtually three of every four flowers had a hole punctured in the calyx and corolla tube, presumably by nectar robbers, and even many unopened flowers containing little or no nectar were pierced. At the first signs of dawn, under an overcast sky, a single, large hummingbird with a conspicuously decurved bill visited virtually every flower, flitting from flower to flower in an irregular fashion. It flew away in the direction of additional flowering stands of *S. divinorum*, and several minutes later returned and again visited most flowers. The nectar in most of the flowers was depleted by the bird, and pollen grains had been deposited on several stigmas. Two bumblebees were in the area but ignored the *Salvia* flowers.

The observations described can in no way be considered evidence for the co-evolution between *S. divinorum* and a species of hummingbird, especially in light of the many supposed melittophilous salvias that are visited by these opportunistic birds (Reisfield 1987). Still, dimensions of the corolla, nectar constituents, and the amount of nectar produced per flower, all suggest the ornithophilous

syndrome. On the other hand, anthropogenic distribution, white corolla color, sporadic and infrequent flowering of populations during most of the year, the great amount of nectar robbing, and apparent complete lack of fruit set, suggest the relationship between plant and pollinator has not been "fine-tuned" by natural selection.

The Barrier To Fertility

Meiocytes from anthers of *S. divinorum* (Reisfield 1242) flower buds were suitable for chromosome counting during the first and second metaphase. No irregularities in pairing were observed, and the species was found to be diploid, with $n=11$ (photograph of squash in Reisfield 1987), which is the number most common in species of subg. *Calosphace*. Nevertheless, many examples of species are known in which chromosome pairing appears normal, but meiosis breaks down in the later stages and pollen grains fail to develop, due to various types and degrees of chromosome non-homology or genic sterility (Stebbins 1958). A failure during the late stages of meiosis or during gametogenesis would normally lead to unviable pollen grains, often reflecting a disharmonious interaction of parental genes, usually indicating hybridity. A scan of pollen from preserved flowers of *Salvia sessei* Benth. (Reisfield 1252), *S. flaccidifolia* Fern. (Reisfield 1218), and *S. mexicana* L. (Reisfield 1244), revealed that virtually all the pollen grains took up the stain. Pollen grains of *S. divinorum* from greenhouse-grown plants (mixed collections) were much less viable, with 882 (56%) of the 1587 pollen grains observed aborted. Pollen from flowers of *S. divinorum* collected at Cerro Quemado (Reisfield 1242) showed a similarly low degree of fertility relative to other *Salvia* species, with 1592 (53%) aborted pollen grains out of 3027 observed. Haplontic and/or diplontic sterility of interspecific hybrids is often similarly manifested, indicating the taxon may be of hybrid origin. Still, the inviability of the haploid stage in the life cycle is only partial, and can not explain why the plants apparently set no seed in Mexico.

Hand pollinations in the greenhouse clearly showed the chief barrier to fertility in *S. divinorum* is not a failure to be pollinated. Of a total of 108 self-pollinations (108 stigmas dusted with pollen from the same plant or genetically identical plants), only 11 mericarps developed fully into dark, indurate, viable nutlets. Since each pollinated flower could potentially yield four nutlets, the 11 fruit represent 2.5% of a total potential yield of 432 fruit. Of 190 cross-pollinations, only 24 (3%) nutlets fully matured from a potential of 760 fruit. Most of the calyces abscised between 5 and 10 days after pollination, and quite often one or two (sometimes more) mericarps were noticed to be developing before the calyx and ovary fell from the plant. Several of the mature seeds were germinated in the UW Greenhouses, and vigorous seedlings developed into plants indistinguishable (though not grown to flowering) from their parents. Since flowering is so sporadic in Mexico, pollination may, in fact, be undependable. Furthermore,

pollen seems not to adhere to the stigma with great efficiency. Still, many viable pollen grains that were deposited on receptive stigmas did not lead to fruit set, indicating some failure after this stage in the life cycle of *S. divinorum*.

It has been suggested that *S. divinorum* is self-sterile (Valdés 1983; Valdés et al. 1987) which, if true, would explain the observed failure to set fruit. Individual populations all seem to be clonal, and plants of adjacent populations could feasibly be genetically identical. Given the anthropogenic distribution of *S. divinorum* throughout the region inhabited by the Mazatec, it is also quite possible that many distinct populations are derived from a single source. Such a situation would explain why artificial cross-pollinations resulted in no greater fruit set than self-pollinations, since the so called cross-pollinations would, in reality, be between genetically identical plants. Even if plants were not genetically identical, a common self-incompatibility factor shared by the functional male and female plants would prevent successful fertilization. This type of self rejection, though, seems to be rare in *Salvia* (few studies have been done), and also could not account for the reduction in pollen fertility.

Further resolution of the problem was obtained by studying pollen germination and tube growth through styles of hand-pollinated flowers. Of 39 styles observed, 13 (33%) had four or more pollen tubes that traversed the entire length of the style, reaching the ovary. Three or more pollen tubes reached the ovary in almost one half the styles observed. Of the 20 styles in which four or more pollen grains or tubes were observable at all, 14 had three or more pollen tubes that reached the ovary. Also, no difference was noted between the self- and supposed cross-pollinations.

In classical genetic self-incompatibility systems, the site of pollen tube inhibition is on the stigma surface or somewhere in the style. A pollen grain may fail to germinate, or produces a tube that grows abnormally and is soon occluded by callose, or produces a tube that is eventually rejected by the transmitting tissue of the style (Heslop-Harrison 1975). Although this type of active inhibition of the pollen tube may not be the only form of genetic mate discrimination in plants (Mulcahy & Mulcahy 1983), the best understood self-rejection systems (oppositional systems) do involve observable changes (e.g., swelling) in the growing tip of the pollen tube. No such abnormalities were observed for *S. divinorum*, and no occlusions were found anywhere in the style. A barrier to seed set is apparently encountered after pollen tubes reach the ovary, at some point between the time the tube enters the micropyle of the ovule and the early development of the embryo. Since calyces often abscise while the included nutlets are developing, post-zygotic embryo abortion or endosperm failure is probable. Whether this is due to inbreeding depression, hybridity, or a late acting (delayed) self-incompatibility reaction is difficult to know with certainty. The latter is unlikely, especially because gametogenesis and other stages of the life cycle are also irregular, and one would think the various aberrations have a common cause. Inbreeding depression

is often the fate of taxa that become closely associated with man, and could potentially cause the observed irregularities.

The various anomalies that characterize *Salvia divinorum* might perhaps be best explained as due to hybridity, but unfortunately, additional evidence is lacking. To none of the almost 500 species that comprise the Neotropical *Salvia* subg. *Calosphace* (Benth.) Benth. does *S. divinorum* show any obvious affinity, nor is intermediacy between two known species evident. Although the various character states of *S. divinorum* are encountered at one place or another within subg. *Calosphace*, I have been unable to identify the two species, out of the hundreds, which, when crossed, might have produced offspring that look like *S. divinorum*. The species is certainly anomalous in sect. *Dusenostachys* Epl. (Epling 1939), to which it was originally assigned (Epling & Játiva 1962).

The Mazatecs say that "La Maria [*S. divinorum*] speaks with a quiet voice," which may explain, in part, why many issues concerning this enigmatic plant remain unsolved. Whether of hybrid origin or an inbred cultigen, questions regarding taxonomic affinities, fruit abortion, native distribution, and pharmacology are yet to be conclusively resolved.

AMENDED DESCRIPTION OF *SALVIA DIVINORUM* EPLING & JÁTIVA-M.

Salvia divinorum Epling & Játiva, Bot. Mus. Leaf. 20: 75-76. 1962. TYPE: MEXICO. EDO. OAXACA: San José Tenango, 8 Oct 1962, Albert Hofmann & R. Gordon Wasson s.n. (HOLOTYPE: LA; ISOTYPES: LA in UC, ECON).

Perennial herb, mostly 0.5–1.5 m tall vegetatively, flowering stems 1–2(–3) m tall, taller stems decumbent for part of their length; stems often trailing along rocky stream banks, sometimes in running water, rooting copiously at the nodes and sometimes along internodes, with broken, trailing, and drooping stems resuming erect growth at stem apices or by axillary branching, the new, vigorous shoots often arising from axils of old, senescent stems, these decaying or dead stems often appearing as woody caudices. *Stems* quadrangular, with flanged angles, hollow, fleshy and crisp, translucent, breaking easily, hirtellous, green. *Leaves* opposite, elliptic to ovate, acuminate to caudate at the apex, attenuate at the base, petioles scarcely differentiated from the blade, 10–25(–30) cm long, 5–10 cm wide, glabrous above, sparingly glandular-punctate below; margins irregularly serrate or crenate-serrate, to entire at the base. *Racemes* simple, erect, 30–40 cm long, with 2–4 cm long internodes; cymules with 3–6(–12) flowers each; rachis hirsute, glabrate. *Bracts* sessile, concave, ovate, rounded at the base, acuminate-caudate at the apex, 1–2(–3) cm long, 0.6–1 cm wide, mostly violet, tardily deciduous. *Pedicels* straight, slender, hirsute, violet, 4–9 mm long. *Calyx* gradually widened above, 10–12 mm long, with subequal lips, glandular-hispid along the veins in bud, glabrate to glandular-puberulent throughout, violet; upper lip 1.5 mm long, with 3 major veins. *Corolla* sigmoid, 28–32 mm long, densely villous with multicellular translucent hairs 0.5–2 mm long especially on

upper and lower lips, glabrous within, white, drying brown in herbarium material, lips becoming tinged blue with age; tube 19–22 mm long, 2 mm high by 1.5 mm wide at the narrowest point near the throat; galea (upper lip) 8–9(10) mm long; lower lip cupped, 5 mm long, 7 mm wide when flattened out, middle lobe emarginate. *Stamens* included within the galea, inserted near the throat, glabrous, white; connectives somewhat rigid, slightly arcuate, 15–16 mm long, 17–18 mm long when flattened out, rudders 10–11 mm long, entire; anthers 2 mm long; pollen white. *Style* 27–32 mm long, densely bearded below the stigma, white; posterior (upper) stigma branch exerted beyond the galea and curling upward, 2 mm long, 2.5 mm long when flattened out; anterior branch sometimes slightly exerted from the galea, somewhat carinate, 1.5 mm long. *Gynobase* horn 3 mm long, 1.2 mm wide, glabrous, white. *Nutlets* when mature 1.8–2 mm long, 1(1.2) mm wide, somewhat pyriform, minutely tuberculate, dark brown, to date never collected in the wild. *Endemic* to the Sierra Mazateca, Oaxaca, Mexico, at elev. of 300–1800 m, in primary and secondary cloud forest and tropical evergreen forest, with many populations cultivated or semi-cultivated (weedy), often spreading vegetatively along streambanks, flowering sporadically from September to May.

Specimens examined: MEXICO. Edo. Oaxaca. Sierra Mazateca: 2 km NNW of Huautla market, 6 Jan 1984, *Reisfield & Solheim* 1077 (WIS); 1 km SW of Huautla market, 15 Jan 1984, *Reisfield & Solheim* 1090 (WIS); ca. 2 km SW of Huautla market, 15 Jan 1984, *Reisfield & Solheim* 1092 (WIS); Huautla, 1960, *Wasson s.n.* (ECON); Huautla, 24 Nov 1962, *Bunnell s.n.* (LA in UC); Huautla, Sep 1957, *Gomez-Pompa 500-E* (GH); Cuauhtemoc, ca. 4 km NE of Santa Maria Chilchorla, 16 Jan 1984, *Reisfield & Solheim* 1093 (WIS); La Soledad, ca. 3.5 km WNW of Ayautla, 13 Feb 1984, *Reisfield & Solheim* 1111 (WIS); 2.5 km W of Ayautla on road to San Juan, 13 Feb 1984, *Reisfield & Solheim* 1112 (WIS); Cerro Alto, ca. 2 km NE of Ayautla, 13 Feb 1984, *Reisfield & Solheim* 1109 (WIS); Cerro Camaron, 0.5 km W of Cerro Quemado Centro, 8 Feb 1984, *Reisfield & Solheim* 1102 (WIS); ca. 0.75 km W of Cerro Quemado, 9 Feb 1984, *Reisfield & Solheim* 1103 (WIS); 1 km W of Cerro Quemado, 9 Feb 1984, *Reisfield & Solheim* 1106 (WIS); 1.5 km W of Cerro Quemado, 9 Feb 1984, *Reisfield & Solheim* 1107 (WIS); ca. 1 km NNW of Cerro Quemado centro, 10 Feb 1984, *Reisfield & Solheim* 1108 (WIS); 1 km W of Cerro Quemado, 27 Oct 1985, *Reisfield* 1242 (WIS); Cerro Quemado, 2 Mar 1980, *Valdés & Paul s.n.* (MICH).

UNITED STATES. California. California State University, Hayward, cultivated from material of uncertain origin, 24 Jan 1980, *Wilcox s.n.* (ECON). Northridge, cultivated at San Fernando Valley State College from material of uncertain origin, *Emboden s.n.* (ECON). Michigan. University of Michigan Botanical Gardens, cultivated from material obtained at Cerro Quemado, 20 Apr 1980, 17 Nov 1980, *Valdés s.n.* (MICH).

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