

PINGUICULA FLOWERS WITH POLLEN IMITATIONS CLOSE AT NIGHT –
SOME OBSERVATIONS ON BUTTERWORT FLOWER BIOLOGY

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Very little is known about flower biology and pollinators of *Pinguicula*, and even less is published about this topic. I will try to fill at least some small part of this gap by reporting some observations I have made during growing a large number of butterwort species in the greenhouse for almost 20 years, as well as from casual observations made on natural populations during several field trips.

Floral growth and motions during anthesis

The flowers of most *Pinguicula* species will more or less open and close on a daily cycle at very early anthesis (the first few days after they opened from bud), because the corolla (especially the corolla lobes) will slowly enlarge and expand by aid of this growth movement. These so-called nastic movements¹ are exactly the same ecological reaction as observed in other flowers which close at night, e.g. in tulips, where flower closure is achieved by directed, one-sided tepal growth (Hess 1983; Sitte *et al.* 1999). The same basis can be assumed for *Pinguicula*: after re-opening, the butterwort's corolla will be slightly larger every morning, until it has reached final size at the end of what could be termed "floral enlargement stage" (that is, at top of anthesis, a maturity stage, when pollen is ripe and the stigma is receptive; in a few *Pinguicula* species, this even goes along with a slight change of flower color, most notably in *P. mirandae*, where the flowers are white in bud and as long as they are in enlargement stage, but they turn pale pink as soon as they are fully sized at maturity stage). At maturity stage of anthesis, the corolla will become immobile and remains open and held at about the same orientation during day and night for the majority of *Pinguicula* species – all except the five species from the southeastern U.S. (*P. caerulea*, *P. ionantha*, *P. lutea*, *P. primuliflora*, and *P. pumila*) and *P. debbertiana* from Mexico (most notably in the white-flowered form of this species). These six species have flowers that keep closing and turning downwards every night, until the corolla finally fades and is shed (although the movement happens to a much lesser extent in aged flowers). While the former five are a group of closely related species forming a natural affinity (*P.* section *Isoloba*; Casper 1966) which evolved from a common ancestor (they constitute a monophyletic lineage; Cieslack *et al.* 2005; Degtjareva *et al.* 2006), *P. debbertiana* is only distantly related to them: this species belongs to the Central American *Pinguicula* clade, members of which have been formerly assigned to various sections. All remaining members of that Mexican affinity (e.g. *P. ehlersiae*, *P. agnata*) have flowers that remain open all day and night. Interestingly, the phenomenon of nocturnal flower closure of certain *Pinguicula* species has not been documented thus far, except

¹ Often, plant movements are commonly called "tropisms", however a tropism is always a direction-dependent response towards the stimulus – e.g. the closure of the snap traps of *Dionaea* or the tentacle movement of *Drosera* upon mechanical and/or chemical prey stimuli, while a nastic movement is a non-directional reaction of the plant – e.g. nocturnal closure of flowers.



Figure 1: “Sleeping” flowers of *Pinguicula debbertiana* (a pale rose-flowered form), photo taken during the night (ca. 2 a.m.) in the greenhouse. For comparison, the flowers of *P. ehlersiae* (species closely related to *P. debbertiana*) do not close at night. The single flower of *P. debbertiana* on the far left which is more open is aged and does not show much of the nocturnal nastic movement anymore.

a single passing mention in a field trip report by Gluch (2005), who states about a population of *P. ionantha* at Franklin County that “[d]ue to the fact that it was still early in the morning the plants were just opening their flowers.”

The “sleeping position” of the flowers of the above-mentioned six species is reached by two different growth movements: Not only do the lobes of the corolla upper and lower lip fold inwardly to overlap and cover the tubular part of the corolla, but also the upper part of the flower scape bends downwards (again, this is a growth movement, as the peduncle elongates during this movement), so that the closed flowers are nodding during night (Fig. 1). The movement of flower closure starts with fading light at dusk, and the flowers will be fully closed about 1-2 hours after sunset in the greenhouse. In the morning, the corollas will slowly start to open again about one hour after sunrise. I have observed this rhythm in the greenhouse (where additional nocturnal cooling and condensation come closer to natural conditions), but also – yet less pronounced – in plants grown indoors under artificial lights (12 hours of light), and even in plants cultivated on a windowsill at more balanced room temperature. However, I was able to prevent closure of flowers of *P. primuliflora* and *P. debbertiana* in a setup under artificial lights running for 24 hours. Thus I assume light change is the primary ecological stimulus to cause the nastic movement (a so-called photonastic movement), howev-

er a supporting temperature-dependent reaction of course cannot be excluded (both temperature and light have been found as ecological stimuli to cause nocturnal flower closure in plants: thermonastic closure of flowers is known, e.g. in tulips, crocus and daisies, while photonastic movement is the cause for flower closure in gentians, water lilies, and cacti, or the opening of nocturnally blooming *Silene* species; Sitte *et al.* 1999). The movement itself is temperature dependent, as the flowers are open earlier on warm, sunny days (whereas the corollas take much longer, about 3-4 hours after sunrise, to fully open on cool, humid, and dull days) – this is reasonable, as the movement itself is caused by growth, which is temperature-dependent in plants, which are poikilothermic organisms.

So why do the flowers of these six species show this nocturnal “sleeping behavior”? For other plants, one reason for flower closure at night is explained by protection of the flower interior (especially the receptive stigma and the dry pollen) from getting wet and inactive by nocturnal condensation (Hess 1983). And indeed, usually these types of flowers will also remain closed on dull, overcast days, or will even close during rain (for example the upright, tubular flowers of many gentian species will already start to close when in shade of a cloud for a few minutes; Sitte *et al.* 1999; pers. obs.). But the situation in *Pinguicula* seems to be different, and I would reject this explanation for the genus, because the upright, tubular flowers as present in many other species of butterworts will not close at night (e.g. *P. agnata*, *P. pilosa*, or *P. ibarrae* all have open, tubular flowers facing sky, nevertheless they do not close at night or during rain; the comparatively open flowers of *P. alpina* are often found “flooded” with rain in the species’ alpine habitats with frequent rain and fog; the same holds true for all other *Pinguicula* species from all sections, except the six above-mentioned species). So, why are these six species showing nocturnal floral motions? It would seem that the conventional explanations for the nightly sleeping pose for these flowers are not successful. At the end of this article, I will propose another hypothesis of why floral sleeping behavior has evolved in exactly these six species. But to get to this conclusion, we will first have to consider a few other features of *Pinguicula* flowers and floral biology.

Pollen dummies and floral cheating

What these six above-mentioned *Pinguicula* species have in common, apart from nocturnal flower closure, is that their flowers show a conspicuous, yellowish, whitish, or greenish, hairy mark protruding from the base of the lower lip, near the corolla entrance (Fig. 2). Quite obviously, these structures serve as stamen or pollen mimicry, i.e. “pseudopollen” lures to attract specific pollinators. However these *Pinguicula* flowers are not rewardless, deceptive flowers, like those of some orchids – all butterworts produce a certain amount of nectar at the tip of their spur as a pollinator reward, including the six species mentioned above (pers. obs.) – their stamen/pollen imitations seems to constitute simply an additional attractive clue to the pollinators, and maybe also prevent the pollinating insects from trying to feed on the real pollen of the plant. In the five south-eastern U.S. species, the pollen mimicry goes along with an almost radial (actinomorphic) appearance of the usually zygomorphic butterwort corolla: there is no evident bilabiate shape of the corolla in the upper and lower lobe (compared to most other *Pinguicula*), as all five corolla lobes are almost identical in shape and size – hence the sectional name *Isoloba*, which means “equal lobes”. Additionally, their flowers have a more or less upright position of the corolla entrance (the open corolla tube is facing sky) – these flowers quite obviously resemble flowers of other, accompanying plant species (some of which offer rich pollen load to their pollinators), such as Ericaceae or Melastomataceae (e.g. *Rhexia*) or *Polygala* (milkweed). Its superficial resemblance with primrose (*Primula*) flowers even led to the specific name of one species, *P. primuliflora*.



Figure 2: The conspicuous yellow to whitish, hairy pollen imitations found at the corolla tube entrance of certain *Pinguicula* species are sterile outgrowths of the corolla palate. The two true stamens of the butterworts are hidden deeply inside on back of the corolla tube interior. These pollen imitations evolved in parallel at least twice in the genus, and are found in all five species of the southeastern U.S. Pictured is *P. primuliflora* (left) and in the only distantly related *P. debbertiana* from Mexico (right).

Interestingly, the orchid genera *Calopogon* (grass pink) and *Pogonia* (pogonia), both which are frequently occurring in the same bog habitats as the five *Pinguicula* species of the southeastern U.S., also have similar stamen/pollen dummies: they display a brush of yellow hairs on the labellum (lip), which mimic pollen. In contrast to *Pinguicula*, *Calopogon* has deceptive flowers not offering any reward to their pollinators (the flowers are nectar-less and the orchid pollinia cannot be consumed by the pollinating insects; Thien & Marcks 1972; Hess 1983; Argue 2012). Apparently, the local pollinators can be easily cheated, as quite unrelated plants apply a similar deceit strategy in the same habitat. While trying to feed/collect this false pollen, the pollinator will pollinate the flower, by touching stigma and anthers, which are hidden inside the corolla tube over the pollinator's head. In *P. debbertiana*, however, the corolla is distinctly bilabiate, and the flowers are held more or less horizontally (while other Mexican species of this affinity have nearly radially symmetrical flowers facing straight upwards, e.g. *P. rotundiflora*, yet they all lack that conspicuous, 3-dimensional pollen mimic which is uniquely found in *P. debbertiana* from that Mexican affinity). However, almost all *Pinguicula* species show one or more contrasting color marks at the base of the corolla lower lobe – the so-called palate – at the entrance to the throat (so-called bulls-eye or nectar guide patterns), and this part of the corolla throat is additionally lined by very specific, club-shaped multicellular glandular hairs (so-called “futterhaare” or “feeding hairs”, see Fig. 3). These are situated on and near the color marks on the palate of all but a few species (one notable exception is the Mexican *P. ehlersiae*, where this part of the corolla is almost glabrous), and their shape and distribution pattern is so unique and often species-specific, that the character of corolla throat indumentum can be used for species identification alone (Wood & Godfrey 1957; Godfrey & Stripling 1961; Casper 1966). From an evolutionary aspect, these hairy nectar guides could represent a pre-adaptation to pollen mimicry – and indeed, I have observed on several occasions various dipterans (Muscidae, Empididae, Bombyliidae, and Syrphidae) dabbing with their proboscis at the yellow spots on the otherwise white corolla of *P. alpina*, and on the white corolla marks on the violet corolla of *P. vulgaris* and *P. leptoceras* in the European Alps, as if they tried to find nectar or pollen there (Fig. 4).

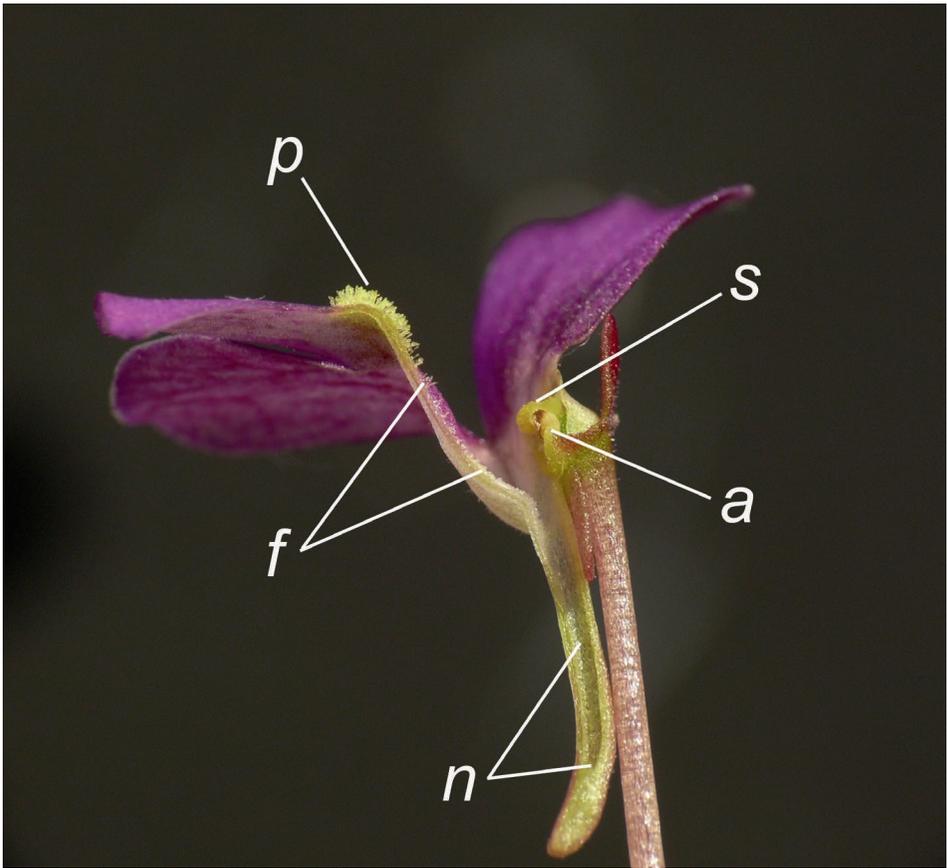


Figure 3: Longitudinal section of a flower of *Pinguicula debbertiana*. *a* = one of the two anthers housing the pollen, *s* = the two lobed stigma, the lower lobe's rear is covering the pollen sacs of the anthers, *f* = feeding hairs, *p* = pseudopollen lure (an outgrowth of the palate at the base of the corolla lower lip's median lobe), *n* = nectar-producing glands inside the spur.

Butterwort pollination and pollinators, insects, and deceit flowers

Reports on natural pollinators of *Pinguicula* are rare, and predominately focus on the European species (e.g. Müller 1881; Molau 1993). Some of the Central American species with bright reddish, large flowers and wide, straight spur have been connected to hummingbird pollination (e.g. *P. laeana*, *P. hemiepiphytica*; Lampard *et al.* 2016), yet this does not account for the Mexican *P. debbertiana*, and for most Mexican species, no pollinator observations have been made until today.

Like the majority of *Pinguicula* species I have been growing and studying in the greenhouse, the six pollen-mimic species discussed here are not able to self-pollinate (the complex arrangement of anthers and bilabiate stigma generally favors cross-pollination in Lentibulariaceae – but the majority of species can be artificially selfed with their own pollen), thus will require pollinator visits in order to set seed in the wild. This agrees with Annis *et al.* (2014), who studied *P. ionantha* in natural habitat – unfortunately, they did not observe natural pollinators, but concluded that pollen vectors are



Figure 4: Species of dagger fly (Diptera: Empididae) first erroneously trying to feed from the white eye-spot of a *Pinguicula leptoceras* flower with its long proboscis (left), then entering the corolla tube guided by the feeding hairs to reach the spur-borne nectar (right). Ötztal Alps, Tyrolia, Austria.

necessary for successful fruit set. A hoverfly (Diptera: Syrphidae) has been observed and pictured in Florida by Makoto Honda in 2008 while it was trying to feed on the false pollen displayed on the corolla of *P. ionantha* (Fig. 5). Syrphids trying to feed on the pollen imitations of the southeastern U.S. species have casually been observed (Th. Franke pers. comms.), and this is quite likely, because syrphid flies are generalist flower visitors, feeding on pollen and nectar of a wide range of plants. And syrphids are also among the known pollinators of certain *Pinguicula* species (reports from the European species only: Müller 1881; Molau 1993). However, they are unlikely to be the main pollinators of the southeastern U.S. species, as they will not enter the corolla tube while dabbing at the false pollen with their mouth parts (Fig. 5). Hence, they will not get in contact with stigma and stamens (which house the real pollen), which are deeply hidden inside the corolla tube (see Fig. 3). Therefore, short-tongued syrphids can be considered casual or even frequent floral visitors of these species, but not their actual or regular pollinators. The entire habit of the flowers (with comparatively thick, robust scapes – especially in *P. lutea* and *P. caerulea*; and freely exposed, visually attractive pollen imitation, and a nectar reward that is hidden deeply at the tip of the spur) makes it quite likely to assume long-tongued larger bees, like bumblebees (*Bombus* spp.) or other large to medium-sized native bees (e.g. Anthophoridae) to be the main natural pollinators of the southeastern U.S. butterwort species. The introduced European honey bees are also able to handle the flowers of the southeastern U.S. *Pinguicula* species (Fig. 5). Schnell (2002) assumes small native bees as natural pollinators, however these could also have sought the flowers as nocturnal roost; this behavior is well known from several bee genera, and especially common in male bees which are often found roosting in tubular flowers, e.g. bellflowers, but also various other flowers which close at night. Especially freshly hatched *Bombus* workers will try a lot of different flowers when foraging for pollen and nectar, and these still “inexperienced” bumblebee workers have been found to be the main pollinators of deceit orchid flowers using pollen imitations in the southeastern U.S. (*Calopogon*, *Pogonia*, *Arethusa*; Thien & Marcks 1972; Heinrich 1975). The workers test these flowers, until they have learned that they will get no pollen reward, and will finally avoid them. In the five *Pinguicula* species, the situation might be slightly different: the pollinator will also experience frustration when foraging for pollen and trying to collect from the imitations, but it will get at least nectar as a reward from these flowers.



Figure 5: The hoverfly *Toxomerus marginatus* (Diptera: Syrphidae; species identified by Ximo Mengual) trying to feed on the yellow pollen imitation on the corolla palate of *Pinguicula ionantha* in Florida (left) and European honey bee (*Apis mellifera*; Hymenoptera: Apidae) nectar-feeding on a flower of *Pinguicula lutea* (right). Photos by Makoto Honda, with kind permission.

Flower colors and breeding system

The flower color variation observed in some populations of the deceptive orchid *Calopogon* has been interpreted as an evolutionary strategy applied by the plants to avoid “learning effects” by the frustrated bumblebees looking for pollen (Heinrich 1975). Indeed, deceptive flowers often show a broad range of coloration and/or flower scent within a single species, in order to minimize that pollinators will learn to avoid a certain flower sight. To which extent this is also true for the “pollen-mimicking” but nectar-rewarding *Pinguicula* species is not clear – at least populations of *P. pumila* are extremely variable in terms of corolla color, size, and shape (Schnell 2002). However, this is most likely connected to the annual life strategy and of the species, in combination with the necessity for outbreeding (flowers of *P. pumila* will not self without artificial pollination, at least not in cultivation). Outbreeders might show more floral variation than congeneric species with the capability for selfing (Lin & Ritland 1997; Fishman *et al.* 2002) – and this apparently also holds true for annual *Pinguicula* species: the annuals *P. pumila* and *P. filifolia* are both not able to self (pers. obs.), hence are in need of flower visitors (or hand-pollination by the grower) to set seed, and both species show a remarkable variation of corolla shape and color within their natural populations (Schnell 2002; Domínguez 2012; Domínguez *et al.* 2014). On the other hand, the annual species *P. lusitanica*, *P. takakii*, and *P. sharpii* are facultatively autogamous, and even without pollination all flowers will self and set seed (pers. obs.) – populations of these three species are relatively uniform in terms of flower shape and color throughout their range. The annual *P. lilacina* is a special case, as plants from some locations will automatically produce seed from selfing in cultivation, while others don’t (pers. obs.; M. Welge pers. comms.) – which might explain that there is certain, local flower variation observed in this species (Casper 1966).

Nocturnal closure seem to be connected to pollen mimics

As mentioned above, *P. debbertiana* is only distantly related to the five southeastern U.S. species, hence a pollen mimicry has evolved at least twice independently in the genus. And coincidentally, a nocturnal closure of the flowers also has developed in parallel in these species. It is quite reasonable to assume a causal connection here, corroborated by the fact that in *P. debbertiana* a slightly different way of corolla closure is applied to achieve the same result, namely a flower tube that cannot be entered at night. Little is known about the natural pollinators of the five southeastern U.S. species (only a few casual floral visitors have been reported so far), and nothing at all regarding pollinators of *P. debbertiana* in Mexico. However, the visual guide of the pollen dummy makes it reasonable to assume diurnal pollinators, probably pollen feeders (such as some diptera, like hoverflies, Syrphidae), or pollen collectors (i.e. bees, Apidae). The hidden nectar offered at the tip of the spur can only be reached by long-tongued insects (like Diptera and Apidae; both have been observed as flower visitors and pollinators in other species of *Pinguicula*; Müller 1881; Molau 1993; pers. obs.; Fig. 4), therefore I would exclude most other insects, such as pollen-feeding beetles as possible pollinators. Anthophilous beetles would also leave their traitorous traces on the corolla, such as gnaw marks and claw marks – however most Mexican *Pinguicula* flowers look relatively undamaged in habitat (see Lampard *et al.* 2016). Yet, perhaps pollen-feeding beetles are not attracted to the “normal” butterwort flower, which has its pollen reward hidden inside the corolla? But do certain nocturnal insects (e.g. certain herbivorous beetles) perhaps cause a potential threat to the pollen dummy of these six *Pinguicula* species, and therefore they hide their conspicuous corolla outgrowths at night? Or do these species just want to protect their nectar from nocturnal flower visitors? If the latter was the case, then why is the trait of nocturnal corolla closure not found in any other butterwort species – and why would a nocturnal insect be an inferior pollinator than a diurnal one? Or, maybe also another likely reason, the nocturnal closure serves to exclude pollinators that are active in the early morning, or to prevent them from learning about the false pollen display too quickly – if other plants with a similar, yet real pollen display are visited first (because the cheaters still have their flowers closed), then this character display will be recognized by the pollinating insects as “reward”, and they will perhaps search for similar flowers more readily. However, if the first few flowers tested for pollen all proved to be “frustration”, then it is likely that the insect might avoid this kind of flower sight for the rest of the day (the sympatrically growing orchids mentioned above have their flowers opened day and night, but would perhaps compensate this by the much longer anthesis time). Detailed field studies on flower ecology of the southeastern U.S. *Pinguicula* species, and on *P. debbertiana*, in natural habitat will be necessary to finally solve these questions – perhaps this article could serve as an incentive to conduct some field studies on the subject? So little is known about the flower biology of most carnivorous plants, thus I would like to encourage you to share your observations.

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