Chapter 2
Cypripedium L. (The Lady's-Slippers), Introduction

Abstract Pollination mechanics, floral attraction, and pollinator behavior are described for the non-rewarding genus *Cypripedium*. The flowers are of a type known as trap blossoms. To obtain their release, traped insects are forced to follow a prescribed sequence of behaviors that lead to pollination of the flower. Insects, usually bees, are attracted by deception based on a false promise of reward, mimicry, or an instinctual response to pheromone-like secretions. Reproductive isolation is critically related to the size of the entrance and especially the diameter of the anther exit hole and the space between the labellar floor and the stigma.

Keywords *Cypripedium* • Trap blossom • Pollinator deceit • Naïve pollinators • Food deception • Pheromones • Reproductive isolation

Among the differences that distinguish the Cypripedioideae from other North American orchids, the most conspicuous is probably the deeply saccate lip of the flowers from which the plants take their common name. This lip or labellum plays a critical role in the pollination of *Cypripedium.* All species of this genus have resupinate flowers of a type known as trap or semi-trap blossoms (van der Pijl and Dodson 1966; Dressler 1981). The flowers temporarily imprison their insect pollinators and force them to follow a prescribed sequence of behaviors in order to obtain their release. An insect of the appropriate size, usually a bee, enters the lip through the obvious large opening or mouth at its top (Fig. 2.1). The lip's slippery inner surface and in-folded margins are often said to prevent it from leaving by the same route (e.g. Summerhayes 1951; Proctor and Yeo 1972). Knoll (1922) and Daumann (1968), however, have shown that bees are unable to exit the lip of the European *C. calceolus* L. even after the in-folded margins are cut away. Bees sometimes escape by chewing through the labellum (Guignard 1886; Stoutamire 1971), but most find a different way out. A foothold is provided by tightly packed hairs (trichomes) on the bottom of the lip (Ziegenspeck 1936; Summerhayes 1951; Stoutamire 1967; Proctor and Yeo 1972). These lead up a pathway ("haarstrassen")
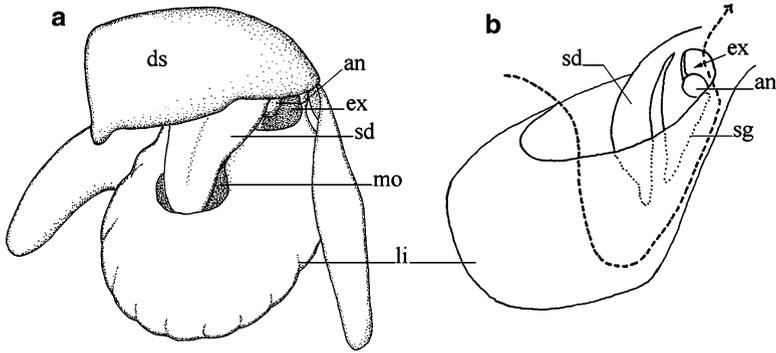
Fig. 2.1 *Cypripedium* flower. (**a**) Oblique view; (**b**) Partial section of lip and column showing route of pollinator through the flower. *an* anther, *ds* dorsal sepal, *ex* exit hole, *li* lip, *mo* mouth, *sd* staminodium, *sg* stigma

toward exit holes at the base or heel of the slipper (Fig. 2.1a, b). The escaping bee may also be attracted along this path by colored lines (false nectar guides) on the lip's inner surface (Arzt 1954) and by light coming from the exit holes or, in some species, from translucent areas ("light windows") in the side of the lip near its base (Webster 1886; Troll 1951; Faegri and van der Pijl 1971). Nilsson (1979) considered evidence for such phototactic behavior to be inconclusive, but Daumann (1968) reported that although bees were well able to find their way out when these "windows" were covered, light gradients were a definite orienting factor. It thus took a bee an average of 11 min to escape the labellum of *C. calceolus* under natural light conditions, but only 2 or 3 min when an external light source was focused on the base of the labellum. When the light was focused on the apex, the time was increased to 30 min.

Nilsson (1981) believes that the hairs inside the labellum may have an additional function. Droplets of oil that are present on their distal tips could absorb body odors (pheromones) from visiting bees and these odors could serve to attract additional pollinators (see below). Because the hairs are strongly light refractive, Ziegenspeck (1936) speculated that they might also stimulate a phototactic response complementing that of the light coming from the base of the labellum.

In its escape the bee must pass two points where the passageway is narrowed. At the first of these it is forced to squeeze under and rub its back against the surface of the stigma (Fig. 2.1b) (e.g. Stoutamire 1967). The stigma in most species is covered by minute, sharp-pointed papillae. These are directed forward and effectively brush the pollen off the insect's back as it passes. The stigma also provides leverage that allows the bee to push down on and slightly depress the labellum, which is elastically hinged to the ovary. The passageway is thus enlarged and can to some extent accommodate variation in the size of the pollinator. (e.g. Wright 1975; Nilsson 1979). Ziegenspeck (1936) considered that an additional function of the basal trichomes might be to reduce friction between the base of the labellum and the insect at this point.

The second narrow passage is the exit hole itself. One exit hole is located on either side of the base of the flower (Fig. 2.1a, b). An anther is so positioned beside each exit that a bee of the proper size cannot force its way out without contacting the anther and carrying away a mass of pollen on the dorsal surface of its thorax (van der Pijl and Dodson 1966; Stoutamire 1967). The pollen itself is sticky, and the non-sectile pollen mass lacks both a viscidium and a stalk. Since the bee contacts the stigma before the anther and usually does not reverse directions, it does not ordinarily transfer pollen to the stigma of the same flower. Rather, pollination is effected when and if the bee, upon escaping from the first flower, is subsequently trapped again, usually in a different flower, and the escape process repeated.

Individual bees do, in fact, frequently visit several flowers in succession (Kipping 1971; Nilsson 1979). Davis (1986), for example, observed five bumblebees follow the prescribed course of entry and exit from flowers of *C. acaule* Ait at a site in Massachusetts. Two were carrying pollen masses on their thoraxes when they entered the flower, and each deposited the pollen on the stigmas. In a study of this orchid in Nova Scotia, O'Connell and Johnston (1998) found a greater than 90% correlation in male and female reproductive success. In other words, over 90% of the flowers that had a pollen mass removed also received one. Visitation rates were low, but once removed, pollen had a 36–51% chance of being transferred to the stigma of another flower. These and similar observations in other lady's-slippers seem to refute the contention that once having endured the ordeals of entrapment and the subsequent lack of reward (see below), bees avoid repeating the experience (Webster 1886; Baxter 1889; cf Gill 1989). Gill (1989, 1996) reported that most pollinator visits occurred during the first few days of the flowering season, perhaps before the bees learned to reject *Cypripedium* flowers.

Although the pollination mechanism promotes cross-pollination, *Cypripedium* reproduces vegetatively, and some transfer of pollen among members of a single clone undoubtedly occurs (Proctor et al. 1996). In addition, a flowering period with two or more simultaneously open flowers on each plant in some cases provides ample opportunity for pollen transfer among flowers of the same inflorescence.

Biologists are uncertain just why bees enter the flowers in the first place. Sometimes the entry is inadvertent. The bees, exploring the outer surface of the lip, tumble into the trap. This has been associated with an inflected rim near the staminodium, the so-called sliding zone (Nilsson 1981). In other cases, entry appears quite deliberate (e.g. Nilsson 1981). Daumann (1968) believes that insects may collect oil from the hairs on the inside of the lip. This has yet to be confirmed. It has also been conjectured that small amounts of nectar are present or that the bees feed on the hairs in the labellum (Stoutamire 1967; Nilsson 1979 and references therein), but according to Ziegenspeck (1936) and Daumann (1968), the hairs are not eaten. It now appears likely that the flower provides no food, and the insects are simply deceived by false nectar guides and the color and odor of the blossoms, which promise nectar or other rewards where none is available (Nilsson 1979). In addition, the staminodium, which projects into the labellum (Figs. 2.1a and 4.1a), is often bright yellow in color with contrasting spots and may appear to be a source of pollen (Vogt 1990).

The possibility that the flowers of some North American species of *Cypripedium* mimic flowers of other, more abundant species that offer a reward must also be considered. Pollinators might then confuse the flowers of *Cypripedium* with those of the rewarding species and visit them by mistake. Sugiura et al. (2002), for example, provided evidence for such a case of floral mimicry involving the Japanese lady-slipper, *C. macranthus* Sw. (as *C. m.* var. *rebunense* (Kudo) Miyabe and Kudo) and a rewarding species, *Pedicularis schistostegia* Vved. (Orobanchaceae).

Fruiting success in *Cypripedium* and other orchids that offer no reward is consistently much lower than in orchids that provide nectar or which mimic plants that do (e.g. Gill 1989; Larson and Larson 1990). Stoutamire (1971) believes that bees learn by experience which flowers offer the best food source and will come to avoid those that offer no reward at all. According to this view, seed production in the lady's-slippers may be dependent upon naïve or possibly "forgetful" pollinators, insects that are newly hatched, new to the area, or shifting from a depleted to a new food source (Delpino 1874; van der Pijl 1966; Dressler 1981; Ackerman 1986). On the other hand, Johnson et al. (2003), in a study on the island of Oland off the coast of Sweden, found that bumblebees already carrying pollen of the non-rewarding orchid *Anacamptis morio* (L.) R. M. Bateman were more likely to visit this orchid than bees carrying no pollen. Inexperienced bees here were therefore not more likely to act as pollinators of a generalized, food deceptive orchid than experienced bees.

Nilsson (1979) contends that, in addition to general food deception, the floral attractants in the European *C. calceolus* are attuned to other instinctive responses in bees and that very little learning is involved. The floral fragrance has an uncommon composition. In addition to a monoterpene alcohol called linalool, which may elicit a feeding response, it contains acetates and alpha-farnesene. The acetates are found in cephalic (from the head) pheromone secretions of *Andrena* Fabricius bees, and farnesene is a component of the abdominal Dufour gland in female Andrenas (Bergstrom and Tengo 1974; Tengo and Bergstrom 1977; Nilsson 1979). Pheromones are used to odor-mark objects, which then attract bees of the same species. The cephalic hormones draw females and may cause the aggregation of males (Tengo and Bergstrom 1977). Farnesene is found as a lining in the nest and also signals the location of the nest site (Bergstrom and Tengo 1974). According to Butler (1965), females of *Andrena flavipes* Panzer deposit farnesene in the soil surrounding the nest site, and the odor provokes instinctive landing responses in both sexes. The opening in the labellum may mimic the opening of the nest tunnel (Catling and Catling 1991). In addition, as already noted, the hairs within the labellum may absorb pheromones directly from visiting bees as a supplement to the artificial pheromones produced by the orchid (Nilsson 1979).

A survey of nine species of *Cypripedium* in North America, however, found that most differ substantially from *C. calceolus* in their fragrance components (Bergstrom et al. 1992; Barkman et al. 1997). Since pheromone profiles can differ intragenerically among pollinators (Tengo 1979), correlation between differences in fragrance chemistry and primary pollinator species would not be surprising. A varying blend of odor constituents may have evolved to stimulate an instinctive or learned pattern of response on the part of a range of pollinators to food, sexual reproduction, or nest location (Nilsson 1979; Gregg 1983; Vereecken and McNeil 2010).

Reproductive isolation in *Cypripedium* is critically related to the size of the flower: the width of the entrance and especially the diameter of the anther exit holes and the space between the labellar floor and the stigma determine the size of the insect involved in the pollination (Stoutamire 1967; Catling and Knerer 1980). The dorsal-ventral thickness of the insect's thorax appears to be of particular importance. In addition, Nilsson (1981) noted that the depth of the labellum in the European *C. calceolus* must exceed the length of the pollinating bee by a minimum of 3–4 mm or the bee can simply crawl back out through the labellar opening. All of these floral characters are clearly under strong selection pressure in relation to the primary pollen vectors.

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