IRIS SPP. FLOWER VISITORS:

POLLINATORS VS. NECTAR THIEVES

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Abstract

In Europe bumblebees and solitary bees are main pollinators of Iris flowers, and green beetles common florivores. Irises without nectar present other strategies such as deception or providing shelter that also ensures pollination. Nectar robbers check for nectar at the base of the tepals avoiding contact with anthers or stigmatic lobes. Decreasing wild populations of pollinating hymenopteran insects raises the question how wild Iris species will be affected long term, since pollinators can act as agents of selection influencing polymorphism of native Iris populations.

Keywords: florivores, tepals, royal iris, reward, pollen

INTRODUCTION

Most plant-pollinator relationships are considered generalist to some extent. Specialization by pollinators for the plants they visit is considered an important driving force for floral diversification and speciation (Wesselingh and Arnold, 2000). Many flowering plants evolved mechanisms to attract flower visitors and to ensure the reproductive success by offering nutritional rewards. But besides pollinators, these adaptations may also attract other visitors that do not provide reciprocal services, instead exhibit nectar robbing or florivory behavior (Ye *et al.*, 2017). Pollinator behavior could also have a role in the origin and maintenance of hybrid zones because the prerequisite for inter-specific hybridization occurrence in natural conditions is mediated by pollinators. Weak or absent selection by pollinators against resulting F_1 hybrids could lead to the formation of an advanced generation of hybrids, while preferences for certain hybrids could result in uneven distribution of offspring among genotypic classes (Wesselingh and Arnold, 2000).

During evolution, most *Iris* species adapted to entomophilous pollination, chromatic patterns of the flowers serving largely the same purpose. The upright tepals called standards and stigmatic

petaloid arch protect the reproductive units of the flower from rain and heat, while the lower tepals called falls, act as landing platforms for insects. Different color patches, veins and hairs found on the falls act as signals for pollinators, leading them towards nectar (Robu, 2005; figure 2a).

Hymenopteran bees are considered principal pollinating insects of *Iris* species. Self-incompatibility of many cultivated and wild irises manifests with different intensities and indicates to the dependence of these plants to their relationship with pollinators. For example, while several *Iris* species distributed in Mediterranean region are largely self-incompatible, in others the selfing rates range between 21.4% and 74.1%. In certain self-incompatible irises was put in evidence the presence of enlarged epidermal cells in ovarian grooves that produce a floccular secretion which provides discriminatory activity to incoming pollen tubes (Pellegrino, 2015). Another mechanism to promote cross-pollination is found in protandrous flowers of *Iris douglasiana* (Uno, 1982) and *Iris alberti* (Robu, 2005). Compared to these species, *Iris cedretii* and *Iris sofrana* ssp. *kasruwana* from Middle East that do not produce nectar, were shown to set fruit if cross pollinated on the first day of the flower opening (Monty *et al*., 2006). The hairs found on falls (Fig. 1a, 1b) of some *Iris* species could have role in helping smaller insects to pollinate while visiting the flowers but also can act as barriers for certain species to enter the flowers on the first day of anthesis. Before it becomes completely mature, the stigmatic petaloid arch is bent close to the falls with hairs further restricting entry for common pollinators such as *Apis mellifera* that was seen to be unable to enter the flower on first day, compared to larger, stronger but more rare - *Bombus hortorum* (Robu, 2005).

Each *Iris* flower consists of three pollination units (Wesselingh and Arnold, 2000) called meranthia (Goldblatt *et al*., 1998), blossoms or gullets (Faegri and Pijl, 1980). The most frequent behavior observed in the pollinators of iris flowers was the tendency to visit only one floral unit of a flower out of the three and then moving to next flower. This was observed in *Iris douglasiana* (Uno, 1982) as well as in *Iris tuberosa* (Pellegrino, 2015). Contrary, for *Iris fulva*, *Iris brevicaulis* and their F_1 hybrids was observed that bumblebees and hummingbirds often visited more than one pollination unit of a flower (Wesselingh and Arnold, 2000).

FLOWER REWARDS

The most sought reward by *Iris* flower pollinators is the nectar, yet there are also *Iris* species that do not present nectar and offer indirect rewards such as shelter.

Pollen is also another flower reward for some visitors.

Although nectar production begins before the flower opens and continues throughout the life of flower, the highest flow is occurring in early stages. Measurements conducted managed to identify that on the first day of opening *Iris douglasiana* flower produced on average 7.58 µl of nectar, on second day 11.63 µl and on the third day 13.27 µl. On the 5th day when flowers wilted no nectar was detected. Mean sugar content of *Iris douglasiana* nectar was 26.9% (Uno, 1982). After 4-10 visits of *Iris douglasiana* flowers, *Bombus* sp. females were observed transferring pollen to their scopae, aspect not observed in visiting bees. The purest pollen loads were obtained from *Bombus occidentalis* with only 6% foreign pollen, compared to *Emphoropsis*, the most frequent visitor of this species that had 55% of pollen from plants other than *Iris douglasiana* (Uno, 1982).

Structures involved in pollination and reproduction of some bearded species from genus *Iris* can be observed in figure 1.

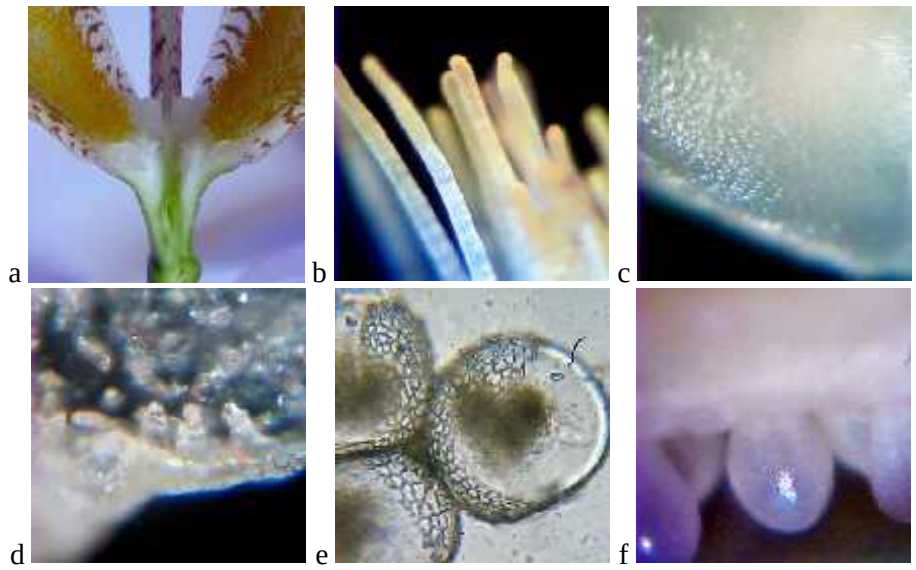


Fig.1. *Iris pallida* flower structures involved in pollination and reproduction: a) section through perigonal tube with base of beard line, b) close-up of lower tepal hairs, c) nectariferous tissue, d) receptive surface of stigmatic lip displaying papillae, e) reticulate exine surface of pollen grains, f) ovules inside ovary (Original)

Iridaceae is unique among monocots because both types of nectaries are present: oil-producing epidermal cells as well as trichomes (elaiophores).

Within subfamily *Iridoideae* perigonal nectaries and

elaiophores take several forms, with highly vascularized nectariferous regions being present on different parts of the flower surface in *Iris* species, as follows:
- nectaries are present at the base of perigonal tube especially within interstaminal regions in *Iris douglasiana, Iris ensata, Iris foetidissima, Iris graminea, Iris pseudacorus, Iris sibirica*
- nectaries are found around the base of the style in *Iris dichotoma*
- nectaries are present in a continuous region from around the base of the perigonal tube to around the style in *Iris chamaeiris, Iris germanica, Iris kolpakowskiana, Iris stolonifera, Iris tectorum, Iris tingitana, Iris warleyensis, Iris xiphioides*
- nectaries are present in a continuous region from around the base of the perigonal tube extending into the base of inner tepals in *Iris sisyrinchium* (Rudall *et al*., 2003).

POLLINATORS

1. IRISES WITH NECTAR

Several studies conducted in Europe mention pollinators of *Iris* species. *Iris sibirica* from *Molinietum caeruleae* phytocoenosis in Poland were reportedly being pollinated by bumblebees (Kostrakiewicz-Gierałt, 2013). *Iris tuberosa* flowers from calcareous, dry grasslands *(Festuco-Brometalia)* of central-south Italy were reportedly visited intermittently during the day between eight in the morning and six in the evening by insects. From these, nine species were identified and recognized as effective pollinators of *Iris tuberosa* flowers, and they belong to five genera of Hymenoptera: *Andrena, Anthophora, Colletes, Lasioglossum* and *Xylocopa. Andrena* was the dominant genus in particular with the species *Andrena nigroaenea*, *A. flavipes*, *A. bicolor*, *A. creberrima* and *A. morio* (Pellegrino, 2015). In China, three bumblebee species (*Bombus friseanus, B. religiosus, B. festivus*) were identified as main pollinators of alpine *Iris bulleyana*, with *Apis mellifera* only as occasional visitor (Ye *et al*., 2017). At an experimental location in California, almost all insect visitors of *Iris douglasiana* flowers were long-tongued nectar-collecting bees, with over 80% of the visitors comprised by *Emphoropsis mirabilis*. The rest of the pollinators belonged to the species: *Bombus occidentalis* ssp. *nigroscutatus, B. bifarius* ssp. *nearcticus, B. vosnesenskii, Anthophora bomboides* ssp. *stanfordiana* (Uno, 1982). At a Louisiana research location, the flowers of *Iris fulva*, *Iris brevicaulis* and their F_1 hybrids were most frequently visited by *Bombus pennsylvanicus*, as well as by ruby-throated hummingbirds (*Archilochus colubris*) and

carpenter bees (*Xylocopa* sp.) (Wesselingh and Arnold, 2000). Another study involving the same two *Iris* species showed that hummingbirds were more effective at transferring pollen among *Iris fulva* flowers while bumblebees were more effective at transferring pollen among *Iris brevicaulis*, compared to effectiveness of interspecies cross-transport (Shaw *et al*., 2017). A different study conducted in United States identified that *Iris nelsonii* is primarily pollinated by hummingbirds, while *Iris hexagona* is primarily pollinated by bumblebees (Taylor *et al*., 2013).
In table 1 can be found the chronologic list by publishing year of some papers that mention *Iris* flowers pollinators.

Table 1

Pollinators of *Iris* flowers

Mentioned visitors	*Iris* species visited	Reward	Country/State	Source
Emphoropsis mirabilis, Bombus occidentalis, B. bifarius, B. vosnesenskii, Anthophora bomboides	*Iris douglasiana*	N, P	USA, California	Uno, 1982
Bombus pennsylvanicus, Archilochus colubris, Xylocopa sp.	*Iris fulva, Iris brevicaulis,* hybrid F_1	N	USA, Louisiana	Wesselingh *et* Arnold, 2000; Shaw *et al*., 2017
Eucera sp.	*Iris atrofusca, Iris haynei, Iris atropurpurea, Iris hermona, Iris bismarckiana, Iris mariae*	S	Israel	Sapir *et al*., 2005
Eucera sp., *Xylocopa* sp., *Andrena* sp.	*Iris cedretii Iris sofrana*	S, O	Lebanon	Monty *et al*., 2006
bumblebees, hummingbirds	*Iris hexagona Iris nelsonii*	N	USA	Taylor *et al*., 2013
bumblebees	*Iris sibirica*	N	Poland	Kostrakiewicz-Gierałt, 2013
Andrena, Anthophora, Colletes, Lasioglossum, Xylocopa	*Iris tuberosa*	N, S	Italy	Pellegrino, 2015
Bombus friseanus, B. religiosus, B. festivus, Apis mellifera	*Iris bulleyana*	N	China	Ye *et al*., 2017
bumblebees	*Iris pumila*	-	Serbia	Radović *et al*., 2017
Bombus sp., solitary bees	*Iris lutescens Iris pumila*	-	France	Souto-Vilarósa *et al*., 2017

Reward: N – nectar, P – pollen, S – shelter, O – other

2. IRISES WITHOUT NECTAR

Reward-less flowers of *Iris pumila* (Willmer, 2011) were reportedly visited and pollinated by bumblebees in Serbia (Radović *et al*., 2017) as well as in France, in addition, deceptive *Iris lutescens*

was visited also by bumblebees, solitary bees and *Vespoidea* exhibiting typical nectar-foraging behavior although neither of this species presents nectar (Souto-Vilarósa *et al.,* 2017). Royal irises (*Iris* subg. *Iris* sect. *Oncocyclus*) do not have nectar either but provide shelter as reward. For *Iris cedretii* and *Iris sofrana* ssp. *kasruwana* from Lebanon, *Eucera* sp. males are the most important flower visitors that shelter in flowers and act as pollinators, while visitors from genera *Xylocopa* and *Andrena* had lower visiting frequency (Monty *et al*., 2006).

FLORIVORES, NECTAR THIEVES AND OTHER FLOWER VISITORS

For the flowers of *Iris fulva, Iris brevicaulis* and their F_1 hybrids the nectar robbers belonged to at least three species of butterflies most frequently *Hesperiidae*, as well as hummingbirds that probed for nectar at the base of the tepals, thereby avoiding contact with anthers or stigmatic lobes (Wesselingh and Arnold, 2000).

In *Iris bulleyana* pollinator visitation and seed production decreased significantly in flowers damaged by florivores identified in sawflies (*Tenthredo* spp.). The short-tongued pollinator *Bombus friseanus* shifted to a robber using the holes made by sawflies to feed on nectar without pollinating (Ye *et al*., 2017).

Flowers of *Iris cedretii* and *Iris sofrana* ssp. *kasruwana* from Lebanon were visited by Coleoptera, Homoptera and Heteroptera with no role in pollination, instead by feeding on flower tissues caused sometimes complete destruction of the flowers (Monty *et al*., 2006). A common florivore of *Iris lutescens* flowers in Europe is *Cetonia hirsuta* (Souto-Vilarósa *et al*., 2017).

In *Iris douglasiana*, *Andrena* spp. and *Bibio necotus* were considered only incidental visitors because they did not enter the flower (Uno, 1982). The visitors of *Iris bulleyana* belonging to genera *Andrena* spp. and *Hydrophoria* spp. did not manage to pollinate because of their size (Ye *et al*., 2017).

In temperate climate of Romania - Cluj-Napoca Agro-Botanical Garden were observed *Iris* flowers visitors belonging to *Apidae, Andrenidae, Syrphidae* and especially in beardless irises: *Formicidae* (figure 2).

POLLINATOR-MEDIATED SELECTION ON FLORAL TRAITS

By assessing phenotypic pollinator-mediated selection on flower color and size for two polymorphic *Iris* species (*Iris lutescens* and *Iris pumila*), was showed that in *Iris pumila* pigment concentration is under selective pressure by pollinators only for one

color morph: blue, while *Iris lutescens* pigment concentration and flower size was under selection independent of pollinators, suggesting that pollinators are not the only agents of selection on floral traits for within-population polymorphism of these species (Souto-Vilarósa *et al*., 2017). For *Iris tuberosa* in Italy no evidence for pollinator-mediated selection on plant and floral size could be identified either (Pellegrino, 2015). However, in a study conducted in United States, was showed that divergent floral morphologies may directly cause a reduced interspecific visitation, reducing gene flow between homoploid hybrid lineage and its progenitors as it was observed for *Iris nelsonii* that is primarily pollinated by hummingbirds, while one of its progenitors – *Iris hexagona*, is primarily pollinated by bumblebees (Taylor *et al*., 2013). More recently following a research conducted in Israel, was found evidence that pollinator-mediated selection is responsible of increased floral size and stem length of *Iris atropurpurea* while the flower color was not (Lavi and Sapir, 2015).

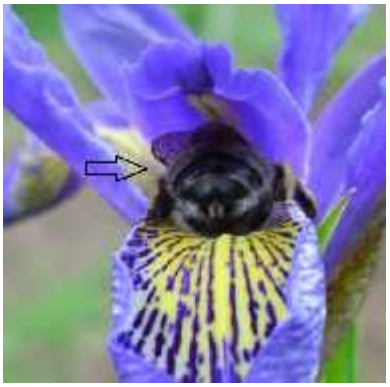
a  b 

Fig. 2. Insect visitors of some apogon *Iris* sp. in Agro-Botanical Garden UASVM Cluj-Napoca: a) *Andrenidae*, b) *Formicidae* (Original)

Results of previous studies reveal the complexity of the interaction existing between *Iris* flowers and their pollinators leaving many open questions as well as rising future challenges for better describing their effect on flower diversity and composition in natural habitats.

It is concluded that pollinator-flower interaction could be a good subject for further research especially in Europe within the context of decreasing wild populations of pollinating hymenopteran insects, for assessing how this phenomenon will affect wild *Iris* populations on long term.

CONCLUSIONS

Irises with nectar are visited and pollinated by a variety of Hymenoptera insects as well as by hummingbirds. Notably in Europe *Bombus* sp. and *Andrena* sp. are important pollinators of *Iris* flowers. Nectar robbers were identified in butterflies most frequently *Hesperiidae*, as well as hummingbirds and sawflies (*Tenthredo* spp.). Studies conducted on *Iris douglasiana* showed that nectar production per flower reached the peak on the third day after opening with a quantity of 13.27 µl while the mean sugar content of nectar was 26.9%. Reward-less flowers of *Iris pumila* and *Iris lutescens* in Europe were most frequently visited by bumblebees and solitary bees exhibiting nectar foraging behavior, while nectar-less royal irises (*Iris* subg. *Iris* sect. *Oncocyclus*) from Middle East are primarily pollinated by *Eucera* sp. males that shelter in flowers. Florivory behavior damaging to flowers can be caused by several taxonomic groups such as *Coleoptera* and *Heteroptera*, but most notably by *Cetonia hirsuta* as reported in Europe.

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