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The importance of butterflies in the pollination of flowering plants

Význam denních motýlů pro opylení kvetoucích rostlin

Bachelor Thesis

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Prohlášení

Prohlašuji, že jsem práci zpracoval samostatně a že jsem uvedl všechny použité zdroje a literaturu.

V Praze, 9.05.2019 Dominik Anýž

Abstract in English

Butterflies are frequent visitors to flowering plants, but their efficiency as pollinators has been often questioned. Although undoubtably inefficient pollinators in many plant species, the role of butterflies in successful pollination of certain plant species has been underestimated. However, their general contribution in the pollination has never been critically reviewed, despite their global species richness and distribution. The aim of this thesis was to summarize the results of studies quantifying pollination effectiveness of visitors to various plant species, with an emphasis on the butterfly pollination syndrome. Pollination efficiencies along with floral preferences were assessed separately for individual families. Whereas papilionids and nymphalids seems to be efficient pollinators of numerous plants, pierids and hesperiids are rather inefficient thieves of nectar. The review also revealed preferences of particular butterfly families in their foraging behavior along with variances in pollination effectiveness.

Key words: butterfly, pollination, pollination syndromes, psychophily, pollination efficiency

Abstrakt v češtině:

Denní motýli jsou častými návštěvníky květů, jejich efektivita opylování kvetoucích rostlin je však často zpochybňována. Přestože řadu rostlinných druhů denní motýli nepochybně neopylují, u řady dalších druhů kvetoucích rostlin jejich roli spíše podceňujeme. Navzdory druhové diverzitě a globálnímu rozšíření denních motýlů nebyl jejich obecný význam v polinačních systémech dosud kriticky shrnut. Cílem této práce bylo shromáždit a zhodnotit výsledky studií zaměřených na efektivitu opylování různých druhů rostlin různými motýlími opylovači s důrazem na psychofilii, neboli motýlí polinační syndrom. U jednotlivých motýlích čeledí jsem zhodnotil účinnost opylování i preference různých vlastností květů. Zatímco otakárci (Papilionidae) a babočky (Nymphalidae) efektivně opylují řadu druhů rostlin, bělásci (Pieridae) a soumračníci (Hesperiidae) spíše kradou nektar z květů. Z výsledků rovněž vyplývá, že jednotlivé motýlí čeledi preferují květy různý rostlin podle jiných znaků.

Klíčová slova: denní motýli, opylovaní, polinační syndromy, psychofílie, efektivní opylení

Contents

1. Introduction

Pollination is a sexual reproduction of most plants. It involves transfer of pollen grains from male reproductive organs, anthers, to female reproductive organs, stigmas. Biotic pollination is performed by animals which are then called pollinators. About 80% of angiosperm plants rely on pollinators for effective pollen transfer, making biotic pollination an essential component for the reproduction of most plants (Ackerman, 2000).

Butterflies (Lepidoptera: Rhopalocera) belong among the most common flower visitors (Willmer, 2011). Nevertheless, there are two very different views on butterflies as pollinators. Often, butterflies are considered as pollinators, especially by people out of pollination research (Capinera, 2008). This is because butterflies are easy to recognize when visiting flowers due to their large and colorful wings. Such visits are then often associated with pollination. The other way around, many pollination scientists would consider most butterflies rather as nectar thieves of many plant species (Willmer, 2011). This may be due to a relatively long-term view of butterflies as nectar thieves in various pollination studies (Adrienne et al., 1985; Wiklund et al., 1979).

My thesis aims to review and summarize available information on butterfly pollination and discuss the overall effect of these insects on pollination of day flowering plants. In this respect, the thesis sets two main goals. Firstly, to assess various aspects of mutual adaptations of both butterflies and plants for their pollination interactions, with the special focus of psychophilous pollination syndrome. Secondly, to review studies on the efficiency of butterflies as pollinators and discuss the validity of their results.

For the first goal I collated studies and general reviews looking into pollination ecology and butterfly pollination along with studies discussing butterfly morphology and preferences of butterflies toward certain plant traits. For the second goal I decided to only consider studies focusing on a single plant species or a single butterfly species. Pollination network studies on the level of entire communities are far too broad to reliably quantify real pollination efficiency. They are thus suitable rather for analysis of flower visitation rather than pollination which is out of the scope of this thesis.

2. Butterflies and pollination syndromes

Lepidopterans are an insect order with the second highest species richness, with over 160,000 species described, i.e. ~16% of the known global species richness of insect (Capinera, 2008). Butterflies (Lepidoptera: Rhopalocera) are a monophyletic group within Lepidoptera (Espeland et al., 2018), which cover over ~10% of lepidopterans, with ~18,000 described species (Espeland et al., 2018). Based on the most recent taxonomical classification, butterflies are divided into seven families: Hedylidae, Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae, Pieridae and Riodinidae (Espeland et al., 2018).

To understand butterfly pollination, it is important to look at the individual characteristics of both the pollinator and the flower, especially to such characteristics playing a fundamental role in their foraging behavior and preferences. Each functional group of pollinators prefers different floral traits based on the pollinator's morphology and their feeding demands. For example, unlike other pollinator groups, adult butterflies utilize only nectar from all floral rewards, unlike bees, for example, which forage also on pollen (Willmer, 2011). Most adult butterflies only need enough nectar to meet their own requirements, whereas bees collect both nectar and pollen to also feed their larvae. Butterflies lack any specialized morphological structures; they carry pollen only passively on parts of their body, which contacted anthers when feeding: i.e. proboscis, head, thorax and sometimes wings. As a result of butterfly disinterest for pollen, butterfly pollinated plants offer only small amounts of it, producing only enough pollen to allow for efficient reproduction, without offering any excess pollen for potential visitors that would feed on it, such as bees (Willmer, 2011). In turn, plants pollinated by butterflies provide a low amount of nectar, as to increase the butterfly visitation rate to individual flowers and thus raise the probability of efficiently being pollinated.

Floral traits such as nectar and pollen quantities in flowers are only two in the array of characteristics I will review, which individual groups of pollinators search for when visiting flowers. Among others, these characteristics include color, odor, time of flowering, or size of the flower. Plants exert these characteristics in order to attract a specific type of pollinator, suggesting the "pollination syndrome hypothesis" (Ollerton et al., 2009). Pollination syndromes are defined as a set of floral traits that has convergently evolved in unrelated plant species to adapt for a specific pollen vector and attract them to their flowers. The syndromes are beneficial for both the plant and its pollinator; the plant provides nutrients necessary for the pollinator and in return the pollinator will more likely visit said plant, raising the plants probability of being efficiently pollinated (Cruden and Hermann-Parker, 1979; Willmer, 2011). The first comprehensive and most cited monograph discussing pollination syndromes is the book Principles of pollination ecology (Faegri and van der Pijl, 1979).

The pollination syndrome hypothesis does not precisely describe all traits that must be present in a certain flower to attract a specific pollinator. It serves rather as a guideline to which characteristics certain visitors prefer. Aside from very specific cases, in which animals and plants have coevolved with one another and are thus fully dependent on each other (an extreme example are figs and their pollinators), most flower visitors are at least generalists in that they do prefer certain traits but are able to forage on diverse plants. Pollination syndromes are set of traits which an animal prefers over generalized pollination. Due to the convolute nature of butterfly morphology and diverse floral traits, I will review them together.

3 Psychophily and butterfly morphology

The 'butterfly pollination syndrome' is called psychophily. This syndrome has been deliberately criticized and its validity has been tested repeatedly (Fenster et al., 2004; Hingston and Quillan, 2000). The consensual psychophilous syndrome is broadly presented in Table 1, which I have compiled based on several different definitions and descriptions (de Araújo et al., 2014; Cruden and Hermann-Parker, 1979; Faegri and van der Pijl, 1979; Hingston and Quillan, 2000; Johnson and Bond, 1994; Willmer, 2011). To prove why the synopsis is necessary, I will use the color definition in the various definitions mentioned. Faegri and van der Pijl, (1979) defined the floral color typical for the psychophilous plants to be red only, which was later followed by Johnson and Bond, (1994). Cruden and Hermann-Parker, (1979) added also orange and yellow flowers to the syndrome definition, whilst Hinston and Quillan, (2000) added yellow and UV colored flowers. Willmer, (2011) widened the floral color of psychophilous plants for violet, blue, orange, red, yellow and UV. Finally, Araújo et al., (2014) again narrowed the definition to the color as pink, red, blue, violet and UV.

I have compiled Table 1 based on the frequency of individual traits described from the various definitions mentioned. The color red was present in all six definitions, yellow, blue, violet and UV were all associated with psychophily two or more times (sometimes), and pink with orange were both only mentioned once in all the definitions (rarely). Two of the six publications defined nectar guides as present (Cruden and Hermann-Parker, 1979; Hingston and Quillan, 2000), resulting them being labeled "sometimes" in Table 1. There was no dispute over the remaining syndrome definitions within the publications used.

Color	Red, sometimes yellow, blue, violet, UV rarely pink and orange
Odor	Sweet but mild
Nectar	Small to medium amount, diluted
Nectar guides	Sometimes present
Shape	Long corolla tube with nectaries at the base, landing platform present.
Flowering	Day flowering
Pollen	Small, limited amount

Table 1 – Main floral traits representing the psychophilous pollination syndrome.

3.1 Butterfly vision and floral color

Butterfly vision is considered among the most complex among animals. As all insect groups, butterflies have compound eyes composed up of individual ommatidia. Most butterflies have three to four color receptors, with wavelength peaks between 300nm and 700nm (Arikawa et al., 1987; Bernard, 1979). A study testing 35 species of butterflies found that most have an absorption maximum in the UV spectrum (Eguchi et al., 1982), allowing to see in it and use it when foraging on flowers.

The fundamental color associated with the psychophilous syndrome is red (Table 1). The first fully comprehensive review on pollination ecology published in 1979 connected the psychophilous syndrome only to the color red, because they did not yet have the methods necessary to study butterfly vision and therefore relied only on observations (Faegri and van der Pijl, 1979). Recent reviews added more colors to the syndrome, mainly violet, yellow, blue and UV (Table 1).

Color is one of the main aspects that has been disputed in the psychophilous syndrome. This is mainly because butterflies are a diverse group and not all have the same receptors able to distinguish the same wavelengths. Some butterflies only have two wavelength maxima (*Parantica* *sita*, Nymphalidae), whereas others can have up to five maxima (*Papilio machaon* Papilionidae) (Eguchi et al., 1982), and even those were found to be in different regions for various species.

Various older studies analyzing color preferences in butterflies managed to identify which colors individual species prefer in laboratory settings (Ilse and Vaidya, 1955; Scherer and Kolb, 1987a; Scherer and Kolb 1987b). These studies used a combination of artificial flowers infused with sugar solutions and recorded the visitation rates of specific butterflies to individual flowers. Ilse and Vaidya (1955) found a strong preference for the colors blue and violet in *Papilio demoleus* (Papilionidae), Scherer and Kolb, (1987a) studied *Pieris brassicae* (Pieridae) and discovered that they were most prone to visit blue, orange and red flowers.

Newer studies utilized modern methods to analyze butterfly color preferences in their natural habitat, and found that butterflies combine many of their senses when foraging, not primarily color. (Pohl et al., 2011; Briggs et al., 2018). For example, *Speyeria mormonia* (Nymphalidae) preferred to visit orange *Dugaldia hoopesii* over both yellow D. *hoopesii* and yellow *Wyethia amplexicaulis*, but showed no preference in color when foraging on both orange and yellow *W. amplexicaulis* (Pohl et al., 2011). The same study also found that *Cercyonis oetus* (Nymphalidae) preferred to visit yellow *W. amplexicaulis* over orange *W. amplexicaulis* and showed no preference between yellow and orange *D. hoopesii* (Pohl et al., 2011).

3.2 Nectar guides

Nectar guides are markings on floral petals, which have the function of leading a pollinator to the nectaries containing the nectar reward (Willmer, 2011). They can range from either a simple shift of color from the flower periphery to its center or they can be intricate markings along the petals' length revealing the way to the reward, depending on the plant species. Nectar guides can differ in colors, including UV.

Several publications comparing studies on butterfly pollination did not include nectar guides to their psychophilous syndrome definition (Ollerton et al., 2009; Willmer, 2011), but there are some that did (Cruden and Hermann-Parker, 1979; Hingston and Quillan, 2000). Nectar guides often seem to be a phylogenetically constrained trait and are therefore one of the characteristics which can hardly be an integral part of any pollination syndrome definition. However, they are apparently important for some functional groups of visitors, such as bees (Willmer, 2011), and flowers with nectar guides were shown to better direct the visiting butterflies' attention toward the nectar reward (Kandori and Ohsaki, 1998; Medel et al., 2003).

3.3 Nectar and pollen

Adult butterflies feed on fluids rich in nutrients, for example sugars (Dierks and Fischer, 2008) or amino acids (Beck, 2007). Most butterfly species consume these fluids in the form of nectar, which is a nutrient-rich liquid created by plants in their nectaries (Willmer, 2011). Floral nectaries are plant tissues secreting nectar from sepals, petals, stigmas or anthers (Willmer, 2011). Some butterflies feed on different resources than flowers, for example fruit (Fermon et al., 2003) or wet soil, from which they can obtain minerals (Beck et al., 1999).

Psychophilous flowers are defined to produce a small to medium amount of relatively diluted nectar, depending on the plant species (Table 1). Studies analyzing plants pollinated by butterflies found nectar volumes to range from 1ul to 12ul (Cruden and Hermann-Parker, 1979; Goldblatt and Manning, 2002; Johnson and Bond, 1994; Mertens et al. submitted). Nectar of psychophilous plants is primarily composed of sucrose (~50%), with other sugars (such as glucose and fructose) or amino acids playing a minor role (Beck, 2007; Dierks and Fischer, 2008; Mertens et al.; Romeis and Wackers, 2000).

The low concentration of nectar in psychophilous flowers is associated with the morphology of butterfly proboscides. Proboscides have evolved from the insect mouthpart maxillae by elongating and interlocking into each other to form a straw like structure, through which a butterfly is able to intake into its mouth by capillary action (Capinera, 2008; Tsai et al., 2014). Lengths of proboscides vary among species of butterfly, ranging from 6 mm (*Apaustus gracilis gracilis*; Hesperiidae) to 45 mm (*Eurybia lycisca*; Riodinidae) (Bauder et al., 2011; Bauder et al., 2014). Butterflies keep their proboscis coiled and extend it only when feeding. It has been suggested, that the butterfly proboscis is an adaptation of the animal for nectar thieving (Wiklund et al., 1979).

Most studies of butterfly proboscis used monarch butterfly, *Danaus plexippus* (Nymphalidae), as the model organism (Monaenkova et al., 2012; Tsai et al., 2014). Tsai et al., (2014) experimentally evidenced that this butterfly species cannot intake nectar solutions of >40% sugar concentration (Tsai et al., 2014). Another study done on the pollination of *Buddleja davidii,* which is a plant frequently visited by butterflies, found sugar concentrations of the flower to range between 17 % to 33.5 % (Chen et al., 2014). Nectar concentrations vary depending on individual plant species, but do not reach sugar percentage of >40%, since butterfly proboscides utilize capillary action and are not able to uptake more concentrated solutions (Tsai et al., 2014).

Pollen in the psychophilous syndrome is defined by a small and limited amount (Table 1). As previously mentioned, most butterflies utilize only nectar when foraging, so butterfly pollinated flowers only need to produce the quantity of pollen necessary for pollination along with a quantity of nectar high enough to attract butterflies, but also to efficiently maximize butterfly foraging behavior. Both the low quantity of pollen and nectar in psychophilous plants are a response to butterfly foraging behavior.

3.4 Shape, size

Psychophilous plants are typically defined by long tubular corollas, with nectar hidden at the base (Table 1). This adaptation lets only visitors with feeding parts long enough to reach it and helps the plants avoid potential nectar thieving. As with other characteristics, the corolla lengths vary, appealing to butterflies of various proboscis sizes (Armstrong, 1979; Bloch and Erhardt, 2008). Along with a long corolla adapted for butterfly proboscides, psychophilous flowers also have a landing platform for the pollinator. Butterflies feed only when sitting, making it a necessity for them to have a place to land.

Some butterfly pollinated flowers exert herkogamy, an adaptation defined by spatial separation of anthers and stigmas (de Araújo et al., 2014). This adaptation has two main functions. The first is to limit the risk of self-pollination, the second function is to increase the possibility of pollen transfer onto the pollinators body from anthers, that are protruding beyond the floral opening (de Araújo et al., 2014).

3.5 Time of flowering

Most butterflies are diurnal insects, i.e. active during the day, therefore psychophilous plants are also defined as being day flowering (Table 1). Certain plants have time of flowering along with nectar secretion synchronized with specific pollinators, which have a high foraging rate in the morning until early afternoon, such as butterflies (Cruden and Hermann-Parker, 1979), suggesting a mutualistic relationship.

3.5 Odor

The psychophilous syndrome is characterized by sweet but mild floral scent (Table 1). Until recently, it has been believed that butterflies favor color over scent in flowers when foraging (Andersson and Dobson, 2003; Ômura and Honda, 2005), but recent studies have confirmed that this assumption is inaccurate and that butterfly preferences are more complex (Ômura and Honda, 2005).

Tang et al., (2013) tested these preferences and found that some butterflies use vision most of the time, but when high concentrations of honey water with a strong odor were presented, the butterflies visited that flower more frequently (Tang et al., 2013). The priority of scent over color was also evidenced in the largest butterfly in the study, *Idea leuconoe* (Nymphalidae), which, preferred to visit flowers with a stronger scent (which suggested a higher sugar reward) to meet its energy needs (Tang et al., 2013).

3.6 Syndromes under discussion

As previously stated, pollination syndromes do not precisely describe all traits that must be present in a certain flower to attract a specific pollinator but serve rather as a guideline to which characteristics certain visitors prefer. This thought considered, most characteristics associated with the psychophilous syndrome can be found accurate. Since most butterflies are diurnal, psychophilous plants are characterized as day flowering (Table 1). Nectar concentration associated with the psychophilous syndrome is defined to be diluted (Table 1), which allows the butterfly visitor to intake it. Odor, even though recently proven to be more important in butterfly foraging preferences that previously thought, is sweet but mild (Table 1). The floral traits that are however inconsistent throughout various definitions are color and shape.

It has been proven that individual butterfly species have wavelength maxima in various peaks of the color spectrum (Eguchi et al., 1982), which leads to diverse species finding different colors appealing. Probably the most influential definition of the butterfly pollination syndrome by Faegri and van der Pijl, (1979) associated only red with the syndrome (Faegri and van der Pijl, 1979). Using only this definition, only a few studies have managed to successfully prove butterfly preferences of psychophilous flowers (Cruden and Hermann-Parker, 1979; Mertens et al. in review). Mertens et al proved butterfly visitation in *Scadoxus cinnabarinus* by *Papilio dardanus,* *Papilio zenobia* (Papilionidae) and three species of Pierids (Mertens et al. in review). Cruden analyzed visitors of *Caesalpinia pulcherrima* and found papilionids and pierids to be efficient pollinators of the species but certain nymphalids and hesperiids to be infrequent and inefficient pollinators.

Recent studies have proven, that butterflies combine their senses when foraging (Briggs et al., 2018; Pohl et al., 2011) affecting their preferences flowers they chose to visit. An example previously used, in which Pohl et al. (2011) found two nymphalids to have opposite preferences can serve as a good example for the variance in certain butterfly families (Pohl et al., 2011). Various publications have assessed color preferences (Briggs et al., 2018; Ilse and Vaidya, 1955; Scherer and Kolb, 1987b) or tested olfactory senses (Ômura and Honda, 2005; Tang et al., 2013) of certain butterflies, but few have assessed psychophily as a whole (Johnson and Wester, 2017; Ollerton et al., 2009).

Among the butterfly families, hesperiids are considered as generalized foragers and most show little interest in psychophilous traits (Adrienne et al., 1985; Herrera, 1987). Papilionids have often been recorded foraging on psychophilous plants (Cruden and Hermann-Parker, 1979; Mertens et al. in review; Sakamoto and Morinaga, 2013; Wester and Johnson, 2017), suggesting that the syndrome is accurate for some of them. Various studies have been carried out to analyze the preferences of nymphalids (Eguchi et al., 1982; Ômura and Honda, 2005; Pohl et al., 2011) and pierids (Eguchi et al., 1982; Scherer and Kolb, 1987a), but no clear preference has yet been observed in either of these families. It is however clear, that the original pollination syndrome definition cannot be assigned to either of these families. Lycaenids, riodinids and hedylids are butterfly families that have not been studied enough to make a conclusion on their individual preferences. I use the second part of my thesis on pollination efficiency of butterflies to gather data regarding individual butterfly families' visitation frequencies to psychophilous flowers (Table 1).

4. Pollination efficiency of butterflies

An efficient pollinator must transfer pollen from anthers onto stigmas in enough quantities to effectively pollinate a certain flower and allow development of as many seeds as possible. In theory, one pollen grain is enough to pollinate one ovule of the stigma and each pollinator usually carry multiple pollen grains. In reality, many pollinators need multiple visits of flowers to efficiently transfer enough pollen grains for efficient pollination (Courtney et al., 1982). Quantities of pollen grains, as well as number of ovules, per flower vary among plant species, and individual plants thus differ in amounts of transferred pollen necessary for efficient pollination (Courtney et al., 1982).

Pollination efficiency of butterflies has been repeatedly discussed in the past. There are multiple reasons why some older studies described butterflies as inefficient pollinators, for instance not enough pollen grains on butterfly proboscis (Wiklund et al., 1979) or on thorax and wings (Courtney et al., 1982), no contact with plant reproductive organs (Leal et al., 2006), or not a high enough visitation rate of flowers (Sugawara et al., 2016). In the rest of this chapter I will review cases in which butterflies were found to be efficient and inefficient pollinators.

4.1. Nectar thieving by butterflies

Inefficient pollination is often confused with nectar thieving., which is defined as a repeated visit of flower by an animal to feed on nectar without contacting its reproductive organs or contributing to its potential reproductive success by some other way. It typically covers mutual morphological or behavioral mismatching of the pollinator and flower. Inefficient pollination, however, usually means transport of an insufficient pollen amount to pollinate the plant by a certain visitor, even though it has the potential to do so. For example, the flower visitor can contact plant reproductive organs, but not deposit enough pollen grains to efficiently pollinate another flower during such visit for various reasons.

Wiklund et al., (1979) even suggested that the butterfly proboscis is a specific adaptation for nectar thieving. Their study based this hypothesis on an insufficient quantity of pollen grains on the proboscides of *Leptidea sinapis* (Pieridae) and the overall morphology of the butterfly proboscis, which is not customized in any way to carry pollen grains (Wiklund et al., 1979). Although the study was later criticized for ignoring pollen grains attached to the butterflies' head and wings (Courtney et al., 1982), the hypothesis of the proboscis as a nectar thieving mechanism became a prominent field of study in the following years (Bauder 2011).

Undoubtably, some butterflies are nectar thieves of certain flowers, one example coming from a study quantifying visitation of *Passiflora coccinea* (Leal et al., 2006) flowers. The study focused on the mutualism between the plant and ants and revealed butterflies as its ineffective pollinators. Five butterfly species, two hesperiids and three nymphalids, were recorded to consume its nectar without contacting the plant reproductive organs. Two more studies, the first studying *Psychotria serpens* (Sugawara et al., 2016) and the second studying *Psittacanthus auriculatus* (Pérez-Crespo et al., 2016) both found butterflies to be nectar thieves of said plants, since they did not contact the flowers reproductive organs.

4.2. Pollination efficiency in butterfly families

Out of the various methods used to determine pollination efficiency, analyzing seed sets of plants visited by pollinators is the only method which directly quantifies visitor effectiveness. However, due to the complexity of this method, various studies use indirect quantification of pollination effectiveness, such as counting of pollen grains deposited on stigmas by visitors, fruit or seed set of the flower, pollen loads on specific visitors, or frequency of visits by animals to specific plants. In this chapter, I assess results of various studies quantifying pollination effectiveness in diverse plant species in which butterflies were found to be visitors and organize results by butterfly families. I use only single butterfly species studies and single plant species studies, in which butterflies were observed. I also examine the visitation rate and pollination efficiency of butterflies to psychophilous plants based on a broadened definition (Table 1).

4.2.1 Hesperiids

The hesperiid family, also called "skipper butterflies", is widely considered to be a group of inefficient pollinators (Schemske and Horvitz, 1984; Adrienne et al., 1985; Herrera, 1987), in many cases even being labeled as nectar thieves (Leal et al., 2006; Li et al., 2014; Pérez-Crespo et al., 2016). Hesperiids are considered more as generalist than specialized foragers, in that they do not show preferences for certain flower traits and visit a vast array of flowers when feeding.

Some studies found hesperiids to be inefficient pollinators because of their body size. A study analyzing pollinators of *Caesalpinia pulcherrima* found two hesperids, *Pyrrhopyge Scylla* and *Phocides pigmalion bellus* to be a frequent visitor of one plant population studies, but not efficient due to their inability to carry large amounts of pollen on their body (Cruden and Hermann-Parker, 1979). Similar results were reached in studies on *Lantana trifolia*, which was frequently visited by *Pyrgus* (Schemske, 1976).

However, the main reason most studies on pollination efficiency have labeled Hespiriids as inefficient pollinators is because, in most cases, they acted as nectar thieves (Adrienne et al., 1985; Leal et al., 2006; Li et al., 2014; Pérez-Crespo et al., 2016; Schemske and Horvitz, 1984). *Phocides urania* did not contact anthers or stigmas of *Psittacanthus auriculates* when visiting it (Pérez-Crespo et al., 2016), *Lobocla proxima* did not make contact with reproductive organs when feeding on *Paeonia delavayi* (Li et al., 2014) and two hesperiid species which were frequent visitors to *Passiflora coccinea* were not observed contacting stigmas or anthers when foraging (Leal et al., 2006). Another study tested fruit set of *Calathea ovandensis*, and even though *Eurybia elvina* accounted for 20 % of all insect visits, it was responsible for only 1 % of all fruit set (Schemske and Horvitz, 1984).

Some studies published in recent years were conducted to disprove the nectar thieving status that the family holds (Ikeuchi et al., 2015; El Ottra et al., 2016). A study analyzing the pollination efficiency of vistors in *Habenaria radiata* observed, that all *Parnara* guttata, which visited the flower, had its pollinia attached to their heads, suggesting efficient pollination (Ikeuchi et al., 2015). Another study analyzing pollination efficiency of *Conchocarpus rubrus* found *Pyrginae* to be efficient pollinators based on their visitation rate along with a high enough pollen count, to successfully pollinate the plant (El Ottra et al., 2016).

Hesperiids were observed visiting two species that could be considered psychophilous and were, at least in some cases considered efficient pollinators (de Araújo et al., 2014; Herrera, 1987). De Araújo et al. (2014) assesed the visitation rates and pollen count on bodies of *Cogia calchas* and *Heliopete ericetorum* and found that although infrequent visitors, they did carry enough pollen to efficiently pollinate *Mandevilla tenuifolia*. A study analyzing pollination efficiency of *Lavandula latifola* observed, that *Thymelicus acteon* and *Hesperia comma* carried enough pollen on their bodies to pollinate the plant, but two more Hesperiid species, *Pyrgus* along with *Spialia Sertorius* did not contact anthers or stigmas in any of their visits (Herrera, 1987).

Hesperiids acted more as generalists, efficiently pollinating very few psychophilous flowers. Their visitation rate to psychophilous flowers was also small and overall hesperiids acted as inefficient pollinators and nectar thieves in most flower species visited.

4.2.2 Nymphalids

Nymphalids are the largest group of butterflies with ~6,000 described species wordwide (Espeland et al., 2018), resulting in them having the highest frequency of appearance in studies quantifying pollination effectiveness which were assessed. Unlike hespiriids, most studies found nymphalids to be efficient pollinators, although they did act as nectar thieves in some plants.

Most cases in which nymphalids were considered efficient pollinators were studies focusing on the pollination of psychophilous plants based on pollen count on the body and contact with reproductive organs (de Araújo et al., 2014; Boyden, 1980; Goldblatt and Manning, 2002; Johnson and Bond, 1994; Schemske, 1976). Schemske (1976) observed three nymphalid species to be efficient pollinators in *Lantana camara*, de Araújo et al. (2014) concluded that *Agraulis vanilla* were the most efficient pollinators in *Mandevilla tenuifolia* and Johnson and Bond (1994) along with Goldblatt and Manning (2002) confirmed that *Aerotpetes tulbaghia* is the primary pollinator in five *Gladiolous* species. Another study measuring pollination effectiveness by counting pollen grains deposited by certain visitors compared with their frequency of visits found nymphalid to be the most efficient pollinators in *Lavandula latifola* out of all butterflies (Herrera, 1987). *Argynnis paphia* and *Fabriciana adippe* had an average pollination effectiveness of 26%, and the most effective of all butterflies was *Pandoriana pandora*, with its' pollination effectiveness reaching 40% (Herrera, 1987).

Various other studies found Nymphalids to be efficient pollinators in plants which did not precisely fit into psychophilous characteristics (Benevides et al., 2013; Valentin-Silva et al., 2016; Wester and Johnson, 2017). Valentin-Silva et al., (2016) analyzed the mutualistic relationship between male *Ithomiinae* butterflies and the plant *Adenostemma brasilianum* and concluded, that these nymphalids were the plants' main pollinators. Two more studies analyzed pollination efficiency of different flowers considered to be ornitophilous (bird pollination syndrome) (Benevides et al., 2013; Wester and Johnson, 2017), which have some similar characteristics to psychophilous flowers. Benevides et al., (2013) found *Heliconius ethilla narcaea* to efficiently pollinate *Passiflora kermesina* based on pollen deposited by butterflies onto stigmas along with frequency of visits and Wester and Johnson (2017) used the same method to characterize *Danaus chrysippus liboria* as an efficient pollinator of *Syncolostemon densiflorus*.

Some studies did label nymphalids as inefficient pollinators or nectar thieves, which were conducted on non-psychophilous plants (Leal et al., 2006; Pérez-Crespo et al., 2016; Sugawara et al., 2016) . Leal et al. (2006) recorded no contact with reproductive organs of three nymphalid species in *Passiflora coccinea*, Pérez-Crespo et al. (2016) found the same results for *Agraulis vanilla* in *Psittacanthus auriculates* and Sugarawa et al. (2016) recorded five nymphalid species to be infrequent visitors with no stigma or anther contact in *Psychotria serpens*. Only one study assessing pollination efficiency of various butterflies on psychophilous plants which found nymphalids to be inefficient pollinators was observed (Cruden and Hermann-Parker, 1979). Cruden and Hermann-Parker, (1979) recorded few visits from four nymphalid species to the plant *Caesalpinia pulcherrima*.

Based on the studies mentioned it can be argued, that nymphalids are prone to visit and pollinate many psychophilous flowers but are not efficient in pollinating all the plants that various species visit.

4.2.3 Papilionids

Although relatively large in body size, “swallowtail butterflies”, as Papilionids commonly called, are a monophyletic group with only ~550 described species (Capinera, 2008; Espeland et al., 2018). Their low species count is the main reason for their relatively low frequency of appearance in various studies measuring pollination efficiency.

Out of the studies in which they were present, Papilinoids were found to be efficient pollinators in four cases (Cruden and Hermann-Parker, 1979; Mertens et al. in review; Sakamoto and Morinaga, 2013; Wester and Johnson, 2017). In two of these cases, Papilionids were discovered to be among the most frequent visitors to psychophilous plants. Mertens et al. (in review) observed two Papilionid species, *Papilio dardanus* and *Papilio Zenobia* to frequently visit *Scadoxus cinnabarinus* and make contact with the flowers’ reproductive organs. Cruden and Hermann-Parker (1979) observed seven papilionids to be most frequent visitors of *Caesalpinia pulcherrima*, along with carrying highest quantities of pollen grains out of all butterfly species.

Two studies which labeled butterflies as efficient pollinators measured pollination efficiency on plants closely related to psychophily (Sakamoto and Morinaga, 2013; Wester and Johnson, 2017). *Syncolostemon densiflorus*, which was primarily pollinated by humming birds (ornitophily), was found to have two efficient butterfly visitors; *Papilio demodocus* and *Papilio nireus lyaeus* (Wester and Johnson, 2017). Sakamoto and Morinaga (2013) measured pollination efficiency in phalaenophilous (moth pollination syndrome) *Clerodendrum trichotomum* and observed that after certain moths, *Papilio Helenus* and *Papilio dehaani* were most frequent visitors that were efficient in pollinating the plant.

Two studies which observed Papilionid visitation labeled them as nectar thieves for specific plants studied (Pérez-Crespo et al., 2016; Sugawara et al., 2016). In both cases, characterization of butterflies as nectar thieves was in result of infrequent visits to studied flowers and lack of contact with reproductive organs by the Papilionids. Sugawara et al. (2016) observed this behavior in the plant *Psychotria sepens* by the butterfly *Papilio bianor* and Pérez-Crespo et al. (2016) recorded similar results in the plant *Psittacanthus auriculatus* by visitor *Papilio multicaudata*.

In the studies where papilionids were observed, they acted as efficient pollinators most of the time. Papilionids were also observed foraging on plants that were psychophilous or are considered to be closely associated with psychophily.

4.2.4 Pierids

Pierids are a medium sized family of butterflies with over 1,100 described species (Espeland et al., 2018). Although their species count is much lower than that of nymphalids, nierids were observed almost as much as swallowtails foraging on flowers in studies analyzing pollination efficiency of visitors in plants.

Most cases in which Pierids were observed to be efficient pollinators were in studies done on psychophilous plants (de Araújo et al., 2014; Cruden and Hermann-Parker, 1979; Mertens et al., in review; El Ottra et al., 2016). Out of the four studies cited, two measured pollination efficiency based on visitation and contact with reproductive organs. Araujo et al. (2014) observed three Pierid species, *Ascia monuste*, *Aphrisa statira* and *Phoebis statira*, to be efficient pollinators of *Mandevilla tenuifolia* and El Ottra et al. (2016) observed the Pierid *Glennia pylotis* to be an efficient pollinator of *Conchocarpus rubrus*. Pollination efficiency was measured by contact with anthers along with pollen count on the butterflies' body in one study, where Cruden and Hermann-Parker (1979) found four species of pierids to efficiently pollinate *Caesalpinia pulcherrima*.

Mertens et al. (in review) used a combination of flower visitation along with average seed germination to measure pollination efficiency and found that *Nepheronia thalassina*, *N. argia* and *Leptosia spp.* were all efficient pollinators in *Scadoxus cinnabarinus*.

Although there were many studies on psychophilous plants in which pierids were found to be efficient pollinators, there were two in which pierids were labeled as inefficient (Herrera, 1987; Spears, 1983). In one study, *Phoebis sennae* were labeled as insufficient pollination of *Ipomoea trichocarpa* not because of lack of visitation or low pollen count on body, but because the time of their visit (Spears, 1983). Spears (1983) suggested, that by the time butterflies started foraging, pollination might have already been achieved by bumblebees, which were active two hours prior to butterflies. Herrera (1987) observed four pierids to be inneficient pollinators of *Lavandula latifola* based on low visitation and low pollen count.

Other studies conducted on various non psychophilous plants observed Pierids to only visit flowers to feed on nectar without contacting the reproductive organs (Benevides et al., 2013; Pérez-Crespo et al., 2016; Sugawara et al., 2016; Wiklund et al., 1979). Instances in which Pierids were considered to be nectar thieves include, for example, *Eurema hecabe* in the flower of *Psychotria serpens*(Sugawara et al., 2016), *Phoebis sennaei* in the plants *Passiflora kermesina* and *Mitostemma glaziovii* (Benevides et al., 2013), or two Pierids, *Anteos clorinde* and *Phoebis sennae* in the plant *Psittacanthus auriculatus* (Pérez-Crespo et al., 2016). Wiklund et al. (1979) characterized *Leptidea sinapis* as a nectar thief of *Viola canina*, *V. riviniana* and *Lathyrus montanus* based on infrequent stigma and anther contact along with low pollen grain quantities on the proboscis.

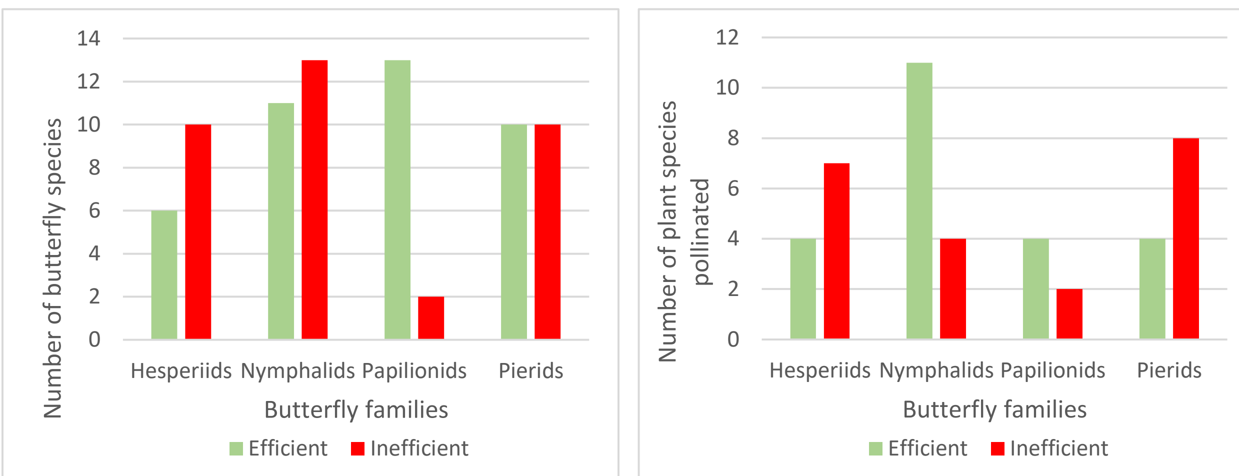
Pierids were observed in almost as many studies as nymphalids, but were not as effective pollinators. They were found to be efficient pollinators and inefficient in various psychophilous plants, along with being observed to me nectar thieves in many cases.

4.2.5 Lycaenids, Riodinids, Hedylids

Three remaining butterfly families had very low to no visitation rates in studies measuring pollination efficiency of visitors in plants. Lycaenids were observered in two studies and were categorized as nectar thieves in both (Herrera, 1987; Sugawara et al., 2016). Sugawara et al. (2016) observed two lycaenids to act as nectar thieves in *Psychotria serpens* and Herrera (1987) observed three lycaenids to be nectar thieves in *Lavandula latifolia*. Due to their short life spans, often observed mud-puddling behavior and generally low nutrition requirements, it can be reasoned that lycaenids are not efficient pollinators. One riodinid species, *Eurybia lycisca* was observed visiting *Calathea crotalifera*, but was labeled as a nectar thieves due to its inability to release the flowers' pollen triger (Bauder et al., 2011). Hedyalis were not observed in any study.

4.2.6 Pollination efficiency data analysis

The studies used measured pollination efficiency by focusing either on one plant species or one butterfly species, producing two results. The first is by number of butterflies observed to be efficient pollinators (Fig. 1a), the second is by the number of plants that were pollinated by diverse butterfly families (Fig. 1b). Lycaenids, riodinids and hedylids were not present in many studies and thus were excluded based on insufficient data.



a) *b)*

Fig. 1.: Comparison of studies showing pollination efficiency of **a)** individual butterfly species splitted by families, and **b)** of individual plant species visited by particular butterfly families. The counts are based on my own review of literature in this thesis (see the main text for details).

Hesperiids were observed to be inefficient pollinators both in cases when measuring the number of butterflies found to be efficient pollinators and in plant species sucessfully pollinated by butterflies (Fig. 1a,b). They were considered as nectar thieves in most studies and based on their floral visitation do not prefer flowers with psychophilous characteristics (see chapter 4.2.1).

Nymphalids were observed to be the most abundent in all studies. Out of them, 11 species were observed to be efficient pollinators and 13 species were observed as inefficient (Fig. 1a).

However, they were found to be inefficient pollinators on only four plant species in total, while sucessfully pollinating 11 plants (Fig. 1b). The reason for the high nymphalid species inefficiency rate as pollinators is their appearance as nectar thieves in various non-psychophilous flowers, which many nymphalid species visited infrequently (see chapter 4.2.2). The high amount of plants pollinated by nymphalids can however be asossiated with the study analyzing pollination efficiency of *Aeropetes tulbaghia*, in which the butterfly was found an efficient pollinator of five different plant species (Johnson and Bond, 1994), but even if this study was not incorporated in the results, nymphalids would still have the highest number of plants pollinated (Fig. 1b). The results show that nymphalids were efficient pollinators in many psychophilous plant while also acting as nectar thieves in various non-psychophilous plants (see chapter 4.2.2).

Papilionids were observed in only six studies mentioned, but were found to be very efficient in terms of individual butterfly species (Fig. 1a,b). Two of the plants visited were psychophilous, which accounted for most of their efficient visits (see chapter 4.2.3) and two plants were described as ornitophilous and phalaenophilous, pollination syndromes closely related to psychophily (Willmer, 2011). In the studies mentioned, papilionids acted as efficient pollinators of most plants they visited, which were often psychophilous or of a syndrome resembling psychophily (see chapter 4.2.3).

Pierids were observed in ten studies, from which ten butterfly species were observed to be efficient pollinators and ten species were observed to be inefficient (Fig. 1a). Pierids were found to be efficient pollinators of some psychophilous plants, but also inefficient pollinators of others along with inefficient pollinators in several non-psychophilous plants (see chapter 4.2.5). Overall, pierids were observed to act more as inefficient pollinators of various plants (Fig. 1b).

5. Summary

Butterflies, as common floral visitors, prefer certain floral traits when foraging. The pollination syndrome hypothesis suggests that diverse plant species have convergently evolved by adapting various traits to attract a specific type of pollinators and thus increase their pollination efficiency. Most traits associated with the psychophilous syndrome can be considered accurate for many nectar-feeding butterflies, but floral color and shape preferences differ among various families and species. From studies analyzing floral preferences of various butterflies, most hesperiids were associated with generalized foraging behavior and the butterfly pollination syndrome failed in their case. For papilionids, as frequent visitors of psychophilous plants, the syndrome was often accurate. Numerous studies have been carried out to assess various nymphalid and pierid species foraging preferences, but the studies did not clearly show any preferences for particular floral traits. Insufficient data on lycaenids, riodinids and hedylids were found to produce conclusive results about their floral preferences.

The efficiency of butterflies as pollinators has been discussed in the past and many pollination scientists have labeled butterflies as inefficient pollinators. To assess butterfly pollination efficiency, I collated various studies quantifying pollination efficiency by butterflies on plants. Hesperiids, as predicted, were generalized foragers and did not visit psychophilous flowers much frequently, nor were their efficient pollinators. Papilionids were found to be attracted by the psychophilous flowers in most cases, and even not always confirmed, papilionids acted as efficient pollinators in most case studies. Nymphalids were observed to visit flowers both with and without psychophilous traits. Out of these cases, nymphalids were efficiently pollinating mainly psychophilous plants, as well as many non-psychophilous plants. However, they did not pollinate many non-psychophilous plants efficiently. From the results it can be interpreted, that nymphalids acted as efficient pollinators in many plant species, whereas often visited flowers with psychophilous traits. Pierids were observed to efficiently pollinate some plant species with the psychophilous traits, but also observed as inefficient pollinators in both psychophilous and non-psychophilous plants. Overall, pierids often acted as inefficient pollinators and were not clearly observed to prefer plants with psychophilous traits.

In conclusion, papilionids and nymphalids were observed to be the most efficient pollinators in the reviewed studies quantifying pollination efficiency of various butterflies pollinators. Both families of butterflies showed preferences for psychophilous floral traits in most cases. Pierids and hesperiids were both observed to act more as inefficient pollinators in most case studies reviewed. Hesperiids were observed to act more as generalized pollinators, rarely visiting psychophilous flowers. Pierids were observed visiting several psychophilous flowers, but overall acted more as generalized foragers.

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