POLLINATION STRATEGY OF **HEDYSARUM SCOPARIUM** FISCH. ET MEY (FABACEAE) IN A DRY HABITAT MEASURED WITH POLLINATION EFFECTIVENESS AND POLLINATOR IMPORTANCE

**ABSTRACT:** *Hedysarum scoparium* is an important xerophyte in the desert region of northwest China and can grow well as a pioneer plant in shifting sand dunes. Flowers of *H. scoparium* provide late season pollen and nectar for several insect groups. *Hedysarum scoparium* benefits from insect pollination in order to set fruit, but it is unknown which flower-visiting insects are the most effective pollinators. In this study, we characterized the pollination effectiveness and the importance of visitors toward flowers of *H. scoparium* in the middle of the Hexi Corridor region, China. Our observations suggest that bees were the most frequent pollinators of *H. scoparium*: 97% of the visits were made by bees. Individuals of three medium size bee taxa (*Apis mellifera*, *Megachile* spp., *Amegilla* spp.) accounted for 94% of all visits. Pollen grains deposited per visit by bees showed equal effectiveness. Of all the bee groups observed, introduced *A. mellifera* was potentially the most effective pollinator since it frequent visitors, and had the highest 'importance value' followed by *Megachile* spp., *Amegilla* spp. Visits by these medium size bees may therefore be important for ensuring *H. scoparium* fruits set.

**KEY WORDS:** *Hedysarum scoparium*, pollination effectiveness, pollinator importance

Plant species are often important components of arid and semi-arid ecosystems. The need for conservation and management of these resources has been recognized. However, plant-insect interactions in such species are often poorly understood, especially the effective pollinators. For entomophilous species, insects would generally be relied upon during the seed production phase. Nevertheless, differences among visitors in morphology, physiology, and foraging behavior may result in differences in their contributions as pollinators (Herrera 1987, Schemske and Horvitz 1984). Because natural selection favors the evolution of floral traits that confer the highest reproductive success, assessing the relative importance to pollination of the different insect visitors to a flower provides a key to understanding the evolution of floral traits (Dieringer 1992).

In this study, pollinator importance was separated into quality and quantity components: the animal's pollination effectiveness, versus its frequency of flower visits. Both parameters have been used to investigate the importance of flower visitors as pollinators in several studies (Fumero-Cabán and Meleñez-Ackerman 2007, Reynolds et al. 2009). Pollinators often vary significantly in effectiveness due to differences in morphology, physiology, and foraging behavior...
Various methods have been used to study the effectiveness of flower visitors as pollinators (Inouye et al. 1994, Bloch et al. 2006, Cauich et al. 2004). One way to quantify pollination effectiveness is to measure the amount of compatible pollen grains (i.e., stigma pollen loads) deposited on stigmas after a single visit (Bloch et al. 2006, Fumero-Cabán and Meleñdez-Ackerman 2007) which represents one of the principal variables that distinguishes “good” from “bad” pollinators (Schemske and Horvitz 1984, Herrera 1987, Fishbein and Venable 1996, Olsen 1997). The importance of a group of visitors as pollinators can be related to their visiting frequencies (Vázquez et al. 2005, Jacobs et al. 2010). Common visitors are sometimes the most important pollinators of a plant (Aizen 2001, Vázquez et al. 2005) though the case that abundant visitors are not always the best pollinators and infrequent visitors can be good pollinators exists (Schemske and Horvitz 1984). Vázquez et al. (2005) analyzed data from several studies and concluded that frequent visitors made the greatest contribution to pollination. Here we combine the visiting frequency of flower visitors and their pollination effectiveness. The combination is a more precise evaluation of the relative importance of each pollinator species because the product reflects the relative contribution of each pollinator group to pollination in a given season (Armbruster 1993).

*Hedysarum scoparium* is an important xerophyte in the desert region of northwest China and can grow well as a pioneer plant in shifting sand dunes. This species has a requirement for insect pollination to produce fruit, since no fruits set in the absence of flower-visiting insects (Pan et al. 2010). Its flowers attract several insect taxa (Pan et al. 2010), which may vary in their contribution to pollination as found in other plants’ studies (Ivey et al. 2003, Fumero-Cabán and Meleñdez-Ackerman 2007). The aims of this study are to determine what the taxonomic composition of floral visitors to *H. scoparium* was at the study sites, how frequently they visited the flowers, how much pollen they deposited on stigmas, and whether pollinators differ in their importance. By quantifying flower visiting frequency, pollen deposition, and pollinator importance of each of the animal visitors, we can test the usefulness of pollination syndromes in predicting the primary pollinators of *H. scoparium*. We evaluate the predictive value of pollination syndromes of *H. scoparium* based on pollinator importance following Fenster et al. (2004). We decided that if the pollinator importance of a single functional group accounted for at least three fourths of total pollinator importance, then the plant manifested specialization on that functional group.

The study area, the experiment zone of Linze Inland River Basin Comprehensive Research Station (39°21’ N and 100°07’ E), located in the middle of Hexi Corridor region in Gansu province of northwest China. The research station is located at the southern edge of Badain Jaran Desert, with an altitude ranging from 1368 to 1380 m. This region is connected with dense moving and denudation residual dunes as well as Gobi. The climate in this region is temperate continental characterized by dry and hot, with an average annual precipitation of 117 mm, and 65% distributed mainly in summer showers of short duration. Mean annual evaporative demands of over 2390 mm. Mean annual temperature is 7.6°C, while the absolute maximum may reach 39°C and minimum –27°C. Mean temperature in the growing season (late May to early October) is 20.4°C.

*Hedysarum scoparium* is a perennial, large shrub usually located in arid and semi-arid desert region. The species is characterized by low densities and patchy distribution in the studied area. Patch size ranges from 2 to 5,000 m² with 1–50 shrubs. *Hedysarum scoparium* is self-compatible, but not autogamous. It produces racemose inflorescences, and nectar-secreted, hermaphroditic flowers with degraded wings that open during daylight hours. Flowering occurs from late May until late September with twice peak flowering in approximately mid-June and mid-August in the middle of Hexi Corridor region. During its second flowering peak, *H. scoparium* is virtually the only dominant species in full bloom (Pan et al. 2010). The geographical range of this species in China includes Hexi Corridor region, the Badain Jaran Desert, the Tengger Desert and the Gurbantunggut Desert.
Pollinator observations were carried out from mid-August to mid-September in 2010, which covered the latter half of the flowering season. Observations were conducted to identify flower visitors and to determine their visiting frequencies weekly on sunny days in the middle of Hexi Corridor region. We hypothesized that this would provide sufficient representation of flower visitation rates throughout the *H. scoparium* flowering period. We observed the area for 15 min every hour from 08:00 to 18:00. A total of 600 min of field observations were made. During observation periods, the species of each flower visitor, the number of visits made by each flower visitor were recorded. Most individual flower visitors were identified in the field, but some specimens were captured and sent to specialists at Ludong University, Yantai, China, for identification.

We grouped the bees visiting *H. scoparium* flowers in the following functional groups: (i) small bees: body length <8 mm, (ii) medium size bees: body length 8–12 mm, (iii) large bees: body length >12 mm.

We calculated the visiting frequency for each pollinator taxon \( \frac{(\text{no. visits made by the pollinator species})}{(\text{no. flowers observed})/(\text{time observed in hours})} \) (Cosacov et al. 2008), and then averaged the value for each pollinator type for plants within each patch. Differences in mean visiting frequencies among the pollinator taxa were analyzed using one-way ANOVA.

Pollination effectiveness was estimated by counting the number of pollen grains deposited after single visits to virgin flowers (Aizen 2001). We considered that the same taxon of pollinators showed consistent pollination effectiveness at different patch and year, respectively. Then pollination effectiveness was observed only in two patches. From 15 August to 10 September 2010, prior to the arrival of pollinators, 137 randomly chosen flowers of *H. scoparium* were emasculated and bagged with fine net bags to allow flowers to open without being visited. Emasculation ensures that only visitor-carried pollen was counted. The bags were removed and each flower was exposed for a single visit. All observations were made between 10:30 and 17:30 hours. Upon visitation, the species of visitor was recorded, and the stigma was collected for pollen counts. In the laboratory, stigmas were mounted on slides and stained with methylene blue solution. Pollen grains were counted under a microscope at 400×. In this way, differences in mean pollen load deposited on stigmas among flower visitors were analyzed using a Kruskal-Wallis test.

The relative importance to pollination by individual species of pollinators was measured as the product of a species’ pollination effectiveness and its visitation frequency. We determined the pollinator importance of each pollinator species by multiplying mean pollination effectiveness of a species by its visitation frequency (Olsen 1997, Fumero-Cabán and Meleñdez-Ackerman 2007). The product represents the number of pollen grains deposited per stigma per hour by each taxon of flower pollinator. We used this index of pollinator importance to evaluate the relative contribution of different flower visitors to plant reproductive success. Differences among flower visitors in pollinator importance were analyzed using one-way ANOVA. All data were analyzed using SPSS software.

A total of 193 flower visits were recorded in a total of 600 min of observations. Bees accounted for 97% of these visits. 96% of bee visits were made by individuals of three medium size bee taxa: *Apis mellifera* L., *Amegilla* spp., *Megachile* spp. These three most abundant visitors are considered as the “principal pollinators”. *Amegilla* spp. visitors, *Megachile kagi ana* Cockerell and *Megachile takoensis* Hedicke were not identified to species in the field, since all species were of similar looks and adopted the similar foraging behavior, respectively. “*Amegilla* spp.” thus refers to this entire group of bees, “*Megachile* spp.” *M. kagi ana* and *M. takoensis*. In terms of total visits to flowers, *A. mellifera* was the most frequent visitor, making 56.5% (109) of total flower visits. *Megachile* spp. was the second most frequent and made 28.5% (55) of total flower visits. Individuals of *Amegilla* spp. made only 9.8% (19) of the flower visits. In addition to these three common pollinators, visits by two other rarer bee species *Megachile spissa lusa* Cockerell, *Xylocopa nasalis* Westwood were recorded. Two additional large bee species (*An thidium septemspinosum* Lepeletier, *Anthophora deserticola* Morawitz), one additional medium size bee species (*Lasioglossum*...
zonulum Smith), two butterflies (Coenonympha amaryllis Cramer, Pieris rapae Linnaeus), one moth (Autographa mandarina Freyer) were observed foraging on H. scoparium occasionally but never on a flower used in the study. Insects other than bees accounted for less than 3% of recorded visits. Most of these were Syrphus vitripennis Meigen (n = 5). Average flower visiting frequencies by different visitors were significantly different from each other (P <0.05; Fig. 1A).

Insect visitors varied in their abundance over the observation. Among the principal pollinators, Amegilla spp. were consistently active throughout the whole period of study. Megachile spp. was prevalent during the first half of the study but was not observed in the last three weeks. In contrast, A. mellifera, introduced in the fourth week, was most prevalent in latter half of the study period. Among the rarer visitors, only X. nasalis showed no change in abundance during the study. M. spissula were observed in the middle of the study period. S. vitripennis was uncommon at the most period of the study but became prevalent in the last week.

There were significant differences in the effectiveness at depositing pollen on stigmas in a single visit by the different flowers visitors of H. scoparium (P <0.05). S. vitripennis deposited < 2 grains per visit on average, was not statistically different from the mean number of pollen grains found in control flowers (Fig. 1B). Other taxa deposited more than four times that number. After excluding control and S. vitripennis from this comparison, no further differences in pollination effectiveness were found among the six remaining bee taxa (P > 0.05, Fig. 1B).

When pollen deposition and visitation frequency were combined, significant differences were found in pollinator importance among flower visitors of H. scoparium (P <0.05; Fig. 1C). Among the principal pollinators, the most abundant taxon, A. mellifera had the highest pollinator importance values. The Megachile spp. species group had the second highest visitation frequency, it proved to have a lower pollinator importance value than half that of A. mellifera (Fig. 1C). The third most important pollinator, Amegilla spp., had a pollinator importance value less than 20% of that of A. mellifera. Other rarer bees (X. nasalis, M. spissula) had a pollinator importance value less than 1%, less than 5% of that of A. mellifera, and were not statistically different from that of S. vitripennis.

We were unable to estimate visiting frequencies and deposition of butterflies and moths for H. scoparium. Thus, pollinator importance was not compared between bees and butterflies and moths.

Of the visitors of H. scoparium, medium size bees were the more important pollinators, with significantly higher estimates of pollinator importance than other visitors combined (e.g., small bees, large bees, hoverflies, butterflies and moths). Total pollinator importance of medium size bees was 20 times greater than that of other bees and hoverflies combined.

Visitation by insects to H. scoparium, was frequent in the patches studied. Composition of floral visitor to H. scoparium included multiple species from diverse guilds of insects (small bees, medium size bees, large bees, hoverflies, butterflies, moths). The end result is that H. scoparium flowers were visited at least by twelve animal species (one small bee, four medium size bees, three large bees, two butterflies, one moth, and one hoverfly). These visitors were not equally effective at depositing pollen because of different morphology, physiology, and foraging behavior. Generally, flowers of H. scoparium in the middle of Hexi Corridor region were visited by eight species of bee-pollinators with different visiting frequency. These bees were frequently observed making contact with the stigma during foraging. Of which, A. mellifera, Megachile spp., Amegilla spp., M. spissula, X. nasalis showed equal effectiveness at depositing pollen after one flower visit (Fig. 1B). However, once their visiting frequencies were taken into account, all pollinators were not equally effective at depositing pollen. Pollinator importance was found to be dictated by the visiting frequency of pollinators. The positive correlation found in this study between the relative pollinator importance and visiting frequency has also been demonstrated in previous works (Olsen 1997, Vázquez et al. 2005, Jacobs et al. 2010). And in agreement with other studies, no correlation was found between pollination effectiveness and visiting frequency (Montalvo and Ackerman 1986, Herrera 1987).
Of the important pollinators, *A. mellifera* is a generalist forager and visits flowers with different pollination syndromes (Roubik 1980, Aizen and Feinsinger 1994). As shown in other studies, *A. mellifera* had lower pollination effectiveness than the native pollinator (Hansen *et al.* 2002, Fumero-Cabán and Meleñez-Ackerman 2007). *A. mellifera* agglutinate the pollen grains with nectar and place them wet into corbiculae on the hind tibia (Simpson *et al.* 1977) that would reduce the chance of pollen being scooped into the stigmas. However, in this study, introduced *A. mellifera* was as effective as other bee-pollinators at depositing pollen because it also packed pollen grains into hairs on its breast except on the hind tibia. And it visited flowers much more frequently than did other pollinators. Once visitation rates were taken into account, *A. mellifera* was proved to be the most important pollinator at depositing pollen. Moreover, where introduced, *A. mellifera* was often considered to be threats to native pollinators abundance and diversity, and thought to disrupt specialized relationships between native pollinators and their plants (Roubik 1980). That was not the case in our study, where the recently introduced *A. mellifera* was found to be good pollinators of *H. scoparium* and the most effective pollinator of this species.

As for *Megachile* spp., *Amegilla* spp., *H. scoparium* flowers were the main source for carbohydrate because this plant species was the only dominant species in full bloom at that time in this area. The workers collect nectar from flowers of *H. scoparium* for their own energy requirements and other colony members. The availability of carbohydrate sources may be important for supporting social bee colonies and influencing their population sizes (Raveret-Pichter 2000). Due to few carbohydrate sources, limited foraging range and temperature decreasing (data not shown), foraging on the sole food source effectively was needed. This strategy is also applicable to *A. mellifera*.

*Hedysarum scoparium* was pollinated almost exclusively by medium size bees in our study patches based on the importance value. It is possible that the common medium size bee species visiting *H. scoparium* formed a single functional pollinating group. Find-
ing floral specialization on one functional group of visitors to support for pollination syndromes is not unprecedented (Schemske and Horvitz 1984, Reynolds et al. 2009). For example, Schemske and Horvitz (1984) demonstrated Calathea ovandensis Matuda specialization on bees while most visitations were made by Lepidopteran visitors. Likewise, Silene virginica Linnaeus and S. stellata Linnaeus strongly corresponded with the predicted major pollinator group respectively (Reynolds et al. 2009). The similar results have also been observed on ivy flowers (Ollerton et al. 2007). Ivy flowers attracted a range of insect foragers and appeared to be generalized, but they exhibited ‘functional specialization’.

The rarer small bee (M. spissula), large bee (X. nasalis) and S. vitripennis, due to their much lower visiting frequencies and pollinator importance, might play a minor role in pollination of H. scoparium. Large bees (A. septemspinosum and A. deserticoa), butterflies (C. amaryllis and P. rapae) and moth (A. mandarina) were relatively uncommon visitors, but might have some limited contribution to pollination of H. scoparium. It was difficult to determine their importance from the limited number of visits by these species. However, we cannot exclude the possibility that these visitors play a larger role as pollinators in different years or in other populations.

This study provided the first quantification of pollination effectiveness and importance of pollinators utilizing H. scoparium flowers. This paper has highlighted the value of medium size bees in particular as pollinators of H. scoparium, and their visits may be important for ensuring fruits production. Medium size bees were the main pollinators of H. scoparium accounting for at least 94% of all visits in the middle of Hexi Corridor region. Following Fenster et al. (2004), H. scoparium manifested specialization on medium size bees. These results suggest that pollination syndromes can lead to accurate predictions about the effective pollinator group of H. scoparium, providing a counter-example to the popular notion that plant-pollinator systems are mostly generalized. However, we do not advocate that pollination syndromes be accepted as evidence that a plant is pollinated by particular animals. This study only provides the contemporary state of H. scoparium having pollinators mainly belonging to a single functional group. The composition of pollinators, the pollination effectiveness, the visiting frequencies and hence importance of pollinators can vary temporally and spatially (Fishbein and Venable 1996, Horovitz and Schemske 2002, Kandori 2002, Ivey et al. 2003). The populations sampled represent only one region in which H. scoparium occurs. It also occurs in the Tenger Desert and the Gurbantunggut Desert (Pan et al. 2010). Insect guilds and their quality and quantity of visits would probably differ in those ecosystems and exhibit different contributions to H. scoparium pollination. Considering the importance of H. scoparium, there is still much to be learned about flower-pollinator interactions.

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