BIRDS OUTNUMBER INSECTS IN VISITING BRASSICA FLOWERS ON VENTOTENE ISLAND (CENTRAL MEDITERRANEAN)

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ABSTRACT. – European warblers migrating across the Mediterranean feed on nectar at stopover sites on islands, thus carrying pollen grains stuck to bills and plumage from one stopover to the next. Plants belonging to a single wild species of the Brassica oleracea group are largely used by songbirds for nectar feeding at Ventotene island and their pollen is commonly found on staging warblers. We studied the potential role of nectar feeding warblers in pollen dispersion of Brassica plants and compared it to that of insects. The study was carried out at Ventotene from the end of March to mid May during the years 2008 and 2009. Direct observations of bird and insect visits to plants showed that the frequency of bird visits was significantly higher than the frequency of flight-feeding insects, while no significant differences were found between the length of bird and insect visits. Plant caging with a 15 mm-mesh net in order to prevent bird visits, but not insect visits, resulted in a significant lowering of fruit production with respect to control plants. In summary, a mutualistic relationship could be hypothesised between songbirds and Brassica plants on Ventotene Island. Warbler species obtain a nutritional benefit through nectar consumption, and can transport pollen grains and potentially pollinate exploited plants across stopover sites, with possible implications for pollen carry-over from genetically engineered agricultural crop plants.

INTRODUCTION

Every spring huge numbers of migrants originating from African wintering quarters cross the Mediterranean to reach their breeding areas in Europe (Hahn et al. 2009). Typically, migrants divide their trip into bouts of flight, during which reserves are depleted, and stopovers, when reserves can be replenished and birds can rest after the demanding muscular exercise (Newton 2008). Some European songbirds, like Sylvia warblers which are generally omnivorous (mostly insectivorous during breeding season and frugivorous during fall migration; Bairlein & Gwinner 1994), feed on nectar at stopover sites both in Africa and on Mediterranean islands during spring migration (Cecere et al. 2010, Salewski et al. 2006). In particular, the nectar feeding behaviour is common and well described on Ventotene (Schwilch et al. 2001), a small island in the Central Mediterranean reached by many migrants after having crossed at least 500 km of open sea (Pilastro et al. 1995). After crossing ecological barriers, migrants usually land at stopover sites in poor physical condition and with reduced digestive organs (Karasov & Pinshow 1998, 2000, Schwilch et al. 2002). At this stage, nectar supplied by flowering plants is a source of water and energy which is easy to find and quick to digest. Birds, in fact, can get a physiological benefit by nectar uptake, which leads to an increase in plasma blood glucose concentration (Cecere et al. 2011b).

As a consequence of nectar feeding, pollen grains of exploited plants stick to the bill and plumage of birds (Ash 1959, Ash et al. 1961, Laursen et al. 1997, Ortega-Olivencia et al. 2005) and are transported between different stopover sites (Cecere et al. 2011a). On Ventotene, plants belonging to the Brassica oleracea group (Gladis & Hammer 2001) grow spontaneously and are largely used by nectar feeding songbirds (Cecere et al. 2010); as a consequence, their pollen are commonly found on the plumage and bill of warblers staying on the island (Cecere et al. 2011a). Given that different cultivars of this group are widely used in agriculture (Messean et al. 2006, OECD 1997), birds could have a role in pollen dispersion and likely in genetic contamination of cultivars.

Plants belonging to Brassica oleracea group are generally pollinated by insects, particularly bees (Nieuwhof 1993), which are usually not abundant on small islands (Whittaker & Fernández-Palacios 2007). Thus we hypothesise that Brassica plants growing on Ventotene rely on pollination by passerines, given the abundance of nectar feeding migrants on the island during the flowering period (Cecere et al. 2011a). We predict that: i) birds outnumber insects in visiting Brassica flowers; and ii) that fruit set
is significantly lower if plants are caged to prevent bird visits during the flowering period.

METHODS

Study site: The study was carried out at Ventotene Island from the end of March to mid May during the years 2008 and 2009. The island (1.3 km²) is 50 km off the Tyrrhenian coast of Italy, it is inhabited by a small community (450 people) and covered by several small fields characterized by crop rotation (cultivated and set-aside fields).

Species of the Brassica oleracea group that provides nectar to warblers at Ventotene, probably belong to B. montana Pourret (= B. oleracea L. ssp. robertiana (Gay) Rouy et Fouc) (Cecere et al. 2011b), although some taxonomic features suggest that they could be also hybrids. Hence, these plants will be considered here as belonging to the Brassica oleracea group and referred to under the collective name of Brassica. Flowers are bisexual, regular, 4-merous with obovate petals, bright yellow, and 6 stamens; the ovary is superior, with 4-50 ovules per ovary. The fruit is a dehiscent, globose, brown, and finely reticulate linear siliqua, containing 10-30 seeds (van der Vossen & Seif 2004). Brassica crops are cross-pollinators, while fertilization by self-pollination is prevented by an incompatibility system (Nieuwhof 1993).

Census of flower visitors: Direct observations of birds and insects visiting flowers of Brassica were made between April 23-May 2, 2009. During preliminary observations we assessed that insects were more abundant during the warm hours and scarcer in the earlier morning and at the end of the day, when often the wind reinforces. Thus, in order to optimize the effort, censuses were carried out between 9:00 and 16:00 (local time, GMT+1), encompassing the daytime interval during which both birds and insects are active. A total of 30 plants were observed for time bouts of 30 min, of which 15 min were dedicated to insect monitoring from a distance of about 2 m (Traveset & Sáez 1997), and 15 min to bird monitoring, by means of 10x42 binoculars from a distance of about 20 m. The order of insect and bird monitoring was reversed at each consecutive observation, and the time-lapse between observations was 20-30 min. After observations from a distance, plants were inspected from nearby for presence of other insects staying on branches or flowers. Each plant visitor was recognized at the species level for birds and at the order level for insects, and the total time spent by each insect at caged and un-caged plants. All observations were carried out between 9:00 and 16:00 (local time, GMT+1).

For this type of analysis, 10 plants were checked for a period of 40 min each. This time period was split into an interval of 20 min with cage and 20 min without cage. The order of the two monitoring protocols was reversed at each consecutive plant, and the time-lapse between them was approximately 180 min.

Data analysis: The frequency of bird and insect visits per minute were compared using a Mann-Whitney test, as well as the length of bird and insect visits. Differences in fruit sets of caged and un-caged plants (for the whole study period, and separately for 2008 and 2009) were assessed by comparing with a Chi-square test the number of fruits and the number of flowers yielding no fruit (which sum equals the total number of flowers). The Mann-Whitney test was also used to compare the frequency of insect visits to plants with or without a moveable cage.

RESULTS

Visits to Brassica flowers by birds and insects

A total of 900 minutes were spent in the census of visitors to Brassica flowers. Table I shows the taxa and the time spent by both birds and flight-feeding insects to visit Brassica plants during the monitoring. The frequency of bird visits (0.089 ± 0.02 visits/min) was significantly higher than the frequency of flight-feeding insects (0.03 ± 0.01 visits/min; Mann-Whitney: U = 285.0, p = 0.006, df = 58), while no significant difference (Mann-Whitney: U = 253.5, p = 0.89, df = 51) was found between the length of bird visits (78.07 ± 12.73 sec/visit) and that of insect visits (95.46 ± 34.75 sec/visit). Apart from flight-feeding insects, on four of the 30 monitored plants, Coleoptera (1-4 individuals) were observed within or nearby flowers, with pollen attached to their hairs. However, all individuals of Coleoptera remained nearly the same flower throughout monitoring (15 min). During night monitoring only earwigs (Dermaptera) were observed on plants, and these occurred on 80 % of plants at an average abundance of 11.7 ± 3.36.

Assessment of bird pollination on Brassica (cage experiment)

A total of 8,453 flowers and 934 fruits were recorded

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on caged plants, while 14,081 flowers and 5,149 fruits were recorded on un-caged plants (Fig. 1). The fruit set of surviving caged plants (11.2%; n. of flowers = 8,310) was significantly lower than that of the corresponding control plants (32%; n. of flowers = 9,514) ($\chi^2 = 1098.49; df = 1; p < 0.0001$). Table II shows the number of flowers and fruits, and the relative fruit set for each surveyed plant.

In 2008 the total number of flowers produced on caged and un-caged plants was similar (7,889 and 8,274, respectively). In 2009 an infestation of Curculionid parasites on caged plants resulted in a very poor flower production compared with control plants (564 vs 5,807). The fruit set of caged plants was significantly lower than that of control plants in each year (2008: $\chi^2 = 866.23; df = 1; p < 0.0001$; 2009: $\chi^2 = 92.45; df = 1; p < 0.0001$), although the subsequent death of 2 of the 3 caged plants in 2009 without producing fruit reduced the experimental comparison to a single pair of caged and un-caged plants. No other parasitized plants were found within a radius of 50 m around each killed plant.

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An average of 11.96 ± 0.85 seeds per fruit was recorded in the caged samples (n. of fruits = 56), while 11.68 ± 0.51 seeds per fruit were recorded in the control plants (n. of fruits = 149). The number of seeds per silique of caged plants was not significantly different from that of control plants (t-test = 0.284; df = 203; p = 0.78).

During 400 min of survey with the moveable cage, one insect (Hymenoptera) was recorded for 11 seconds on a caged plant, another insect (Diptera/Hymenoptera) was observed for 18 seconds on the same plant without cage, and two insects (Diptera/Hymenoptera) were observed on free plants for a few seconds. The frequency of insect visits to plants with and without moveable cage was not significantly different (Mann-Whitney: U = 40.0, p = 0.28, df = 18). However, such a limited number of observations did not allow us to assess whether cages influenced insect visits. Neither Lepidoptera nor other animals were observed on Brassica plants either during this manipulation, or during the observation periods for bird and insect visits. All insects recorded had a size which potentially allowed them to pass through the cage netting (X. violacea was the biggest species, with a diameter of less than 10 mm).

**DISCUSSION**

In this study we show that flowering plants of Brassica growing on Ventotene are visited mainly by birds, although it is characterized by entomophilous features. Moreover we provide preliminary and intriguing indications of a possible bird pollination of Brassica. To our knowledge, among European plants, bird pollination is only described for Anagyris foetida in Southern Spain (Ortega-Olivencia et al. 2005). Daylight visits by flight-feeding insects on Brassica are much less frequent. Although Coleoptera carrying pollen were sometimes observed on flowering plants, they were relatively sedentary. During the night, only earwigs were observed on plants, which are unlikely to play a role in pollination, given the absence of hairs or other structures suitable to carry pollen. No other animals, such as lizards or small mammals, were observed resting or climbing on Brassica plants.

The greater number of bird visits could be due to the coexistence of two main factors on the island. First, island ecosystems usually have a lower incidence of insects than mainland ecosystems (see, for instance, Traveset 2001). Because of the scarcity of many common continental pollinators, plants become either super-generalists (Olesen & Jordano 2002) or establish relationships with unusual pollinators, such as lizards (Olesen & Valido 2003). In many cases, birds occupy a niche otherwise occupied by insects and pollinate plants characterized by entomophilous rather than ornithophilous features (Anderson 2003, Castro & Robertson 1997). Second, a prevalence of bird over insect pollination could also derive from the huge number of occasional nectarivore warblers staging on Ventotene regularly during spring migration, when Brassica flowers are more abundant (Cecere et al. 2011a).

During bird visits to flowers for nectar feeding, large amounts of pol-

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**Table II – Numbers of flowers and fruits recorded in each surveyed plant († plant parasitized by Curculionid).**

<table>
<thead>
<tr>
<th>Year</th>
<th>Plant</th>
<th>n. flowers</th>
<th>n. silique</th>
<th>fruit set</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>Caged 1</td>
<td>5071</td>
<td>833</td>
<td>16.43 %</td>
</tr>
<tr>
<td>2008</td>
<td>Caged 2</td>
<td>78</td>
<td>4</td>
<td>5.13 %</td>
</tr>
<tr>
<td>2008</td>
<td>Caged 3</td>
<td>2740</td>
<td>59</td>
<td>2.15 %</td>
</tr>
<tr>
<td>2009</td>
<td>Caged 4*</td>
<td>143</td>
<td>0</td>
<td>0 %</td>
</tr>
<tr>
<td>2009</td>
<td>Caged 5</td>
<td>421</td>
<td>38</td>
<td>9.03 %</td>
</tr>
<tr>
<td>2009</td>
<td>Caged 6*</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>Control 1</td>
<td>7093</td>
<td>2393</td>
<td>33.74 %</td>
</tr>
<tr>
<td>2008</td>
<td>Control 2</td>
<td>1126</td>
<td>93</td>
<td>8.26 %</td>
</tr>
<tr>
<td>2008</td>
<td>Control 3</td>
<td>55</td>
<td>15</td>
<td>27.27 %</td>
</tr>
<tr>
<td>2009</td>
<td>Control 4</td>
<td>2028</td>
<td>607</td>
<td>29.93 %</td>
</tr>
<tr>
<td>2009</td>
<td>Control 5</td>
<td>1240</td>
<td>539</td>
<td>43.47 %</td>
</tr>
<tr>
<td>2009</td>
<td>Control 6</td>
<td>2539</td>
<td>1502</td>
<td>59.16 %</td>
</tr>
</tbody>
</table>
len grains stick to the plumage and bill of nectarivore warblers, which could potentially play a role as pollen vectors for *Brassica* (Cecere et al. 2011a). The comparison between control plants and plants caged from birds supports this hypothesis, since the fruit sets of the caged plants were much lower than those of un-caged ones, while no difference was found between the number of seeds per siliqua. Considering that plants belonging to the *Brassica oleracea* group are largely cultivated and also genetically engineered (Clive 2010), warblers could be also considered as potential vectors of genetically modified organisms (GMO). Further studies are needed to assess whether such a prevalence of birds over insects in flowering plant visits is more widespread in Mediterranean stopover sites.

Given the evidence, a mutualistic relationship is hypothesised between songbirds and *Brassica* plants on Ventotene Island. Some warbler species, as confirmed for the Garden Warbler, obtain a nutritional benefit through nectar consumption, with nectar uptake leading to an increase in plasma blood glucose concentration (Cecere et al. 2011b). On the other hand, birds can transport pollen grains and potentially pollinate the exploited plants. In addition, we have anecdotal evidence that birds may help to protect *Brassica* from parasites. In 2009, two caged plants were heavily attacked and eventually killed by a parasitic Curculionidae; in contrast, adjacent control plants regularly visited by insect-feeding birds did not show parasitism.

The mutualistic relationship between birds and plants growing on islands can drive changes in phenotypical characteristics and phenotype of plants. Nectar composition, for instance, has been demonstrated to be evolved rapidly in bird-pollinated plants on the Canary Islands from their entomophilous ancestors (Dupont et al. 2004). Bird pollination in the orchid *Angraecum striatum* on Reunion Island (Indian Ocean) led to atypical unscented and short-spurred flowers, very different from the ones of moth-pollinated congenic species growing in Madagascar (Micheneau et al. 2006). As regards phenology, many trees have the time of fruit production which coincides with fall bird migration patterns on different islands (Noma & Yumoto 1997, Parrish 1997). On Ventotene, *Brassica* flowering period corresponds to spring migration of many nectar feeding birds (Cecere et al. 2011a).

Anyway, further studies are still needed to assess whether the pollination by Sylvia warblers draws the flowering period of *Brassica* on the island and changes in not-evident flower characteristics, such as nectar composition.

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