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Proboscis Length and Resource Utilization in Two Uruguayan Bumblebees: *Bombus atratus* Franklin and *Bombus bellicosus* Smith (Hymenoptera: Apidae)

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Abstract

Bumblebees (*Bombus* sp.) are eusocial insects with an annual life cycle whose use as pollinator of crops has gained great importance in modern agriculture. Several authors have found that resource use in *Bombus* species is usually based on the correlation between the proboscis length of the bumblebees and the corolla depth of the flowers. The aim of this study was to determine proboscis length of *Bombus atratus* and *B. bellicosus*, two Uruguayan bumblebees, and verify the resource exploitation testing two cultivated species, the red clover and the bird's foot trefoil. Bumblebee foraging activity was recorded in two culture conditions: in a red clover and bird`s foot trefoil mixed meadow, and in contiguous plots of these legumes, and the proboscis length of collected foragers was determined. Both species of bumblebees exploited red clover and bird`s foot trefoil although they did it in different proportions in all instances tested. The results indicated that the choice of the resources in *B. atratus* and *B. bellicosus* was influenced by their proboscis length. *Bombus atratus* has a longer proboscis and preferably visited red clover, possibly obtaining nectar easier and faster than *B. bellicosus*, which has a shorter proboscis. *Bombus bellicosus* used both resources without any clear preference.

Introduction

Bumblebees (*Bombus* sp) are univoltine eusocial insects (Michener 1974, 2007) whose use as crop pollinators has gained great importance in modern agriculture, especially in those cases where honeybees are not efficient (Velthuis 2002, Goulson 2003, Heinrich 2004, Velthuis & van Doorn 2006). They must be able to exploit a great diversity of plant species because colony life is generally longer than the flowering duration of a single species (Heinrich 1976a, 2004). Except by a few very specialized artic species, bumblebees are polilectic, i.e. they obtain food from a variety of plant species (Michener 1974, 2007, Heinrich 1976a, 2004, Goulson 2003). At any time and facing a varied floral offer, the choice of the plant species will be influenced by several factors, some inherent to the bumblebee and others to the plants. Among the former there are morphological characteristics such as weight, size, wing length and specially proboscis length, which will determine the ability of a bumblebee to use certain plant species (Harder 1983, 1985).

There is a number of reports on resource use in *Bombus* species based on the correlation between the proboscis length of the bumblebees and the corolla depth

of the flowers (Heinrich 1976a,b, 2004, Inouye 1978, 1980, Pyke 1982, Graham & Jones 1996). This is explained by differences in the efficiency with which bumblebees can extract nectar from the different plant species. Nectar is the only source of energy for bumblebees, polen is the protein food and is essential to mantain the larval growth, but without nectar adult bumblebees perish in a few hours (Heinrich 2004). It is then expected that growth, survivorship and reproduction of bumblebee colonies depend on the rate of net energy gain of the colony (Pyke 1982, Heinrich 2004). Thus, foragers will seek to maximize their net profit working in those plant species in which they obtain the greatest amount of nectar per unit of time with the least energy expenditure (Heinrich 2004).

Proboscis length determines the depth at which a bumblebee can reach nectar in a flower as well as the handling time of each flower and therefore the number of flowers visited per unit of time (Holm 1966, Inouye 1980, Harder 1983, 1985, Graham & Jones 1996). Bumblebees probably learn through experience to visit flowers that are best suited to the length of their proboscis (Heinrich 2004). Variation between species in this morphological feature determines differences in floral preferences, with long proboscis species specializing on long corolla flowers and short proboscis species on short corolla flowers (Holm 1966, Heinrich 1976a,b, 1979, Inouye 1978, 1980, Harder 1985, Graham & Jones 1996). So, when different species of bumblebees coexist, the morphological differences between them as well as the great diversity of flower structures and sizes of the different plant species, provide an opportunity for differentiation in resource use. The resulting resource partitioning would diminish interspecific competition (Heinrich 1976b, Inouye 1978, 1980, Pyke 1982, Harder 1985, Graham & Jones 1996).

There are four species of bumblebees reported from Uruguay: *Bombus atratus* (Franklin), *B. bellicosus* (Smith), *B. brasiliensis* (Lepeletier) and *B. morio* (Swederus). The first two are widely distributed in the country, while the others have been recorded only in one location each (Abrahamovich & Díaz 2002, Abrahamovich *et al* 2004). The two most common species are easily distinguishable because of their different coloration. They also differ in mean worker body size, since *B. atratus* was larger in average than *B. bellicosus* (13.6 mm and 10.6 mm, respectively) (Moure & Sakagami 1962 in Cortopassi-Laurino *et al* 2003).

In Uruguay, red clover (*Trifolium pratense* L.) and bird's foot trefoil (*Lotus corniculatus* L.) are two of the most cultivated forage legumes for livestock feeding. These Fabaceae present a similar general floral structure in which sexual structures of flower are enclosed in the keel petals that form the corolla, at the bottom of which are found nectaries (Izaguirre & Beyhaut 1997). Despite this general similarity, these plants have large differences in several floral characters. The red clover inflorescence shows a head formed by numerous pink florets that have a deep tubular corolla 7.5 mm to 12.4 mm long and 1.6 mm to 2.5 mm wide (Carámbula 1981). On the other hand, the bird`s foot trefoil presents a more open corolla of less than 10 mm long and form inflorescences in clusters of 4-6 yellow-orange flowers (Izaguirre & Beyhaut 1997). These morphological differences make a good scenario to explore possible differences in resource utilization by bumblebees.

The aim of this study was to determine proboscis length of *Bombus atratus* and *B. bellicosus*, two Uruguayan bumblebees, and verify the resource exploitation testing two cultivated species, the red clover and the bird's foot trefoil.

Material and Methods

Field work was carried out at the Centro Regional Sur (CRS) of Facultad de Agronomía (34º 36' S, 56º 13' W) in March 2005 and at La Estanzuela Experimental Station of the Instituto Nacional de Investigación Agropecuaria (INIA-LE) (34º 20' S, 57º 41' W) in March 2007. The only bumblebee species found in both locations were *B. atratus* and *B. bellicosus*, which were identified using the available literature (Abrahamovich *et al* 2005).

At the CRS, bumblebee visits to flowers were recorded in a 2 ha mixed meadow of red clover and bird`s foot trefoil. Foraging activity of bumblebees was observed when both plant species were in full bloom. A transect was designed so as to allow covering the entire meadow surface without passing the same zone more than once. It was walked several times during the morning and afternoon of two days, totalizing 640 min of record. During the walk, the species of each bumblebee observed and the plant species in which it was foraging was recorded.

At INIA-LE, bumblebee visits were recorded in small contiguous plots of red clover and bird`s foot trefoil located in two distant areas of the Station. Area 1 consisted of three plots: one red clover plot of 35 x 20 m, and one bird`s foot trefoil plot at each side, one of 20 x 20 m at the ending of blooming and the other of 60 x 30 m which was starting a second bloom. The plots were walked simultaneously in the morning and the afternoon totalizing 135 min of record. In Area 2, foraging bumblebees were observed in a red clover plot of 45 x 25 m and in a bird`s foot trefoil plot of 40 x 40 m, which were at approximately 3 km away from the previously described plots. These plots were walked at different times of the day, resulting in 180 min of record.

At the end of the data collection period, foraging bumblebees of both species were captured on bird`s foot trefoil and red clover and were maintained at -20ºC. The proboscis of each individual was extended and its length was measured from the prementon to the tip of the glossa

using a digital caliper under magnifying lens.

To test whether differences on distribution of *B. atratus* and *B. bellicosus* on red clover and bird`s foot trefoil differ from the one expected by chance, a Chi square test in a 2 x 2 contingency table (Zar 1997) was used. To determine differences of bumblebee species proboscis length, and intraspecific differences according to the plant species in which they were foraging on, a t-test ($\alpha = 0.05$) was used.

Results

Both species of bumblebees exploited red clover and bird`s foot trefoil in the studied areas, although they did it in different proportions at each of the three instances tested.

In the mixed meadow at the CRS, a total of 400 visits of *B. atratus* and 49 visits of *B. bellicosus* were recorded. While *B. atratus* visited mainly red clover, *B. bellicosus* visited both resources in similar proportions ($\chi 2 = 66.61$, $P < 0.001$) (Fig 1).

At INIA-LE, 104 visits of *B. atratus* and 137 visits of *B.bellicosus* were recorded in Area 1. Both species visited mainly red clover, although the proportion of visits of *B. bellicosus* to bird`s foot trefoil was significantly higher than that of *B. atratus* ($\chi 2 = 28.06$, $P < 0001$) (Fig 2a). Additionally, interspecific differences in resource utilization remained in the same in Area 2, with most of the visits of *B. atratus* to red clover, while most of the visits of *B. bellicosus* were to bird`s foot trefoil ($\chi 2 = 24.91$; $P < 0.001$) (Fig 2b).

The proboscis length of *B. atratus* (8.4 ± 0.65 mm, N = 34) was longer than that of *B. bellicosus* (7.9 ± 0.79 mm, N = 25), providing significant interspecific differences ($t = 2.90$; $P < 0.01$) (Fig 3).

There were no differences in proboscis length between individuals of *B. atratus* collected on red clover and those collected on bird`s foot trefoil ($t = 0.86$; $P = 0.39$) (Fig 4a). By contrast, the intraspecific analysis of *B. bellicosus* in the two legumes did show differences: the proboscis length of foragers caught on red clover was longer than those collected on bird`s foot trefoil ($t = 2.84$; $P < 0.01$) (Fig 4b).

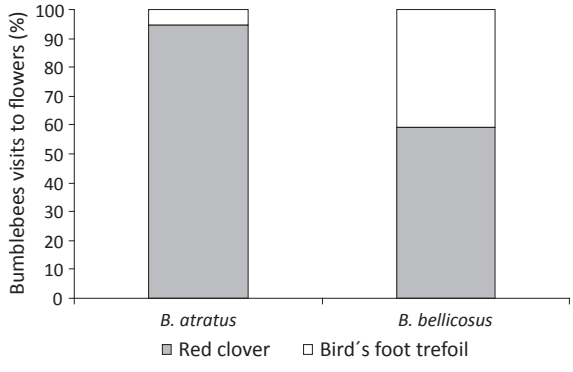

Fig 1 Proportion of *Bombus atratus* and *B. bellicosus* visits to red clover and bird`s foot trefoil in a mixed meadow in the Centro Regional Sur of Facultad de Agronomía in March 2005 ($\chi 2 = 66.61$; $P < 0.001$).

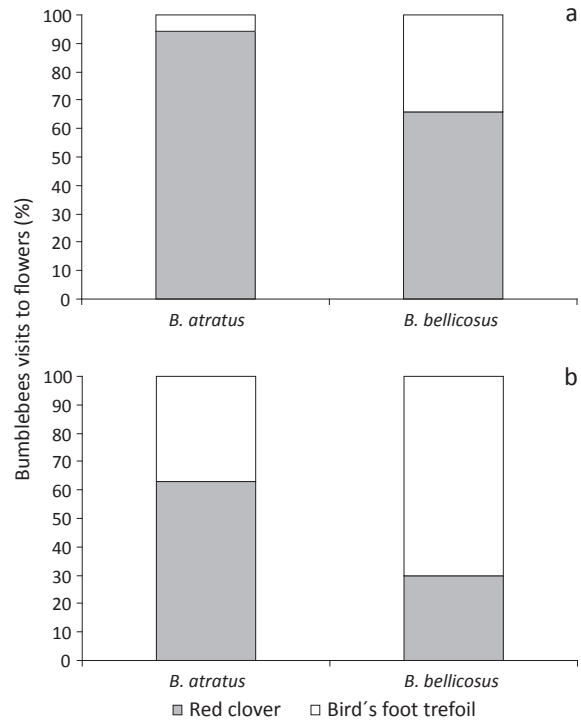

Fig 2 Proportion of *Bombus atratus* and *B. bellicosus* visits to red clover and bird's foot trefoil on contiguous plots of this legumes in La Estanzuela Experimental Station of the Instituto Nacional de Investigación Agropecuaria in March 2007. a) Area 1 ($\chi 2 = 28.06$; $P < 0001$), b) Area 2 ($\chi 2 = 24.91$; $P < 0.001$).

Discussion

The results showed that *B. atratus* and *B. bellicosus* visited red clover and bird`s foot trefoil differentially and suggested that those differences could be associated with proboscis length: bumblebees with longer proboscis preferred red clover and those with shorter proboscis used bird`s foot trefoil. This could indicate that differences in corolla depth between these plant species were enough to cause the observed pattern of resource utilization. In this scenario, *B. atratus* (with a longer proboscis) possibly obtained nectar easier and faster from deeper flowers than *B. bellicosus*, which has a shorter proboscis. The pattern observed in *B. bellicosus* represented an intraspecific resource partitioning that could also be explained by differences found in the length of the proboscis. Intraspecific resource partitioning related mainly to differences in proboscis length has been reported in several *Bombus* species (Inouye 1978, Morse

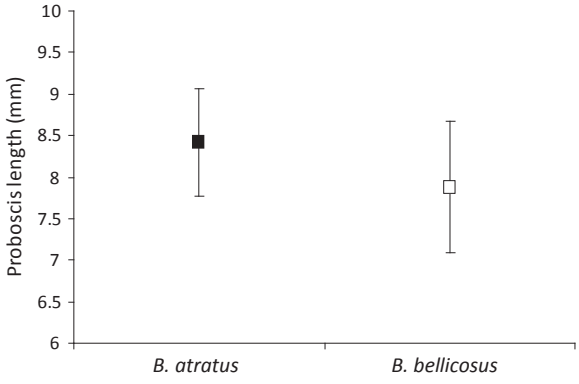

Fig 3 Mean proboscis length of foraging bumblebees *Bombus atratus* and *B. bellicosus* (t = 2.90; P < 0.01) collected on red clover and bird`s foot trefoil at La Estanzuela Experimental Station of the Instituto Nacional de Investigación Agropecuaria in March 2007.

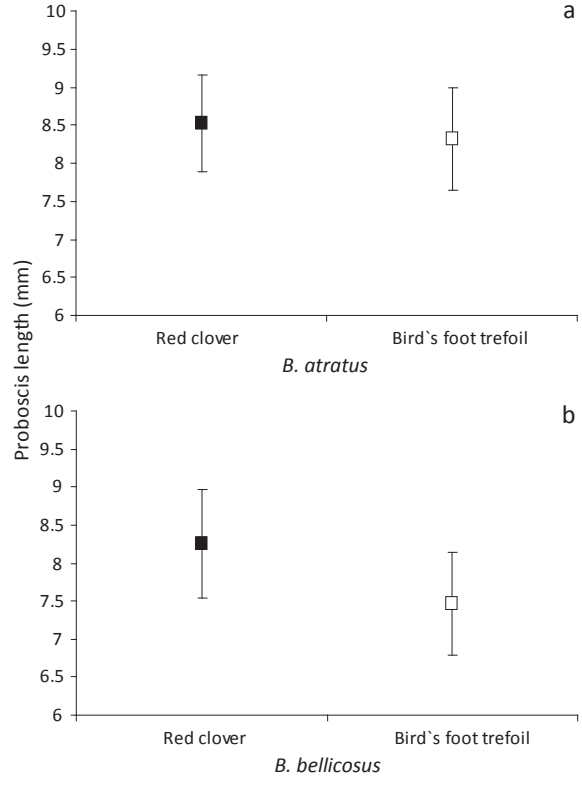

Fig 4 Mean proboscis length of foragers collected on red clover and bird`s foot trefoil. a) *Bombus atratus* (t = 0.86; P = 0.39). b) *B. bellicosus* (t = 2.84; P < 0.01). Bumblebees were collected at La Estanzuela Experimental Station of the Instituto Nacional de Investigación Agropecuaria in March 2007.

1978, Johnson 1986, Heinrich 2004). Proboscis length is highly correlated with body size (Morse 1978, Harder 1985, Heinrich 2004), so differences in worker body size within a *Bombus* species, and even within a colony, allow individuals to specialize in different plant species. This would enable bumblebee colonies to efficiently exploit the diverse range of resources available throughout the life cycle of the colony (Inouye 1978, Heinrich 1979, 2004, Kearns & Thomson 2001).

Several factors would indicate that during the analyzed periods, red clover was a more rewarding resource for bumblebees than bird`s foot trefoil. First, red clover was the resource most frequently visited by bumblebees and, in general, the most visited resource in a given area is often the best source of nectar (Heinrich 1979). Red clover inflorescence is formed by numerous flowers so, as long as these contain nectar, a bumblebee can visit several flowers without incurring high costs of time and energy in flight (Heinrich 2004). Finally, due to the type of inflorescence, it is likely that the number of individual flowers of red clover was significantly greater than that of other resources, and the amount of potential reward of a particular plant species is also a function of the total number of available flowers (Heinrich 1979). However, the energetic advantage that red clover could provide would be diminished by the high rate of nectar consumption due to the large number of visits it receives.

A decrease in nectar level in the flowers will affect more severely short proboscis individuals since they will have to struggle to reach the nectar, increasing the time of manipulation and therefore the costs that could outweigh the benefits (Holm 1966, Inouye 1980, Harder 1983, 1985, Graham & Jones 1996). This would lead short proboscis individuals to visit other plant species in which they obtain a greater net energy gain than in those of long corolla, despite the lower daily production of nectar (Heinrich 1979). Thus, smaller *B. bellicosus* individuals would be displaced from red clover by *B. atratus*, and even by their larger conspecifics, with a higher foraging efficiency in this plant species.

Several authors agree that exploitative competition is the mechanism responsible for resource partitioning in bumblebees since direct competition is rarely observed (Inouye 1978, Heinrich 1979, 2004, Graham & Jones 1996), although there are a few documented cases (Morse 1978, Plowright & Laverty 1984). In this study, competition intensity between *B. atratus* and *B. bellicosus* did not seem to reach such a level to cause absolute resource partitioning. In this sense, Rodríguez-Gironés & Santamaría (2006) using a mathematical model to analyze foraging behavior of bumblebees predicted that when two coexisting bumblebee species differ in their proboscis length, differences in their ability to exploit two flower types that differ in the corolla depth will lead to resource partitioning. In such condition, at least one species would specialize in a single type of flower, while the other (no matter which one) would act as a specialist when nectar competition is intense or as a generalist when competition is weak. In the present study, *B. atratus* behavior would approach to that of a specialist whereas *B. bellicosus* behavior would do so to

that of a generalist; nevertheless the allocation of these roles was not clear.

Although proboscis length is undoubtedly a very important factor in the selection of resources by bumblebees, it is certainly not the only one. Foraging efficiency of bumblebees and, therefore, the choice of resources are also affected by bumblebee body size (Harder 1983, 1985, Goulson 2003, Heinrich 2004), wing length (Harder 1985), learning capacity (Heinrich 1976a, 2004, Laverty 1994, Raine & Chittka 2007), daily volume of nectar produced per flower (Pyke 1982, Harder 1983, Goulson 2003, Heinrich 2004), communication between members of the colony and the state of the colony in relation to its nutritional requirements (Heinrich 1979, 2004, Goulson 2003), among other characteristics. There are several factors affecting distribution of bumblebees on the different resources available in a given area, making difficult explanations for the observed patterns. In the case studied, differences in the length of the proboscis would explain much of the general pattern of distribution of species on the evaluated resources, but further research on the discussed issues are required.

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