



Recruitment to forage of bumblebees in artificial low light is less impaired in light sensitive colonies, and not only determined by external morphological parameters



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ABSTRACT

Bumblebees of *Bombus terrestris* are essential pollinators in natural and managed ecosystems. Their foraging ability relies on the individual morphology, task allocation within the colony, and external factors, such as light intensity. The foraging activities of commercial bumblebees can sometimes be impaired, especially in the artificial and weak light intensities of greenhouses at high altitudes. Here we investigated whether the eagerness (or willingness) to forage of bumblebee colonies in different light conditions is correlated with the light sensitivity of bumblebees colonies and/or different external morphological parameters. The initial foraging capacity of bumblebee colonies correlated with their light sensitivity. However, light sensitive bumblebee colonies did not necessarily had a higher foraging activity at lower light intensities. Differences in initial foraging capacity and light sensitivity among colonies could not be explained by the external morphological parameters. In conclusion, our data illustrated that the recruitment to forage in artificial low light is less impaired in light sensitive colonies, and that not only the external morphology parameters determine the light sensitivity of bumblebees and their eagerness to forage in weak light conditions. The data obtained here create a better understanding of which criteria are able to select towards light sensitive bumblebees and their link with the foraging capacity of these bumblebees.

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1. Introduction

Bumblebees are essential pollinators in natural and managed ecosystems (Heinrich, 1979; Goulson, 2003). Like honeybees, bumblebee workers have specialized morphological structures for the collection of nectar and pollen such as a *corbicula* and adapted mouthparts (Michener, 1999; Inouye, 1980; Thorp, 2000). Due to their thermoregulation system, bumblebees are capable of foraging on days when it is too cold to forage for other pollinations (Heinrich, 1975, 1979; Goulson, 2010). The foraging abilities of bees rely also on their sensory systems, the visual and the olfactory system, which consist out of two apposition compound eyes and three ocelli (Wcislo and Tierney, 2009) and several pore plate sensillae on their antennae as described for *Bombus terrestris* L. by Spaethe et al. (2007). An increase in size of the morphological parameters of both sensory systems increases the ability to detect and discriminate between different flowers which in turn can increase their foraging efficiency (Chittka et al., 1999).

As bumblebees are social insects, the food influx of a colony is affected by how the work is allocated among all members of the col-

ony (Goulson, 2003). Typically, bumblebee colonies consist of hundred workers that differ in size (Goulson, 2010). The size differences within a colony are related to a specific task, a phenomenon known as alloethism (O'Donnell et al., 2000; Jandt and Dornhaus, 2009). Small workers are found more inside the nest where they fulfill all kinds of nest tasks, whereas large workers are more likely to become foragers (Goulson et al., 2002; Spaethe and Weidenmüller, 2002; Jandt and Dornhaus, 2009). However, this size-dependent division of labor is not strict and task-switching is possible (Jandt and Dornhaus, 2009). For instance, when there is a shortage of foragers, the smaller bees can be recruited or start foraging to comply with the nutritional needs of the bumblebee colony (Dornhaus and Chittka, 2005; Molet et al., 2008; Kitaoka and Nieh, 2009).

Bumblebee foraging activity depends also on external factors such as food quality (Chittka et al., 1997; Roldán-Serrano and Guerra-Sanz, 2005; Goulson, 2010) and environmental conditions like temperature, humidity, weather conditions and light intensity (Corbet et al., 1993; Peat and Goulson, 2005; Goulson, 2010). The latter parameter turned out to be of importance in relation to foraging activity and foraging initiation of bumblebees in greenhouses (Blacquière et al., 2007; Roman and Szczęśna, 2008; Johansen et al., 2011).

Bumblebees (*B. terrestris*) are used worldwide in greenhouses for the pollination of different crops (Velthuis and van Doorn,

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2006). Although commercial bumblebees perform better in the artificial light environment of the greenhouse than honeybees, they also show some problems particularly when the artificial light environment of a greenhouse deviates from the natural light environment in intensity and spectral composition (Moradin et al., 2001; Blacquière et al., 2006, 2007; Johansen et al., 2011). Indeed, under these reduced artificial light conditions the activity of the bumblebees is decreased (Roman and Szczęśna, 2008).

Here in this project, we wanted to determine which parameters of individual bumblebees are linked with the lower performance of colonies in artificial light conditions. Therefore, we used eight queen-right bumblebee colonies from a mass-rearing program and developed a new bioassay which determines the number of workers triggered to forage in two different standardized light intensities. Furthermore, we measured different external morphological parameters and the light sensitivity of 15–20 individual bumblebees of each of those colonies. In this way, we obtained more insights in the plasticity or variability of these parameters within the same colony and between colonies. The data obtained may help to improve the criteria for selecting towards light sensitive bumblebees and their link with the foraging capacity of these bumblebees.

2. Material and methods

2.1. Laboratory conditions for maintenance of bumblebee colonies

For this project we used eight commercial queen-right colonies of *B. terrestris* from a mass-rearing program at Biobest (Westerlo, Belgium) (Fig. 1a and b). These colonies were provided with commercial sugar water (BioGluc, Biobest) and pollen (Apihurdes, Spain) *ad libitum*. All experiments were performed in a controlled laboratory environment at 28–30 °C and 60–65% air humidity.

2.2. Determination of the initial foraging capacity under different light conditions

We developed a new bioassay to determine the initial foraging activity (F_a), which is the number of bumblebees leaving the colony in a time period of 1 h divided by the total number of workers in the colony at that moment. With the use of this bioassay we measured both the initial foraging activity of a colony in weak and strong light conditions (F_a^w and F_a^s , respectively). The initial foraging capacity, F_c , was calculated as the ratio of the initial foraging activities at weak and strong light intensity, F_a^w/F_a^s . This parameter (F_c) is a measure for the ability of a colony to keep its baseline initial foraging activity even with a decrease in light intensity.

In detail, for the eight different queen-right colonies we measured the foraging activity by placing each colony individually in a meshed fly cage (60 × 60 × 60 cm, BugDorm-2, MegaView Ltd, Taichung, Taiwan) in strong light intensity (F_a^s , 14,000–14,500 lux or $2.2\text{--}2.3 \times 10^{20}$ photons $\text{m}^{-2}\text{s}^{-1}$) and weak light intensity (F_a^w , 4000–4500 lux or $5.3\text{--}6.0 \times 10^{19}$ photons $\text{m}^{-2}\text{s}^{-1}$). Light was provided by a Halogen Floodlight (PowerPlus Light, Varo, PowLI023, W400/500) which was placed at 30 cm in front of the entrance of the colony. The light intensity was measured with a calibrated luxmeter (Taschen-Luxmeter LM37, Karlsruhe, Germany) at the opening of the colony. As foragers are only active during the period of day, due to a robust internal circadian clock (Stelzer et al., 2010; Stelzer and Chittka, 2010), both measurements were performed on the same day between 10 a.m. and 12 p.m., alternating weak and strong light intensities as first measurement. All foragers were placed back in the colony before the light conditions were changed.

The foraging test was performed 20 times for each colony, following a three days cycle of overnight starvation, one day of mea-

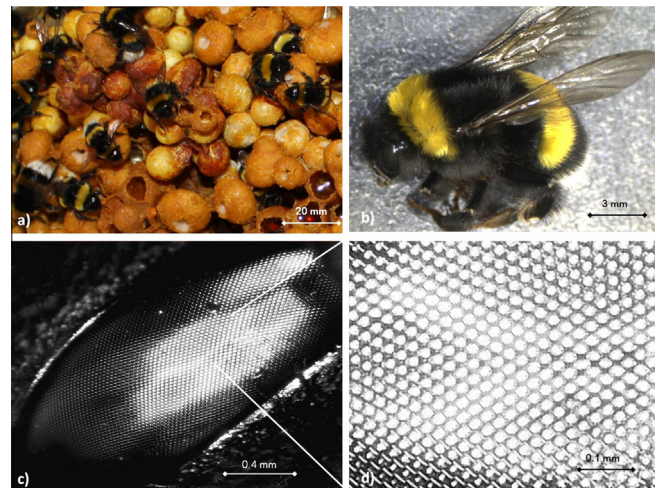


Fig. 1. Panel with (a) *Bombus terrestris* colony, (b) *B. terrestris* worker, (c) compound eye and (d) ommatidia.

surement and a day of recuperation. In the latter step, colonies were allowed to feed on sugar water. Colonies were starved overnight to trigger each nest towards maximal foraging. During the experiment we determined F_c and measured F_a^w and F_a^s , while the colonies developed from a workforce of 20 until 99 workers. For each colony these values of F_c , F_a^w and F_a^s were placed in different classes based on the size of the workforce in the colony at the moment of measurement (with class 1: a workforce from 20 until 29 workers; class 2: a workforce of 30 until 39 workers; ...; class 8: from 90 until 99 workers). Thereafter we calculated F_c , F_a^w and F_a^s as the mean (\pm SE) over all the classes.

After logarithm transformation of the measured F_c , F_a^w and F_a^s values, the data were tested for normal distribution and analyzed by one-way ANOVA followed by a *post hoc* Tukey test.

2.3. Measurement of different morphology parameters of bumblebee workers

For 15–20 workers of the eight different bumblebee colonies, we determined eight morphology parameters: (i) thorax width (intertegular span) of workers; (ii) total fresh weight; (iii) dorsal-ventral length of compound eye; (iv) width of compound eye; (v) total surface of compound eye; (vi) diameter of facet; (vii) total numbers of ommatidia of compound eye; and (viii) diameter of median ocellus.

Each bumblebee and its left compound eye were photographed with a Leica DFC295 (Leica Microsystems Ltd, Switzerland) digital camera mounted on a Leica S6D microscope by using the software LAS vs 3.6.0 (Leica Application Suite). Measurements of all the morphological parameters were done on the images with the free software program Image J (<http://rsb.info.nih.gov/ij/index.html>) (Fig. 1c and d). Worker size was measured as the thorax width (Goulson et al., 2002) and the total surface of the compound eye (S) was estimated by using the formula of measuring an ellipse surface as described by Jander and Jander (2002). We calculated the diameter of a facet as the mean of a row of 10 facets measured in three dimensions (w , y and z) (Kapustjanskij et al., 2007) and always at the centre of the compound eye (Jander and Jander, 2002). The ommatidia surface, a hexagon, was calculated using the formula $S = 3\sqrt{3}/2 \times z^2$ with z as the radius of the ommatidia. Ommatidia numbers were then estimated by dividing the eye surface

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