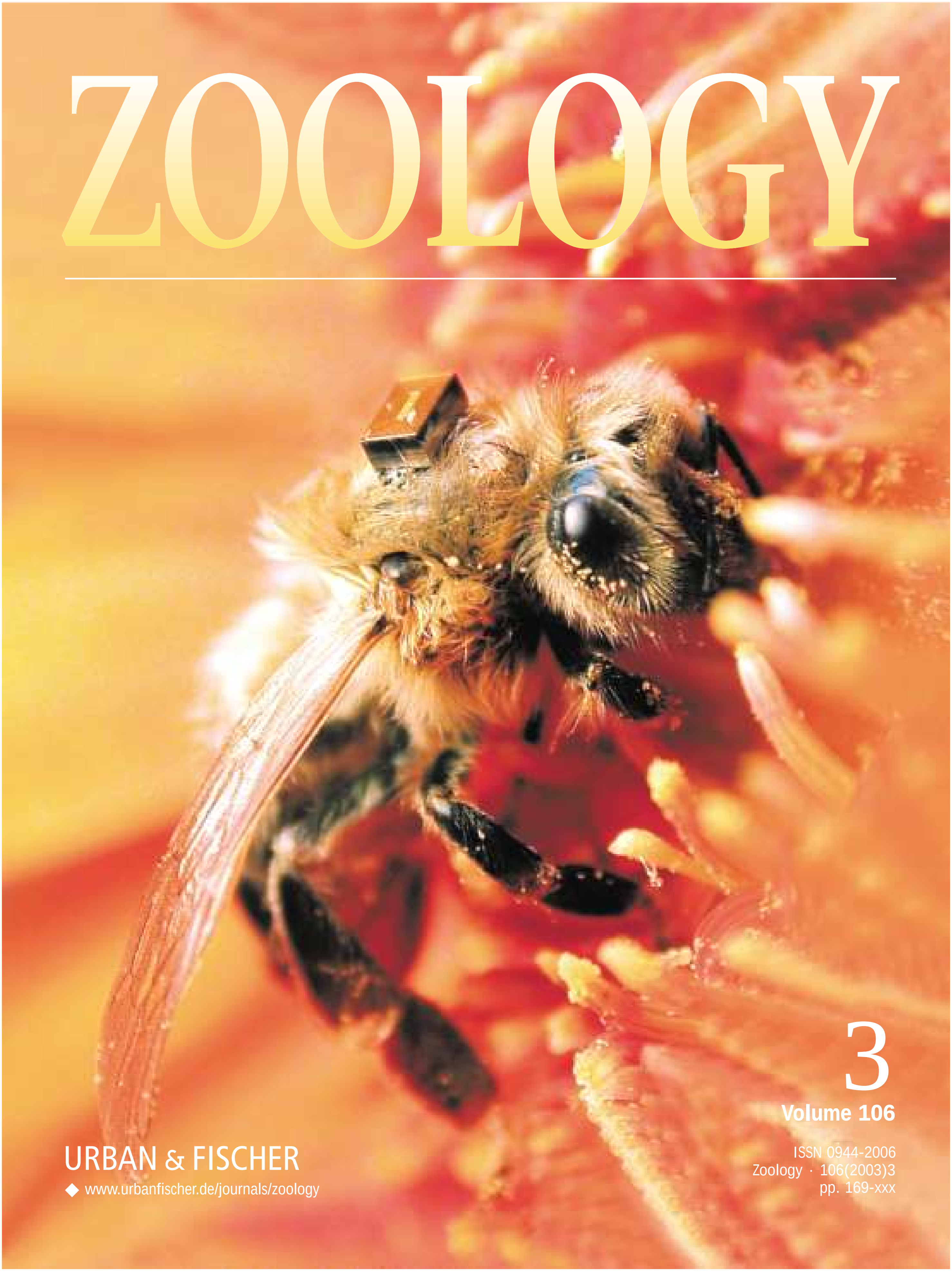

# ZOOLOGY

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Volume 106

URBAN & FISCHER

◆ www.urbanfischer.de/journals/zoology

ISSN 0944-2006
Zoology · 106(2003)3
pp. 169-xxx

Zoology **106** (2003): 169–171
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http://www.urbanfischer.de/journals/zoology

ZOOLOGY

# Automatic life-long monitoring of individual insect behaviour now possible

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Received July 18, 2003 · Revised version received August 8, 2003 · Accepted August 8, 2003

## Summary

Automatic tracking and identification of individuals has the potential to revolutionize the study of insects, especially social insects, by opening up options for questions which could not be asked before. To achieve this we developed a reliable and cost-sensible RFID (Radio-Frequency Identification) based solution that automatically recognises a virtually unlimited number ($18 \times 10^{18}$ possible ID numbers) of individual insects down to the size of bees and ants. The data are collected automatically for any desired time span (if interesting, up to the entire life of the individual), pre-processed and saved in a database for further analysis. The usage of database techniques allows parallel data processing with a virtually unlimited number of parameter connections. ID numbers can be linked to any simultaneously recorded parameters of interest, e.g. spatial and temporal information as shown here for a bumblebee colony.

**Key words:** individual insect behaviour, RFID tags, automatic life-long monitoring

## Introduction

Individual identification is essential in many observations and experiments on animal behaviour and physiology. This was one of the revolutionizing insights of Karl von Frisch in his experiments with honeybees (von Frisch 1967). Traditionally used in insect studies are "manual" techniques like recognition of coloured number tags or colour labelling either by direct observation or by video recording. These techniques have their limits regarding the number of individuals which can be monitored simultaneously and the time span during which this can be done; and they require a disturbance of the insects (e.g. shining light into a colony for video-recording).

Radio Frequency Identification (RFID) technology is a major leap forward and offers multiple advantages (Finkenzeller 2002): RFID tags can be read at remarkable speed, in our case in less than 50 milliseconds, and without contact through a variety of substances such as glue, plastic, in total darkness and continuously life-long. The online processing of data offers real-time access to the results and automatic control of any devices during the experiment (e.g., feeder visit frequency of a selected individual can now regulate food availability by controlling an automatic food flow).

Such an RFID approach is most useful in the study of insect behaviour due to the potentially large number of individuals and high frequency of parallel or sequential events. The development of ever smaller microchips provides us now with the desired tag sizes ($1.6 \times 1.0 \times 0.3$ mm including antenna).

## Setup

We used customary passive RFID tags, weighing 2.4 mg (Fig. 1), a weight easily carried by most social insects (honeybees are able to carry up to 70 mg (Nunez 1982)). Former RFID tags needed large extending antennae (1–2 cm) unsuitable for the study of undisturbed insect behaviour. Passive RFID tags function without a

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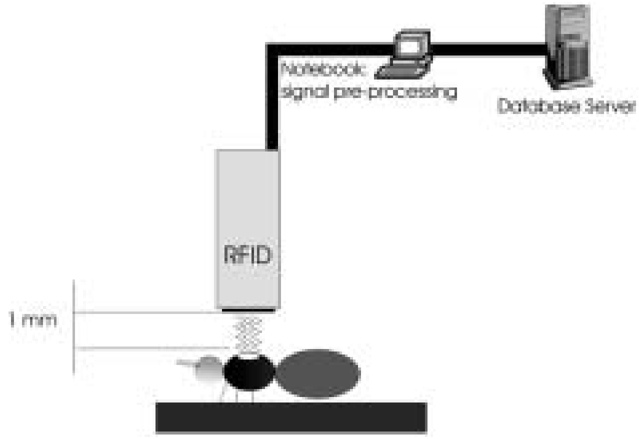


**Fig. 2.** Experimental set-up. A small insect carrying a 2.4 mg tag passes underneath the scanner which identifies the insect and sends the data along with real-time recording to a notebook.

◀ **Fig. 1.** Honeybee (*Apis mellifera*) equipped with a transponder which is glued to the centre of the dorsal thorax in such a way that the movement of the wings is not hampered.

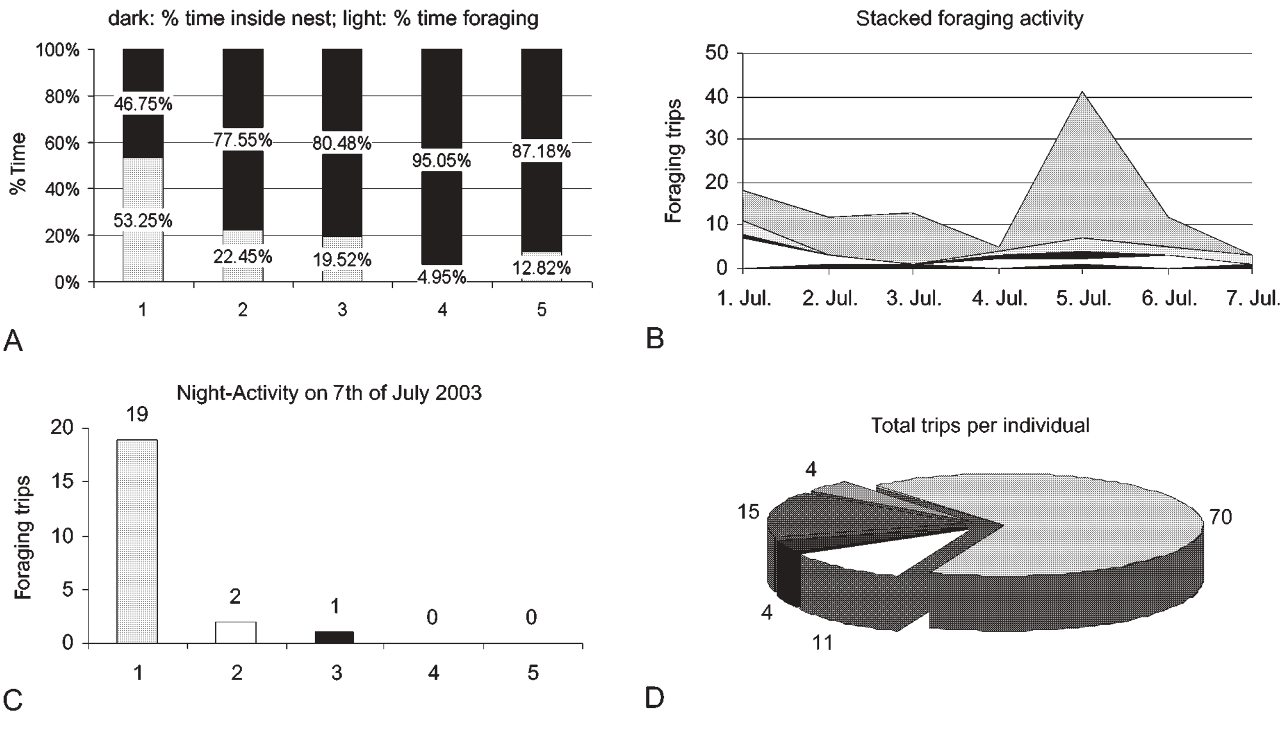


**Fig. 3.** (**A**) Time of foraging and within the nest for 5 bumblebees (no. 1–5). (**B**) Stacked foraging activity for 5 bumblebees from July 1–7, 2003. (**C**) Leaving the nest during night time (0:00 until 6:00 a.m.) of 5 bumblebee foragers on July 7, 2003. (**D**) Total number of trips per individual for 5 bumblebee foragers from July 1–7, 2003.

power source, obtaining operating power from the reading process. Therefore, passive tags are much lighter, easier to produce and cheaper than active tags and offer a virtually unlimited operational lifetime. Each tag is programmed with a unique identification number.

We designed a specialized reader mountable at points of interest (here: nest entrance) and reading up to 20 individuals per second. The working distance of the current reader generation is 3 mm, the sector watched by the whole reader spans a tunnel of 20 × 5 mm. In passing the reader, the bee is monitored twice, thus determining the direction and speed of movement. The orientation of the tag relative to the reader does not influence the readings. The reader software ("Beegroup ID2DB" © Beegroup, Sebastian Streit, 2003) records the ID number, speed and direction of movement and the exact time of the event to a database. We created an easy-to-use interface ("Beegroup DB2Use" © Beegroup, Sebastian Streit, 2003) for designing the groups of interest (e.g. races, patrilines, raising conditions), monitoring the experimental progress online and analysing the data.

The complete system described here (Fig. 2) is available through the *Beegroup Würzburg*.

## Proof of concept

To demonstrate the significant advantages of this new tool for the study of insect behaviour we tagged a number of 5 bumblebees of the current foraging force of a *Bombus terrestris* colony with a total size of about 70 individuals and recorded data continuously over a period of one week.

We developed a tunnel system which (A) forced the bees to pass under the reader, (B) ensured normal in-and-out-traffic (as compared to parallel colonies of the same size housed in identical hives), (C) guaranteed for avoidance of jamming and (D) produced a low number of mistakes, i.e. insects escaping the detector while entering or leaving the hive (a visual check demonstrated that one bee out of 300 passing went unrecorded by the detector).

The traditional tools of data collection need a lot of manpower which limits the degree of complexity. Direct observation or video recording makes disturbance of the colony necessary, if only by shining light into an otherwise dark nest. In order to answer questions which come to mind later, experiments often have to be done again because certain aspects may not have been considered. Furthermore, the search for patterns not recognized by eye-inspection of raw data, a technique called *data-mining* in fields like economy (Han and Kamber 2000) where enormous quantities of data are collected, is now also possible in the study of insect behaviour.

With the new system there is no limit to the questions which can be asked even afterwards due to the unlimited specification and combination of the data.

To demonstrate the power of the system we present four examples of possible questions – two we had formulated as initial questions at the beginning of the experiment (Fig. 3 A and B), and two more developed after completion of the experiment during data processing that could not have been answered if the observations would have been made using any of the traditional techniques (Fig. 3 C and D). The collected data contain information which can be brought to light with the technique shown here, which allows to follow up on biologically relevant questions even a long time after the actual data recording. Furthermore, a wide spread of this system among scientists would allow for linking of data and comparing data from different labs on different species or under different observational conditions.

## Acknowledgements

Financial support by the DFG-Graduierten Kolleg GK 200 and the SFB 554.

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