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Digging behaviour and responses to photic and gravitational cues as elements of escape behaviour of bumblebees

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Abstract. We have investigated escape behaviour of workers of two bumblebee species, *Bombus terrestris* and *B. pascuorum*, when confined to test tubes plugged with soil and either exposed to sunlight or kept in darkness. In both these situations *B. terrestris* performed better (i. e. escaped after a shorter time) than *B. pascuorum. B. terrestris* (but not *B. pascuorum*) also performed better in darkness than in tubes exposed to sunlight. This implies that in both situations *B. terrestris* showed higher readiness to dig than *B. pascuorum*, and that in tubes exposed to sunlight only *B. terrestris* showed high readiness to display photopositive behaviour as well. *B. pascuorum* displayed, however, photopositive behaviour in another escape situation: when released in a dark room in front of a vertical array of four sources of white light. In that situation, *B. pascuorum* also displayed the tendency to fly upwards, based most probably on responses to gravitational cues.

Key words: *Bombus terrestris*, *Bombus pascuorum*, bumblebees, escape tactics, digging behaviour, photopositive behaviour, gravity responses, nesting ecology

INTRODUCTION

In Hymenoptera, escape behaviour triggered in response to confinement involves often photopositive behaviour (von Hess 1913, 1916, 1920, Lutz 1924, Bertholf 1931a, b, Heintz 1959, Morgan 1981, Godzińska 1983), most probably because light is a fairly reliable token signal of open space. Hymenoptera may also respond to confinement by attempts to remove obstacles obstructing their way. Such behaviour was already described by Verlaine (1925) in social wasps *Paravespula germanica* (L.) trained to run a maze made of cardboard. In this study the wasps kept to bite holes in the walls of the maze instead of learning the correct way to run it.

Behavioural patterns displayed by various Hymenoptera while confined to relatively small containers started to be interpreted in terms of escape behaviour by Godzińska (1983). Subsequently, Godzińska (1988) and Godzińska and Korczyńska (1992) studied escape behaviour displayed by workers of two bumblebee species, *Bombus pascuorum* Scopoli and *B. terrestris* L. in a situation of confinement to a test tube plugged with sand or closed by a thin paper membrane. The other, transparent end of the tube was always oriented in the direction of the sun, so that the tested bee could choose between two alternative escape tactics: on one hand, photopositive behaviour, on the other hand, digging or biting. In contrast to the majority of situations encountered by bumblebees in their natural environment, in this particular experimental situation only digging or biting were the correct escape tactics: photopositive behaviour could not result in a successful escape.

In both those experiments escape behaviour of *B. terrestris* and *B. pascuorum* showed significant differences. As a rule, *B. terrestris* started to display digging/biting behaviour sooner than *B. pascuorum*, and were interrupting digging/biting less frequently. Moreover, in tubes plugged with sand the values of the total time spent on digging and the latency from the beginning of the test to the final escape out of the tube were both significantly lower in *B. terrestris* than in *B. pascuorum*.

In terms of its proximate mechanisms, better escape performance of B. terrestris confined to test tubes plugged with sand might have resulted either (1) from higher readiness of workers of that species to dig, and/or from higher efficiency of their digging behaviour, or (2) from their lower readiness to show photopositive behaviour, or, lastly, (3) from both these factors. To throw more light on that question, in the Experiment I we have been comparing the behaviour of workers of *B. terrestris* and *B. pascuorum* tested in two experimental situations: in a test tube plugged with sand and exposed to sunlight, and in an identical tube kept in darkness. The comparison of the total test time (= the time from the beginning of the test to the final escape of the bee out of the tube) recorded for *B. terrestris* and *B. pascuorum* in these two situations enabled us, in particular, to test two hypotheses concerning the relative importance of the photopositive behaviour for the overall escape performance shown by workers of these two species in tubes plugged with sand. Thus, (1) absence of significant differences between the total test time recorded for workers of a given species at different illumination conditions would imply that photopositive behaviour does not contribute in a significant way to their escape behaviour in a test tube plugged with sand. At the same time, (2) presence of significant interspecific differences between the total test time recorded in darkness would imply that better performance of *B. terrestris* in tubes exposed to sunlight did not result exclusively from the fact that workers of that species were less photopositive than *B. pascuorum*.

The results of the Experiment I revealed, among others, that photopositive behaviour does not contribute in a significant way to the overall escape behaviour shown by workers of *B. pascuorum* when confined to a test tube plugged with sand. *B. pascuorum*, however, were already known to display photopositive behaviour when tested in another escape situation, namely, when confined to the so called escape box: a relatively large (21 x 21 x 31 cm) box made of non-transparent plastic. In this box, a set of circular exit holes pierced in the walls (and closed by large transparent test tubes to prevent the escape of the tested bee after its first choice) was acting as an array of light sources (Godzińska 1983). The workers, queens and males of *B. pascuorum* tested in such escape boxes were entering frequently these exit holes, showing that in that experimental situation they responded positively to light cues. They also displayed a tendency to fly upwards and in the direction of the sun.

The tests using the escape boxes were carried out in the field and the boxes were illuminated by sunlight. Hence, it was impossible to determine whether the tendency to fly upwards observed in these boxes involved solely photopositive behaviour, or both photopositive behaviour and responses to gravitational cues. To throw more light on the relative role of these two response types in the escape behaviour of *B. pascuorum*, in the Experiment II we tested workers of that species in yet another escape situation: they were released in a dark room in front of a vertical array of four sources of artificial white light. The centre of that array was placed opposite the starting platform on which the tested bees were released. In such a way we could distinguish between photopositive behaviour and upward flights not based on the photopositive responses and presumably involving the responses to gravitational cues.

METHODS

Experiment I

Experiment I was carried out in the field at several sites near Mrozy and Krześlin (Siedlce District, central-eastern Poland) during August 1983. We used as subjects workers of *Bombus terrestris* L. and *B. pascuorum* Scopoli taken from among wild living foragers visiting various wild and cultivated plants, mostly *Galeopsis tetrahit* L., *Melampyrum nemorosum* L. and *Trifolium pratense* L. The bees were captured to a small glass beaker (3 cm in diameter, 10 cm long). Immediately after capturing, each bee was transferred to a clean test tube, 1.5 cm in diameter and 15 cm long. The tube was then plugged with a 2 cm layer of slightly moist sandy soil. It was then wrapped in aluminum foil to assure darkness, and placed horizontally so that its sand-plugged end was oriented away from the sun. We recorded the latency from the introduction of the bee to the tube to its final escape (= the total test time T). Thirty workers of each species were tested.

The behaviour of *B. terrestris* and *B. pascuorum* tested in tubes kept in darkness (experimental situation A) was then compared with the behaviour of workers of the same two bumblebee species - 64 workers of *B. terrestris* and 111 workers of *B. pascuorum* - tested in tubes exposed to sunlight (experimental situation B). In that situation, the tube was also placed horizontally so that its sand-plugged end was oriented away from the sun. The data on the escape behaviour of *B. terrestris* and *B. pascuorum* in tubes exposed to sunlight have already been published as a part of a previous paper of one of us (Godzińska 1988); however, we would like to stress that all the data compared in the Experiment I were collected in the same sites during the same period.

The tests were carried out on sunny days only, at the air temperature ranging from 21 to 23°C. To prevent possible heat exhaustion of the tested bees, the tubes were shadowed from above. As demonstrated in preliminary tests (Godzińska 1988), such a simple precaution is very efficient in preventing overheating of the tested bees. After the end of each test the bee was marked with a quick drying acetone-based paint to avoid recapturing and testing it for the second time, and was released free at a place where it was captured.

Statistical analysis of the data was carried out by means of the two-way Mann-Whitney U test.

Experiment II

Experiment II was carried out at a single site in Krześlin (Siedlce District, central-eastern Poland) in August 1992. We have used as subjects 20 workers of *B. pascuorum* taken from among wild living foragers visiting a large field of the dwarf dahlias. They were captured to a small glass beaker (3 cm in diameter, 10 cm long) and immediately transferred to a test tube plugged with cotton. They were then taken to a dark room in the nearby house in a basket wrapped with several layers of black cloth to assure darkness.

The tests were carried out in a large dark room (4.6 m x 4 m x 2.1 m). The window of that room was covered with several sheets of aluminum foil to cut out daylight; however, the walls of that room were painted white and they reflected a certain amount of artificial light used during the tests. The tests were conducted at the ambient temperature of 28°C.

At the beginning of each test, the tested bee was released on a circular starting platform (35 cm in diameter) placed in front of a vertical array of four sources of artificial white light (7 cm in diameter spherical frosted electric bulbs of 60 W). The bulbs were arrayed as a 45 x 45 cm vertical square composed of two rows and two columns. The starting platform was placed at the distance of 1.5 m from that array, exactly opposite its centre, so that all light sources were equidistant from the tested bee when it was taking off. The platform was also placed equidistantly in relation to the floor and the ceiling of the room, at the height of 1.05 m. Bees were released at the distal border of the platform in relation to the array of light sources, and as a rule they were walking over the whole platform before taking off at its border closer to the array of bulbs. To avoid any possible modulating effects of chemical cues left by other bees on the escape behaviour of the tested individual, the starting platform was covered with a sheet of paper which was changed for a fresh one for each tested bee.

During each test, we were recording only the first choice of the bee, as only at its first choice the bee was situated opposite the centre of the array of light sources, equidistantly in relation to each bulb, and equidistantly in relation to the ceiling and the floor of the dark room. Behavioural events recorded by us were classified into the following categories:

1. Flight to one of the light sources (often followed by a sinuous flight in the close vicinity of that light source). The position of chosen light source was noted.
2. Flight between two or more light sources: the bee was flying towards the array of light sources, but without choosing any particular target light. The position of the point of the arrival of the bee to the array of light sources was noted.
3. Flight above the upper row of light sources.
4. Upward flight. The bee was flying upwards towards the ceiling of the room and then usually kept flying just below it.
5. Upward flight preceded by a short downward flight.

We have never recorded any behavioural event not falling into one of the above categories (for instance, persistent downward flight, flight directed away from the array of light sources, flight directed at the right angle from the array of light sources etc.).

After its first choice, the bee was allowed to continue to fly until it landed spontaneously and then it was recaptured into a test tube and tested again in the same way. Each bee was thus tested ten times. After the series of ten tests, each bee was marked with paint and released free at the site where it had been captured. Bees (*n* = 2) which were not active enough to complete the series of ten successive tests were not taken into account in the analysis of the data.

The preferences of position shown by the tested bees at their first choice directed to one of the light sources were tested by means of the binomial test.

To check whether the behaviour of bees was significantly changing as a function of time (or, more strictly, as a function of the number of the successive tests), the data obtained for all tested individuals on all their successive tests were analysed using Friedman two-way analysis of variance by ranks. We carried out two such analyses, described below.

In the first case the behaviour of bees was classified into four ranked categories: 1 = flight to one of the light sources, 2 = flight between two or more light sources, 3 = flight above the upper row of the light sources, and 4 = upward flight.

In the second analysis we did not make a distinction between two principal forms of the photopositive behaviour observed in our experiment: flights directed to one of the light sources, and flights between two or more light sources. Instead, we have analysed the possible modifications of the relative importance of readiness to display photopositive behaviour in general, and readiness to fly upward (based most probably on responses to gravitational cues). The behaviour of bees was now classified into 5 ranked categories: 1= flight directed to the bottom row of light sources, 2 = flight between two rows of light sources, 3 = flight directed to the upper row of light sources, 4 = flight directed above the upper row of light sources, and 5 = upward flight. In both analyses, three cases in which an upward flight was preceded by a short downward flight were classified as an upward flight.

During the second analysis, we also calculated for each bee the total score (S) calculated as a sum of scores obtained by it during the whole session of ten successive tests. The values of S ranged from 22 to 47. Thus we could create a ranking order of the tested bees based on the increasing values of S indicating mainly the relative importance of the tendency to fly upwards.

RESULTS

Experiment I

The values of T (= the latency from the beginning of the test to the final escape of the bee) recorded for workers of *B. terrestris* and *B. pascuorum* tested in tubes plugged with sand and exposed to sunlight or kept in darkness are shown in Table I.

In both experimental conditions the values of T obtained for *B. terrestris* were highly significantly smaller than those obtained for *B. pascuorum*. Thus, we may refute the hypothesis according to which *B. terrestris* perform better than *B. pascuorum* in tubes exposed to sunlight only because the first species is less photopositive. On the contrary, our present data demonstrate that exactly the opposite is true. *B. terrestris* performed significantly better in darkness than under sunlight which implies that in tubes exposed to sunlight photopositive behaviour contributes in an important way to overall performance of this species. In contrast to that, in the case of *B. pascuorum* values of the total test time do not differ significantly between the two experimental conditions (darkness versus light). It shows that photopositive behaviour does not contribute in a significant way to the overall escape behaviour of *B. pascuorum* in tubes exposed to sunlight.

TABLE I

Median values ± range of the latencies from the beginning of the test to the final escape out of a test tube (in seconds) obtained for workers of *Bombus terrestris* and *Bombus pascuorum* tested in tubes plugged with sand and either kept in darkness (A) or exposed to sunlight (B)

	Bombus terrestris			*Bombus pascuorum*			*P*
	n	Median	Range	*n*	Median	Range	
A. Darkness	30	62.5	15-136	30	258.5	25-790	***
B. Light[1]	64	144.5	17-282	111	265.0	30-1276	***
P		***			NS		

n, sample size. Statistics: two-tailed Mann-Whitney *U* test. *** $P < 0.001$; NS: $P > 0.05$, [1] (from Godzińska, 1988).

B. terrestris performed better than *B. pascuorum* both in darkness and under sunlight. In darkness, in absence of any photic cues, better performance of *B. terrestris* must have resulted from higher readiness of workers of that species to display digging behaviour, and/or from higher efficiency of their digging. In tubes exposed to sunlight better performance of *B. terrestris* must have resulted from the same factor, too. In that latter situation photopositive behaviour and digging behaviour have mutually counteracting consequences, as photopositive behaviour guides the bee away from the sand-plugged end of the tube. Hence, in tubes exposed to sunlight the effects of higher readiness of *B. terrestris* to show photopositive behaviour must have been counterbalanced by their higher readiness to dig, and/or by higher efficiency of their digging behaviour, to result in a better performance of *B. terrestris* also in that experimental situation.

Experiment II

Main results of the Experiment II are shown in Fig. 1. As can be seen, all the individuals tested in the Experiment II showed photopositive behaviour (= directed their flights towards the experimental array of light sources or just above the upper row of light sources) at least twice during the session of ten tests.

In the majority of the cases (19 out of 20; 95%) photopositive behaviour of the tested bees involved at least once a flight oriented to one of the light sources, followed as a rule by a sinuous flight in its vicinity. Light sources situated in the top row of the array were approached more frequently than those situated in its bottom row (in total, 76 and 33 choices, respectively). Bees also approached more frequently light sources situated in the right column of the array than those situated in its left column (77 and 32 choices, respectively). However, in the present study we do not analyse these pooled data to avoid the error of the pseudoreplication (cf. Machlis et al. 1985, Leger and Didrichsons 1994). Such an error may be avoided, among others, by taking into account only one choice per bee, for instance, its first flight directed to one of the light sources. However, if to analyse only the data concerning the initial choices of each bee, the preference for the top row of the array (11 out of 19 choices; the bee No. 20 never directed its flight to one of the light sources) is not significant (binomial test: NS). Similarly, instead of the preference for the right column of the array, we have observed a non-significant trend to approach more frequently light sources situated in its left column (12 out of 19 choices; binomial test: NS). We will return to the question of position preferences shown by workers of *B. pascuorum* in response to an array of four lights in a separate publication (in preparation) in which we have analysed the behaviour of a larger sample of bees.

The flight directed towards the array of light sources but not oriented to any particular light source was performed at least once by 11 bees (55% of the total number of the tested individuals). However, only one of these individuals (the bee No. 20) displayed solely this form of photopositive behaviour (on 3 tests) and was never observed to fly to some particular target light source. Flights directed to the centre of the array of light sources were relatively rare: only four such flights were recorded during the whole experiment (Fig. 1).

The flight directed above the upper row of light sources was performed at least once by 8 individuals (40%

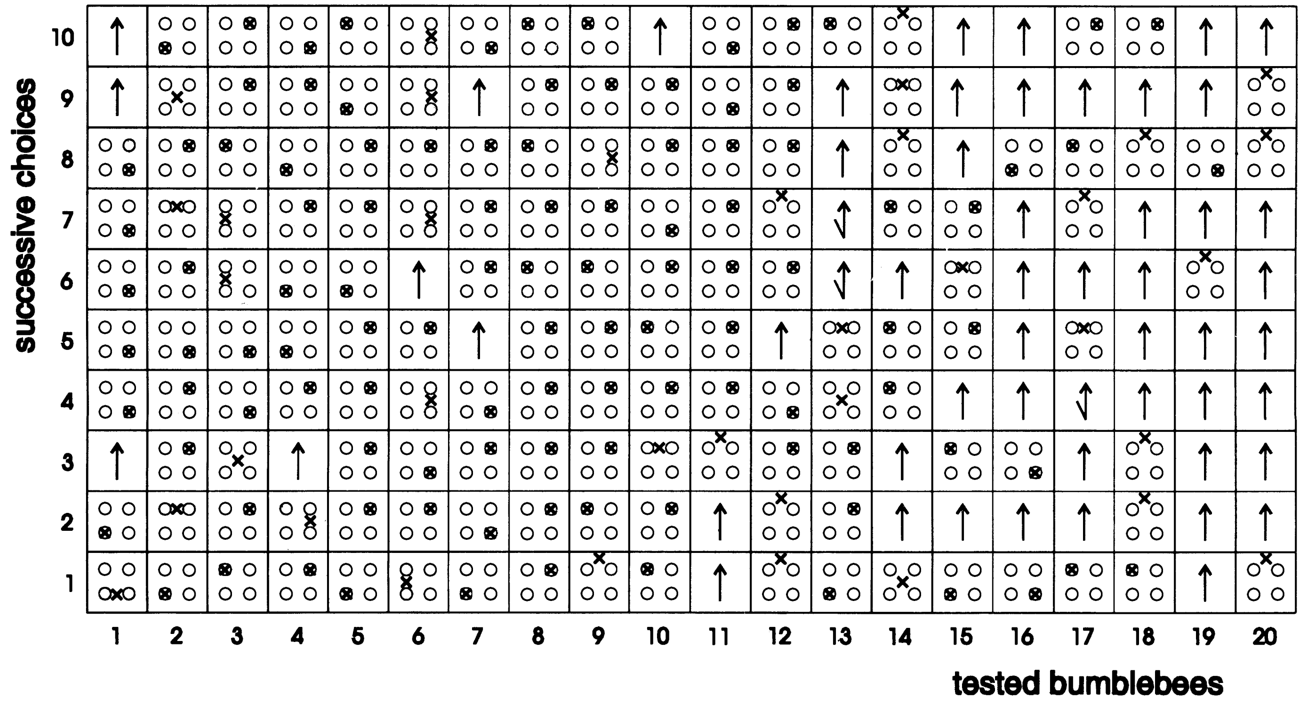

Fig. 1. Responses to a vertical array of four sources of artificial white light shown by 20 workers of *B. pascuorum* on 10 successive tests. Each square represents one test. Arrays of four circles represent tests on which the bee flied in the direction of the experimental array; the point of the arrival of the bee to the array is indicated by an x. Vertical arrows represent the tests on which the bee flied upwards (toward the ceiling of the room).V-shaped arrows represent an upward flight preceded by a short downward flight. The bumblebees are ranked according to the order based on the total score S obtained by each of them during the session of ten tests. For further explanations see the text.

of the tested bees); in total, such type of flight was recorded 15 times. The flight directed below the lower row of light sources was never recorded.

Fifteen individuals (75% of the tested bees) at least once directed their flight toward the ceiling of the dark room. Such upward flights were as a rule followed by sinuous flight below the ceiling. During the session of ten tests the number of tests when bees flied upwards ranged from 1 to 8. None of the bees flied upwards on all ten tests. In three cases (two successive tests of the bee no. 13 and one test of the bee No. 17; see Fig. 1) an upward flight was preceded by a very short downward flight.

As demonstrated by these data and shown in Fig. 1, the behaviour of the tested bees showed very high level of inter-individual variability.

Five bees (labeled as 2, 3, 5, 8 and 9; 25% of the total number of the tested individuals) were never observed to fly upwards: they were flying in the direction of the array of light sources on all their tests. Two of these bees (labeled as 5 and 8) were always flying to some light source (although not always the same one).

On the other hand, three bees (labeled as 16, 19 and 20) were flying mostly upwards (upward flight was observed on more than a half of their tests). None of the bees, however, employed solely the tactic of upward flight, and in the majority of the cases (12 out of 15) the bees observed at least once to fly upwards were displaying both forms of the photopositive behaviour - flight to one of the light sources, and flight between the light sources or above the upper row of light sources - on their other tests. Only three bees (No. 7, 16 and 20) differed in this respect from the majority of the tested individuals (Fig. 1).

The behaviour of the tested workers of *B. pascuorum* showed important intra-individual variability as well: as a rule, the bees behaved differently on various tests. Even the two bees labeled as 5 and 8 which were always flying to some light source were not choosing constantly the same target. The bee No. 5 was flying to three different light sources, and the bee No. 8 was alternating its choices between two light sources, switching five times from one to the another (Fig. 1). Out of 11 individuals which flied upwards on more than one test, 9 individuals were switching repeatedly from photopositive behaviour to upward flight (up to 6 times in the case of the bee No. 17; Fig 1). The behaviour of the bee No. 17 was particularly variable: it displayed the whole spectrum of five behavioural options observed in this experiment (flight oriented to one of the light sources, flight oriented between light sources, flight directed above the upper row of light sources, upward flight, and upward flight preceded by downward flight).

No significant differences were found between the behaviour of the bees on their successive tests (Friedman two-way analysis of variance by ranks: NS in the case of both analyses). Thus, we can conclude that although the behaviour of the tested individuals is as a rule fairly variable, it does not change in a predictable way as a function of time.

DISCUSSION

The results of the Experiment I show that the mechanisms underlying the escape behaviour of *B. terrestris* and *B. pascuorum* displayed in response to confinement in a test tube plugged with sand and exposed to sunlight differ significantly in at least two respects: (1) expression/absence of photopositive behaviour, and (2) readiness to carry out digging behaviour and efficiency of digging. In the situation used in our Experiment I, *B. terrestris* showed high readiness both to display photopositive behaviour and to dig. In contrast, in the case of *B. pascuorum* photopositive behaviour did not contribute in a significant manner to the overall escape performance, and the readiness to carry out digging behaviour was significantly lower than in *B. terrestris*. As revealed by the comparison of the values of the cumulative time spent on digging recorded for *B. terrestris* and *B. pascuorum* in the tubes exposed to sunlight, the digging behaviour of *B. pascuorum* is also significantly less efficient than that of *B. terrestris* (Godzińska 1988).

As already pointed out in our earlier studies on the responses of *B. terrestris* and *B. pascuorum* to confinement (Godzińska 1988, Godzińska and Korczyńska 1992), the differences in the escape behaviour of these two bumblebee species reflect differences in their nesting habits. *B. pascuorum* is a surface-nesting species founding nests in small mounds of withered leaves, grass and/or moss. In contrast to that, *B. terrestris* nests mostly in abandonned burrows of rodents and in other underground cavities connected with the outside world by long tunnels (Jacobs-Jessen 1959, Alford 1975). The ability to clear quickly and efficiently soil obstructing a narrow passage is crucial for the survival of colonies of underground-nesting bumblebees, but it is relatively less important for the surface-nesting bumblebees. Better escape performance of *B. terrestris* in test tubes plugged with sand may thus reflect some preprogrammed behavioural adaptation(s) to subterranean nesting. It might also have resulted, at least partly, from the experience in removing soil. Such experience might have been acquired by the tested workers of *B. terrestris* prior to the experiment, while they were clearing away soil obstructing their nest tunnels. Lastly, both these groups of factors (preprogrammed and acquired) might have contributed to inter-specific differences in the escape behaviour observed in our experiments.

In the Experiment I photopositive behaviour and digging behaviour acted as two alternative and mutually antagonistic tactics of escape. However, in the majority of situations encountered by bumblebees in their natural environment (in particular, during clearing away of soil obstructing their nest tunnels), photopositive and digging behaviours do not have counteracting consequences. On the contrary, they act in a mutually backup way. Thus it is not surprising that workers of *B. terrestris*, an underground-nesting species, show not only higher readiness to dig, but are also more photopositive than *B. pascuorum*. The importance of photopositive behaviour for the orientation of *B. terrestris* in their nest tunnels was postulated already by Jacobs-Jessen (1959); however, she has not studied that question experimentally.

The results of the present study concerning the relationship between readiness to display digging behaviour and nesting habits of two bumblebee species have an interesting parallel to studies of King and Weisman (1964), King et al. (1968), and Layne and Ehrhart (1970) on digging behaviour of various species of deermice (*Peromyscus*). In these studies, various parametres characterizing digging behaviour were also related to ecological variables, in particular, to the nesting habits of tested species (ranging form ubiquitous nesting through dwelling in burrows excavated by other animals to subterranean nesting with construction of highly characteristic, species-specific type of burrow). Interestingly, the results of these studies depended strongly on the method applied to study digging behaviour. Thus, King and Weisman (1964) found no correlations between the nesting habits of five stocks (species and subspecies) of *Peromyscus* and their sand digging behaviour measured by the amount of sand removed from a dispenser by a subject confined to a relatively small cage during 24 h. In contrast to that, such correlations were found by King et al. (1968) in the experiment in which the deermice were confined to a larger cage and allowed to dig during 15 min only. Lastly, Layne and Ehrhart (1970) observed digging behaviour of four species of deermice in a still larger open-field box during five minute periods only, and found still more numerous correlations of various parametres characterizing digging behaviour of the studied species with their nesting habits. As argued by Layne and Ehrhart (1970), in deermice high level of the motivation to escape appears to obscure interspecific differences in digging behaviour related to the differences in the nesting ecology. It provides such strong motivation for digging that animals of all species perform close to the upper threshold of their possibilities. Our present study demonstrates that in bumblebees, unlike as in deermice, interspecific differences in digging behaviour correlated with the differences in nesting habits are well expressed also in animals strongly motivated to escape.

The proximate mechanisms underlying the expression of digging behaviour of bumblebees are so far very little known. Several studies demonstrated, however, that digging behaviour of bumblebee queens and workers may be induced by parasitoid infection. Such induction of digging behaviour was observed in bumblebee queens parasitized by the nematode *Sphaerularia bombi* Dufour (Poinar and van der Laan 1972, Lundberg and Svensson 1975) and in workers of *B. terrestris* parasitized by the larvae of conopid flies (Conopidae, Diptera) (Müller 1994). Digging behaviour of parasitized bumblebees ensures dispersal and transmission of the nematode larvae (Lundberg and Svensson 1975) and enhances hibernation success of the pupae of conopid flies (Müller 1994). The research on digging behaviour of parasitized bumblebees led, among others, to a hypothesis that the induction of digging behaviour in parasitized bumblebees may be caused by the decrease in the juvenile hormone level. It is not known, however, if parasitoids are manipulating host hormone production or producing their own substances (Palm 1948, Müller 1994).

In other Hymenoptera, digging behaviour and its mechanisms were studied mostly in ants. These studies were focused mostly on the effect of group size on digging performance (Chen 1937a,b, Sakagami and Hayashida 1962, Sudd 1971, 1972b, Imamura 1982, Klotz 1986), on stimuli releasing and guiding digging (Lafleur 1940, Wilson 1958, Markl 1965, Blum and Warter 1966, McGurk et al. 1966, Spangler 1968, Hangartner 1969, Haskins 1970, Haskins et al. 1973, Hubbard 1974, Markl and Hölldobler 1978), and on mechanisms underlying excavation of soil nests (Sudd 1969, 1970a, b, 1971, 1972a, b, 1975, Franks et al. 1992). Digging may also act as a hunting tactic (Maschwitz and Mühlenberg 1975).

The Experiment I demonstrated that photopositive behaviour constitutes an important component of the escape behaviour triggered in tubes plugged with sand only in *B. terrestris*. However, the results of that experiment did not prove that *B. pascuorum* confined to a test tube do not respond to photic cues: it was only demonstrated that photopositive behaviour did not contribute in a significant way to the overall escape performance shown by *B. pascuorum* in that situation. So far we cannot exclude the possibility that *B. pascuorum* do respond to photic cues, but that these responses do not result in a significant increase of the total test time in respect to the values of that index recorded in darkness. Further direct tests are necessary to find out whether and to what degree *B. pascuorum* indeed disregard photic cues when confined to a test tube.

Nevertheless, our present data demonstrate that the escape performance of *B. pascuorum* in test tubes exposed to sunlight was not influenced significantly by the effects of their responses to photic cues (Experiment I), whereas bees of the same species were strongly photopositive when tested in the escape boxes (Godzińska 1983) or when released in a large dark room (Experiment II). Hence, our data suggest that in *B. pascuorum* the readiness to display photopositive behaviour in response to confinement may depend greatly on the size of a container to which a bee is confined. Bertholf (1931a) described a similar modulating effect of the size of the container used to confine the tested subjects on responsiveness to light cues shown by the honeybees (*Apis mellifera* L.). To quote Bertholf (1931a), a honeybee confined to a small container usually displays "the tendency [...] to race continuously around the sides of the container, apparently in a frantic effort to escape and to disregard the light almost entirely. [...] Every effort to confine the bee to narrow paths or make it go through small openings resulted in almost complete substitution of contact reactions for light reactions [....]. To remedy this difficulty the container must be made so large that even with its great activity the bee will seldom strike the sides, but will be able to roam in comparative freedom over a large space".

However, the tendency to show photopositive behaviour only when confined to a sufficiently large container is not a general rule among flying Hymenoptera. As we demonstrated in the present study, workers of *B. terrestris* are strongly photopositive also when confined to a test tube. Berthold and Benton (1970), Vollbehr (1971) and Menzel and Greggers (1985) investigated successfully photopositive behaviour of honeybee workers running within narrow glass tubes (9, 10 and 16 mm in diameter, respectively). The discrepancy between the findings of Bertholf (1931a), and those of Berthold and Benton (1970), Vollbehr (1971) and Menzel and Greggers (1985) might have resulted from the differences in the motivational state of the tested individuals. As stressed by Menzel and Greggers (1985), closer examination of the results of the studies on honeybee photopositive behaviour reveals many other important discrepancies as well, most probably because in these studies different response types were all labelled as phototaxis. Hence, these authors recommend the use of experimental procedures which could control not only the sensory parametres but also the motivational ones. Our present data also strongly suggest that various bumblebee species may differ in a major way in respect to conditions necessary for the expression of photopositive behaviour. Hence, they indicate an importance of the comparative approach in the study of even such elementary and stereotyped behavioural traits as the photopositive behaviour.

Photopositive behaviour of bumblebees has been investigated in a few studies so far. Jacobs-Jessen (1959) demonstrated that foragers of *Bombus hortorum* L. and *B. hypnorum* L. are photopositive when departing from the nest but photonegative when returning to it. Moreover, she demonstrated that in *B. hypnorum* the readiness for photopositive behaviour depends on the time of the day: it is higher in the morning than in the evening. She also demonstrated that workers of *B. terrestris* and *B. hypnorum* may respond to sunlight by the so called photomenotaxis (Kühn 1919), also called the light compass response (Fraenkel and Gunn 1961, Schöne 1984). It involves the choice of movement direction at a temporarily fixed angle to light rays. More recently, Godzińska (1983) demonstrated that workers, males and queens of *B. pascuorum* display photopositive behaviour while attempting to escape out of the escape box (see introduction).

Much more numerous studies were devoted to the photopositive behaviour shown by the honeybees (among others, von Hess 1913, 1916, 1920, Minnich 1919, Lutz 1924, Bertholf 1931a, b, Műller 1931, Urban 1932, Heintz 1959, Jacobs-Jessen 1959, Jander 1963, Berthold and Benton 1970, Vollbehr 1971, Labhart 1974, Kaiser et al. 1977, Menzel and Greggers 1985). In contrast to our Experiment II, in which bees were tested while flying, photopositive behaviour of honeybees and bumblebees was investigated as a rule in subjects tested while walking or running (Minnich 1919, Bertholf 1931a, b, Urban 1932, Heintz 1959, Jacobs-Jessen 1959, Berthold and Benton 1970, Vollbehr 1971, Labhart· 1974, Kaiser et al. 1977, Menzel and Greggers 1985),

A synthetic review of early studies devoted to the mechanisms of the photopositive behaviour of the honeybee is provided by Fraenkel and Gunn (1961). These studies demonstrated, among others, that honeybees can respond to an array of two or more light sources by orientating to only one target and disregarding other light sources. This type of oriented response to external stimuli, so called telotaxis (Kühn 1919, Fraenkel and Gunn 1961, Schöne 1984), involves the processes of fixation and target orientation and is assumed to be evolutionarily more advanced than so called tropotaxis, a response type based on the comparison of the intensity of stimulation on the two sides of animal's body (Kühn 1919, Fraenkel and Gunn 1961, Schöne 1984). In the two-light experiment, positive phototropotaxis guides an animal between the two lights, and only at the last moment the animal turns to one of them. Positive phototelotaxis guides the animal directly to one of the lights (Fraenkel and Gunn 1961). According to Fraenkel and Gunn (1961), honeybees may display both phototropotaxis and phototelotaxis. The honeybee may even display phototropotaxis during the first part of its track towards an array of lights and then switch to phototelotaxis (Fraenkel and Gunn 1961).

The results of the Experiment II demonstrate that escape behaviour of workers of *B. pascuorum* may also involve various forms of photopositive behaviour, and among them flights directed to some particular light source and flights directed between the light sources or towards the centre of the whole array. However, we cannot interprete these respective responses in terms of the "telo-tropo alternative" (Schöne 1984). The distinction between these two response types requires additional tests and may be very difficult, in particular if the same animal is able to display both these responses, as it was shown in the case of the honeybee (Fraenkel and Gunn 1961, Schöne 1984). Moreover, phototropotaxis and phototelotaxis are not two mutually exclusive alternative tactics of responses to light. For instance, even if the honeybee tested in the two-light experiment moves toward one of these lights in a way characteristic for the phototelotaxis, a trajectory of its movement is usually slightly deviated towards the second light (Jander 1963). Finally, it must be taken into consideration that a light source may be approached not only as a result of the positive phototaxis but also as a result of the light compass response (Fraenkel and Gunn 1961) which is known to be displayed also by bumblebees (Jacobs-Jessen 1959). Because our Experiment II was not designed to analyse in detail flight trajectories of tested bees, exact mechanisms underlying various forms of photopositive behaviour shown by them during that experiment cannot be identified. Besides it, as pointed out already by Mast (1938), and then by Schöne(1984) and Wehner (1992), interpretation of animal behaviour in terms of various types of taxes may lead to oversimplifications and hinder the discovery of further details which could throw more light on its real neurobiological mechanisms; therefore, it is not recommended (Wehner 1992) or recommended to be applied with caution (Schöne 1984).

Moreover, behavioural patterns displayed by *B. pascuorum* in the Experiment II cannot be reduced solely to any form of the photopositive behaviour: they formed a series of grades representing the whole spectrum of the possible outcomes of the joint action of the photopositive behaviour and the tendency to fly upwards. The latter tendency involved most probably the responses to gravitational cues. We may exclude the possibility that bees oriented their flights to a small amount of light reflected form the ceiling of the dark room because they always flied directly upwards and they never directed their flights to any other part of the ceiling or to the walls of the dark room (also reflecting some light).

The ability to respond to gravitational cues is well documented in bumblebees. Jacobs-Jessen (1959) demonstrated that workers of *Bombus hortorum* L. respond differently to gravitational cues when departing from the nest and when returning to it. In the first situation they move most frequently upwards, in the second one they move predominantly downwards. The data of Jacobs--Jessen also showed that responses of workers of *B. sylvarum* L. to gravitational cues depend on the time of the day and may be modulated by the illumination level. The readiness of *B. sylvarum* to move upwards showed a peak in the morning and then gradually decreased. It was enhanced by strong light and suppressed by darkness. Jander and Jander (1970) also demonstrated that the choice of direction made by walking queens of *B. terrestris* L., *B. pascuorum* Scopoli and *B. lapidarius* Müll. may be influenced by both their responses to gravitational cues and positive oriented responses to light.

In the majority of studies on the photopositive behaviour of various bees, bumblebees were tested while walking. As far as we know, our present study provides the first data on joint influence of responses to light and to gravitational cues on the choice of direction of flight made by bumblebee workers motivated to escape. However, in our experiment bees were released at the border of platform placed distally in relation to the array of light sources, and as a rule they were walking over the whole platform before taking off. Hence, also in our study we cannot exclude that they were choosing the direction of their subsequent flight while still walking.

The influence of both photic and gravitational cues on the choice of direction of movement was also reported in many other insect species (Heintz 1959, Jander 1963, Jander and Jander 1970). Heintz (1959) reported that honeybees confined to a dark box crawled upwards rather than downwards while responding positively to light. However, Jander and Jander (1970) found out that in the honeybee and some other bees of the genus *Apis*, the effects of photopositive behaviour may entirely mask much weaker effects of responses to gravitational cues.

As far as we know, our study provides the first analysis of inter-individual variability in responses to these two types of cues (photic and gravitational) displayed by bee foragers motivated to escape

As pointed out by Bell (1990), insects are able to integrate directional cues from more than one modality. Hence, studies dealing with only one modality may inaccurately portray the mechanisms underlying their behaviour. The results of the Experiment II suggests strongly that flight patterns displayed by *B. pascuorum* when released in front of an array of sources of light involve complex integrative responses to cues of various modality, in particular, to photic and gravitational cues. Most probably, these patterns involved even more complex phenomena. For instance, we cannot provide any simple explanation for the preference for the right side of the array of light sources shown by *B. pascuorum* in that experiment: the bees could have responded to yet another cue which has not been controlled.

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