

## **Influence of Intermittent Rewards in Learning to Handle Flowers in Bumblebees (Hymenoptera: Apidae: *Bombus impatiens*)**

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CHITTKA L [Zool II, Biocentre, Univ, D-97074 Würzburg]: **Influence of Intermittent Rewards in Learning to Handle Flowers in Bumblebees (Hymenoptera: Apidae: *Bombus impatiens*).**- Entomol Gener 26(2): 085- 091; Stuttgart 2002-08. - - - [Article]

Bumblebees, *Bombus impatiens* Cresson 1863 had to learn to associate colour signals with particular motor patterns needed to extract sucrose solution from artificial flowers. The influence of reward variance on learning such motor skills was tested. Learning speed does not depend on the amount or variance of rewards when bumblebees learned only one novel task. If, however, bumblebees have to switch between 2 flower types while familiarising themselves with their morphology, increased reward variance does slow down the rate of learning, while reward amount has no influence over the range tested.

**Key words:** flower constancy - memory - motor learning - reward variance - specialization

CHITTKA L [Zool II, Biozentrum, Univ, D-97074 Würzburg]: **Bedeutung diskontinuierlicher Belohnungen im Lernprozeß von Hummeln beim Manipulieren von Blüten (Hymenoptera: Apidae: *Bombus impatiens*).**- Entomol Gener 26(2): 085-091; Stuttgart 2002-08.- [Abhandlung]

Hummeln der Art *Bombus impatiens* Cresson 1863 wurden trainiert, Farbsignale mit motorischen Programmen zu assoziieren, um Zuckerlösung aus künstlichen Blüten zu extrahieren. Der Einfluß der Belohnungsvarianz auf das Lernen solcher motorischen Fähigkeiten wurde getestet. Die Geschwindigkeit des Lernens ist unabhängig von Belohnungsmenge und Varianz, wenn die Hummeln nur *eine* neue Aufgabe lernen. Wenn die Tiere jedoch während des Lernens *zweier* neuer motorischer Aufgaben zwischen diesen Aufgaben wechseln müssen, lernen sie bei variablen Belohnungen deutlich langsamer, wohingegen die Menge der Belohnung über den getesteten Bereich ohne Einfluß bleibt.

**Schlüsselbegriffe:** Belohnungsvarianz - Blütenstetigkeit - Gedächtnis - motorisches Lernen - Spezialisierung

## 1 Introduction

The question of whether bumblebees respond to variance (not just the mean) of nectar amounts in flowers has received considerable interest [WELLS & WELLS 1983, REAL 1991; WADDINGTON 2001]. In previous studies, the flower types examined only differed in reward variance and signal, but not in morphology. Flowers visited by generalist bumblebees, however, vary strongly in morphology, and bumblebees have to make a considerable investment into learning how to manipulate flowers [CHITTKA 1998; CHITTKA & THOMSON 1997; CHITTKA et al 1999; LAVERTY 1994]. The influence of reward variance on learning floral morphology has previously been left unconsidered. It is potentially important, however: natural flowers vary considerable in reward, and the activities of pollinators add to this variance, so that a large proportion of flowers encountered by an individual bee may be empty [HEINRICH 1979, CHITTKA & SCHÜRKEN 2001, SCHÜRKEN & CHITTKA, 2001]. For a bee first learning how to handle a flower, a missing reward may mean that the flower is indeed empty, or that the bee has failed to locate the reward. Thus, a plant species' quality as a nectar source is confounded with the bee's ability to manipulate flowers of that species, making the outcome of learning difficult to predict.

To contribute to the elucidation of this problem, artificial, computer-monitored flowers with rewards of adjustable magnitude and variances are used in this study. Bumblebees, *Bombus impatiens* Cresson 1863, are trained to collect sucrose solution from these flowers to measure the influence of these parameters on the acquisition of the necessary motor skills to extract the reward from the flowers.

## 2 Material & Methods

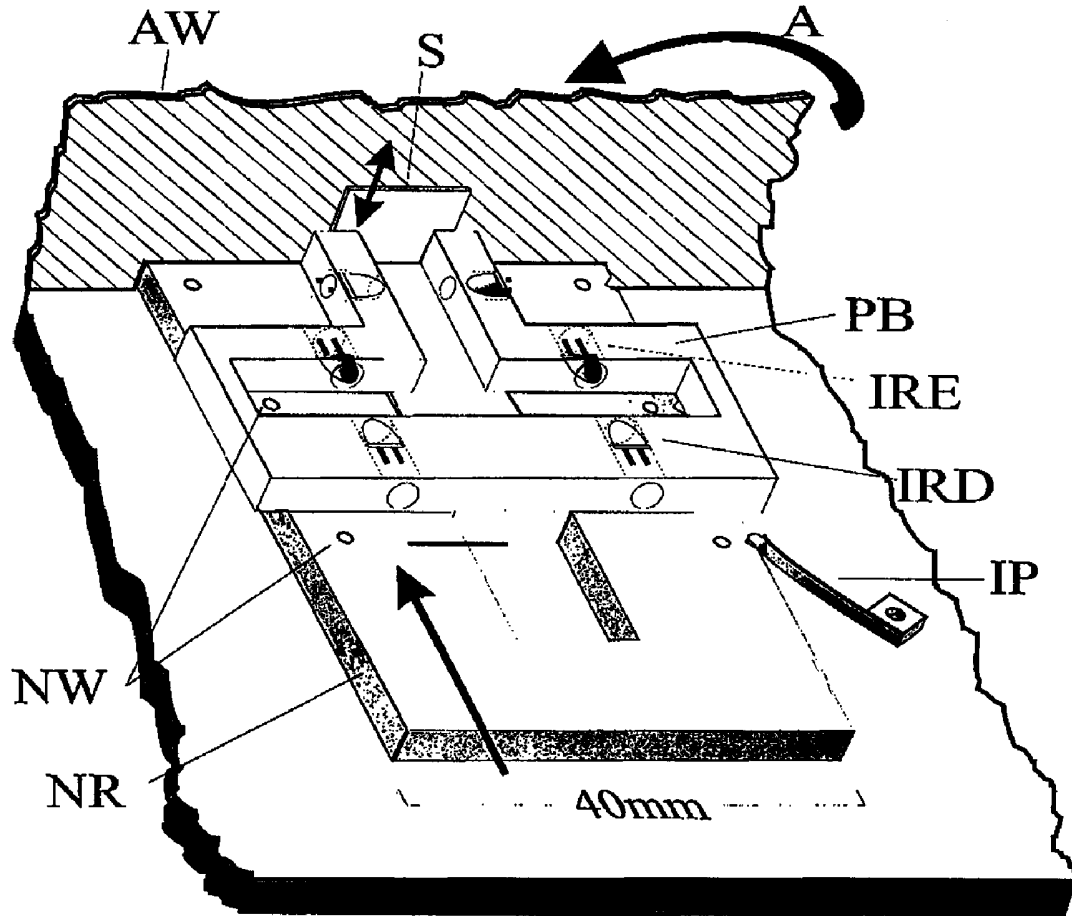
**The bumblebees:** Bumblebee colonies (*Bombus impatiens* Cresson 1863) were obtained from Koppert Biological Systems, Michigan, USA. They were kept in a nest box which was connected to a flight arena of 60cm × 40cm (28 cm height) by means of a transparent plastic tube. Shutters in the tube allowed to control which bumblebees entered the arena. Between experiments, bumblebees could forage freely from a Petri dish with holes in the top, which contained 50% (volume/volume) sucrose solution, and which was located in the centre of the flight arena. The only foraging experience the bumblebees had prior to training was extracting sucrose solution from this openly accessible feeder. Thus, they were naive not just with respect to the experimental task, but with respect to foraging from any structure that might resemble a flower.

**The flowers:** In the flight arena, bumblebees had access to 4 artificial "flowers" which were blocks of polyethylene with channels milled into them. The entrances to the flowers were in one of the walls of the flight arena. The entrances were arranged side by side, with distances of 10cm between them. The entrance hole was square (6mm × 6mm) and each hole was surrounded by a yellow or blue coloured square (3cm × 3cm). The inside of each flower had the shape of a T-maze (Fig1). The entrance tunnel measured 14mm, and both arms were 17mm in length; all tunnels had the same width as the entrance of the flowers. The rationale for this simple flower design was that left and right turns constitute 2 tasks that are different but exactly equal in difficulty. In natural flowers and in the T-mazes, the bumblebees have to learn to move their bodies in a particular way to get access to the reward, and these learned movements must be guided by sensory stimuli (e.g. colours) that define a flower type. The reward was offered in the right arm of the flower when the entrance bore a blue mark, and in the left arm when the entrance was yellow.

The entrance and each arm of the mazes were controlled by infrared light barriers that signalled a computer when the bee broke the beam at each of 3 locations, so that behaviour could be evaluated on-line. Access to the flowers was controlled by manual shutters which slid into a 1mm crevice between the colour cardboard and the actual entrance tunnel of the flowers. Under each maze arm was a sliding plexiglas rail with a linear series of nectar wells, 1.5mm in diameter and 3mm deep, drilled into the plexiglas. If a well was emptied, the rail was advanced so that the next reward was made available.

In this way, the entire floor of both arms of the maze was replaced, so that if bumblebees should leave odour marks on the floor, these could not be used during subsequent visits. The insides of the flowers were cleaned with ethanol after every other foraging bout. Each plexiglas rail featured 25 wells, which contained variable, but small amounts of 50% sucrose solution.

Bumblebees had to visit multiple flowers (typically several dozens) to fill their honey stomach once. In the experiments with variable rewards, every second well was left empty. Bumblebees could choose freely which flower to exploit next, with the restriction that they could not directly return to the flower just visited. Because of this, the sequence of rewards in the tests with variable rewards was actually quasi-random. The maximum number of empty or full flowers visited in sequence was 4, if a bee visited all 4 flowers consecutively.



**Fig 1:** Schematic drawing of one of the 4 'flowers', with the roof removed. During experiments, each flower is covered with a red translucent plastic sheet. A - flight arena (behind the wall); AW arena wall (which bears the colour marks at the entrance holes of the mazes); IRD infrared detectors; IRE infrared emitters; NR 'nectar' rail, sliding under the arms of the T-maze, so that the rail forms the floor of the maze in the arms. The nectar rail slides under the floor of the flight arena. The single-headed arrow marks the direction in which the nectar rail is advanced. NW - nectar wells; P spring-loaded indexing pawl that engages regularly spaced notches on the nectar rail, allowing fresh nectar wells to be rapidly and precisely clicked into position between visits; PB polyethylene block which constitutes the walls and the central (white) part of the maze floor; S manual shutter to control access to the flower (the double headed arrow shows the directions of movement).

**Training and test schedules:** Bumblebees were trained and tested individually. For each bee, the whole procedure comprised 600 trials (flower visits), of which the first 400 were completed on the first day, and the remaining trials on the subsequent day. During this procedure, no other bumblebees were allowed in the flight arena, and the experimental bee was never fed at any food sources other than the experimental flowers. A single bee was selected from those feeding from the plexiglas feeder. No other bumblebees were then allowed in the arena, and training could begin. A detailed description of how bumblebees were enticed to visit the mazes can be found in [CHITTKA & THOMSON 1997].

Bumblebees of groups A-F (with 6 individuals each, except group E which contained only 5 bumblebees) were trained on 6 different schedules, with 600 trials each. Groups A-C were trained on only one task, with the following particulars. Group A was given 2.5  $\mu$ l as a reward for each flower visit. Group B was rewarded with twice the amount (with 5  $\mu$ l) on every visit. Group C was rewarded, on average, on every second trial (with 5  $\mu$ l, so that the mean reward for groups A and C was the same).

Groups D-F were confronted with both tasks, and had to switch between tasks after every single flower visit. To force them to switch between floral types, both flowers of the previously visited type were made inaccessible. Group D was trained on 2 tasks with forced alternate trials, with 2.5  $\mu$ l on each trial. Group E received twice that amount on every visit. Group F was trained on 2 tasks, but only every second flower visit (on average) was rewarded with 5 $\mu$ l.

In the groups which were trained to both tasks, bumblebees had to be prevented from simply using the position (and not the colour) of a flower entrance to identify the correct direction. To this end, the blue entrance marks were exchanged for yellow ones (and vice versa), and the rails containing the rewards were exchanged accordingly, so that blue flowers would again contain the reward on the right side, and yellow ones would offer them on the left. This was done after every other foraging bout. If bumblebees initially turned into the wrong arm of the maze, they were allowed to correct this error and feed in the opposite arm.

### 3 Results

Bumblebees which were trained to only one task (groups A-C) start out with 20-25% errors during the first 10 trials. Their performance improves rapidly, and after about 50 trials, a saturation level of <5% error is reached (Fig 2a). Bumblebees of group A and C perform as well as bumblebees of group B; thus, cutting the rewards by half does not alter the learning curve of bumblebees. Neither do the intermittent rewards of Group C impair the learning rate: even though these bumblebees were rewarded on only every second trial, they learn the single task as well as the bumblebees which were rewarded on every single trial.

Bumblebees trained on both tasks (Fig 2b: groups D-F) start with similar accuracy as bumblebees trained only on a single task (~20% errors). Subsequently, bumblebees trained on 2 tasks display a temporary increase in error rates, as was observed by CHITTKA & THOMSON [1997]. After about 80 visits, performance strongly improves, until saturation is reached after 200-300 trials, with an overall level of 5-10% errors between 300 and 600 visits. However, bumblebees of group F (intermittent rewards) take substantially longer to reach saturation than both groups trained on 2 tasks with continuous rewards (group D, E).

To compare performance statistically, the Chi-square goodness of fit test ( $df = 1$ ) was applied to bins of 30 trials each. The error score of group F was significantly higher than group D in trials 151-180 ( $\chi^2 = 11.21$ ;  $p = 0.0008$ ), 181-210 ( $\chi^2 = 8.23$ ;  $p = 0.0041$ ), 211-240 ( $\chi^2 = 3.99$ ;  $p = 0.0457$ ), 241-270 ( $\chi^2 = 4.86$ ;  $p = 0.0275$ ) and 271-300 ( $\chi^2 = 5.77$ ;  $p = 0.0163$ ). In comparison with group E, the error score of group F was significantly higher in trials 121-150 ( $\chi^2 = 4.44$ ;  $p = 0.0352$ ), 151-180 ( $\chi^2 = 7.34$ ;  $p = 0.0068$ ), 181-210 ( $\chi^2 = 12.16$ ;  $p = 0.0005$ ), 211-240 ( $\chi^2 = 9.67$ ;  $p = 0.0019$ ), and 241-270 ( $\chi^2 = 7.35$ ;  $p = 0.0067$ ).

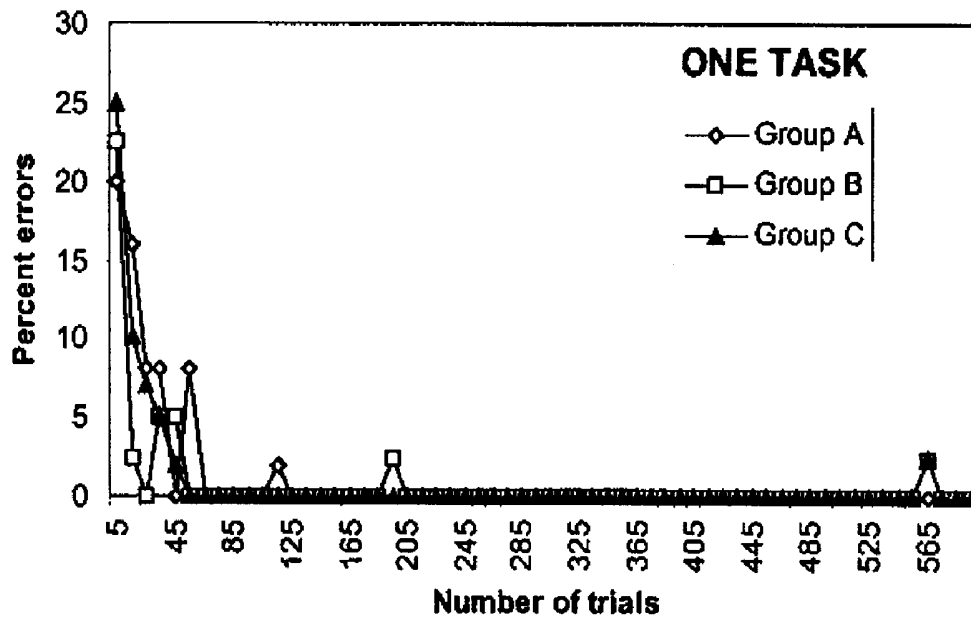


Fig.2a

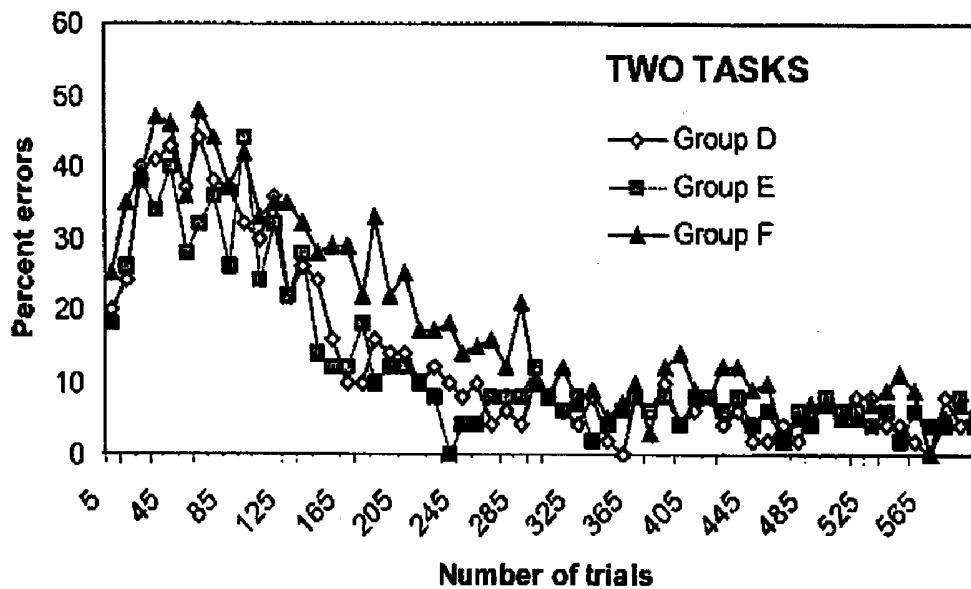


Fig 2 b

**Fig 2:** Error scores for bumblebees of groups A-E. Error scores were determined by categorizing the 600 ordered visits into 60 bins of 10 consecutive visits. The number of errors within each bin were evaluated. (a) Bumblebees trained on only a single floral handling task. Group A: reward 2.5 $\mu$ l on every floral visit; group B: 5  $\mu$ l on every visit; group C: 5 $\mu$ l on every other visit. There were no statistically significant differences between any of the groups during any phase of training. (b) Bumblebees trained on 2 different tasks. Group D: reward 2.5 $\mu$ l on every floral visit; group E: 5  $\mu$ l on every visit; group F: 5 $\mu$ l on every other visit. Group F performed significantly worse than groups D and E during trials 121-300.

## 4 Discussion

The dynamics of motor learning of a single task do not depend on the amount of reward, nor on whether or not the rewards are intermittent. This is interesting because in other tasks, such as associating rewards with sensory stimuli such as colours, learning speed does depend on reward levels [McCLELLAN BUCHANAN & BITTERMAN 1988]. Apparently, in motor learning, what matters is the number of times the motor pattern is actually performed, not how well and how often it is rewarded. Similar phenomena are known from rat spatial learning [BLODGETT 1929, TOLMAN & HONZIK 1930].

Even in those bumblebees that had to learn 2 tasks (groups D-E), learning is not slowed down if bumblebees were rewarded with half the reward amount. However, intermittent rewards while learning 2 opposite tasks (group F) slows bumblebees substantially relative to those which received equal average amounts, but were rewarded on every single trial (group D). This is likely because bumblebees encounter a 1:1 ratio of re-enforcement and extinction trials on each task, so that they might "conclude" that each non-rewarded, correct movement was actually an error. On the other hand, bumblebees do not only learn during the correct sensori-motor associations during rewarded trials. If that were the case, they should have reached saturation in performance at twice the number of trials as group D. This, however, was not the case: bumblebees of group F reached saturation after ~300 trials, and bumblebees of Group D and E at ~200. These results cast a new light on the tendency of generalist bumblebees to specialise temporarily on a limited number of plant species while foraging [CHITTKA et al 1999]. Such specialisation might be especially favoured when bumblebees first learn to manipulate flowers with high reward variance.

## 5 Literature

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DELAFLANE K S & MAYER D F [2000]: **Crop Pollination by Bees.** [XV+344 Seit, 48 Fig, 56 Tab, 155 x 230 mm, Balacr-Hartkart.Ebd].- Publ: CABI Publish, CAB International, NewYork-Wallingford, Oxon OX10 8DE UK, 2000; ISBN: 0-85199-448-2; Pr: Brit £ 60,-- / US \$ 100,-- / € 117,83. --- [EGR-Nr 2776]

Bestäubung durch Insekten ist für die Mehrzahl aller höheren Pflanzen entscheidend für eine erfolgreiche Reproduktion. Dies gilt auch für schätzungsweise 70 Prozent aller Kulturpflanzen, deren Ertrag nur gesichert ist, wenn genügend geeignete Bestäuber vorhanden sind. Die weltweite Zerstörung von naturnahen Lebensräumen und die Intensivierung der Landwirtschaft ist auch an den Bestäubern, in gemäßigten Breiten primär soziale und solitäre Bienen, nicht spurlos vorbeigegangen. In ausgeräumten Landschaften ist ein signifikanter Rückgang der Diversität und Abundanz von wildlebenden Bienen zu verzeichnen. Auch die Haltung von Honigbienen ist aufgrund von Krankheiten wie der Varroatose deutlich zurückgegangen. Vor diesem Hintergrund einer potentiellen Bestäuberkrise geben die Autoren einen breiten Überblick zur Bestäubung von Kulturpflanzen durch Bienen, der sowohl biologische und ökologische Grundlagen wie auch sehr angewandte Aspekte berücksichtigt.

Im ersten Kapitel versuchen die Autoren den ökonomischen Wert von Bienenbestäubung insgesamt sowie für die wichtigsten Kulturpflanzen in Europa und Nordamerika zu quantifizieren. Die allgemein gut gelungene Zusammenstellung verdeutlicht jedoch, wie groß der Forschungsbedarf auf diesem Gebiet noch ist. Die nächsten Kapitel vermitteln grundlegende ökologische Kenntnisse zu wildlebenden solitären Bienen, Habitatsprüchen und Gefährdungsursachen. Die folgenden Abschnitte beschäftigen sich detaillierter mit den Bestäubereigenschaften und potentiellen Einsatzmöglichkeiten von Honigbienen, Hummeln und solitären Wildbienenarten wie Blattschneiderbienen (*Megachile*) und Mauerbienen (*Osmia*).

Dieser sehr praxisnah geschriebene Teil des Buches gibt auch Hinweise zur Haltung von Bestäubern. Sehr aktuell sind hier insbesondere die Informationen zum Einsatz solitärer Wildbienenarten. Zumindest in Europa wurden bisher Wildbienen als eine Alternative zu Honigbienen kaum beachtet. Der zweite Teil des Buches behandelt in alphabetischer Reihenfolge die wichtigsten Kulturpflanzen mit Angaben zur Bestäubungsbiologie, geeigneten Bestäubern und empfohlenen Bestäuberdichten für optimale Erträge. Dieser Teil des Buches zeigt eine recht große Überlappung mit dem Standardwerk auf diesem Gebiet [FREE 1980: Pollination of Crops], auch wenn die einzelnen Kulturpflanzen deutlich kürzer abgehandelt werden und neuere Literatur mit berücksichtigt wurde.

Insgesamt ist es ein gelungenes Buch zu einem interessanten Thema. Die Kombination von Grundlagenkenntnissen und sehr angewandten Inhalten macht es sowohl für Studenten und Wissenschaftler aus den Bereichen Agrarwissenschaften und Gartenbau interessant als auch für Praktiker, die sich mit diesem Thema näher beschäftigen möchten.

INGOLF D STEFFAN-DEWENTER [Georg-August-Universität Göttingen]