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Effect of Reinforcement Duration on Response Rate of Stingless Bees (*Melipona quadrifasciata anthidioides*)

Efeito da Duração do Reforço na Taxa de Respostas de Abelhas sem Ferrão (Melipona quadrifasciata anthidioides)

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Abstract

Studies with bees typically observe one response to the experimental apparatus per visit, as the bee returns to the hive after filling its honey vesicle. This reduces the sample of the behavior of interest, posing analytical and methodological challenges for studying certain processes. The present study aimed to explore a procedure and its theoretical analysis to promote larger samples of behavior per visit in an experimental chamber. The procedure consisted of fractionating the amount of syrup consumed to increase the number of bar-press responses per visit by *Melipona quadrifasciata anthidioides* stingless bees. Experimental subjects were taught to press a bar for access to a 30-s syrup solution (average time to fill the honey vesicle). Once stability was achieved, the syrup was fractionated (i.e., reduced) based on duration. Durations of 6, 10, 15, and 30 s were applied as consequences for bar pressing. When syrup access was fractionated, experimental subjects continued responding to the bar during the same visit, returning to the hive after vesicle filling. Fractionation of access to syrup increased the response rate. We suggest that manipulating the duration of syrup introduces a second-order schedule, similar to a token system. This exploration has implications for behavioral management in experiments aimed at investigating operant behavior in meliponas bees.

Keywords: automated equipment, bar-press response, bees (*Melipona quadrifasciata anthidiodes)*, duration reinforcement, second-order schedule.

Resumo

Estudos com abelhas tipicamente mensuram uma resposta ao aparato experimental por visita, quando a abelha retorna à colmeia após preencher a sua vesícula melífera. Nesse procedimento, a amostra do comportamento de interesse se torna limitada, possibilitando desafios analíticos e metodológicos para o estudo de determinados processos. O presente estudo teve como objetivo explorar um procedimento e sua análise teórica para promover maiores amostras de comportamento por visita em uma câmara experimental. O procedimento consistiu em fracionar a quantidade de xarope oferecida visando aumentar o número de respostas de pressão à barra por visita das abelhas sem ferrão *Melipona quadrifasciata anthidioides*. Os sujeitos experimentais foram ensinados a pressionar uma barra para acesso a uma solução de xarope de 30 segundos (tempo médio para preencher a vesícula melífera). Uma vez alcançada a estabilidade, o acesso ao xarope foi fracionado (i. e, reduzido) com base na duração. As durações de 6, 10, 15 e 30 s foram aplicadas como consequências para a pressão à barra. Quando o acesso ao xarope foi fracionado, os sujeitos experimentais continuaram respondendo à barra durante a mesma visita, retornando à colmeia após o preenchimento da vesícula. O fracionamento do acesso ao xarope aumentou a taxa de respostas. Sugerimos que a manipulação da duração do xarope introduza um esquema de segunda ordem, semelhante a um sistema de *tokens*. Esta exploração tem implicações para o manejo comportamental em experimentos que visam investigar o comportamento operante em abelhas meliponas.

Palavras-chave: abelhas (*Melipona quadrifasciata anthidioides*), duração do reforço, equipamento automático, esquema de segunda ordem, resposta de pressão.

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To study discrimination learning in bees, researchers have the evaluated responding during discrete trials. During a discrete trial, the participant has only one opportunity to respond. When only one response is measured per visit[1], as a discrete trial, it becomes difficult to identify whether the choices between discriminative stimulus (S+) and delta-stimulus (S-) is correct (i.e., whether it occurs under S+ control) or by chance. In free operant procedures, responses are measured continuously and overtime, allowing bees to respond to the operandum more than once per visit, thus producing multiple choices to the same stimulus. Such free-operant procedures have the potential to increase the data reliability and the predictability of discriminatory behavior. Thus, experimental procedures that allow for multiple responses per visit are important for studies of simultaneous discrimination in bees, such as those conducted in the laboratory at the Federal University of São Carlos - UFSCar (e.g., Moreno et al., 2005; Moreno, 2007; Moreno et al., 2012; Moreno et al., 2014; Scienza et al., 2019).

During experiments with bees, subjects forage for syrup solution until their honey vesicle [2] is full, thereafter returning to the hive, and completing one discrete trial. A possible procedural solution to collect multiple samples of behavior per visit is to fraction access to sucrose solution, requiring subjects to respond multiple times before filling their vesicle. Previous studies have examined the effects of reinforcement magnitude (Catania, 1963; Gentry & Eskew, 1984; Jenkins & Clayton, 1948; Todorov, et al., 1984) on response rates. For example, Jenkins and Clayton (1948) investigated the relationship between pigeons' pecking behavior on an illuminated disk and the duration of access to food, using an ABA design. Their results revealed that response rates were systematically higher when the duration of access to reinforcement was 5 s, compared to 2 s, regardless of presentation order. Subsequent studies also demonstrated the same effects in choice procedures employing concurrent schedules of reinforcement (Catania, 1963; Gentry & Eskew, 1984).

However, other studies did not show a clear effect of reinforcement magnitude on behavior (Bonem & Crossman, 1988). For example, Lowe et al. (1974, Experiment 1) and Staddon (1970) found that longer reinforcement durations increased post-reinforcement pause in fixed-interval schedules. Furthermore, studies comparing the effects of reinforcement frequency and duration have consistently shown greater sensitivity of behavior to reinforcement frequency (Davison & Nevin, 1999; Todorov et al., 1984). It is important to note that, in studies on reinforcement magnitude, the manipulated reinforcer is immediately consumed by the organism after its production.

A peculiarity of bees foraging in experimental chamber is that most of the material collected is not ingested, but deposited in the hive (cf. Pessotti, 1969). Previous studies conducted with *Melipona Quadrifasciata* in the Laboratory of Learning Psychology at UFSCar (e.g., Moreno et al., 2012; Moreno et al., 2014; Scienza et. al., 2019) found that, at each visit, this species feeds in the syrup solution for approximately 30 s before returning to the hive and completing a foraging bout.

Pessotti (1969) observed that when sucrose available subjects were limited, bees remained within the experimental chamber, performing additional operant responses until filling their honey vesicles. This procedural adjustment consisted of retracting the feeder a few seconds after a bee's target response. Utilizing an electromagnet, the feeder was programmed to descend after 15 s, a duration determined by the capacity of the equipment to sustain an electrical impulse. Pessotti discovered bees did not simply leave after obtaining an incomplete syrup portion but stayed to engage in further responses. Once fully replenished, the bees would return to the hive and, a few minutes later, return to the chamber. This procedure effectively mimicked a free operant experimental condition, in which bees

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[1] The term Visit was defined as the period in which the bee remained in the experimental equipment and emitted at least one response before flying back to the hive (Pessotti, 1969).

[2] Structure of the bee's body responsible for transporting the collected nectar (Grimaldi & Engels, 2005).

could increase their response frequency by working for incremental rewards. This procedure opened the possibility to manipulate syrup's duration to promote a free operant context.

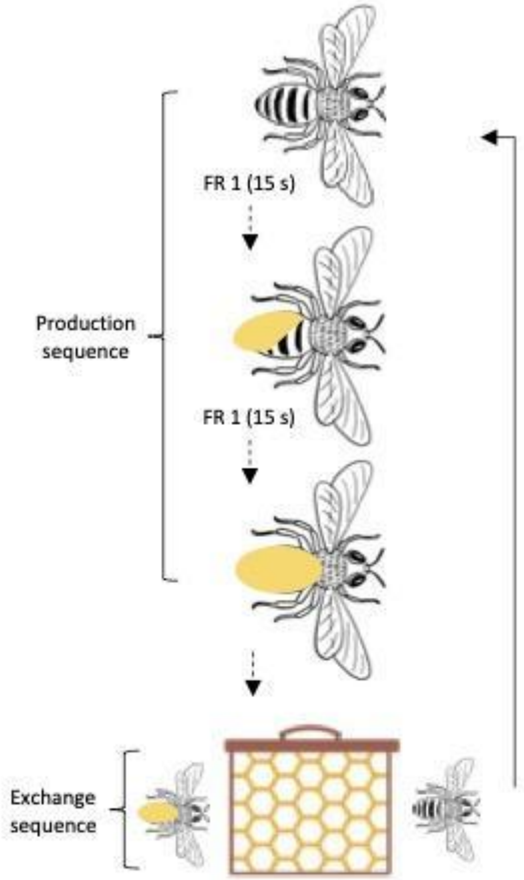
Considering that bees engage in an experiment where filling their honey vesicles depends on more than one response sequence, each producing a fractioned syrup duration, the whole foraging bout until depositing the material in the hive can be described as second-order schedule, analogous to a token system. That is, “a *second-order schedule* treats a pattern of behavior engendered by a schedule contingency as a unitary response that is itself reinforced according to some schedule of reinforcement” (Kelleher, 1966, p. 476).

Thus, responses for each schedule requirement functions as a unitary link to the next schedule. Each component produces its own stimulus, and the set of units provides the opportunity to obtain reinforcement. In a token system, three components could be distinguished: (a) token-production, the first-order schedule which is treated as a unitary response, (b) exchange-production, the second-order schedule which establishes how many first-order units are necessary for the subject have the opportunity to access reinforcement, and (c) token-exchange, which establishes the opportunity to exchange tokens for other reinforcers (Hackenberg, 2009). In the case of bees, working schedules of reinforcement to produce fractions of the syrup availability represents a token-production (a), and the exchange-production (b) as the deposit of the syrup in the hive.

Figure 1 illustrates a second-order schedule of reinforcement performed by a bee during a foraging bout. During the token-production sequence (top panels), “tokens'' (fractions of the syrup) are presented according to a Fixed Ratio 1 (FR 1) schedule. Each FR 1 completion produces 15 s syrup availability (i.e., token). After two FR 1 completions, the vesicle is filled (exchange-production schedule – FR 2), initiating an exchange period (bottom panels). If the portions of syrup production are fixed (e.g. FR1), then the fractionation of syrup solution enlarges the exchange-production schedule component.

Figure 1

Diagram of an FR2 (FR1) Like Token Reinforcement Schedule



Note. Each panel depicts a bee with a Roney vesicle of 15 token stimuli (fraction of nectar in the vesicle). Bottom panel shows an exchange period. See the text for more information.

The present study aimed to explore whether the acquisition of syrup sustains the bee's behavior within certain parameters. To this end, the duration of the syrup was fractionated, allowing for the complete filling of the honey vesicle bee, to ensure the continuous presence of the bee in the experimental situation. This approach aims to enable a more precise and reliable tracking of behavior as a function of its consequences, a methodological procedure to maintain bees' responses in an experimental chamber. This would enable confirmatory answers of choice, which would increase the reliability of the data We tested the impact of fractionating the syrup duration (specifically, access time to syrup) on the responding rates of bees. In essence, our objective was to assess whether durations shorter than 30 s would facilitate the collection of larger samples of behavior per visit. To achieve this, we employed an automated apparatus operated by a pressure response to a metallic bar situated on the landing platform. This response was precisely defined, and the feeder was exclusively activated when the bar pressure closes the electrical circuit, ensuring reliability in recording the responses and accuracy in determining the time the feeder was available. *Melipona quadrifasciata anthidioides* bees were trained to respond to the operandum, manipulated to be 30 s (representing duration of syrup; Moreno et al., 2005; Moreno, 2007; Moreno et al., 2012; Moreno et al., 2014; Scienza et al., 2019), which was adequate for filling the honey vesicle. Subsequently, we systematically reduced the durations to 15, 10, and 6 s to investigate the corresponding changes in responding rates.

Method

Subjects, Hive and Environment

The subjects were six forager stingless bees of the *Melipona quadrifasciata anthidioides* species, experimentally naïve and from the same hive. The hive was installed on the sill of a 2 m x 2.8 m window in the experimental room of the Learning Psychology Laboratory at the UFSCar. The window remained closed but had an opening of 23.2 cm in height x 22 cm in length, providing bees with free access both to the outside field and the experimental room. The room had a glass door that remained closed to the outside environment and was artificially illuminated by a 32-watt fluorescent lamp. The temperature, relative air humidity, and room brightness were measured daily using the 4IN1 Multi-Function Environment Meter. The room temperature ranged from 19 to 26°C, relative air humidity ranged from 60 to 80% RH, and brightness ranged from 200 to 450Lux. All stages of the experiment were conducted with one bee at a time (cf. Pessotti, 1969). A new bee was recruited only when the procedure was completed with the previous one. The experimental sessions took place daily and lasted several hrs., as long as the bees remained working (i.e., returning from the hive to the experimental apparatus).

Equipment

The design of the apparatus used in this experiment was inspired by Pessotti (1969) and Escobar and Santillán (2017). The operant chamber (8 cm x 9 cm 20 cm) was built using acrylic walls and a silver stainless steel floor. An aluminum operant bar (8 mm x 5 mm x 1 mm) placed on the apparatus floor was connected to a sensor that was dislocated. The sensor was connected to an Arduino UNO. This electronic circuit was covered by a tower that had a white LED used to signal responses. The syrup access mechanism consisted of a small ladle attached to a servo motor SG90 that delivered the solution through a circular hole next to the bar (Figure 2). Programs in Visual Basic and Arduino IDE controlled chamber components and all experimental events.

Procedure

Subject Selection and Identification

Before the first experimental session, a plastic recipient with syrup (sugary solution, concentrated to 50%, with lemongrass essence - *Cymbopogon citratus*) was placed next to the hive entrance and a bee that landed on the recipient was marked on its back of the thorax[3] with gouache paint (cf. Pessotti, 1967). Each experimental subject was assigned a unique color to facilitate individual identification.

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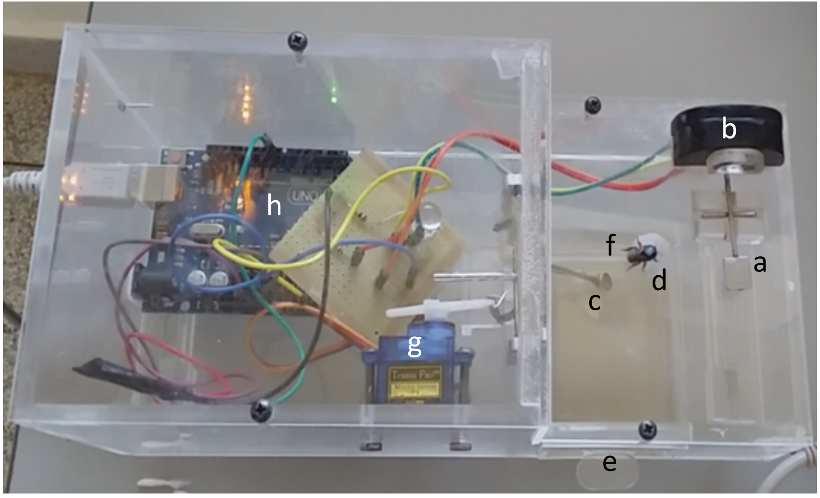
[3] Region of the body of the bee located between the wings (Grimaldi & Engels, 2005).

Figure 2

Experimental Box and its Components (Adapted from Pessotti, 1969)



Subtitle

a) Metal bar whose opposite end activated the photoelectric cell
b) White light LED photoelectric cell tower
c) Metal ladle for syrup presentation
d) Feeder fountain hole
e) Removable syrup container with handle
f) Bee to the edge of the feeder
g) Servo Moto
h) Arduino Uno board

Note. Photograph of the equipment by the author. Notice the bee (f) to the edge of the feeder (d).

Shaping of Landing Response to the Apparatus and Feeding

The plastic recipient was gradually moved from the hive toward the experimental chamber and placed 1.5 m away. When one of the bees was systematically foraging on the apparatus, the plastic recipient was removed, and a few drops of syrup were deposited on the surface of the chamber. Subsequently, the chamber surface was cleaned, and the syrup was available only on the feeder, which was activated by the experimenter. Considering that this training was conducted individually (Moreno et al., 2005; Moreno, 2007; Moreno et al., 2012; Moreno et al., 2014; Pessotti, 1969; Scienza et al., 2019), no more than three bees were marked at the time, and one of them was used as a subject. Intruders were prevented from reaching the feeder. This procedure was implemented to avoid other subjects in the experimental area.

Bar Pressing Shaping

Once the subject learned to collect the syrup from the feeder, access was restricted to 30 s contingent on responses of walking towards the bar, followed by touching it, then pressing it. Once the bee pressed the bar regularly, the session remained active for 1 h, before the next step.

Manipulation on Syrup's Duration

The duration of syrup was manipulated in descending order (30, 15, 10, and 6 s) for four bees (D1, D2, D3, and D6) and in ascending order (6, 10, 15, and 30 s) for two other bees (D4 and D5). In each syrup duration, two experimental sessions were conducted for three bees and three sessions for the other three bees, 6 to 10 sessions of 30 min per day were performed.

The criterion for changing the duration was at least two 30 min sessions in each duration and visual inspection of the cumulative record of responses. The inspection of the cumulative record provided information on the consistency and systematicity of the response, represented by the number of responses and similar reinforcements in each visit and by the constancy of the intervals between visits. The measure of interest was the response rate (R/min). The calculation of the response rate was obtained by summing the number of responses emitted, divided by the session time (30 min). However, the session time included both the time on the apparatus and the time going in the hive. So, a response rate including only the time of the bee in the apparatus was also calculated, which we called *visit fee.* The rate including the two times (in the apparatus and in the hive) was called the *total fee*.

Warming up Phase

This phase was carried out at the beginning of every daily experimental session, while the syrup's duration to the feeder was manipulated. It consisted of exposure to 30 s duration, for a minimum period of 15 min. After the warm-up period, the current duration was resumed.

Results

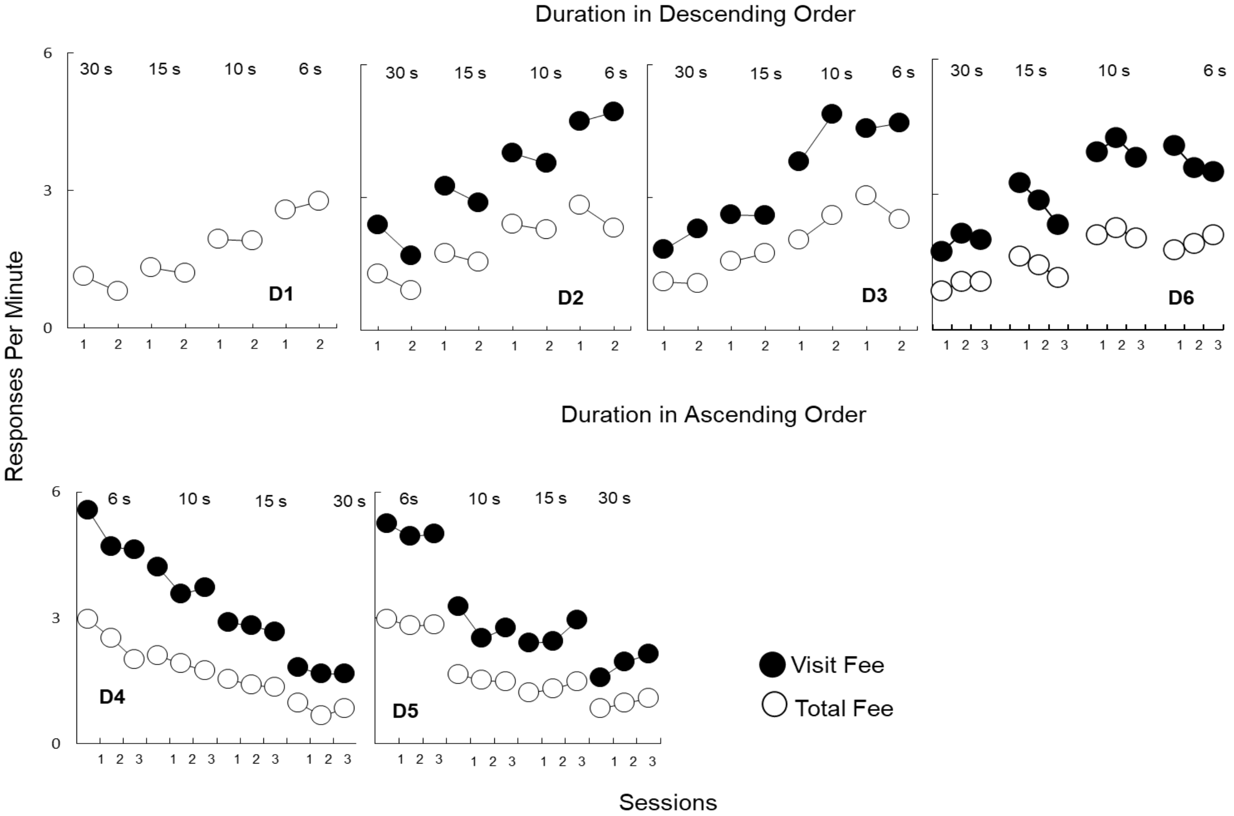
Figure 3 shows the response rate (R/min), of six bees under duration of syrup 30, 15, 10, and 6 s over successive experimental sessions. For all bees, the total fee (empty circles) and the visit fee (full circles) showed an inverse function of the duration of syrup. As the duration decreased, the response rate increased proportionally (and vice versa, for the bees that started with the shortest duration). The total fee ranged from approximately 1.0 R/min to 2.6 R/min; the visit fee varied between approximately 2.0 R/min and 5.0 R/min. The longest time of the bee in the experimental apparatus was 20 min and the shortest was 12 min, ranging from 40 to 66.7% of the session time which was 30 min.

Response rates (R/min) were relatively stable under each duration to syrup, but in some cases upward trends were observed (see D2 bees, 6 s; D3, 10 s; and D5, 15 and 30 s) or downward trends (D1 bees, 30 s; D2, 30, 15 and 10 s; D6, 15 and 6 s and D4, 6 and 10 s.

In general, no differences in rates were observed concerning the order of the duration of syrup manipulation. Bees (D4 and D5), for which duration was manipulated in ascending order (6, 10, 15, and 30 s) and bees (D1, D2, D3, and D6) for which duration varied in descending order (30, 15, 10 and 6 s) had approximately equal response rates (Figure 3).

Figure 3

Rate of Responses (R/min) in Syrup's Duration Throughout the Experimental Sessions

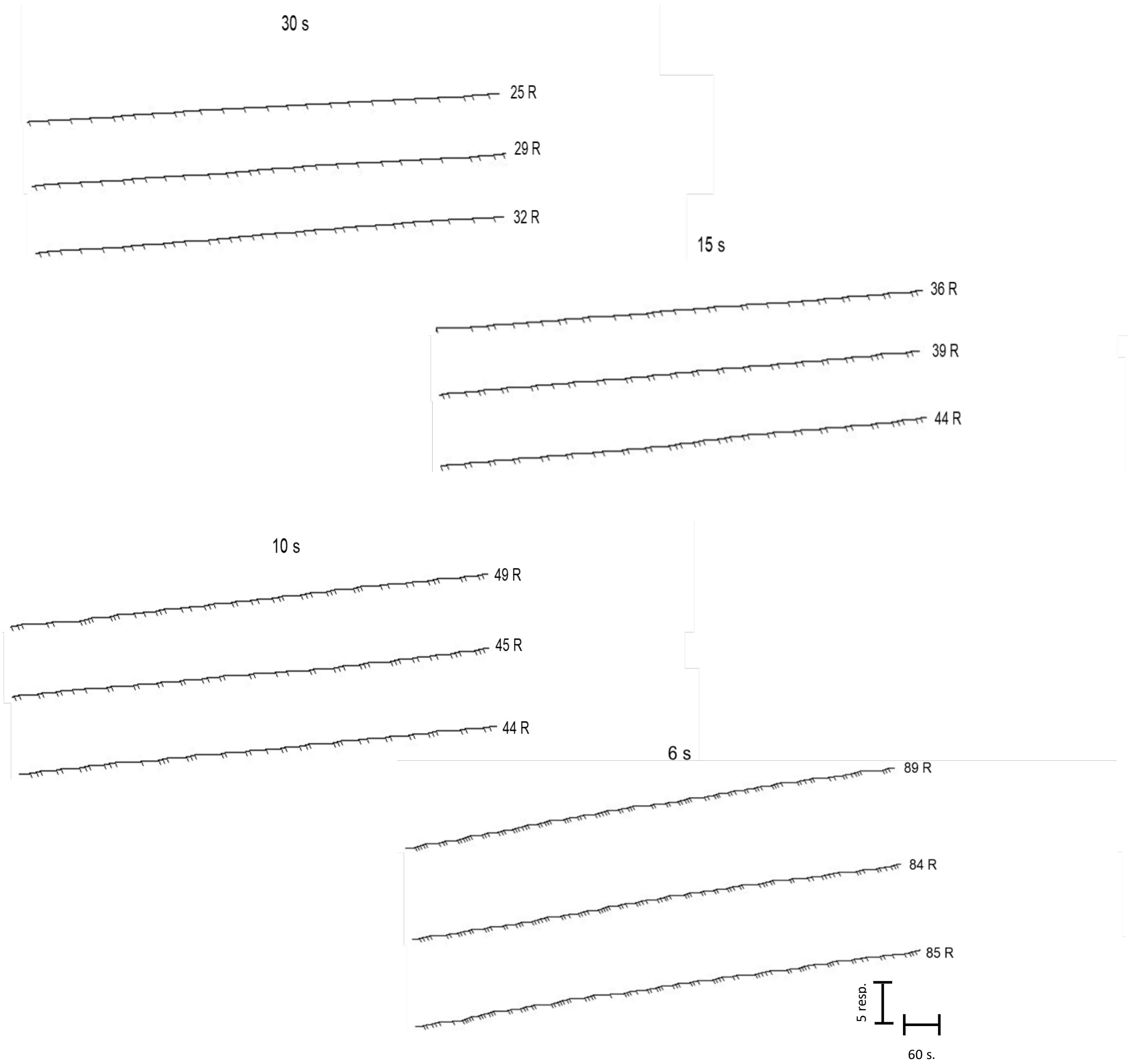


Note. For bee D1, the time in the hive was not measured, which made it impossible to calculate the visit fee. The filled circles represent the visit fee, and the empty circles represent the total fee. The duration of syrup is indicated at the top of the figure.

To exemplify the patterns of responding in the different durations, Figure 4 shows the cumulative record of responses of the D5 bee. For each duration, there is a presentation of the curves related to the three experimental sessions (30 min each) to which this bee was exposed. The numbers to the right of each curve indicate the total responses in the session during the 30 min.

Figure 4

Accumulated Records of D5 Bee Responses in Each Experimental Condition



The records indicate that, in general, as the syrup's duration was reduced from 30 to 6 s, there was also an increase in the number of bar-press responses emitted per session, indicated by the vertical lines along the accumulated curves. The larger intervals between the vertical lines correspond to the time of the bee's exit and return to the hive. In duration of 30 s, an average of 29 responses were emitted, with 1 to 2 responses in each visit. For 15 s, an average of 40 responses were emitted, with 2 to 3 responses in each visit. For 10 s, an average of 46 responses was observed, with 2 to 3 responses per visit, and for 6 s, an average of 86 responses. When the duration was 6 s, there was a significant increase in the number of responses emitted per visit compared to the other durations, but there was also a greater inconstancy in the pattern of the response, represented by the greater variability in the number of responses per visit, between 2 and 6 responses per visit (Figure 4).

Table 1 corresponds to the number of visits of the bees to the experimental apparatus through the duration of syrup during the 30-min sessions. In general, the mean number of visits was 19.3, ranging from 16.2 (minimum, bee D3) to 21.8 (maximum, bee D5) and remained relatively constant among the different experimental conditions. For the 30 s duration, the number of visits ranged from 15 (minimum) to 23 visits (maximum), and for the 15 s duration, this variation was between 15 and 24 visits. For 10 s, between 14 and 22 visits and for 6 s between 12 and 24 visits. Bee D2 had the lowest number of visits (12) in the second 6 s session and bee D5 had the highest number of visits (24) in the first 15 s session and the second 6 s session.

Table 1

Number of Visits to the Experimental Apparatus in the Last Two or Three 30-Minute Sessions for Each Reinforcement Duration

	Reinforcement Duration (s)												
	30			15			10			6			
Session Bees	1	2	3	1	2	3	1	2	3	1	2	3	Average
D1	17	19	-	20	17	-	22	18	-	21	17	-	18.9
D2	19	18	-	20	20	-	21	20	-	16	12	-	18.2
D3	17	17	-	17	15	-	16	14	-	14	20	-	16.2
D4	19	15	20	22	19	18	22	19	16	20	18	16	18.7
D5	23	22	23	24	20	21	19	21	19	23	24	23	21.8
D6	23	20	20	21	20	19	21	21	21	21	20	18	20.4
Average	19.7	18.5	21.0	20.7	18.5	19.3	20.2	18.8	18.7	19.2	18.5	19.0	19.3
Grand Total	19.7			19.5			19.2			18.9			19.3

Discussion

The present study explored and refined a methodological procedure to enhance collection of behavioral samples of bees responding to operant conditioning tasks. An automated apparatus equipped with a bar and feeder was used. The procedure consisted of fractionating the duration of the syrup delivered and measuring the rate of bar-press responses emitted by bees for each duration.

For all six bees, the response rate (R/min) was an inverse function of the reinforcement duration (Figure 3), both for the calculation considering the total session time (Total Fee), and for the calculation considering the visit time (Visit Fee). Given that the bee of the species used in the study (*Melipona quadrisfascita anthidioides*) needs approximately 30 s to fill it its honey vesicle at a syrup (sugary solution, concentrated to 50%, with lemongrass essence - *Cymbopogon citratus*) (Moreno et al., 2012; Moreno et al., 2014; Pessotti, 1961, 1964a, 1964b, 1965a, 1965b, 1967a, 1967b, 1969, 1971, 1972, Pessotti & Carli-Gomes, 1981a, Pessotti & Lé'Sénéchal, 1981b, Scienza et. al., 2019), reducing the duration of reinforcement increases the opportunity for responses to occur in each foraging bout.

The aim of the present study was achieved - i.e., subjects remained active during the experimental sessions with shorter durations reinforcers and producing larger behavioral samples. However, reducing the syrup's duration may have affected other variables, for example increasing the response effort (Foster & Hackenberg, 2004), and potentially influencing responding. This trend was noticeable in the 6-s duration. Although the response rate increased in the 6-s duration, there was greater inconsistency in the response pattern, represented by variability in the number of responses per visit compared to the other durations, which present greater systematicity in the response pattern (see Figure 4).

In the present study, each bar-press response produced access to a fraction of the syrup, possibly producing a second-order schedule, akin to a token system (see Figure 1). To access 30 s syrup and fill the honey vesicle, bees were required to produce multiple fractions (i.e., tokens). Thus, as the duration of the syrup decreases, the number of tokens increases. For example, in the duration of 30 s around 1 token is needed and in the duration of 6 s around 5 tokens for the collection of the 30 s of syrup and deposit (exchange of tokens) in the hive (Figure 3). Therefore, the amount of syrup deposited in the hive at each visit will always be around 30 s, regardless of the value of the duration in force. Table 1 illustrates the constancy of visits between the different durations in each 30 minutes of data collection. Bee D6, for example, performs 20 visits in the second and third sessions of 30 s, in the second session of 15 s and in the second session of 6 s. For all other bees, the values are similar, varying on average between 16.25 and 21.83.

The procedure employed in the present experiment can be described as a second-order schedule, with token-exchange serving as the depositing of material in the hive. Thus, the reinforcer for foraging would be the depositing of material in the hive (Figure 1), which restores the condition of deprivation achieved through the response chain to fill the honey vesicle. There is, however, evidence to suggest that each duration fraction also functions as a primary reinforcer. For example, in the study by Hammer (1997), when conducting classical conditioning between an odor (neutral stimulus) and depolarization of a neuron [4](unconditioned stimulus) in honeybees, the conditioned proboscis extension response [5]of the bee was observed in the presence of the odor (conditioned stimulus).

A limitation of this study refers to the number and duration of sessions. There were 2 to 3 daily sessions of 30 min each. One possibility for future studies would be to use a greater number of sessions with longer durations, which would make it possible to obtain more samples of bee behavior. Other measurements and data analysis could also be carried out, such as analysis of pauses (post-reinforcement and beehive pause) and analysis of rates every 5 or 15 min of the session, to observe in more detail the trends in responding as the foraging time passes. The criterion for changing duration of syrup access was at least two 30-min sessions in each duration and visual inspection of cumulative response records (as exemplified in Figure 4). Future studies could use, in addition to visual inspection of the response rate, some quantitative criterion (e.g., percentage of change in the mean rate of the last three sessions) (Bourret, & Pietras, 2013; Sidman, 1960).

Future studies could also benefit from a more precise measure of consumption time. Consumption time was calculated by multiplying the number of reinforcers obtained by the duration of the reinforcement. To calculate the response rate, we divided the number of responses emitted by the session time minus the reinforcement consumption time. Due to a limitation of the program / equipment, the reinforcement consumption time continued to count even when the bee had flown to the hive and the feeder was no longer available. The bee needs an average of 30 s of reinforcement duration to fill the honey vesicle, but it can occur sporadically, consuming a little more or a little less syrup. A way to improve the measure of consumption time is to place a photocell in the dipper mechanism so that the duration of the reinforcement would count only when the bee was present at the dipper mechanism consuming the reinforcement, this strategy would allow for a reliable measure of consumption time.

Our results add to other studies showing that bees, including melipona, are capable of learning complex behaviors such as pressing the bar. This confirms and replicates the studies carried out by Pessotti (1961, 1964a, 1964b, 1965a, 1965b, 1967a, 1967b, 1969, 1971, 1972), Pessotti and Carli-Gomes (1981a), Pessotti and Lé'Sénéchal (1981b), Moreno et al. (2012), Moreno et al. (2014), and Scienza et al. (2019), among others, demonstrating the acquisition of arbitrary responses, such as the bee entering an acrylic tube (Grosmann, 1973), entering a hole (Sokolowski et al., 2010), carrying a small ball to a defined location (Loukola et al., 2017), and pulling a rope (Alem et al., 2016). The present study also contributes to interdisciplinary efforts, involving, for example, neurosciences (the neural bases of operant behavior, given the advantages of a smaller nervous system, which allows identifying specific circuits), and the interaction with environmental variables. This includes studies investigating the effects of pesticides on behavior and the nervous system of bees (e.g., Cabirol & Haase, 2019; Mitchell et al., 2017; Muth & Leonard, 2019; Piiroinen & Goulson, 2016; Siviter et al., 2018).

In addition, this experiment can be an important instrument for the systematic study of the operant behavior of bees, presenting the original contribution of demonstrating experimentally that the behavior of collecting syrup by the melipona persists until the fulfillment of its honey vesicle before its return to the hive.

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[4] For more details on classical conditioning by neuronal depolarization, see the study by Hammer (1993).

[5] PER (proboscis-extension response) is a type of bee response that is evaluated for its responsiveness to sucrose in classical conditioning experiments (Hammer, 1993/1997).

Declaração de conflito de interesses

Os autores declaram que não há conflito de interesses relativos à publicação deste artigo.

Contribuição de cada autor

A contribuição de cada autor pode ser atribuída como se segue: Lívia ajudou na construção do equipamento, coleta de dados, análise de dados, confecção de figuras, redação do texto do artigo em português e inglês; Rafael contribuiu com a construção do equipamento, programação em Visual Basic e Arduino e redação do texto em inglês, Marcelo Keller contribuiu com a coleta de dados e confecção de figuras, Lucas Codina contribuiu com a análise de dados e redação do texto em inglês, Marcelo Henriques contribuiu com a escrita do texto na versão em inglês e confecção da Figura 1, Kalliu contribuiu com toda a revisão do texto em inglês, Lucas Couto contribuiu com a programação em Visual Basic e Arduino e Deisy além de contribuir com a supervisão de todas as etapas do trabalho, também contribuiu com as correções do texto. Todos os autores também contribuíram com leituras e discussões sobre o procedimento.

Direitos Autorais

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