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Delay discounting in bumblebees (bombus terrestris): Methodological development and refinement

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Abstract

Whereas the ecological analysis hypotheses suggests that self-control is adaptive and context dependent, and influenced by long-term fitness, the metabolic hypotheses suggests that self-control negatively correlates organism's metabolic rate. Bumblebees have a high metabolic rate and are highly eusocial. Thus, examining delay discounting in bumblebees may provide valuable knowledge about interspecies variation in self-control, and inform assumption made by both hypotheses. To do that, delay-discounting procedures accounting for bumblebee's particularities as a specie should be developed. The present study aimed to test and refine a procedure made to study preference reversal as a measure of self-control in bumblebees (bombus terrestris). Preference reversal was investigated by systematically increasing the delay to access a sweeter reinforcer (5, 10, 15, 20, 25, 30, 35, 40, 45. 50, 60, 70… seconds) in an adjusting delay procedure. Five bumblebees were tested to find the point where they showed preference reversal by choosing less sweet immediate reinforcer. On average, the bumblebees showed preference reversal at 50 s delay in phase 1 and at 55 s in phase 2. Intertrial interval were stable across conditions, showing no systematic variation when delays were increased. Procedural solutions regarding the definitions of choice and method of adjusting delays are discussed.

Keywords: Delay discounting, self-control, impulsivity, interspecies variation, bumblebees, bombus terrestris.

Delay discounting in bumblebees: Method development and refinement

Both humans and animals are continually confronted with choices varying in consequence dimensions - e.g., amount, delay, probability, quality. For example, a customer choosing between buying one pair of jeans with a better quality or one with poorer (i.e., quality) or a child choosing between receiving one lollipop now and one tomorrow (i.e., delay). Predicting choice behavior is relatively simple when the options vary in only one dimension. Organisms commonly prefer larger over smaller reinforcers and reinforcers available sooner rather than later (Chung & Herrnstein, 1967; Green & Myerson, 2004). However, challenges to predict choice may arise when the options vary on more than one dimension (Green & Myerson, 2004). For example, a child choosing between receiving one lollipop now or 3 lollipops tomorrow. In such example, the child needs to make a trade-off between two dimensions of consequences, amount-, and delay receipt. In intertemporal choices, organisms frequently chose smaller sooner reinforcers over larger later reinforcers (Green & Myerson, 2004). The tendency of choosing smaller sooner over larger later consequences is commonly understood through delay discounting (Tesch & Sanfey, 2008).

From a delay discounting perspective, the subjective value of a reinforcer is increasingly discounted from its nominal value when the delay is added between the moment of choice and the point of reinforcer access (Ainslie, 1974; Green & Myerson, 2004; Green et al., 1997; Mazur, 1987). For example, if an experimenter presents subjects with two options, both with consequences far in the future, between receiving $100 in six months (smaller sooner) or $200 in nine months (larger later), subjects may choose the larger later option. Inferred from the choice, the subjective value of the larger later option is higher than of the smaller sooner. However, subjects may choose differently with the passage of time. If the experimenter presents the same subjects with the option between receiving $100 today (smaller sooner) or $200 in three months (larger later), they may choose the smaller sooner

alternative. From the discounting perspective, this phenomenon is referred to as preference reversal (Green et al., 1997). The subjective value of the smaller sooner reinforcer is discounted more steeply than the subjective value of the larger later reinforcer when the delay to both reinforcers decreases equivalently (Green & Myerson, 2004). Another example is a student who chose to go to party on Saturday instead of reading to a final exam, even though the student reported a preference for reading for the exam days before the party. Simplified, delay discounting describes a process of devaluing a future behavioral outcome (Madden & Johnson, 2010, p. 13).

Procedures to study delay discounting

Delay discounting procedures often evaluate the point where a smaller sooner reinforcer (SS) and larger later (LL) reinforcer have approximately the same subjective value to a choosing organism (Odum, 2011). Preference may be determined by presenting subjects with a series of two optioned choices between a smaller sooner reinforcer (SS) and a larger later reinforcer (LL). When examining delay discounting in humans, participants are often presented with a series of choices between options with hypothetical outcomes (Odum, 2011). For example, Rachlin et al. (1991) asked participants to make a series of hypothetical choices between $1,000 available today or $1,000 available after delays between 1 month to 50 years. The amount of the reinforcer available immediately (i.e., "today") was systematically decreased until the options were $1 today or $1,000 with a specific delay and increased back up again at the specific delay to determine the point where participants responded indifferently between the two options at different delays.

However, there are procedural limitations of examining delay discounting in human subjects. Firstly, skepticism about the validity of procedures with hypothetical consequences has been drawn (Madden & Johnson, 2010, p. 30). When the consequences are hypothetical, the participants are not actually experiencing the delay or the consequences, and the assumed

effect rely on self-reported verbal responses, often difficult to verify through observations (Critchfield & Kollins, 2001). Secondly, there are concerns about possible effects of mediating variables influencing choices, for example cultural values (Ainslie, 1975). Such limitations are presumably reduced by examining delay discounting with non-human subjects.

Examining delay discounting in non-human subjects have several advantages. Firstly, procedures and general principles derived from studies of delay discounting in non-human generalizable to humans (Ainslie, 1975). In fact, several delay discounting procedures used with humans have been derived from procedures used with non-humans (Madden & Johnson, 2010, p. 23) and results from experiments with non-human subjects correlate with results from experiments with delay discounting procedures in human subjects (Critchfield & Kollins, 2001). Secondly, studying delay discounting in non-human subjects allows higher control of the manipulated variables and potential confounding variables (Madden & Johnson, 2010, p. 23). Delay discounting procedures with non-human subjects have historically been examined in operant chambers; using pigeon pecks to keys or a rat lever presses as organisms and responses studied (McDiarmid & Rilling, 1965; Richards et al., 1997).

Although there is a variety of delay discounting procedures used with non-human subjects, they often employ common methodological features. Madden and Johnson (2010, p. 24) describes three common procedural features of non-human procedures. First, alternatives should be centered so the distance between the two options is equivalent (i.e., distance between available choices). secondly, forced-choice trials are often used to ensure that the subject experiences the two programmed contingencies before free-choice trials (i.e., during forced-choice trials, only one of the two options is available). Lastly intertrial intervals (ITI) are often controlled for to ensure that the time between choice opportunities is held constant,

and independent of the subject chose. If ITI is not controlled for, the sequence of response

and reinforcer becomes shorter in the smaller sooner option relative to in the larger later.

Thus, the reinforcer rate increases if the subject chose the smaller sooner reinforcer instead of

waiting the required time of the delay to receive the larger later reinforcer. The goal of

control ITI is to assure that rates of reinforcement are not different among available

alternatives.

The Evenden and Ryan (1996) procedure is amongst the most used delay discounting

experiments in non-human subjects. Evenden and Ryan (1996) exposed rats with a series of

choices between a sooner option with one pellet available immediately and a larger later

option of three pellets available after a delay. The subjects were exposed to the task for

several sessions consisting of five blocks with eight trials in each, over several days. The

delay to the larger later option was gradually increased over blocks in each session. Each

block consisted of two forced-choice trials, one on the smaller sooner reinforcer and one on

the larger later reinforcer, and six free-choice trials. The dependent measure was presented as

the percentage of choices of the larger later option in each block. The Evenden and Ryan

(1996) procedure provides measures of both the sensitivity to delay and amount of the

reinforcer in each session, as well as providing a stable baseline across days.

Even though the procedure developed by Evenden and Ryan (1996) is the most

commonly used to study delay discounting in non-human subjects, drawbacks to the

procedure have been pointed out. Since the procedure consists of a discreet trial format with

two options that are different in amount while the delay is non-adjusting in blocks, it should

result in stable responding with an exclusive preference for the option with the highest value

(e.g., Herrnstein, 1981). However, it is rarely the case that subjects show exclusive preference

after the first block with no delay in this procedure, which might be explained by a carryover

effect from choices in earlier blocks and sessions (Fox et al., 2008). A second drawback

might be that the carryover effect can potentially be a confounding variable affecting the measures of sensitivity to the amount of the reinforcers (Madden & Johnson, 2010, p. 28). The sensitivity to the amount of the reinforcers is usually measured when both reinforcers are delivered without delay, commonly in the first block of each session. However, it is rarely the case that the option with the larger reinforcer is exclusively chosen during the blocks with no delay (e.g, Cardinal et al., 2001). Since the no-delay block usually is the first block tested in each session, choice might be affected by the avoidance of the larger delayed reinforcer in the preceding session (Madden & Johnson, 2010, p. 28). Lastly, it is a time-consuming procedure. Procedural improvements might reduce the problematic drawbacks. For example, including periodically control sessions with no delay on the larger reinforcer or an increased number of forced trials in each block can potentially enhance sensitivity to changes in delay (Madden & Johnson, 2010, p. 28).

Adjusting delay procedure or adjusting amount procedure may be a viable alternative (Odum, 2011). For example, a delay discounting procedure pioneered by Mazur (1987) consisted of presenting pigeons with choices between access to food for 2 s after 2 s fixed delay (SS) or access to food for 6 s after an adjusted delay (LL). Delay was manipulated by adjusting delay to the larger reinforcer according to the pigeon's choice. When the pigeon chose the smaller sooner option, delay to larger reinforcer was increased. When the pigeon chose the larger later option, delay to larger reinforcer was decreased. The time between choices was held constant by varying the intertrial interval (ITI) to keep the rate of delivered reinforcers independent of choice. The delay was widely adjusted in the beginning, and the restrained to be adjusting in a more constrained range before a criterion for stability was met. The procedure ended when the subject responded indifferently between the two options, thus when the pigeon choice of smaller sooner was proportional to choices of larger later. The value to the larger later reinforcer was determined at a range of fixed delays to the smaller

sooner reinforcer. Mazur (1987) found that the delay to the smaller sooner reinforcer increased over conditions, the pigeons responded indifferently between the two options with an increased duration on the delay to the larger later reinforcer.

Similar to the adjusting-delay procedure is the adjusting-amount procedure, developed by Richards et al. (1997). Instead of adjusting the delay to the reinforcers while the amount is held constant, the amount of the reinforcers is adjusted while the delay is held constant in an adjusting-amount procedure (Madden & Johnson, 2010, p. 26). Richards et al. (1997) presented rats with a choice between a small amount of water available after a constant delay and an even smaller amount of water available immediately. If the rat chose the small amount reinforcer with the constant delay, the amount to the smaller-sooner reinforcer was increased. If the rat chose the reinforcer with the smallest amount, the amount of the smaller-sooner reinforcer was decreased. Both the adjusting-delay and adjusting-amount procedures produce approximately similar estimates of discounting curves (Green et al., 2007).

Interspecies variation in self-control

Delay discounting procedures have been used to examine self-controlled and impulsive choices, which can address many socially important issues (Odum, 2011). Choosing a larger later reinforcer can be defined as a self-controlled and choosing a smaller sooner reinforcer can be defined as an impulsive choice (Ainslie, 1974). Showing impulsive behavior is a central part of many socially important issues, for example gambling, ADHD, substance abuse (Barkley et al., 2001; Forzano & Corry, 1998; Kirby & Petry, 2004; Weatherly, 2010). From an evolutionary perspective, these choice patterns have evolved because of uncertainty in tradeoffs in available consequences, for example in terms of delay, probability and amount. Thus choices of smaller sooner option over later and uncertain consequences may have been selected during phylogenesis (Green et al., 1994). For example, choosing a delayed option may be more adaptive when the future is more certain and it is less

unnecessary with immediate reinforcers (Logue, 1988). Natural selection may also explain

interspecies variation in degrees of self-control. Two complementary hypotheses have been

used to interspecies variation in self-control – explain this variation have been suggested

ecological analysis hypothesis and the *metabolic hypotheses* (Mayack & Naug, 2015).

Logue (1988) proposed that the ecological analysis hypothesis explain self-control

when contextually adaptive choices contribute to organism's fitness in the long-term. From

this perspective, self-control choices are adaptive not only at the individual level but also at

the group level (Gailliot & Baumeister, 2007). Self-controlled choices can for example

contribute to abstain from behaving in harmful or maladaptive ways and complying with

different rules in the society (Gailliot & Baumeister, 2007). It can therefore be viewed as

beneficial for cooperation by exerting self-controlled choices, and it might be a part of the

reason for its selection (Baumeister et al., 2005). However, the ecological analysis hypothesis

has been criticized for its limitations to explain cross-species variations in self-control

(Mayack & Naug, 2015).

Alternatively, the metabolic hypothesis states that self-control is determined by

organisms' metabolic rate (Tobin & Logue, 1994). Tobin and Logue (1994) looked into the

result of several studies on self-control in pigeons, rats, and humans to examine whether

variation in the degree of self-control could be described by a common framework. Self-

control was measured as the deviation from a prediction based on the matching law, and the

specie body size, as well as the metabolic rate. The result indicated that variation in the

degree of self-control between the species examined seemed to negatively correlate with their

metabolic rate. The human subjects had low estimates of metabolic rate and showed a

significantly higher degree of self-control compared to the two other species. Both the rats

and pigeons had similar estimates of metabolic rate that were significantly higher than of the

human subjects, and both species showed similar degrees of self-control that were lower than

the human subjects. It has been proposed higher metabolic rate leads to quicker deprivation, thus influencing self-control (Tobin & Logue, 1994). Therefore, Tobin and Logue (1994) argued that species with a high metabolic rate might benefit more from making impulsive choices because of a higher and frequent requirement to meet their nutritional needs. However, Tobin and Logue (1994) pointed out that it is not sufficient to conclude whether self-control can be determined by metabolic rate from their study, as they only examined three species. Even so, the rats and pigeons were so close in body size that they only provided two data points for analysis and the result might also be affected by other variables, for example previous experience with delay or language ability (Tobin & Logue, 1994).

To evaluate the extent to which the ecological analysis and the metabolic hypotheses explain self-control, different species must be compared. The majority of experiments with delay discounting in non-humans have been conducted with pigeons and rats (Madden & Johnson, 2010, p. 23). Recently, other species have been tested in delay discounting procedures, for example monkeys, apes, and ravens (Kabadayi & Osvath, 2017; Osvath & Osvath, 2008; Petrillo et al., 2015). However, examining delay discounting in species with higher metabolic rates (e.g., social insects) can potentially broaden the understanding of interspecies variation in self-controlled behavior. For example, Wendt and Czaczkes (2017) presented individual ants with a spatial discounting task consisting of a low-quality reinforcer close to the nest and a high-quality reinforcer located far away from the nest. The result indicated that the ants chose the higher-quality reinforcer located far away from the nest, and when the quality was the same on both reinforcers, they chose the closest option. Social insects that both have a high metabolic rate and are eusocial can put the ecological analysis hypothesis and the metabolic hypotheses to the test. According to the metabolic hypothesis, it can be predicted that social insects with high metabolic rates show more impulsive behavior by choosing a smaller sooner reinforcer over a larger later reinforcer. However, the prediction

from the ecological analysis hypothesis seems to contradict since it predicts that social insects
will show more self-controlled behavior since they are highly social.

Examining delay discounting in bees can potentially broaden the understanding of
interspecies variation in self-controlled behavior. They are small in size and have a high
metabolic rate (Heinrich, 1977). At the same time, bees are highly eusocial and work for the
good of their hive. The beehive consists of a queen that produces new eggs, and the majority
of the bees in the hive are sterile female workers who conduct different tasks to keep the hive
functioning to pass on their genes (Cheng et al., 2002). In addition, bees can complete a high
number of trials in one session, and it is easy to manipulate important variables controlling
bee behavior. easy to manipulate. It can be argued that it is beneficial to begin by establishing
basic knowledge about how to apply delay discounting procedures in bees and examine if
there are potential species-specific considerations that should be controlled for before moving
on to the bigger questions concerning interspecies variation.

There is a small number of studies investigating delay discounting in bees. For the
proposes of the present thesis, a literature search of papers examining delay discounting in
bees was conducted, yelling only two relevant papers (Cheng et al., 2002; Mayack & Naug,
2015). The literature review was conducted May 2021, in the following databases: PsychInfo,
Web of Science, and PubMed. The search was conducted with the keyword combination
"bee*" or "bumblebee*" or "honeybee*" and "delay discounting" or "delay of gratification"
or "self-control" or "reinforcer delay" or "impulsiveness". The articles were reviewed
according to two criteria: (1) consisting of a delay discounting procedure where the delay was
manipulated and (2) bees as subjects. 20 out of 24 papers did not meet the criteria and were
therefore excluded. Only two articles met the criteria and were included (Cheng et al., 2002;
Mayack & Naug, 2015). The reference lists of the two included articles and articles citing
them were searched for to examine if they referred to or were cited by studies using delay

discounting procedures in bees. A search in the reference lists of the two included articles and for articles citing the two articles did not yield new studies applying delay discounting procedures in bees. However, Mayack and Naug (2015) referred to Cheng et al. (2002).

Mayack and Naug (2015) investigated if starvation influenced impulsivity in individual honeybees in an associative conditioning procedure by measuring choice as a proboscis extension response (PER) reflex. PER is a reflex where honeybees, in response to antennal stimulation with sucrose solution, extend their proboscis (Smith & Burden, 2014). Mayack and Naug (2015) used a PER assay procedure by conditioning honeybees to associate two odors as a conditioned stimulus (CS) that partially overlapped in time with two different reinforcers consisting of either a small volume or large volume of 50 % sucrose solution as an unconditioned stimulus (US). The honeybees were exposed with conditioning trials where the two reinforcers were presented without delay, as well as conditioning trials where a small reinforcer was delivered after 1 s and a larger reinforcer 5 s after CS presentation. Hereafter honeybees were starved for 6, 18, or 24 h and presented with a two-alternative forced-choice PER assay consisting of presenting them with the two conditioned odors on each side of the bee's head to record its response of the direction it turns its head and extends its proboscis.

The result in Mayack and Naug (2015) indicated that the honeybees that had associated the larger reinforcer with a delayed reinforcer showed more impulsive choices as function of deprivation time. The authors argue that the individual energetic state affects impulsive choices according to the metabolic hypothesis even in eusocial animals like the honeybee. Mayack and Naug (2015) suggest that the metabolic and ecological analysis hypotheses are necessarily mutually exclusive, even though they seem to be conflicting in predicting the degree of self-controlled choices. The authors suggest that when bees display self-controlled choices, it might be because they discriminate that there is no risk of

starvation in a specific context and not just because self-controlled behavior is beneficial for the hive. However, examining delay discounting with an associative conditioning procedure is distinct from the way choices are commonly made in other delay discounting procedures, which poses limitations in comparing such results to the literature.

Cheng et al. (2002) procedure is similar to those adopted by other delay studies with non-human animals. They examined self-control in honeybees that could freely fly when performing the experimental task. In three experiments, honeybees were trained to feed from two alternatives forced-choice trials. In the first alternative the reinforce was smaller but delivered immediately. In the second alternative the reinforcer was larger but delivered after a delay. In the first experiment, individual honeybees were presented with a series of choices between a sweeter 5 s delayed reinforcer (50 % sucrose solution) and a less sweet immediate reinforcer (25 % sucrose solution). In the second and third experiment, the choices were between two reinforcers with 33 % sugar concentration, but with manipulation of the amount available. In the second experiment, honeybees were either assigned to a no-delay group without delay to a larger reinforcer or a delay-group with delay to the larger reinforcer. Honeybees in the on-delay group were presented with a series of choices between a larger reinforcer just inside the entrance of a small plastic box and a smaller reinforcer just inside the entrance of a small plastic box. In the delay group, honeybees were presented with a series of choices between a larger reinforcer at the end of the plastic box, and a smaller reinforcer just at the entrance of the second plastic box. In the third experiment, two reinforcers were covered by plastic cups as in the first experiment, the plastic cup was removed after 0.1 s on the small reinforcer and after 6 s on the larger reinforcer.

The results in Cheng et al. (2002) indicated that the honeybees showed far more self-control than predicted by the metabolic hypotheses; they preferred the larger or sweeter delayed reinforcer over the smaller or less sweet immediate reinforcer. Besides, the

ecological analysis hypothesis was not sufficient as an alternative explanation for the result

because it seemed to be unclear and lacking quantitative precision (Cheng et al., 2002).

Unlike the metabolic hypothesis, this hypothesis does not have a quantitative independent

variable. Although, there are some limitations to their procedure. In the first experiment,

honeybees were free to leave the sweeter reinforcer during the countdown of the delay. It was

not specified if the delay was reset when honeybees left the sweeter reinforcer, or if the

countdown continued and the reinforcer was delivered even if she left the marked area. In the

third experiment, when a honeybee chose smaller reinforcer, they always flew to the larger

reinforcer to drink until saturated before returning to the hive. However, it was still recorded

as choosing the smaller reinforce, even though they always fed from the larger reinforcer.

In the present study, several procedural improvements were made to the Cheng et al.

(2002). A simple adjusting delay procedure was used to determine the point where

bumblebees show preference reversal and examine if their behavior was affected by gradually

increasing the delay to a sweeter reinforcer. Individual bumblebees were presented with a

series of two-optioned choices between a less sweet reinforcer available immediately and a

sweeter reinforcer available after an increasing delay in a Y-maze. Delay to sweeter

reinforcer was manipulated by gradually increasing the duration between response to the

sweeter option and the delivery of sweeter reinforcer until she showed preference reversal.

Delay was reset and the sweeter reinforcer was not delivered if the bumblebee left the

sweeter reinforcer before the required delay was counted down. A choice was defined as a

response in which the bumblebee committed to drinking until saturated and returned to the

hive. Therefore, it was not registered as choosing the less sweet reinforcer if the bumblebee

responded to the less sweet reinforcer before she waited the required delay to the sweeter

reinforcer and drank it until saturated before she returned to the hive. The aim of the study

was to contribute to the understanding of delay discounting in bees through method development and refinement of delay discounting procedures in bumblebees.

Method

Subjects

Five bumblebees, *Bombus Terrestris*, housed in a wooden box (length = 30 cm, width = 22 cm, height = 20 cm) were selected to participate in daily experimental sessions. The beehive was purchased from a local producer (Bombus Natur AS) and moved to a wooden box 4 days before the experiment began. The bumblebees measured an average length of 1.96 cm (*SD* = .18) and width of 0.92 cm (*SD* = .19). All bumblebees were experimentally naïve. They were fed indoors with 45 % sucrose solution (45 % sugar, and 55 % water) and pollen. The hive was kept at the temperature of 25°C $\pm$ 2 which is optimal for the highest foraging activity and colony traffic (Kwon & Saeed, 2003), and with a relative humidity of 60 % $\pm$ 10 % for optimal colony development and highest nectar collection activity (Nasir et al., 2019; Peat & Goulson, 2005; Yoon et al., 2002). According to the Norwegian regulations on the use of animals in experiments (Forskrift om bruk av dyr i forsøk, , § 2), no applications or approvals are required for experiments on bumblebees. The experiment was conducted with ethical considerations concerning the bumblebee's welfare.

Apparatus

A beehive was connected to a flying arena by a plastic tube in-between the hive and one of the walls in the flying arena. The only light entering the hive was from the entrance to the flying arena to facilitate foraging behavior since light facilitates positive phototaxis. Positive phototaxis is when an organism moves towards a light source (Merling et al., 2020). A landing platform was located at the wall parallel to the wall with the entrance to the hive. The landing platform was connected to an entrance channel leading to a Y-maze. A sliding door was placed at the end of the entrance channel that could be opened and closed to control

bumblebee's access to enter the Y-maze. The Y-maze was a Y-shaped wooden box covered by plexiglass to ensure sufficient indoor lighting, similar to that used in Giurfa et al. (1999). The Y-maze consisted of a decision chamber and two separate arms closed off by two back walls each with an entrance hole in the middle connected to a small plastic container. Both arms were in the bumblebee's field of view when entering the decision chamber. The back walls were covered with stimuli of white laminated paper with a 10 cm diameter black circle drawn around the entrance to the arms, the stimuli are shown in figure 1. A plastic lid was placed in both containers, and sucrose solution was delivered with a syringe through a small hole in the top of the plastic container. The experimental apparatus and its measurements are shown in figures 2 and 3.

White light was turned on during the experiment and feeding. Infra-red light was turned on when moving the bumblebees, marking them, cleaning the hive, or changing the apparatus. Infra-red light is visually dark for the bumblebees (Scheiner et al., 2013, p. 19). 30 percent disinfection was used to clean the apparatus between each experimental trial.

Data collection

Two computer programs were applied for data collection. The countdown function on the clock app in iPhone 11 was used to measure the time of delay on the sweeter reinforcer (Apple, 2021). A program coded in Microsoft Visual Studio (Microsoft corporation, 2021) was used to register the selected bumblebees activity during the experimental session by pressing a button for each activity. The activities registered were the trial number, duration of trial, duration of time in the flying arena, duration of time in the decision chamber, duration of time inside the left arm, duration of time inside the right arm, number of reinforcers delivered in the left arm and the right arm. A third program, also coded in Microsoft Visual Studio, was used to transform the output from the second program into txt-files that could be opened in excel.

Design

A *within-subject design* with an adjusting delay procedure was used to determine the point where bumblebees show preference reversal and examine how their behavior was affected by the increasing delay to the sweeter reinforcer. Five bumblebees were presented with a series of two-optioned choices, the less sweet sucrose solution was available immediately and the sweeter sucrose solution was available after an increasing delay. Three bumblebees completed the experiment with a blue stimulus located at the wall to the left arm and a yellow stimulus at the wall to the right arm. The remaining two bumblebees completed the experiment with the yellow stimulus located at the wall to the left arm and the blue stimulus at the wall to the right arm.

The most active bumblebee was selected in a selection procedure. The selected bumblebee completed forced-choice trials to ensure that she had experience with the contingencies for both reinforcers (Madden & Johnson, 2010, p. 24). A criterion for assuming preference for sweeter sucrose solution over less sweet sucrose solution had to be met in a preference test before the adjusting delay procedure was introduced. Thereafter she was exposed to a two-phased adjusting delay procedure where delay to the sweeter sucrose solution was gradually increased while the less sweet sucrose solution was delivered immediately. Delay was gradually increased until she showed preference reversal in phase one. Before phase two began, the delay was decreased to 0 s in one forced-choice trial with the sweeter reinforcer. In phase two, delay to the sweeter reinforcer was increased until she showed preference reversal a second time and the experimental session was ended. The data was recorded as the duration of delay each bumblebee showed preference reversal at in both phases, number of times the delay were reset, number of visits to the arm with the less sweet reinforcer and intertrial interval (ITI).

Procedure

The procedure and parameters used were carefully selected based on seven pilot experiments. Six of the pilot experiments with the reinforcer magnitudes used by Mayack and Naug (2015), that is 33 % sucrose solution during feeding, 25 % as the less sweet reinforcer, and 50 % as the sweeter reinforcer, indicated that the reinforcer magnitudes were not optimal percentages to use in the present experiment. The bumblebees used significantly more time to complete the forced-choice trials with the less sweet reinforcer compared to those with the sweeter reinforcer. They only tasted the less sweet reinforcer, flapped their wings, and switched a lot between both arms in the forced-choice trial with the less sweet reinforcer. Their behavior indicated that the less sweet reinforcer might have been loss aversive and not just a reinforcer of a lower percentage than the sweeter reinforcer. The sweeter sucrose solution was 17 % higher than the 33 % sucrose solution used during feeding, however the less sweet sucrose solution was only 8 % lower than the sucrose solution used during feeding. Therefore, the sucrose solutions used in the present study consisted of a 45 % sucrose solution during feeding, 35 % as the less sweet reinforcer, and 55 % as the sweeter reinforcer. Similarly, as in Mayack and Naug (2015), a choice was registered as the first reinforcer the bumblebee responded to in four of the pilot experiments. The bumblebees did often not drink the first reinforcer they responded to and drank the other option afterward. It can therefore be argued that it is more ecologically valid that a choice was registered as a response in which the bumblebee committed to drinking until saturated and get back to the hive. Which is often, but not always, the last response in a trial.

In four of the pilot experiments, the other option was made unavailable after the bumblebees' first response, either by closing the entrance to the arm she entered first or the entrance to the opposite arm. However, this was problematic because they did not engage with the less sweet reinforcer and instead of showing preference reversal they stopped going out of the hive. Therefore, the bumblebees in the present study were free to switch between

the two options and choice was defined as the bumblebees committed to drink a reinforcer

until saturated before returning to the hive instead of defining it as the first response which

was done in (Cheng et al., 2002).

Magazine training and feeding

Bumblebees were trained to drink sucrose solution from the two arms in the Y-maze.

Shaping was performed by gradually moving the placement of a sucrose solution with

successive approximation to the Y-maze entrance. Small droplets of sucrose solution were

placed in the tube in-between the hive and the flying arena to recruit bumblebees to the flying

arena. Hereafter a lid with sucrose solution was gradually moved over the flying arena,

through the entrance channel, and into the Y-maze. Two lids with sucrose solution were

gradually placed closer to both arms in the Y-maze and removed when the active foragers

drank sucrose solution from inside the two arms. The shaping procedure was conducted with

all active foragers over a period of 2 weeks. Hereafter, new bumblebees were regularly

recruited from the flying arena to the Y-maze by placing small droplets of sucrose solution

contingent on responses closer to the target behavior. After shaping, bumblebees were

magazine trained to drink 45 percent sucrose solution (45 % sugar and 55 % water) from

inside the two arms in the Y-maze with white stimuli on the walls for five days.

The bumblebees that were actively drinking from the two arms were marked with

individual numbers for identification. Marking was done by using a queen marking number

kit to glue a paper disk with an individual number to the thorax of the bees. When new

bumblebees were trained to drink sucrose solution from the Y-maze, they were also marked.

The data collection was conducted on Mondays, Wednesdays, and Fridays, and started

at 8 a.m. The hive was deprived of sucrose solution for 22 hours by only giving them access

to drink sucrose solution from the Y-maze from 8 a.m. to 10 a.m. the day before data

collection. On the days of the data collections, the bumblebees were given free access to

drinking sucrose solution from the Y-maze for two hours after the experimental session

ended. They were feed from the Y-maze from 8 a.m. to 3 p.m. on Saturdays. The hive would

acquire all its nutritional needs during feeding for 2 hours after ended data collection, during

feeding for two hours the days between data collection and feeding for seven hours on

Saturdays. Pollen was placed inside the hive in a bottle cap every day.

Experimental session

One bumblebee was selected to participate in an experimental session at the beginning

of each day of the data collection. All active foragers were given free access to drinking a 45

percent sucrose solution inside the two arms in the Y-maze. To select the most active

bumblebee for that day, the first bumblebee to complete five trials was selected. One trial was

defined as the bumblebee entering the Y-maze, drinking sucrose solution until saturated from

one of the two arms and returning to the hive. Individual bumblebees could only be selected

to participate in an experimental session one time. If the first bumblebee to complete five

trials had participated in an experimental session before, the next bumblebee to complete five

trials was selected instead. All active foragers, except for the selected bumblebee, were

removed and placed in a temporary container, with access to sucrose solution, during the

experiment sessions to ensure that the selected bumblebee made independent choices. If a

bumblebee that was not actively foraging during the selection and therefore not removed,

entered the Y-maze during the experimental session, she was also removed.

Forced-choice trials and preference test. The left arm in the Y-maze was assigned

with a less sweet reinforcer and the right arm was assigned with a sweeter reinforcer. The less

sweet reinforcer consisted of a 35 % sucrose solution (35 % sugar and 65 % water), and the

sweeter reinforcer consisted of a 55 % sucrose solution (55 % sugar and 45 % water). The

yellow and blue stimuli were introduced as visual cues to facilitate discrimination between

the two reinforcers. The circle on the wall around the entrance to the two arms was filled with

yellow on one arm and blue on the second arm. Three bumblebees completed the experiment

with the blue stimuli as a visual cue for less sweet reinforcer on the left arm and the yellow

stimuli as a visual cue for sweeter reinforcer on the right arm. The two remaining bumblebees

completed the experiment with the yellow stimulus as a visual cue for less sweet reinforcer

on the left arm and the blue stimulus as a visual cue for sweeter reinforcer on the right arm.

The blue and yellow colors were in the bumblebees range of color vision (Meyer-Rochow,

2019).

The selected bumblebee was introduced to the less sweet reinforcer and the sweeter

reinforcer in four forced-choice trials. The four forced-choice trials were completed to ensure

that she had experience with the contingencies for both the less sweet and the sweeter

reinforcer (Madden & Johnson, 2010, p. 24). The forced-choice trials consisted of two trials

with less sweet reinforcer and two with sweeter reinforcer presented in an alternated

sequence, starting with the less sweet reinforcer. Even though both arms were available to

enter, the reinforcer was only delivered in one of the two arms on each trial. During the

forced-choice trials with the less sweet reinforcer, the less sweet reinforcer was delivered

immediately after she entered the left arm. During the forced-choice trials with the sweeter

reinforcer, the sweeter reinforcer was delivered immediately after she entered the right arm.

The trial did not end until the bumblebee entered the arm with the forced reinforcer, drank

until saturated, and returned to the hive.

After the forced-choice trials were completed, a preference test was conducted to

ensure that she showed a preference for the sweeter reinforcer over the less sweet reinforcer

before the experimental conditions were introduced. The selected bumblebee was presented

with a minimum of six free-choice trials with the choice between entering the left arm with

the less sweet reinforcer delivered immediately and entering the right arm with the sweeter

reinforcer delivered immediately. The bumblebee was free to switch between the two

reinforcers, however a choice was defined as a response in which the bumblebee committed

to drink until saturated and get back to the hive. That was often, but not always, be the last

response in the trial. For example, the bumblebee might sample from the less sweet reinforcer

before committing to drink the sweeter reinforcer until saturated and return to the hive. It can

be argued that it is more ecologically valid when the bumblebees could switch freely between

the options because it is resembling their foraging behavior of drinking the sucrose solution

from individual flowers. The criteria set for assuming preference of the sweeter reinforcer

was that the selected bumblebee chose the sweeter reinforcer in at least five of the last six

consecutive free-choice trials. If she chose the less sweet in three coherent trials or if she did

not meet the criteria in the first six trials, she was exposed for one forced-choice trial with the

sweeter reinforcer before continuing with the free-choice trials until the criteria were met.

Adjusting delay procedure. After the criteria for assuming preference of the sweeter

reinforcer was met, the selected bumblebee was presented with a two-phased adjusting delay

procedure where the delay to the sweeter reinforcer was gradually increased while delay to

the less sweet reinforcer was held constant at 0 s. The less sweet reinforcer was delivered

immediately after the selected bumblebee entered the left arm, and the sweeter reinforcer was

delivered with an increasing delay after she entered the right arm in the Y-maze. Figure 5

illustrates the choice options. The delay countdown to the sweeter reinforcer began when she

made physical contact with the entrance to the right arm and reset if she lost contact with it.

In both phases, the delay to the sweeter reinforcer was increased until she demonstrated

preference reversal by choosing the less sweet over the sweeter reinforcer. In the first phase,

the delay to sweeter reinforcer was gradually increased over blocks consisting of 2 trials to

ensure that the criterion for delivery of the reinforcer was not increased too quick. The delay

began with 5 s in the first block, and if she chose the sweeter reinforcer in both trials, the

delay was increased with 5 s in the next block. The increasement continued with 5 s each block if she chose the sweeter reinforcer in both trials until the delay was 50 s. Hereafter, the delay was increased by 10 s for each block. The first phase of the adjusting delay procedure ended when she showed preference reversal by switching to choose the less sweet reinforcer delivered immediately over the sweeter reinforcer delivered after a delay.

Before phase two of the adjusting delay procedure began, delay to sweeter reinforcer was decreased to 0 s in one forced-choice trial with the sweeter reinforcer. In phase two, the adjusting procedure started with one trial with 5 s delay on the sweeter reinforcer followed by one trial with 10 s delay on the sweeter reinforcer. If preference reversal was shown when the delay was 50 s or higher in phase one, the delay was increased with 10 s every trial in phase two. If preference reversal was shown when the delay was less than 50 s in phase one, the delay was increased with 5 s every trial in phase two. When delay was increased to the same duration as it was in the block preceding the one she showed preference reversal in phase one, the delay was increased every second delay until show showed preference reversal a second time and the experimental session with the selected bumblebee that day ended. 30 percent disinfection was used to clean the apparatus between each experimental condition and airdried to remove possible scents from preceding trials. A flowchart of the procedural steps is shown in figure 4.

Results

Five bumblebees (ID: 29, 23, 21, 30, and 39) were exposed for an adjusting delay procedure to determine the duration of delay they showed preference reversal at and examine how their behavior was affected by increasing delay to the sweeter reinforcer. The selected bumblebees used an average of 6 h and 11.5 min ($SD = 43.2$ min) to complete the experimental session.

The bumblebees did not show any systematical side preferences during the selection, three bumblebees showed a preference for the right arm and two showed a preference for the left arm. All subjects met the criteria of choosing the sweeter reinforcer five out of the last six free-choice trials in the preference test. Two subjects (21 and 30) met the criteria without extra trials. The remaining three subjects (29, 23, and 39) choose the less sweet reinforcer in three free-choice trials and were therefore exposed to one forced-choice trial on the sweeter reinforcer before they met the criteria in the following six free-choice trials.

The point of preference reversal was registered as the delay in the last trial the bumblebees chose the sweeter reinforcer before switched to choose the less sweet reinforcer. Figure 6 visualizes the point where each individual bumblebee showed preference reversal in phase 1 and phase 2, in addition to the mean duration of delay subjects showed preference reversal in both phases. The point of preference reversal was similar in phase 1 and phase 2 between all bumblebees except for in bumblebee 21. A visual analysis of figure 6 indicates that the point of preference reversal in phase 1 appeared to be replicated in phase 2, corresponding with a non-significant statistical difference between the mean of delay the subjects showed preference reversal at during phase 1 ($M = 50$, $SD = 16.96$) and phase 2 ($M = 55$, $SD = 14.14$) in a paired sample t-test, t(4) = -.845, $p = .446$, $d = 13.23$. Two out of five bumblebees replicated the point of preference reversal perfectly by switching to choose the less sweet reinforcer at the same duration of delay in phase 2. Two of them replicated the point of preference reversal sufficiently in phase 2 by switching at 10 s lower than and higher than in phase 1. Whereas the remaining bumblebee did not replicate it because she showed preference reversal at an importantly lower delay in phase 1 than in phase 2.

A visual analysis of delay reset and time waited in right arm in relation to the required time of delay for the sweeter reinforcer to be delivered, was conducted. Delay reset and time waited in the right arm over trials with increasing delays in both phases is visualized in

Figure 7. The result indicated that the subjects showed a general pattern of managing to wait the required time of delay for sweeter reinforcer to be delivered without resetting the delay until preference reversal, however with a few exceptions. The exceptions were that delay was reset once in two trials for subject 29 in phase 1, once in one trial for subject 21 in phase 1 and two times in one trial in phase 2, and once in one trial for subject 30 in phase 1. In phase 1, the delay interval was reset several times in the trials the bumblebees showed preference reversal, whereas in phase 2 two out of five bumblebees reset the delay interval. Bumblebee 23 waited 1 s less than the required time for delivery of the sweeter reinforcer in the trial she showed preference reversal in phase 1. Bumblebee 21 and 39 waited 1 s less than the required time for delivery of the sweeter reinforcer in the trial they showed preference reversal in phase 2.

A visual analysis of the number of visits to the arm with the less sweet reinforcer in each trial was conducted to examine switching between options when the delay was gradually increased, see figure 8. In trials prior to preference reversal, the bumblebees had few visits to the arm with the less sweet reinforcer in both phases, typically around one visit. In the trials the bumblebees showed preference reversal, they had a significantly higher number of visits to the arm with the less sweet reinforcer.

The Intertrial interval (ITI) was calculated as the amount of time the bumblebees used from the start of one trial, registered as entered the Y-maze, to the start of the next trial. Thus, the ITI depended on the bumblebee and when it returned to the Y-maze from the hive between trials. The mean intertrial interval in seconds from the trials prior to preference reversal in phase 1 and phase 2 from bumblebee 23, 21, and 30, in addition to the mean intertrial interval from the four bumblebees in both phases, is visualized in figure 9. The intertrial interval from bumblebee 29 was not reported because time outside of Y-maze was not registered. Four outliers were identified with a criterion of more than 3 z-scores above or

underneath the mean. The four outliers were removed because they represent something else than ITI and they are included in figure 10. The bumblebees were on average exposed to the procedure for 6 hours, and it is only natural that they showed deviant behavior, for example staying longer in the hive or flying in the flying arena. The figure shows that intertrial intervals were generally stable over increasing delays. Which is consistent with a statistically non-significant Pearson's correlation test between intertrial interval and delay from all four bumblebees in the trials prior to preference reversal neither in phase 1, $r(89) = .034$, $p = .750$, nor in phase 2, $r(41) = -.156$, $p = .329$. A paired sample t-test showed significantly higher intertrial interval from the four bumblebees in phase 2 ($M = 523.98$, $SD = 83.3$) than in phase 1 ($M = 375$, $SD = 35.9$), t(38) = -5.063, $p < .001$, $d = -.811$, 95% CI [-1.16, -.444].

Intertrial interval in each trial prior to preference reversal with the increasing delay in phases 1 and 2 from bumblebee 23, 21, 30, and 39 is visualized in figure 10. The outliers are included in figure 10. The intertrial interval from bumblebee 29 was not reported for the same reason as it was not reported in figure 9. The bumblebees showed relatively stable intertrial intervals over increasing delays in the trials prior to preference reversal in both phases. Bumblebee 23, 21, and 39 show one outlier that was visually higher than the other ITI in phase 1, corresponding to the significant statistical higher ITI in phase 2 than in phase 1, ITI was visually higher in phase 2 than in phase 1 in figure 10. In addition, ITI varied more in phase 2 than in phase 1.

Discussion

The aim of the present study was to develop and refine a procedure to investigate delay discounting in bumblebees. Preference reversal was tested by systematically increasing delay to a sweeter reinforcer in an adjusting delay procedure, and determining the point that bumblebees switched from choosing the sweeter delayed reinforcer to choosing the less sweet reinforcer. The overall results show that the bumblebees demonstrated high levels of self-

control by choosing the sweeter delayed reinforcers over the less sweet immediate reinforcers

when delays were substantially increased (see figure 6). The bumblebees average point of

preference reversal was at 50 s in phase 1, which was replicated in phase 2 with average

preference reversal at 55 s. Switching between the two options was measured as the number

of delays reset and overall visits to the alternative with the less sweet reinforcer. Delay resets

(figure 7) and overall visits to less sweet alternative (figure 8) shows that bumblebees visited

the less sweet arm of the apparatus relatively more frequent at preference reversal trials.

The results on the present study corroborate with those found by Cheng et al. (2002), bees

(honeybees and bumblebees) choose sweeter and delayed over less sweet and immediate

reinforces. These results support the argument that bees are able to emit self-controlled

choices. However, Cheng et al. (2002) study only manipulated a single delay duration (5 s),

whereas the present study systematically increased delayed duration to determine the point of

preference reversal. Cheng et al. (2002) found that not all of the honeybees showed a

preference for the sweeter delayed reinforcer, which was not the case in the present study.

This difference is likely due to procedural and measurement disparity between both studies.

In Cheng et al. (2002), a choice was defined as the first alternative visited by bees, even with

they switched to and fed from delayed and sweeter alternative before returning to the hive.

The bumblebees in the present study often visited the less sweet alternative and sampled the

less sweet reinforce by poking it with their proboscis. They would then switch to the sweeter

and delayed alternative and committing to the feeding before return to the hive (see figure 8).

Therefore, the reason that some Cheng et al. (2002) honeybees did not show a preference for

the sweeter reinforcer may be due to their definition of choice. In the present study, a choice

was instead defined as the choice the bumblebee committed to feed until saturated before

returning to the hive, which often was, but not always, the last response in the trial. This

definition resembles a more natural foraging behavior because they can respond to both

reinforcers before choosing one of them, which can be viewed as more ecologically valid.

Even though the intertrial interval (ITI) was not adjusted in the present study, the

bumblebees showed stable ITI across experimental sessions, with no differences for trials

with longer delays. The means of ITI in phase 1 and phase 2 were similar, however with

more observable variability in phase 2. The ITI in phase 2 might have been more variable

because as fewer data points were collected, and delays were increased more drastically. ITI

is commonly adjusted in delay discounting procedures with animals to control for differences

in the total reinforcer rate by ensuring that the time between choice opportunities is held

constant independent of the bumblebees choice (Madden & Johnson, 2010, p. 24). However,

Hayden (2016) pointed out that when adjusting the ITI, it is assumed that the animals are

aware of events happening after a reinforcer is delivered, even though there is little evidence

supporting this assumption. For example, ITI did not seem to affect the degree of delay

discounting when ITI was manipulated in monkeys (Pearson et al., 2010), starlings (Bateson

& Kacelnik, 1996), and rats (Goldshmidt et al., 1998; Sjoberg et al., 2021). Sjoberg et al.

(2021) even pointed out that it may not be necessary to adjust the ITI in delay discounting

procedures with animals when they seem unaffected by ITI. The ITI in the present study did

not vary with increases in delays, indicating that preference reversal was not affected.

However, further research is needed to determine if it is necessary to adjust ITI in delay

discounting procedures with bumblebees. For example, by examining if bumblebees show

preference reversal at different points if ITI. For that, a waiting are may be added before the

Y maze entrance, where ITI is manipulated by restricting access to both reinforces.

The present study contributes to the understanding of delay discounting in bumblebees by

refinement of the procedure and suggesting procedural improvements to choice definition.

The results indicate that choice should be defined as the reinforcer the bumblebee committees

to feed until saturation and not just as the first alternative visited. The reason to such

definition of choice is because they frequently visit the less sweet alternative before choosing

alternative and committing to feed until saturation and return to the hive (see figure 8). The

bumblebees would therefore be able to freely visit both alternatives before committing to a

choice, which increases ecological validity by better resembling a natural foraging behavior.

However, the delay should be reset if the bumblebees leave the sweeter reinforcer before the

delay is counted down. That is to ensure that they do not leave before the countdown to

respond to the less sweet reinforcer and return to the sweeter reinforcer being delivered

without them actually waiting the delay. In this way, each choice chamber function as an

distinct "artificial flower", the first with less caloric value but that can be accessed

immediately after the visit, and the second with a higher caloric value, but that the bumblebee

should wait to feed after every visit (reset criteria).

Future research may provide insights on bumblebee delay discounting by finding

indifference points, rather than preference reversal. That would require to test bumblebees

over several days. In the present experiment, the bumblebees were only exposed to an

adjusting delay procedure in one experimental session, even though sessions consisted of an

average of 6 hours. Even though the present procedure did not consist of several sessions, the

point of preference reversal was replicated in phase 2. A procedural improvement might be to

find a way of determining preference reversal more precisely by including more data points.

Testing delay over several session would also allow testing a procedure similar to Evenden

and Ryan (1996), thus finding a more accurate estimates of the point of preference reversal.

Challenges to test bumblebees over several days include their short lifespan and difference in

activity levels along the days.

Even though testing the ecological analysis hypotheses and metabolic hypotheses were

not the focus of the present study, the results can still be looked at in relation to the

hypotheses. The result in the present study indicated that the bumblebees typically preferred the sweeter delayed reinforcer, corroborating the ecological analysis hypotheses (Logue, 1988). However, the present procedure did not allow for testing the metabolic hypothesis (Tobin & Logue, 1994). That is because only the delay, and not the metabolic state of bumblebees, was manipulated. Mayack and Naug (2015) examined if honeybees showed different degrees of self-control on different levels of starvation in an association procedure by measuring choice as a proboscis extension response (PER) reflex. Their result indicated that the honeybees showed less self-control when starved. Importantly, Mayack and Naug (2015) pointed out that the ecological analysis hypothesis and the metabolic hypothesis do not necessarily need to be mutually exclusive. Although the bumblebees tested in the present study typically preferred the sweeter delayed reinforcer, their metabolic needs were likely met, as the distance needed to enter the experimental apparatus was insignificant. It is challenging to deprive each individual bumblebee, as we are actually depriving the whole hive, allowing for caloric storage in the hive. Therefore, depriving it would have to be examined by starving bumblebees before every trial, which would be difficult. A solution to test the metabolic hypothesis might instead be to expose the bumblebees with varying body size to a delay discounting procedure to examine if there is a correlation between body sizes or and the point of preference reversal.

Limitations to the present study must be acknowledged. The inconsistency in delay increasement might have affected the point the bumblebees showed preference reversal at, thus contributing to the noisy data. However, more research with more data points is needed to increase reliability. In addition, questions concerning if the choices were reinforced when bumbles accessed the sucrose solution or when they unload the sucrose solution back in the hive can be raised (Cheng et al., 2002). If it is reinforcing when they are unloading the solution back in the hive, both reinforcers would have been delayed. Therefore, the choice

might not have been between a less sweet immediate reinforcer and a sweeter delayed

reinforcer. Instead, it might have been between a less sweet sooner reinforcer and a sweeter

later reinforcer that both are further in the future than assumed. If so, it could potentially have

affected the point the bumblebees showed preference reversal at because the subjective value

of the sweeter later reinforcer might have been discounted more steeply than the subjective

value of the less sweet sooner reinforcer when the delay to both reinforcers was higher than

assumed (Green & Myerson, 2004). This can for example be studied by determining if the

point of preference reversal varies when manipulating the delay to both reinforcers.

Research on delay discounting in bumblebees may provide useful information to the

understanding of interspecies variation in self-control. Because bumblebees are both highly

eusocial and small in size, examining delay discounting in bumblebees can put the ecological

analysis hypotheses and the metabolic hypotheses to the test. Before examining the questions

concerning interspecies variation in self-control and the two hypotheses, it is beneficial to

establish basic knowledge about how to apply delay discounting procedures in bumblebees.

Few studies have examined delay discounting in bumblebees, therefore the present study

aimed to contribute to the understanding of delay discounting in bumblebees through

methodological development and refinement of a delay discounting procedure. By taking the

procedural refinements and suggested procedural improvements into consideration when

designing delay discounting procedures with bumblebees as subjects in future research, basic

research on procedures should be applied in bumblebees can be established. Thus, making it

possible to examine the bigger questions concerning interspecies variation in self-control

through experiments with bumblebees.

References

Ainslie, G. (1975). Specious reward: A behavioral theory of impulsiveness and impulse

control. *Psychological Bulletin, 82*(4), 463-496. https://doi.org/10.1037/h0076860

Ainslie, G. W. (1974). Impuls Control in Pigeons. *Journal of the Experimental Analysis of

Behavior, 21*(3), 485-489. https://doi.org/10.1901/jeab.1974.21-485

Apple. (2021). *Clock.* In (Version iOS 14) [Mobile App]. App Store.

https://support.apple.com/no-no/guide/iphone/iph96b1e460/ios

Barkley, R. A., Edwards, G., Laneri, M., Fletcher, K., & Metevia, L. (2001). Executive

functioning, temporal discounting, and sense of time in adolescents with attention

deficit hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD).

Journal of Abnormal Child Psychology, 29(6), 541-556.

https://doi.org/10.1023/a:1012233310098

Bateson, M., & Kacelnik, A. (1996). Rate currencies and the foraging starling: The fallacy of

the averages revisited. *Behavioral Ecology, 7*(3), 341-352.

https://doi.org/10.1093/beheco/7.3.341

Baumeister, R. F., Dewall, C. N., Ciarocco, N. J., & Twenge, J. M. (2005). Social exclusion

impairs self-regulation. *Journal of Personality and Social Psychology, 88*(4), 589-

604. https://doi.org/10.1037/0022-3514.88.4.589

Cardinal, R. N., Pennicott, D. R., Lakmali, C., Sugathapala, Robbins, T. W., & Everitt, B. J.

(2001). Impulsive choice induced in rats by lesions of the nucleus accumbens core.

Science, 292(5526), 2499-2501. https://doi.org/10.1126/science.1060818

Cheng, K., Peña, J., Porter, M. A., & Irwin, J. D. (2002). Self-control in honeybees.

Psychonomic Bulletin & Review, 9(2), 259-263. https://doi.org/10.3758/BF03196280

Chung, S.-H., & Herrnstein, R. J. (1967). Choice and delay of reinforcement. *Journal of the Experimental Analysis of Behavior, 10*(1), 67-74. https://doi.org/10.1901/jeab.1967.10-67

Critchfield, T. S., & Kollins, S. H. (2001, Spring). Temporal discounting: basic research and the analysis of socially important behavior. *Journal of Applied Behavior Analysis, 34*(1), 101-122. https://doi.org/10.1901/jaba.2001.34-101

Evenden, J. L., & Ryan, C. N. (1996). The pharmacology of impulsive behaviour in rats: The effects of drugs on response choice with varying delays of reinforcement. *Psychopharmacology, 128*(2), 161-170. https://doi.org/10.1007/s002130050121

Forskrift om bruk av dyr i forsøk. (2015). *Forskrift om bruk av dyr i forsøk* (FOR-2015-06-18-761). https://lovdata.no/forskrift/2015-06-18-761/§6

Forzano, L. B., & Corry, R. J. (1998). Self-control and impulsiveness in adult human females: Effects of visual food cues. *Learning and Motivation, 29*(2), 184-199. https://doi.org/https://doi.org/10.1006/lmot.1998.1001

Fox, A. T., Hand, D. J., & Reilly, M. P. (2008). Impulsive choice in a rodent model of attention-deficit/hyperactivity disorder. *Behavioural Brain Research, 187*(1), 146-152. https://doi.org/10.1016/j.bbr.2007.09.008

Gailliot, M. T., & Baumeister, R. F. (2007). The physiology of willpower: Linking blood glucose to self-control. *Personality and Social Psychology Review, 11*(4), 303-327. https://doi.org/10.1177/1088868307303030

Giurfa, M., Hammer, M., Stach, S., Stollhoff, N., Müller-Deisig, N., & Mizyrycki, C. (1999). Pattern learning by honeybees: Conditioning procedure and recognition strategy. *Animal Behaviour, 57*(2), 315-324. https://doi.org/10.1006/anbe.1998.0957

Goldshmidt, J. N., Lattal, K. M., & Fantino, E. (1998). Context efffects on choice. *Journal of the Experimental Analysis of Behavior, 70*(3), 301-320. https://doi.org/10.1901/jeab.1998.70-301

Green, L., Fry, A. F., & Myerson, J. (1994). Discounting of delayed rewards: A life-span comparison. *Psychological science, 5*(1), 33-36. https://doi.org/10.1111/j.1467-9280.1994.tb00610.x

Green, L., & Myerson, J. (2004). A discounting framework for choice with delayed and probabilistic rewards. *Psychological Bulletin, 130*(5), 769-792. https://doi.org/10.1037/0033-2909.130.5.769

Green, L., Myerson, J., & McFadden, E. (1997). Rate of temporal discounting decreases with amount of reward. *Memory & Cognition, 25*, 715-723. https://doi.org/10.3758/BF03211314

Green, L., Myerson, J., Shah, A., Estle, S., & Holt, D. (2007). Do adjusting-amount and adjusting-delay procedures produce equivalent estimates of subjective value in pigeons? *Journal of the Experimental Analysis of Behavior, 87*, 337-347. https://doi.org/10.1901/jeab.2007.37-06

Hayden, B. Y. (2016). Time discounting and time preference in animals: A critical review. *Psychonomic Bulletin & Review, 23*(1), 39-53. https://doi.org/10.3758/s13423-015-0879-3

Heinrich, B. (1977). The physiology of exercise in the bumblebee: Bumblebees are limited in their superb athletic performance by extremes of air temperature, but by regulating their body temperature they achieve considerable control. *American Scientist, 65*(4), 455-465. http://www.jstor.org/stable/27847968

Herrnstein, R. J. (1981). Self-control as response strength. In C. M. Bradshaw, E. Szabadi, &

    C. F. Lowe (Eds.), *Quantification of steady-state operant behavior* (pp. 3-20).

    Elsevier.

Kabadayi, C., & Osvath, M. (2017). Ravens parallel great apes in flexible planning for tool-

    use and bartering. *Science, 357*(6347), 202-204.

    https://doi.org/10.1126/science.aam8138

Kirby, K. N., & Petry, N. M. (2004, Apr). Heroin and cocaine abusers have higher discount

    rates for delayed rewards than alcoholics or non-drug-using controls. *Addiction,*

    99(4), 461-471. https://doi.org/10.1111/j.1360-0443.2003.00669.x

Kwon, Y. J., & Saeed, S. (2003). Effect of temperature on the foraging activity of Bombus

    terrestris L. (Hymenoptera: Apidae) on greenhouse hot pepper (Capsicum annuum

    L.). *Applied Entomology and Zoology, 38*(3), 275-280.

    https://doi.org/10.1303/aez.2003.275

Logue, A. W. (1988). Research on self-control: An integrating framework. *Behavioral and*

    Brain Sciences, 11(4), 665-709. https://doi.org/10.1017/S0140525X00053978

Madden, G., & Johnson, P. (2010). A delay-discounting primer. In G. Madden & W. K.

    Bickel (Eds.), *Impulsivity: The behavioral and neurological science of discounting*

    (pp. 11-37). American Psychological Association. https://doi.org/10.1037/12069-001

Mayack, C., & Naug, D. (2015). Starving Honeybees Lose Self-control. *Biology Letters,*

    11(1). https://doi.org/doi:10.1098/rsbl.2014.0820

Mazur, J. E. (1987). An adjusting procedure for studying delayed reinforcement. In M. L.

    Commons, J. E. Mazur, J. A. Nevin, & H. Rachlin (Eds.), *Quantitative analyses of*

behavior Vol. 5. The effect of delay and of intervening events on reinforcement value (pp. 55-73). Lawrence Erlbaum Associates, Inc.

McDiarmid, C. G., & Rilling, M. E. (1965). Reinforcement delay and reinforcement rate as determinants of schedule preference. *Psychonomic Science, 2*(1-12), 195-196. https://doi.org/10.3758/bf03343402

Merling, M., Eisenmann, S., & Bloch, G. (2020). Body size but not age influences phototaxis in bumble bee (Bombus terrestris, L.) workers. *Apidologie, 51*(5), 763-776. https://doi.org/10.1007/s13592-020-00759-0

Meyer-Rochow, V. (2019). Eyes and vision of the bumblebee: A brief review on how bumblebees detect and perceive flowers. *Journal of Apiculture, 34*, 107-115. https://doi.org/10.17519/apiculture.2019.06.34.2.107

Microsoft corporation. (2021). *Microsoft Visual Studio Community 2019.* In (Version 16.9.3) [Computer software]. Microsoft. https://visualstudio.microsoft.com/vs/

Nasir, M., Ata ul, M., Ahmad, M., Saeed, S., Aziz, M. A., Imran, M., & Sheikh, U. A. A. (2019). Effect of different temperatures on colony characteristics of Bombus Terrestris (Hymenoptera: Apidae) [Article]. *Pakistan Journal of Zoology, 51*(4), 1315-1322. https://doi.org/10.17582/journal.pjz/2019.51.4.1315.1322

Odum, A. L. (2011). Delay discounting: I'm a k, you're a k. *Journal of the Experimental Analysis of Behavior, 96*(3), 427-439. https://doi.org/10.1901/jeab.2011.96-423

Osvath, M., & Osvath, H. (2008). Chimpanzee (Pan troglodytes) and orangutan (Pongo abelii) forethought: self-control and pre-experience in the face of future tool use. *Animal Cognition, 11*(4), 661-674. https://doi.org/10.1007/s10071-008-0157-0

Pearson, J., Hayden, B., & Platt, M. (2010, 2010-December-29). Explicit Information

    Reduces Discounting Behavior in Monkeys [Original Research]. *Frontiers in*

    Psychology, 1(237). https://doi.org/10.3389/fpsyg.2010.00237

Peat, J., & Goulson, D. (2005). Effects of experience and weather on foraging rate and pollen

    versus nectar collection in the bumblebee, Bombus terrestris. *Behavioral Ecology and*

    Sociobiology, 58(2), 152-156. https://doi.org/10.1007/s00265-005-0916-8

Petrillo, F. D., Micucci, A., Gori, E., Truppa, V., Ariely, D., & Addessi, E. (2015). Self-

    control depletion in tufted capuchin monkeys (Sapajus spp.): does delay of

    gratification rely on a limited resource? *Frontiers in Psychology, 6*.

    https://doi.org/10.3389/fpsyg.2015.01193

Rachlin, H., Raineri, A., & Cross, D. (1991). Subjective probability and delay. *Journal of the*

    Experimental Analysis of Behavior, 55(2), 233-244.

    https://doi.org/10.1901/jeab.1991.55-233

Richards, J. B., Mitchell, S. H., De Wit, H., & Seiden, L. S. (1997). Determination of

    discount functions in rats with an adjusting-amount procedure. *Journal of the*

    Experimental Analysis of Behavior, 67(3), 353-366.

    https://doi.org/10.1901/jeab.1997.67-353

Scheiner, R., Abramson, C. I., Brodschneider, R., Crailsheim, K., Farina, W. M., Fuchs, S.,

    Grünewald, B., Hahshold, S., Karrer, M., Koeniger, G., Koeniger, N., Menzel, R.,

    Mujagic, S., Radspieler, G., Schmickl, T., Schneider, C., Siegel, A. J., Szopek, M., &

    Thenius, R. (2013). Standard methods for behavioural studies of Apis mellifera.

    Journal of Apicultural Research, 52(4), 1-58. https://doi.org/10.3896/ibra.1.52.4.04

Sjoberg, E. A., Ramos, S., López-Tolsa, G. E., Johansen, E. B., & Pellón, R. (2021). The

    irrelevancy of the inter-trial interval in delay-discounting experiments on an animal

model of ADHD. *Behavioural Brain Research, 408*, 113236.

https://doi.org/10.1016/j.bbr.2021.113236

Smith, B. H., & Burden, C. M. (2014). A proboscis extension response protocol for

investigating behavioral plasticity in insects: Application to basic, biomedical, and

agricultural research. *Journal of Visualized Experiments*(91).

https://doi.org/10.3791/51057

Tesch, A. D., & Sanfey, A. G. (2008). Models and methods in delay discounting. *Annals of*

the New York Academy of Sciences, 1128(1), 90-94.

https://doi.org/10.1196/annals.1399.010

Tobin, H., & Logue, A. W. (1994). Self-control across species (Columba livia, Homo

sapiens, and Rattus norvegicus). *Journal of Comparative Psychology, 108*(2), 126-

133. https://doi.org/10.1037/0735-7036.108.2.126

Weatherly, J. N. (2010). Temporal discounting and gambling: A meaningful relationship?

Analysis of Gambling Behavior, 4(1), 27-37.

https://repository.stcloudstate.edu/agb/vol4/iss1/4

Wendt, S., & Czaczkes, T. J. (2017). Individual ant workers show self-control. *Biology*

Letters, 13(10). https://doi.org/10.1098/rsbl.2017.0450

Yoon, H. J., Kim, S. E., & Kim, Y. S. (2002). Temperature and humidity favorable for colony

development of the indoor-reared bumblebee, Bombus ignitus. *Applied Entomology*

and Zoology, 37(3), 419-423. https://doi.org/10.1303/aez.2002.419

Figure 1

Stimuli
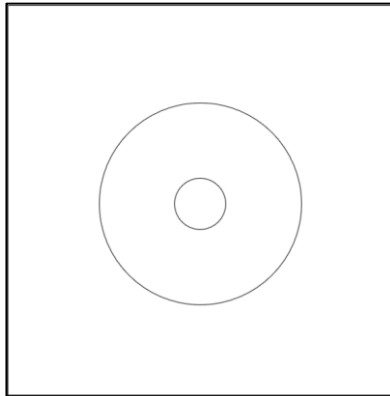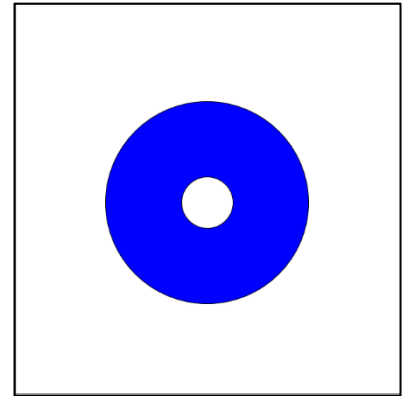
Stimulus 1   Stimulus 2   Stimulus 3
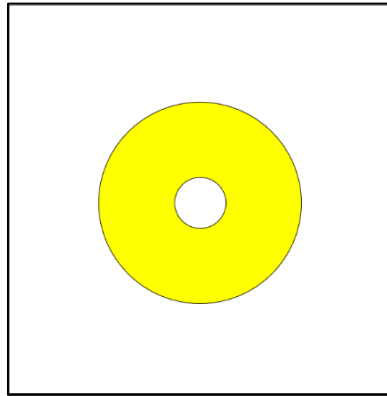
Note. The figure displays an overview of stimuli (20 cm x 20 cm) placed on the back walls around the entrance to the two arms in the Y-maze. All three stimuli were white laminated paper (20 cm x 20 cm) with a 10 cm black circle around the entrance. Stimulus 1 was used on both arms during feeding and selection. Stimuli 2 and 3 were used during data collection, one on each arm.

Figure 2

Apparatus

Note. This figure shows a 2D model of the apparatus. The flying arena consists of a rectangular box with white acrylic walls and floor with a glass lid (length = 120 cm, width = 100 cm, height = 50 cm). A landing platform (3 cm x 1 cm) was located underneath the entrance to the hive on the wall, and green marks are placed around the entrance to signal the hive entrance and facilitate navigation. A squared hole (30 cm x 70 cm) in the wall parallel to the wall with the hive entrance is covered with a brown cardboard with one white landing platform (length = 5 cm, width = 7 cm, height 2 cm) in the middle of the cardboard. A sunburst pattern with green stripes was drawn on the cardboard around the top half of the landing platform for easier navigation. The landing platform was connected to a white plastic entrance channel coved by plexiglass (length = 14 cm, width = 7 cm, height = 2 cm) leading to a Y-maze.

Figure 3

Y-maze
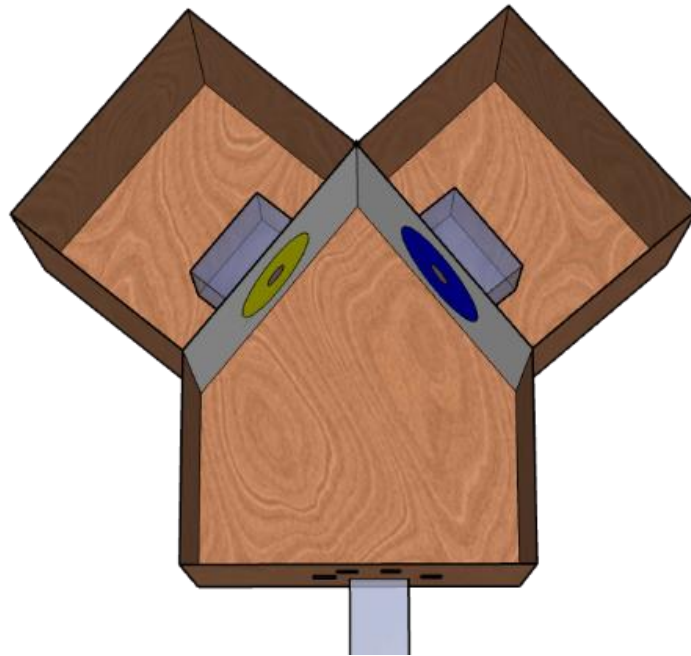
Note. The bumblebees entered the Y-maze through a hole (diameter = 2 cm) in the frontal wall connected to the end of the entrance channel. The first room they entered was a decision chamber where they were faced with two back walls (20 cm x 20 cm) on the arms of the Y-maze. Each back wall had a hole with 2 cm in diameter in the middle that is connected to a small plastic container (length = 5 cm, width = 6 cm, height = 6 cm).
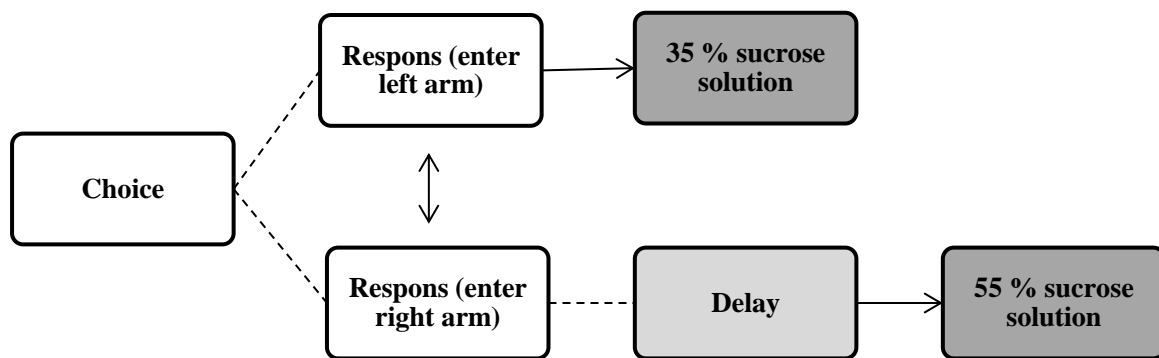
Figure 4

Procedural steps
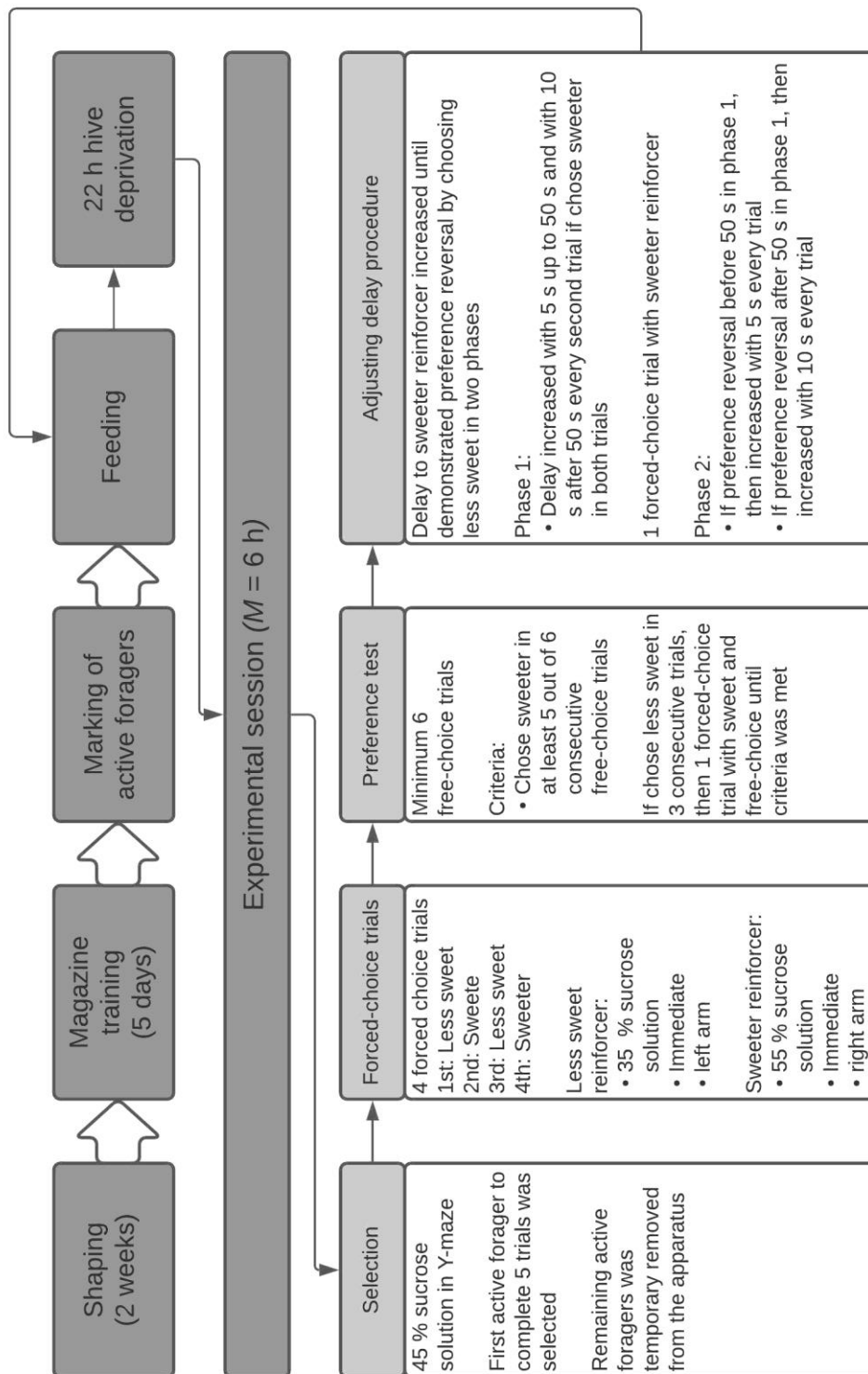
Note. Flowchart of the procedural steps.

Figure 5

Choice options

Note. Illustration of the choice options in one trial.
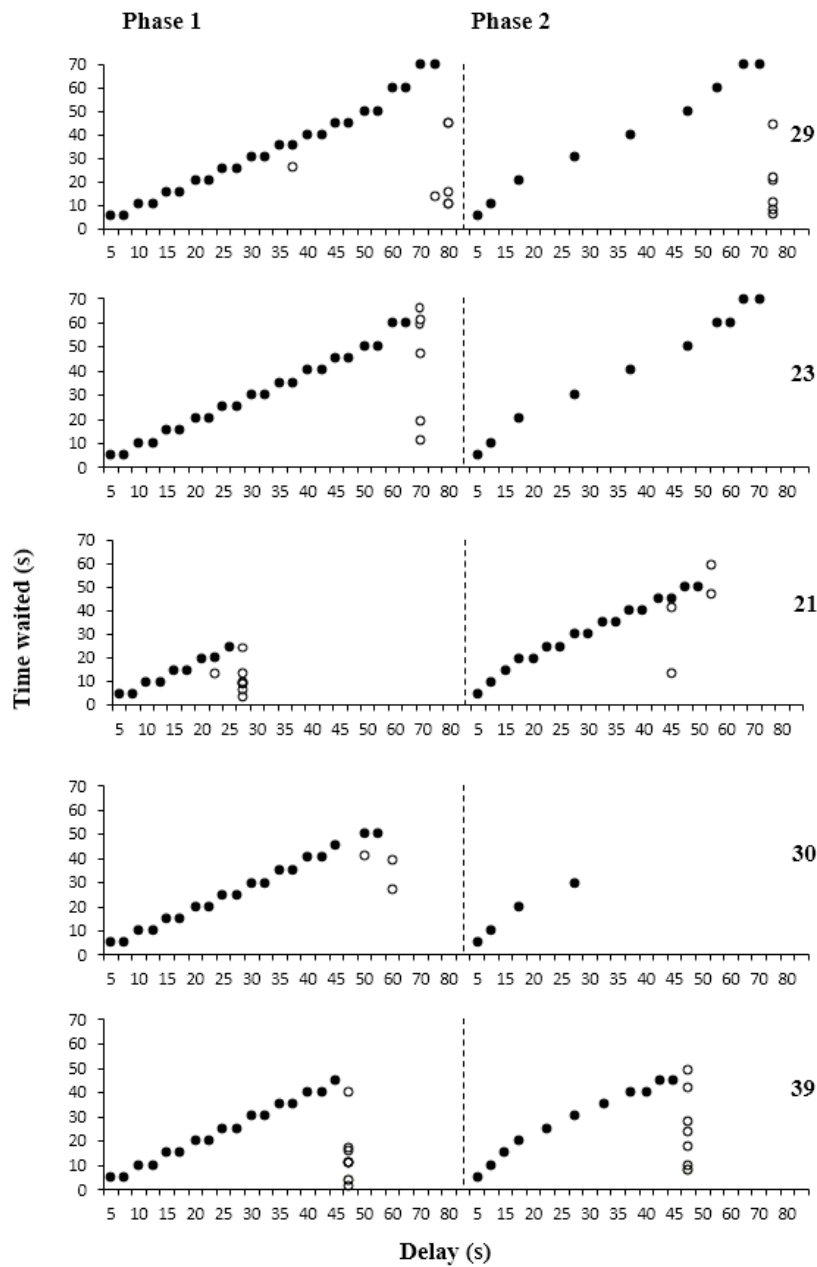
Figure 6

Preference reversal
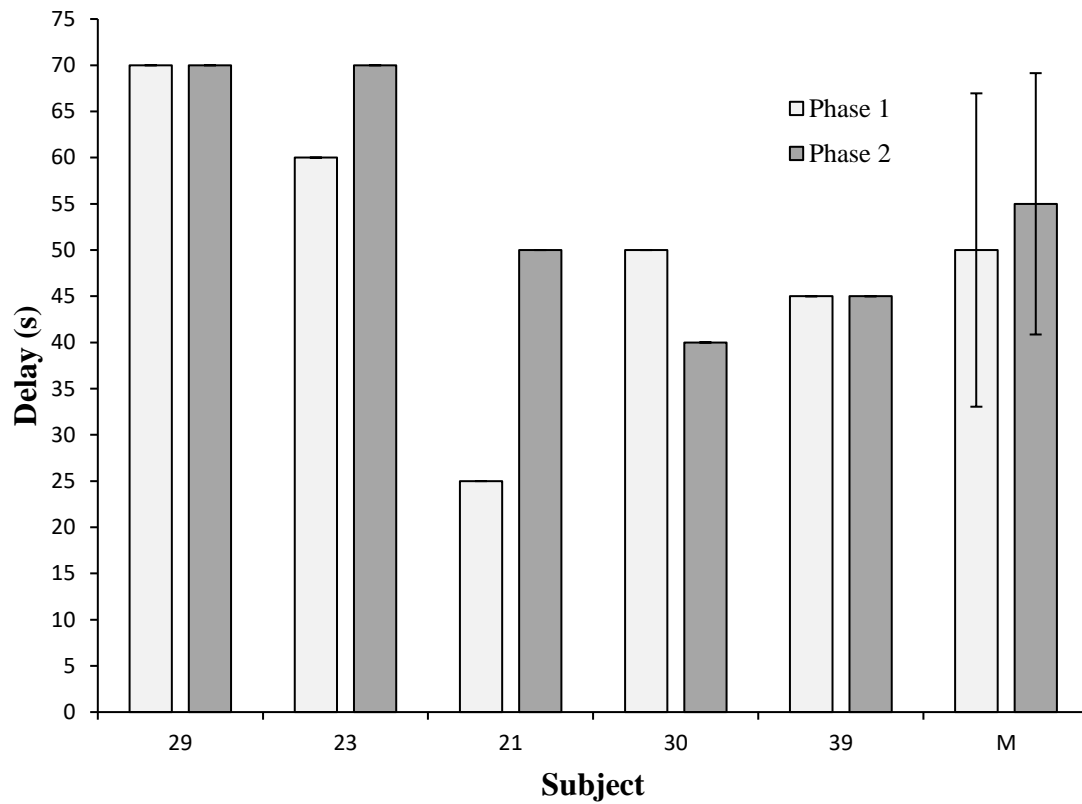
Note. Preference reversal registered as the delay to sweeter reinforcement in the last trial each

subject chose sweeter delayed reinforcement in both phases. Mean and SD of the delay

subjects showed preference reversal at in phase 1 ($M = 50$, $SD = 16.96$) and phase 2 ($M = 55$,

$SD = 14.14$) is visualized as subject M.

Figure 7

Delay reset

Note. Markers with solid fill represents when the selected bumblebee waited the required duration of delay and sweeter reinforcement was delivered. Markers with no fill represents when the selected bumblebee waited less than the required duration of delay, sweeter reinforcement was not delivered, and delay was reset. Markers with no fill that have a thicker line indicate that delay was reset more than once with the same time.
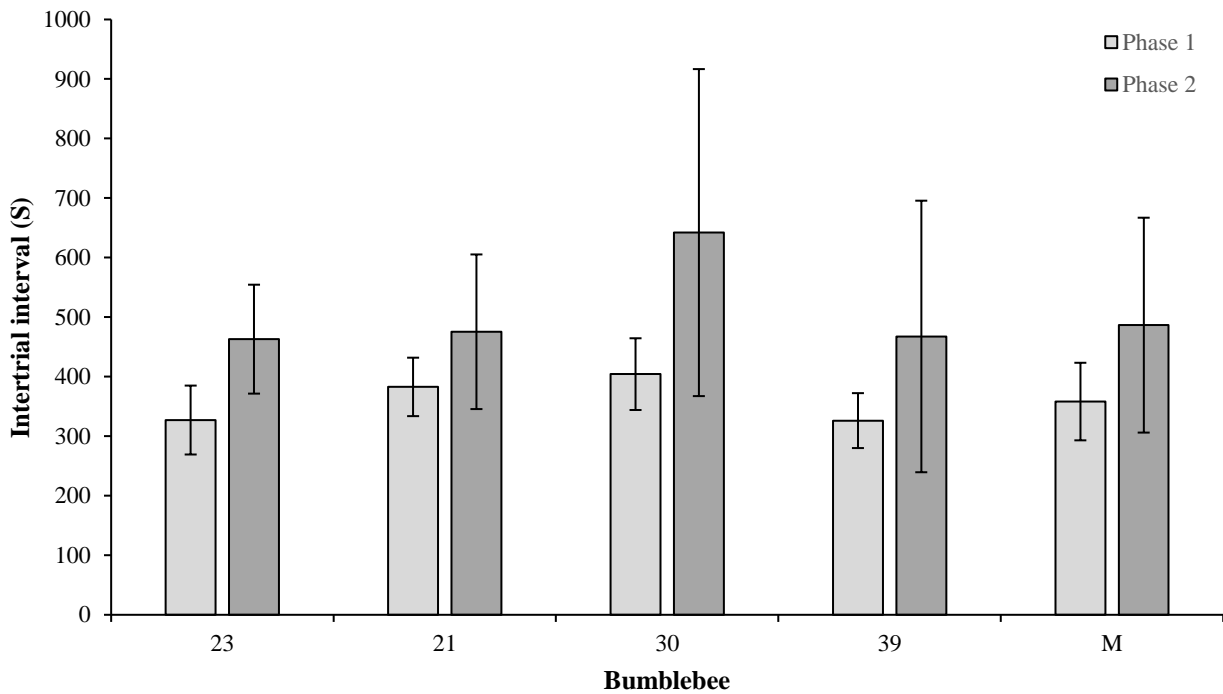
Figure 8

Visits to the arm with less sweet reinforcer
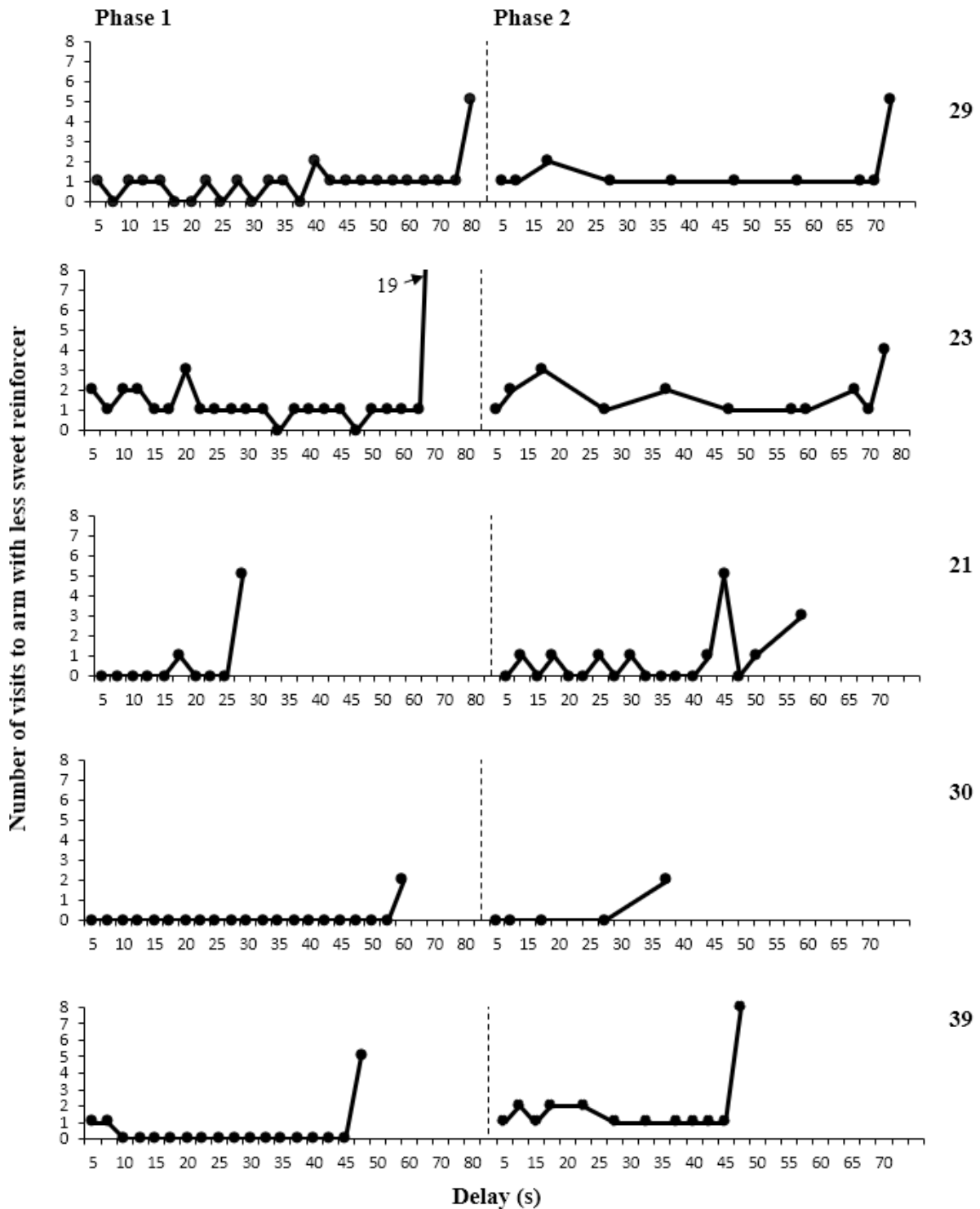
Note. The last datapoint in phase 1 from subject 23 represents 19 visits to arm with less sweet reinforcement during the second trial with 60 s delay.

Figure 9

Intertrial interval: Phase 1 and 2 average

Note. Mean intertrial intervals in seconds from trials prior to preference reversal in phase 1

and phase 2 from bumblebee 23, 21, and 30. M represents the mean of the intertrial intervals

from the four bumblebees in both phases. Error-bars represent standard deviation. 4 outliers

were removed with a criterion of z-scores more than 3 above or underneath the mean.

Figure 10

Intertrial interval: Individual overview
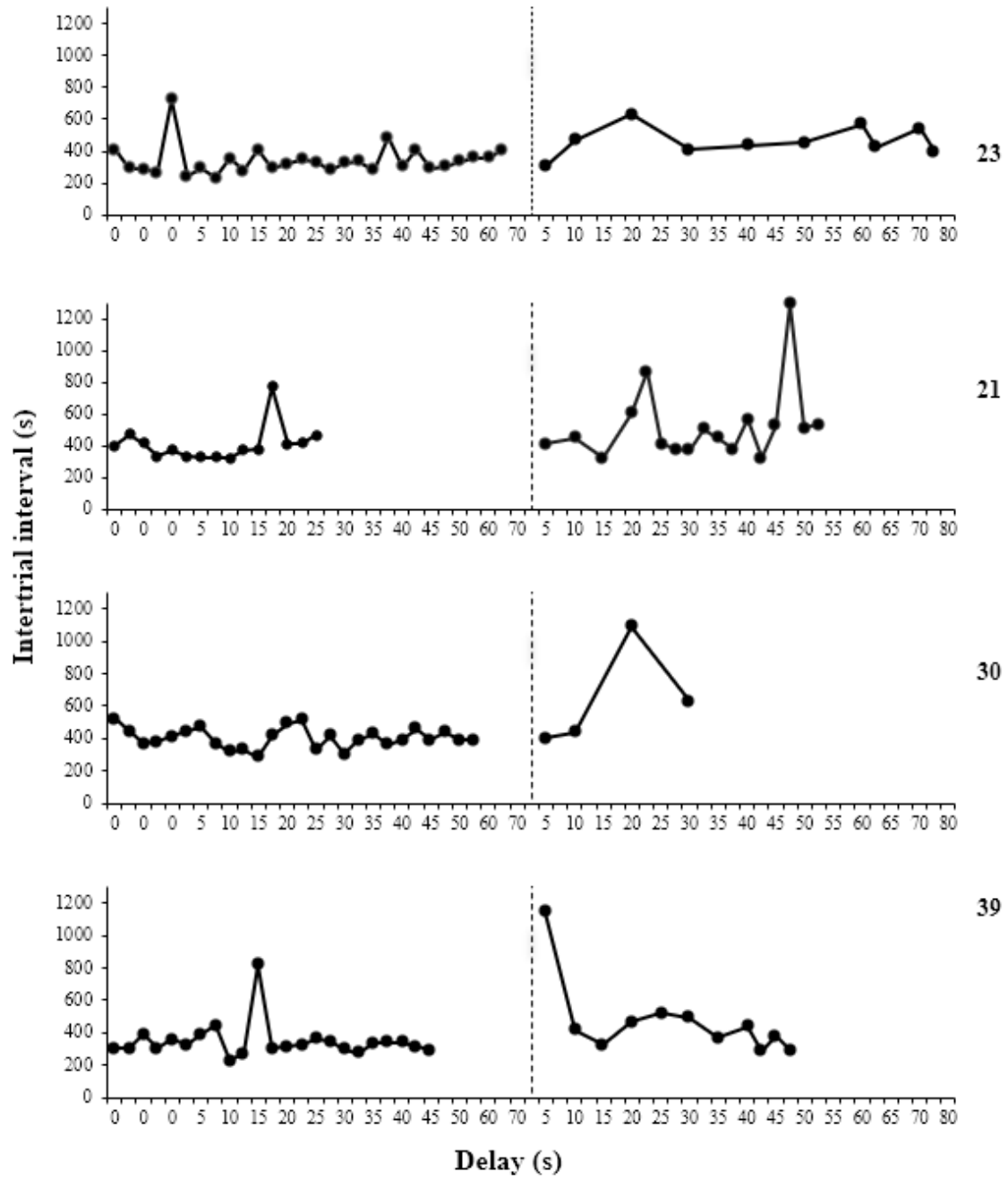
Note. Intertrial interval in seconds at in trials prior to preference reversal with increasing delay from subject 23, 21, 30 and 39.