



## Assessing restricted stimulus control in typically developing preschool children and bees (*Melipona quadrifasciata*)

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### Abstract

This study established a simple simultaneous discrimination between a pair of two-element compound visual stimuli in children (Experiment 1) and bees (*Melipona quadrifasciata*, Experiment 2). The contingencies required discriminative control by the compound and the question was whether the accurate stimulus control reached at this level would hold for each individual element of the compound. After baseline reached stability, probe trials assessed stimulus control by each single element of both S+ and S-. Average data showed that children (Exp. 1) tended to show stimulus control by a single element of the S+ compound. In Experiment 2 three of four bees showed stimulus control by both elements of S+ and did not respond or responded only infrequently to the elements of the S-. The children's decline in discrimination accuracy in probe trials, along with its maintenance during the baseline, replicated previous findings showing the development of restricted stimulus control (RSC). The precise stimulus control shown by the bees indicated that all elements correlated with reinforcement acquired stimulus control over their behavior; this confirms the extensive literature on visual discriminative learning in bees, but due to the small number of subjects it is premature to say that bees do not develop RSC.

**Keywords:** discriminative learning, compound stimuli, restricted stimulus control, preschool children, bees.

Received 11 July 2013; received in revised form 02 February 2014; accepted 26 February 2014. Available online 27 June 2014.

### Introduction

Restricted stimulus control (RSC) refers to discrimination learning with limitations in the range of controlling stimuli or stimulus features among those correlated with reinforcement (Dube et al., 2010). Any stimulus has many dimensions and a discrimination training may result in stimulus control by either all or only a subset of the potentially controlling dimensions or features. When reinforcement is contingent on a response in the presence of a multi dimensional stimulus, the contingency will be met even if the behavior occurs under the control of only one dimension of that stimulus (Dube & McIlvane, 1999).

Reynolds (1961) first described this kind of partial stimulus control in a study with pigeons, using compound stimuli. Two pigeons were exposed to a multiple variable interval (VI) extinction schedule. Pecking a key illuminated by a white triangle displayed on a red background was intermittently reinforced with food, whereas pecking the key illuminated by a white circle on a green background produced no food. Response rates became high and stable during the S+ element of the multiple schedules (triangle plus red). During the extinction element (S-: circle plus green) the birds rarely pecked the key. In the test phase that was conducted during extinction, the members of each compound stimulus (i.e., triangle+, circle-, red+, and green-) were displayed separately in intervals of 30 s each across the experimental session. One pigeon showed high response rates under the control of the triangle (+) and low response rates in the presence of the elements of the S- (circle-, green-), but also in the presence of the red element of the S+. The other pigeon showed high response rates to the red (+) element and low response rates to the other elements. Although the S+ stimulus was a compound stimulus (triangle on red) one pigeon “attended” to the triangle only,

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whereas the other pigeon attended to the red color only. Attention here simply describes the functional relation. The pigeons emitted responses under the control of a specific feature or property of the compound “white triangle on a red background” stimulus that correlated with reinforcement.

Many studies have sought to identify and describe restricted stimulus control (RSC; e.g., Bailey, 1981; Bickel, Stella, & Etzel, 1984; Dickson, Wang, Lombard, Dube, 2006; Domeniconi, de Rose, & Huziwara, 2007; Dube et al., 2010; Hale & Morgan, 1973; Litrownik, McInnis, Wetzel-Pritchard, & Filipelli, 1978; Lovaas & Schreibman, 1971; McHugh & Reed, 2007; Reynolds & Reed, 2011; Stromer, McIlvane, Dube, & Mackay, 1993).

Restricted stimulus control is often reported in individuals with intellectual or neurodevelopmental disabilities, such as individuals with autism spectrum disorder (for review, see Ploog, 2010). However, typically capable humans may also show RSC.

RSC in developing children at preschool ages was initially investigated by Eimas (1969). The participants in that study included 270 boys and girls in kindergarten and the 2<sup>nd</sup> and 4<sup>th</sup> grades, aged 5.4 years, 7.6 years and 9.5 years, respectively. Half of the participants were presented a red T-shaped form (S+) and a green triangle (S-). In the probe trials that tested for control by shape, both stimuli were presented in their original forms but in the same color (yellow). In the probe trials that tested the control by color, the participants were presented a red square and green square. Kindergarten children attended to both color and shape in the first task. In another task, in which the stimuli presented three relevant cues, the children attended to shape and size but interestingly failed to attend to color. In the task that presented color, shape, size, and borders as relevant dimensions, responding by the kindergarten children appeared to be controlled only by shape. Children in the 2<sup>nd</sup> grade failed to attend to color and borders, whereas the children in the 4<sup>th</sup> grade only failed to attend to the borders.

Bailey (1981) reported RSC in older children (mean age 7 years) described as “learning disabled children” (with social or learning difficulties, but a normal IQ). Similar to Eimas (1969), Bailey also employed a simultaneous discrimination procedure. The positive compound might be a card that contained the elements “sun”, “bird” and “shoe” displayed side-by-side. The negative compound might be a card that contained the elements “book”, “cat”, and “girl”. The test trials involved the presentation of one positive element together with one negative element. Eight of 16 participants showed RSC. Six participants responded to two of three positive elements, whereas two participants responded to only one positive element.

In a recent study, Reed, Stahmer, Suhrheinrich, & Schreibman (2013) investigated the occurrence of RSC in typically developing children between the ages of 19 and 50 months (mean age = 34.1 months). The

participants were trained with a simultaneous procedure to discriminate between two geometric shapes (wooden blocks) of different colors, a green cube and a pink pyramid. The stimulus designated as S+ (green cube or orange pyramid) was randomized across participants. The acquisition criterion was 80% correct choice responses. Discriminative control by shape was tested in 10 trials that presented a cube and a pyramid of the same color (i.e., green). Discriminative control by color was tested in 10 trials that presented two new blocks with the same shape, but with different colors (i.e., a T-shaped green block and a T-shaped pink block). RSC (or overselectivity) was defined as responding to one feature (shape or color) in at least 80% of the test trials of one type (i.e., at least eight of 10 trials), and responding to the other feature at chance levels, (i.e., 25-75% of the test trials of the other type), in which the number of responses was equal to or greater than three and equal to or smaller than seven. The criterion of at least 80% correct responses to one feature was chosen based on a 95% cut-off for a binomial distribution. In the training phase, 34 of 37 participants met the learning criterion. The group that showed RSC (eight participants) was significantly younger than the group that did not show RSC. Notably, the authors found that only the participants who were older than 36 months consistently responded to all of the positive features of a compound stimulus.

As pointed out by McHugh & Reed (2007), who reported RSC in older individuals, “the overselectivity effect bears on issues fundamental to understanding the circumstances under which stimulus control will occur” (p. 369), and the investigation of those conditions should be extended to normally developing humans (e.g., Eimas, 1969; McHugh & Reed, 2007; Reed & Gibson, 2005) and to nonhuman subjects (e.g., Brino et al., 2011; Brino, Galvão, Barros, Goulart, & McIlvane, 2012) under various procedures and variables. Research on stimulus control with nonhumans may help to determine the variables that are involved in the occurrence of RSC and perhaps may result in the development of additional procedures that can assess and control such variables.

Extensive literature shows that bees can easily discriminate between visual stimuli (Backhaus, 1993; Backhaus, Menzel, & Kreissl, 1987; Dyer & Chittka, 2004; Giurfa, Núñez, Chittka, & Menzel, 1995; Giurfa, Zhang, Jenett, Menzel, & Srinivasan, 2001; Gumbert, 2000; Hempel de Ibarra, Giurfa, & Vorobyev, 2002; Hertz, 1938; Lehrer, Horridge, Zhang, & Gadagkar, 1995; Lunau, Wacht, & Chittka, 1996; Menzel & Backhaus, 1991; von Frisch, 1967; Wu, Moreno, Tangen, & Reinhard, 2013), even when the visual stimuli are presented on a computer monitor (Brown, McKeon, Curley, Weston, Lambert, & Lobowitz, 1998; Cooke, Couvillon, & Bitterman, 2007). Such discriminative ability extends to the genus *Melipona* (Menzel, Ventura, Werner, Joaquim, & Backhaus, 1989; Moreno, de Souza, & Reinhard, 2012; Pessotti, 1972), which was used in

the present study. Bees can also master discriminative tasks that involve stimuli of different sensory modalities such as colors and odors (Giurfa et al., 2001; Reinhard, Srinivasan, Guez, & Zhang, 2004; Reinhard, Srinivasan, & Zhang, 2004; Srinivasan, Zhang, & Zhu, 1998), and tasks that involve arbitrary relations between colors and temperatures (Dyer, Whitney, Arnold, Glover, & Chittka, 2006).

Gould (1996) showed that the elements of compound stimuli may exert different degrees of control over the behavior of bees. The author taught *Apis mellifera* to collect a sugar solution in artificial blue flowers that were pepper-scented triangular shapes (S+) distributed among unrewarded yellow artificial flowers (S-). The honeybees consistently chose the correct stimulus (positive compounds). In the test phase, the stimulus elements (i.e., color, shape, and scent) were recombined and presented without sucrose (i.e., extinction). The results indicated that all of the positive elements exerted control but to different degrees. The scent exerted the highest degree of control, followed by color and shape. However, when shape was the only element presented, the bees correctly chose the positive element, suggesting discriminative control by shape. Although this study was not specifically designed to assess RSC, the results indicated that bees can attend to different features of a complex (compound) stimulus.

Bees may not attend only to different features of a complex stimulus, such as in the study by Gould (1996): the mere presence of a characteristic can also increase the discriminative control exerted by another characteristic. Kunze & Gumbert (2001) trained a group of 12 bumble bees (*Bombus terrestris*) to discriminate among colored flowers. The flowers defined as positive (S+) and negative (S-), received the same scent and differed only in color. Another group of 12 bumble bees received the same discriminative training, but the flowers were not scented. In both groups, color was the only feature relevant for discrimination. After 150 trials, the first group showed a mean percentage of 95% correct responses, whereas the other group showed less than 80% correct responses. The authors suggest that the scent is perceived before colors in foraging situations (Giurfa, Vorobyev, Kevan, & Menzel, 1996), and perceiving scent first may facilitate color discrimination. Kulahci, Dornhaus, & Papaj (2008) showed that bumble bees had more accurate discrimination of complex stimuli with color and scent compared with the discrimination of stimuli with just one sensory modality (color or scent). However, this study did not test the responses to the elements (color and scent) after the discrimination was established, and it was not possible to specify the controlling variables more precisely. Results like those of Gould (1996) led to one of the main questions of the present study: would bees respond to both positive elements of a compound stimulus after established visual simple discrimination? Or would they show restricted stimulus control?

For this purpose it was decided to test the experimental protocol simultaneously with children and bees. The rationale was that there is a large variability in procedures and parameters used to assess the controlling relations in discriminations with compound stimuli that make it difficult to compare the effects in different species (and even within-species).

The procedure was a simple simultaneous discrimination task displaying two pairs of compound stimuli, as in Eimas (1969), Bailey (1981) and Reed et al. (2013); thus, the study would enlarge the data of children on tests of control by single elements of compound discriminative stimuli and extend the investigation to bees (*Melipona quadrifasciata*). After establishing a stable baseline, probe trials assessed stimulus control by each single element of both the S+ and S-.

Experiment 1 – Assessing elemental (and RSC) stimulus control in typically developing preschool children

## Methods

### Participants

Seven typically developing preschool children (three boys and four girls), aged 27-53 months, participated in the experiment (Table 1). They were selected based on child and experimenter availability. Formal consent was obtained from their parents. The project was approved by the Ethics Committee on Human Research of the University (protocol no. CAAE 0156.0.000.135-07).

Table 1 presents the participants main characteristics. Four children showed verbal age-equivalent scores in the Peabody Picture Vocabulary Test-Revised (Dunn & Dunn, 1981) below their chronological age, with differences of 4 months (Ane), 5 months (Nico), and up to 1 year (Lili and Carol). Three of the other children (Rafa, Guto, and Yara) had verbal age-equivalent scores that were similar to their chronological ages. Two children (Lili and Rafa) had prior experience in simple discrimination studies. Three children used a computer mouse to respond to the stimuli on the screen, and the other children touched the screen directly.

**Table 1.** Participants, gender, chronological age, equivalent age in the Peabody Picture Vocabulary Test – Revised (PPVT-R, Dunn & Dunn, 1981), prior experimental history (with simple discrimination tasks) and use of a computer mouse or touch screen.

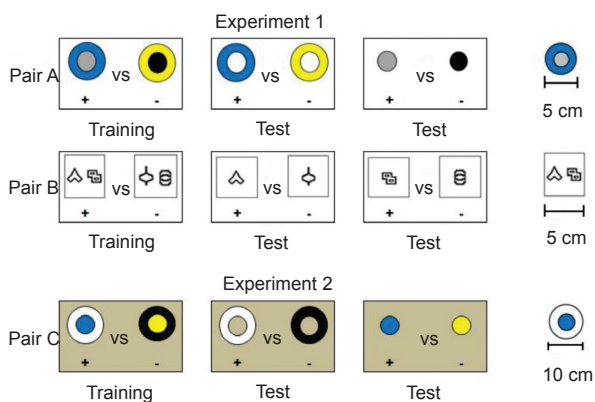
Participant	Gender (F / M)	Chronological age (years: months)	Equivalent age (years: months)	Previous experimental history	Mouse or touch screen
Lili	F	4:5	3:5	Yes	Mouse
Carol	F	4:3	3:3	No	Mouse
Rafa	M	4:1	4:2	Yes	Mouse
Guto	M	3:11	4:1	No	Screen
Nico	M	3:6	3:1	No	Screen
Ane	F	2:6	2:2	No	Screen
Yara	F	2:3	2:2	No	Screen

### Setting and materials

The sessions were conducted in the children's school in a room specially designated for data collection. The tasks were presented via a desktop computer (Macintosh Performa 6360) equipped with a 15" touch-screen monitor (Mitsubishi Microtouch) and software developed by Dube (1991; MTS version 11.6; Dube & Hiris, 1996) to manage the procedure and record the data.

### Stimuli and consequences

Two pairs of compound stimuli were used in Experiment 1 (Pairs A and B, see Figure 1, upper and central panels). Each stimulus of Pair A consisted of two concentric disks (elements). The logic of using this stimulus pair was to reproduce stimuli used by Reynolds (1961), with one element "inserted" into another element. In Pair B, each stimulus consisted of two shapes (two black shapes displayed side by side on a white background), similar to the stimuli used by Dube, & McIlvane (1997). The use of Pair B had the goal of controlling for some aspects of Pair A: arranging elements "side by side" instead of "one inside the other" and using black shapes, instead of colored geometric shapes.



**Figure 1.** Stimuli used in Experiment 1 with children (Pairs A and B) and in Experiment 2 with bees (Pair C). Two compound stimuli (one S+ and one S-) were simultaneously displayed in each training trial; the S+ was located on the left on half of the trials and on right on the other half. During the probe trials the two stimuli displayed simultaneously were single elements of the compound stimuli (one from a positive compound stimulus and the other from a negative compound stimulus), as shown on the central and on the rightmost displays of each stimulus pair.

Each compound stimulus was presented in one of two locations, the lower left or lower right corner of the computer monitor and S+ and S- were displayed equally often in each location.

Correct responses were followed by the presentation of colored stars on the screen, a series of ascending tones, and by praises and a token delivered by the experimenter. Incorrect responses produced the intertrial interval (ITI 0.5 s). The presentation of the token and the back-up reinforcers will be described in the section 5 about the pretraining.

### Experimental design

The present experiment in children was designed according to a within-subject experimental design. Each participant was initially exposed to a simple simultaneous discrimination between two compound stimuli until a learning criterion was reached; over this baseline, a "probe technique" (Sidman, 1960) evaluated whether or not each element of the compound S+ (but not of S-) acquired discriminative control of responding. Baseline sessions were followed by probe sessions in which probe trials were randomly interspersed between baseline trials. After establishing stable baseline, probe trials assessed stimulus control by each single element of both the S+ and S-.

This design was used with the stimulus Pair A and replicated with stimulus Pair B (see stimuli in Figure 1).

### General procedure

Each participant was individually exposed to a pre-training phase, in preparation for the baseline and the probe sessions. The participants underwent sessions 3-5 days per week. The session duration was approximately 5 min.

### Pretraining

The four younger children who had no prior experience working on a computer (Guto, Nico, Ane, and Yara) were taught to touch a stimulus (e.g., a picture of a car or doll) on the screen. The older child without experience, Carol, was taught to use the mouse for moving the cursor and to press a button on the mouse to select a stimulus on the computer screen. Pretraining was omitted for the two children who had prior experience with computerized discrimination tasks (Lili and Rafa).

The procedure began with a block of five consecutive trials in which the same familiar picture was presented in any of two locations of the computer monitor screen (lower left or lower right corners). Correct responses produced the planned consequences. Verbal and physical prompts were provided, if necessary. The second block had 15 trials and two familiar pictures (e.g., "car" and "telephone") were presented; one picture was defined as the S+, and the other was defined as the S-. In the first five trials, only the S+ was presented. In the remaining 10 trials, the S+ and S- were presented simultaneously. At this point, choice responding was differentially reinforced as in the subsequent training phase. The third training block had 20 trials, and stimuli were two other familiar stimuli (e.g., "bear" or "doll"). Blocks 2 and 3 were repeated until the participant reached the criterion of 90% correct responding.

The token system was first introduced during the first pretraining session. The experimenter presented the child with a cup and a few tokens (plastic chips) and said, "I will put a chip here inside [pointing to the cup] each time you choose the correct one [pointing to the computer monitor]. If you fill this up [pointing to the cup] we will play with one of these toys [pointing

to some toys]. Did you understand? Let's get started!" Upon the completion of each session, across the experiment, the tokens were exchanged for access to educational games (e.g., dominos, puzzles, and memory games), stationery items (e.g., pencil, eraser, crayons, color pen, and rulers), children's story books, drawings, dolls, and others.

### ***Simple simultaneous discrimination training (Baseline A)***

Baseline sessions consisted of a single block of 24 trials of simple simultaneous discrimination. In each trial, two compound stimuli (S+ and S-) were displayed in the lower corners of the computer screen. The right/left locations of the stimuli were counterbalanced across trials in a semi-random order, provided that a stimulus would not appear in the same location for more than three consecutive trials.

The first response to one of the compound stimuli was recorded as the selection response. Selections of the compound stimulus that was defined as the S+ were automatically followed by the programmed consequences and by a 0.5 s intertrial interval (ITI; blank screen). Incorrect responses were followed by the presentation of a 2 s blackout screen and the ITI. The learning criterion was defined as a maximum of four incorrect responses (or a minimum of 83.3% correct responses) and at least 20 consecutive correct responses in the session. Baseline sessions were repeated until reaching the criterion.

### ***Probes of stimulus control by elements of the compound stimuli A***

Probe sessions consisted of eight probe trials that were randomly interspersed between 20 baseline trials. Each probe trial displayed two elements: one element of a positive compound stimulus and one element of a negative compound stimulus (Figure 1). Each element was probed on four trials per session. The elements in each stimulus pair were the same across the four trials (S<sub>1</sub><sup>+</sup> and S<sub>1</sub><sup>-</sup>; S<sub>2</sub><sup>+</sup> and S<sub>2</sub><sup>-</sup>). The right/left locations of the stimuli were counterbalanced across trials. Two probe sessions were conducted with each stimulus pair (thus, each element was probed on a total of eight trials).

After probing the stimuli in Pair A, the same baseline and probe procedures were conducted with stimulus Pair B.

### ***Data analysis***

Interobserver agreement analysis was omitted because the computer program automatically controlled training and testing procedures and data recording. Considering the small number of probe trials, the main type of analysis was conducted using grouped data. A permutation test was used to assess the statistical significance of the mean difference between the choices of one positive element (S<sub>1</sub><sup>+</sup>) and choices of the alternate positive element (S<sub>2</sub><sup>+</sup>). A significant mean

difference would indicate stimulus control by only one positive element (RSC), whereas the absence of such difference would indicate equivalent stimulus control by both elements of the compound (see Appendix).

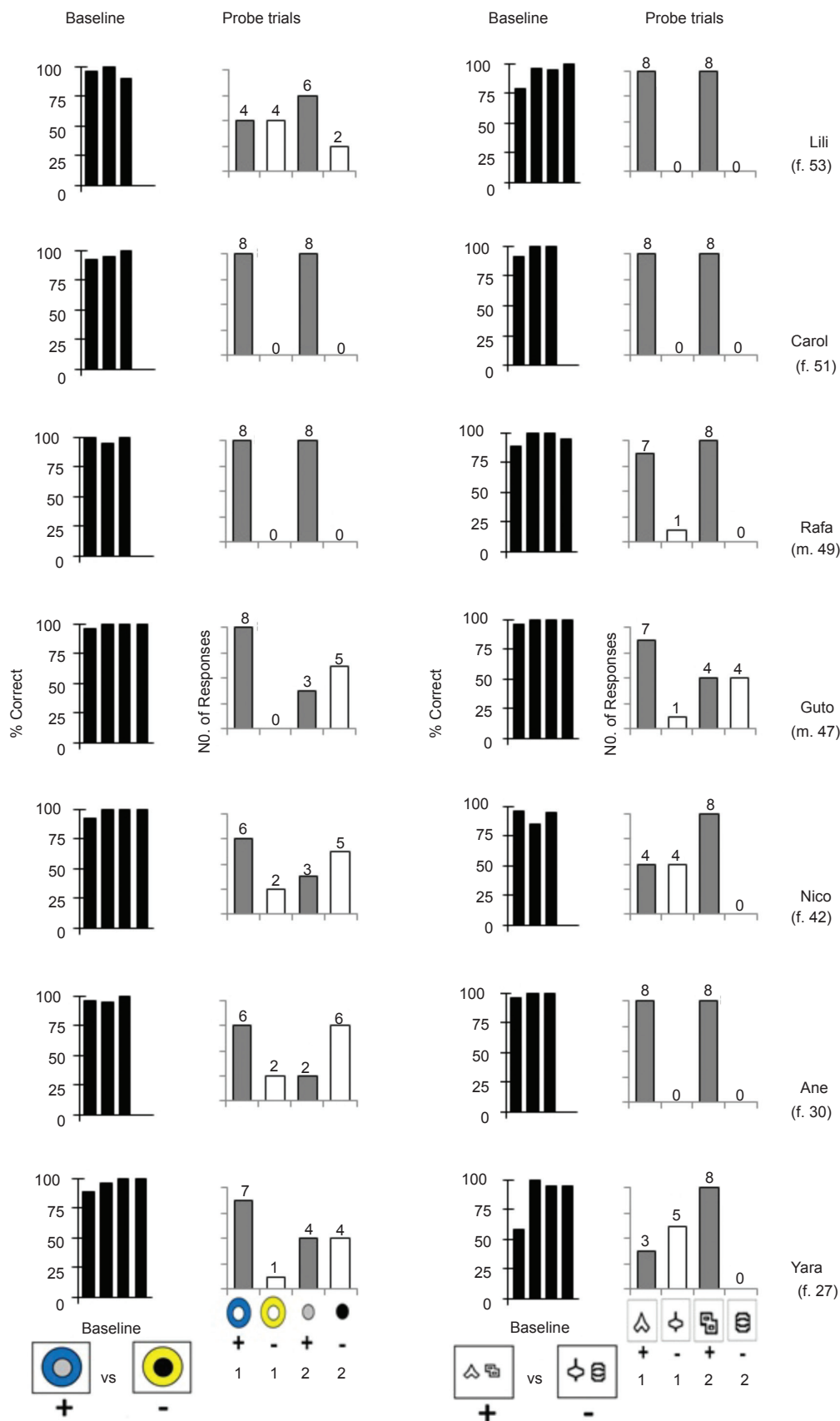
The second type of analysis was based on individual data. Individual performances were analyzed and are presented in figures for visual inspection. The criterion of stimulus control by a particular element was defined as at least seven responses out of eight trials. Based on a binomial distribution, the probability that such proportion is due to chance is less than 0.031 (analysis adapted from Reed et al., 2013). However, due to the small number of probe trials, between two and six responses to one element was characterized as "inconclusive", instead of "due to chance".

## **Results**

The participants needed between one and two sessions to learn the simple discrimination task. The permutation test demonstrated that, as a group, the children responded under restricted stimulus control on probe trials testing for elemental stimulus control. For Pair A the mean difference between the choices of each positive element of a positive compound was 2.43. The probability that this difference was caused by chance was less than 0.005 (see Appendix). For Pair B, the mean difference between the choices of one positive element and choices of the alternate positive element was 1.86 ( $p < 0.005$ ).

Regarding individual data, Figure 2 shows the percentage of correct responses on baseline trials (black bars) and the number of responses (out of 8) to each element of the compound S+ (gray bars) and S- (white bars) on probe trials, for individual participants (fictitious name, gender and chronological age indicated at the right of each line). Graphics on the left columns represent data for Stimulus Pair A; graphics on the right represent data for Stimulus Pair B. As described before, the stimulus control by one element during probe trials was defined as at least seven selection responses to one element out of eight trials.

In probes with Pair A (colored concentric disks), Carol and Rafa responded to both positive elements (+) on all trials (i.e., to the blue outer disk as much as to the gray inner disk), and did not select any element of the S- (Figure 2), thus showing accurate stimulus control by single elements of the compound stimuli. Guto and Yara consistently responded to one positive element (the blue outer disk), but showed variable responding to the other positive element (the gray inner disk), distributing their responses between this element and the negative element presented simultaneously. Lili, Nico and Ane showed a somewhat inconsistent responding pattern during the probe trials. Responding to each element (positive or negative) occurred on two to six trials. Notably, however, they tended to respond more frequently to the blue outer disk (the element of the positive compound stimulus from Pair A), whereas the gray inner disk



**Figure 2.** Percentage of correct responses on baseline trials (black bars) and the number of responses (out of 8) to each element of the compound S+ (gray bars) and S- (white bars) on probe trials, for individual participants (fictitious name, gender and chronological age indicated at the right of each line). Graphics on the left columns represent data for Stimulus Pair A; graphics on the right represent data for Stimulus Pair B.

(the other element of the positive compound stimulus) appeared to exert much less control. Three of these participants (Guto, Nico, and Ane) emitted a total of twice as many responses (i.e., 16 responses) to the black inner disk (negative element) than to the gray inner disk.

On probes with elements of Pair B (black shapes on a white background), Lili, Carol, Rafa, and Ane responded to both components of the S+ (see Figure 2, two right columns), indicating equivalent (not restricted) stimulus control, according to the present definitions. Guto, Nico and Yara, however, consistently responded to one of the positive elements, but distributed their selection responses among the positive and the negative element of the other pair presented on probe trials. The positive element that acquired stimulus control varied among participants: S<sub>1</sub><sup>+</sup> for Guto; S<sub>2</sub><sup>+</sup> for Nico and Yara. That is, results with these three participants indicate RSC in probes with Pair B.

## Discussion

All seven children met the combined learning criterion of a maximum of four incorrect responses in a 24-trial block, with the requirement that the last 20 consecutive responses must be correct, and they met this criterion in one or two sessions. Notably, performance in the baseline trials during the probes phase remained stable, with mean accuracy of 97.5%. This performance indicates that the learning criterion was appropriate to guarantee baseline accuracy.

The learning criterion defined for this experiment is comparable to the criteria used in previous studies. For example, Lionello-DeNolf, McIlvane, Canovas, Souza, & Barros (2008) presented six preschool children (aged 35-51 months) with a simple discrimination and reversal-learning task with three sets of visual stimuli. The sessions consisted of 30 trials and the accuracy criterion was no more than two errors per session. The number of training sessions required for meeting the learning criterion with each stimulus set ranged between one and four. Whitehurst (1969) trained 60 preschoolers (aged 5-6 years) in a simple discrimination task. The learning criterion was 10 consecutive correct responses in a 60-trial session. The participants met the criterion in average of 38.7 trials. In a study by Lovaas & Schreibman (1971) a group of five typically developing children (mean age, 6.4 years) learned a simple discrimination and all of them reached the learning criterion (90% of correct responses in one session) in the first session. Similarly, other studies reported baseline acquisition on the first training session (Bailey, 1981; Koegel & Wilhem, 1973; Lovaas et al., 1971).

Despite the accuracy during baseline, averaged data of participants in this experiment showed that the children responded under the control of only one of the two positive elements during the probe trials (significant mean difference, according to the permutation test). This is in accordance with the current definition of selective or restricted stimulus control. Individual data

confirm this result: three participants (Guto, Nico and Yara) responded under control of one, but not the other component of the compound S+, on at least one occasion (with stimulus Pair A or stimulus Pair B). However, three other participants (Carol and Rafa with Stimulus Pairs A and B, and Ane with Pair B) showed perfect stimulus control by both elements of S+ (i.e., it is possible that they acquired the discrimination under the control of the compound, and they also discriminated its elements).

The observation of some instances of RSC in this experiment extended and replicated the findings reported by Bailey (1981), Eimas (1969), and Reed et al. (2013). All of these studies reported the consistent occurrence of RSC in children. Notably, the current data appear to confirm the results of Reed et al. with regard to the age at which RSC will more reliably occur. Reed et al. reported that the age of the participants who exhibited RSC varied between 22 and 40 months, and RSC occurred consistently for participants younger than 36 months old. Three of the four younger participants responded under RSC in one task (Nico) or on both tasks (Guto and Yara). However, such a correlation between age and the occurrence of RSC was not found in the present study (*correlation coefficient* R<sup>2</sup> was 0.24). Future studies should replicate the present experiment using a larger number of participants, within a larger age range, and planning a larger number of test trials, in order to better investigate whether or not such correlation exists.

Another aspect to be considered is the possibility that performances on tests for discriminative control by elements of a compound stimulus may vary considerably and depend on the physical characteristics of the elements, number of elements or properties to be tested, and the sensory modality involved (Eimas, 1969; Reed et al., 2013). One might speculate that more participants in the current study would display RSC if trained with more complex compounds, for example, with three or more elements. Although the number of elements was not manipulated, we used two stimulus sets (for two different discriminative tasks) in order to control for color of the elements of each compound, and the position of the elements. Therefore, elements of each compound of Pair A were arranged as colored shapes, “one inside the other”, similarly to the compounds used by Reynolds (1961), whereas the elements of each compound of Pair B were black shapes displayed “side by side”. It appears that such manipulation did not affect the results. Baseline performances, as well as the results on probes with Pairs A and B were quite similar.

Another variable that should be considered is the effect of training and testing with one stimulus pair on the results of the training and testing with a second stimulus pair. Participants were first trained and tested for RSC with stimulus Pair A. They were later trained and tested with stimulus Pair B. It might be expected that the experience with Pair A could have somewhat

influenced responding to all positive elements in the second task, decreasing the likelihood of exhibiting RSC. But, as mentioned above, results with the first stimulus pair did not differ reliably from results with the second pair. In fact, these data suggest that two participants exhibited RSC with stimuli in the first task and three other participants exhibited RSC in the second task.

Despite the concerns mentioned above, which should be empirically addressed in future studies, the procedure used in Experiment 1 proved to be adequate for establishing accurate simple discrimination baselines and for assessing RSC. Therefore, it seems well-suited to assess RSC in other species. Experiment 2 addressed this question by employing a similar procedure with bees.

## Experiment 2 – Assessing elemental (and RSC) stimulus control in bees

### Methods

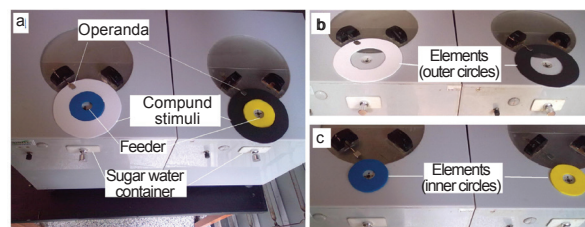
Sessions were conducted in a laboratory setting at the Universidade Federal de São Carlos, Brazil. In this facility, a beehive was mounted on a wall next to a window, which allowed the bees to forage outside or find a sucrose reward (50% sugar solution) in an experimental apparatus located indoors.

### Subjects

The subjects were four experimentally naive foraging bees (*Melipona quadrifasciata*), all of which were randomly selected from the same hive.

### Apparatus

The apparatus was adapted from Pessotti (1969, 1981) by Insight Equipamentos Eletrônicos (Ribeirão Preto, São Paulo, Brazil) for operant studies with bees (see Figure 3). It consisted of two identical Plexiglas operant chambers (27 x 27 x 27 cm) fixed on top of a table, set side by side, and 150 centimeters away from the hive. Both operant chambers, painted in a silver color, were interfaced to a computer (PC Intel Pentium 4), equipped with a custom-made software (Insight Equipamentos Eletrônicos), for controlling events and data recording. The experimental area was set on the top of each chamber, into which the bee would fly and land. Each chamber had two tiny bars (only one used in the experiment) and a feeder. Pressing the lever activated an infrared sensor; the sensor signal was used to record the operant response and to operate the feeder. The two feeders were about 30 cm apart. Below each feeder there was a sucrose recipient equipped with a spoon. When the feeder was automatically operated, the spoon was elevated to the feeder opening. On each box, a stimulus (handed by the experimenter) was placed near one of the bars, covering the feeder; the stimulus had a central opening that overlapped with the feeder's opening. On each trial, two stimuli could be displayed simultaneously, one over each box.



**Figure 3.** Setup of Experiment 2. a) Two experimental boxes for operant studies with bees, connected to a computer that controlled all events and recorded data. Each box had two bars (operanda) and a feeder located on the open surface on top of it. A stimulus was placed over the feeder (with a central opening as large as the feeder), near one of the bars; on each trial, two stimuli were displayed simultaneously (one over each box), one S+, one S-; b) and c) Stimuli used on probe trials (elements of the compound stimuli shown in a).

### Stimuli

The stimuli of Pair C were used with bees (see lower panel of Figure 1). The stimuli were rubber-made (EVA – Ethylene Vinyl Acetate) colored disks of two sizes: 10 cm diameter outer disks and 5 cm diameter inner disks. The whole stimulus, including the inner and the outer disk, was designated a “compound” stimulus, whereas any individual disk (inner or outer) was designated an “elemental” stimulus (or element, for short). As in Experiment 1, the logic of using these stimuli was to reproduce stimuli used by Reynolds (1961), with one element “inserted” into another element. Pair C differed from Pair A (Exp. 1) with regard to the color of one element (gray with children; white with bees); also, Pair A was displayed vertically, on a computer screen, and Pair C was presented horizontally, over the experimental box. White, black, blue, and yellow were used (instead of white, green and red, as in Reynolds’) because these colors have been successfully discriminated by bees (e.g., Giurfa et al, 2001; Pessotti, 1969, 1981).

### Experimental design

The design of this experiment was similar to Experiment 1 in the most important aspects: a baseline of simple simultaneous discrimination was established until each subject met the learning criterion; the stable baseline was then used to probe for elemental stimulus control. Different from children, the bees were exposed to a single stimulus pair (Pair C).

### Procedure

#### Pretraining

Each bee was individually marked and trained. A bee was first taught, by successive approximations, to collect sucrose that was dropped on the top of the chamber surface near the feeder. Subsequently, a bee was taught to collect the sucrose in the feeder; the shaping procedure was then used to teach bar pressing in one of the experimental boxes. That chamber was then made inaccessible, and the same procedure was



repeated using the alternate chamber until each trained bee flew regularly between the hive and apparatus. Typically, pretraining lasted between 1 and 2 h.

#### *Simple simultaneous discrimination training*

When the bees were flying regularly to the apparatus, the discriminative stimuli (Pair C) were introduced, one on each box. During training, every operant response in a chamber that presented the positive compound stimulus (S+) resulted in 55 s of access to the feeder, followed by a 10 s ITI. The bar press released the feeder and a spoonful of sucrose was elevated to the feeder. Typically, a bee collected the reward and returned to the hive in less than 55 s. Responses in the chamber that presented the negative compound stimulus (S-) resulted in a 10 s timeout (both bars became inoperative) followed by a 10 s ITI. The locations of the stimuli were interchanged manually during the ITI, as specified in a protocol that listed the sequence of trials and the distribution of S+ and S- across trials. The right/left locations of the stimuli were counterbalanced across trials in a semi-random order, provided that the same stimulus was not presented in the same location on more than three consecutive trials.

Training was conducted in blocks of ten trials and the learning criterion was at least nine consecutive correct responses in a block (i.e., no error or a single error on the first trial of the 10-trial block). Typically, one training session lasted between 2 and 6 h (average: approximately 4 h) and comprised from 6 to 17 blocks (60 to 170 trials). Because the bees were not confined to the experimental setting, variations in the duration of the sessions and number of training blocks depended on how regularly one bee would fly between the hive and the apparatus. Throughout the experiment it was observed that the learning criterion (i.e., nine correct responses out of 10) was not sufficiently stringent to ensure baseline accuracy in probe blocks. Therefore, some of the bees received additional training blocks after the criterion was met.

#### *Testing elemental stimulus control*

Following the criterion in training with Pair C, the bees were exposed to probe blocks, comprised of 8 baseline trials and two probe trials. Baseline trials always displayed two compound stimuli (one S+ and one S-), whereas the probe trials always presented a single element of the S+ in one chamber and a single element of the S- in the other chamber. Half of the probe trials presented only inner disks (one positive and one negative), and the other half presented only outer disks (Figure 3). During the probe trials, the disks were presented simultaneously for 20 s on top of the chambers and responses had no programmed consequences (extinction). After 20 s the stimuli were removed and another baseline trial was presented. A probe trial was never followed by another probe trial.

If during a probe block the bee failed to maintain accuracy on baseline trials (at least 75% correct

responses), baseline training was reintroduced until the criterion was reached again. Otherwise, successive probe blocks were conducted until four probe blocks (eight probe trials) were completed.

Each bee was exposed to two to three daily sessions in this phase. As in the training phase, the average session duration was approximately 4 h.

#### *Data analysis*

Similar to Experiment 1, a permutation test (with aggregated data from all bees) was used to assess the statistical significance of the mean difference between the choices of one positive element and choices of the alternate positive element. A significant mean difference indicates stimulus control by only one positive element (RSC; see Appendix). For the individual analysis, the demonstration of stimulus control by each positive element was defined as three or four selection responses out of four.

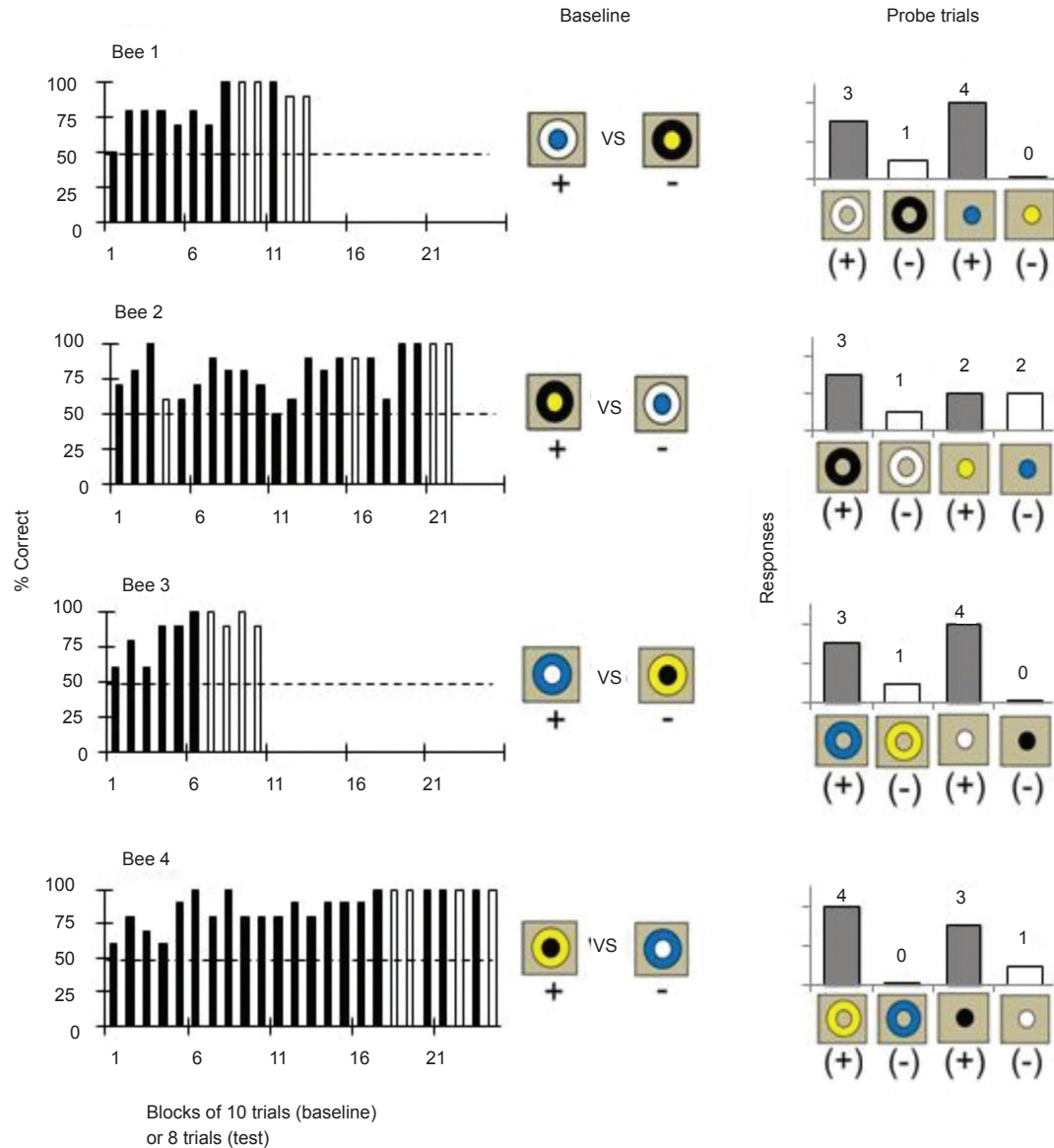
## **Results**

All of the bees acquired the simple discrimination between compound stimuli despite differences in the number of trials required to reach the learning criterion. Three bees (Bees 1, 2, and 3) completed the entire procedure (training and probes) in two sessions. Bee 2 completed the procedure in three sessions.

The permutation test conducted with the aggregated data of all bees confirmed that the bees did not respond under restricted stimulus control. The mean difference between the choices of one positive element and choices of the alternate positive element was 1.0, which was not significant, indicating the occurrence of stimulus control by both positive elements.

Figure 4 shows the performance of individual bees on baseline trials and on probe trials. The left columns show the percentage of correct baseline responses across successive blocks of trials. Black bars represent the percentage of correct responses in the training (N= 10 trials per block) and white bars represent the percentage of correct responses in the baseline trials that comprised the probe blocks (N=8) along with probe trials (N=2). The probe trial results are shown separately in the right columns, as the total number of responding under the control of the positive element of a compound stimulus (maximum of four trials per trial type). Gray bars represent the number of responses to positive elements of S+, whereas white bars represent the number of responses to negative elements of S-. The two bars on the left correspond to responding to the outer circles; the two bars on the left present responding to the inner circles (see Figures 3 and 4).

Bee 1 met the 90% correct response criterion within eight 10-trial baseline blocks. On probe trials with the two outer disks presented simultaneously, this bee responded three times to the white disk (the positive element), and one to the black disk (the negative element). When the probes presented the two inner



**Figure 4.** Performances of individual bees (*Melipona quadrifasciata*) on baseline (left columns) and probe trials (right columns) in Experiment 2. Baseline was measured as the percentage of correct responses in successive 10-trials training blocks (black bars) and on probe blocks (white bars). In probe trials (elemental stimuli presented under extinction) the measure was the number of responses to each element of the compound stimuli. In each trial (baseline and probe), two stimuli were presented simultaneously.

disks, all four responses occurred on the blue disk (the positive element). Thus, seven responses (out of eight possibilities) occurred under the control of the positive elements (S+). Bee 2 reached the criterion rapidly, within three blocks, but when a probe block was conducted, baseline responding decreased to 50% and training blocks were resumed. Responding on baseline trials was inaccurate and variable responding for many blocks; for this reason, the criterion for this bee was tightened. After the criterion was finally reached on Block 14, a second probe block was conducted and followed by four training blocks before the probes were completed. On

the probe trials, the bee distributed five responses to the positive elements, and three responses to the negative elements, in a clear indication of lack of accuracy in the simple discrimination baseline. Bee 3 acquired the simple discrimination by the end of 40 trials. In the probe trials, seven responses were to positive elements (S+), and only one response was to a negative element (S-). Bee 4 showed a relatively slow acquisition, but once criterion was reached, the bee selected the positive elements on seven of eight probe trials.

In summary, three of four bees selected both positive elements of the compound discriminative stimulus (the

outer circle and the inner circle of the S+) on most trials (except for one choice of one of the negative elements), showing stimulus control by both elements of the compound; the other bee (Bee 2) did not show accurate discrimination of any element.

## Discussion

Four bees learned to discriminate between two compound stimuli and then were tested for control by its elements. The test indicated that three bees responded to both positive elements, whereas the results with another bee (Bee 2) were inconclusive. This subject exhibited somewhat discrepant baseline performance, and its responding in the probe trials was also the least consistent with the training reinforcement contingency. Nevertheless, the responses to negative elements occurred on the first two blocks of testing, and only responses to positive elements occurred on the last two blocks of testing, suggesting that the responses to the negative elements resulted from an initially inaccurate baseline. Nevertheless, these data do not suggest reliable stimulus control by the positive elements; therefore, they also do not suggest RSC. In summary, under conditions similar to the present study, bees (*Melipona quadrifasciata*) will not show restricted stimulus control.

The present results are consistent with those described by Gould (1996), showing that the responding in bees occurred under the control of all defined aspects of a positive compound stimulus (i.e., a particular odor, color, and shape). The results with bees contrasted with the results reported by Reynolds (1961) who observed RSC in two pigeons. Our results with bees are not entirely comparable to those reported by Reynolds, however, because we used simultaneous discrimination training and testing rather than successive discrimination training and testing as Reynolds did. Successive discrimination training in bees is a procedural resource yet to be developed.

In fact, the present study was the first especially designed to assess elemental stimulus control in operant discrimination in bees (but see Sommerlandt, Rösler, & Spaethe, 2014, for an example of elemental stimulus control under a Pavlovian paradigm with bumble bees), and our data are not very comparable to data from other studies. For example, Gould (1996) primarily examined how stimuli from different modalities (odor, color, and shape) gained priority in the control of responding and did not address the matter of RSC. In the testing procedure developed by Gould, the bees might be presented with two compound stimuli that are rearrangements of both positive and negative elements. For example, one test trial might present a positive shape plus a negative color vs. a negative shape plus a positive color. Specifically in this case, Gould was interested in which property (color or shape) would show privileged discriminative control.

Had the baselines in this experiment been more accurate, then results of the RSC probes would have been

more consistent with the programmed contingencies of reinforcement (i.e., positive elements would exert more reliable discriminative control). In fact, the results for baseline acquisition were somewhat unexpected for this experiment. Prior studies of visual discriminative learning in bees indicated that less than 100 training trials were required to establish a robust discriminative baseline (Giurfa et al., 2001; Moreno et al., 2012; Pessotti, 1972). For example, in the study by Moreno et al., 16 bees received simple discrimination training with horizontal vs. vertical patterns and blue vs. yellow colors. All of the bees met the learning criterion (100% correct responses in a block) after approximately 60 trials (i.e., less than 2 h of training). Why the bees in the present experiment (especially Bee 2) performed inaccurately in the training phase is not clear. Nevertheless, future studies should define more stringent criteria for baseline acquisition.

## General discussion

The purpose of the present study was to assess whether one or two elements of compound discriminative stimuli had acquired control over the responding of preschool typically developing children (Experiment 1) and of bees (Experiment 2) during a simple simultaneous discrimination task. These experiments were similar with regard to the training paradigm. Both used a simple simultaneous discrimination procedure task. The visual stimuli used with the children were also similar to the stimuli used with bees.

As may be expected, the experiments differed from each other with regard to some other aspects, such as duration of sessions, due to the specificities of working with very young children and with bees. Average data from Experiment 1 suggest that children responded under RSC, whereas in the Experiment 2, with bees, three of four animals responded under control of all positive elements.

Results from Experiment 1 with children extend and replicate findings reported by Bailey (1981), Eimas (1969), and Reed et al. (2013). It is important to notice that RSC might present barriers to achieving educational goals. For example, as pointed out by Dube and McIlvane (1999), RSC may preclude acquisition of stimulus-stimulus relations (repertoires involved in symbolic behavior). For that reason, several other studies have sought to develop procedures to manage RSC and overcome this limitation in discriminative learning (e.g., Allen & Fuqua, 1985; Doughty & Hopkins, 2011; Dube & McIlvane, 1997, 1999; Huguenin & Touchette, 1980; Koegel, Schreibman, Britten, & Laitinen, 1979; Lovaas, Schreibman, Koegel, & Rehm, 1971; Ray, 1969; Ryan, Hemmes, & Brown, 2011; Walpole, Roscoe, & Dube, 2007). Accordingly, the present findings might be important for suggesting relevant variables in the development of procedures to manage RSC.

Although average data analysis has indicated the occurrence of RSC in Experiment 1 and the absence

of RSC in Experiment 2, one must be cautious about interpreting individual results, due to the small number of probe trials, particularly in the experiment with bees. Typically in experiments of discriminative learning, as in the present study, bees are not confined to a closed arena. Although bees are taught to collect a sugar solution presented in artificial flowers inside the experimental setting, they are allowed to forage outside the laboratory. Such specificity of working with bees makes it particularly difficult to run the large number of nonreinforced probe trials that might be necessary for an appropriate test of elemental stimulus control. After a few nonreinforced responses, bees might definitively leave the experimental setting. Such difficulty explains the decision to run only eight probe trials with each bee during the probes phase. Given that such a number of probe trials was insufficient for conclusive analysis, alternative test procedures should be considered. For example, future experiments should consider employing reinforced probe trials.

One important limitation of the present study was that the testing protocol did not control for the rejection effect by negative elements (see Goulart, Mendonça, Barros, Galvão, & McIlvane, 2005, for an account of select-control and reject-control relations in simple discrimination repertoires). In the testing procedure, half of the probe trials presented one element from the positive compound stimulus together with one element from the negative compound stimulus, whereas the other half of the probe trials presented the alternate positive element together with the alternate negative element. If trained with AB+ vs. CD-, then one subject would be tested with A vs. C and B vs. D, but would never be tested with A vs. D, or B vs. C. Because the effects of positive and negative elements might be confounded in probe trials, such a test design may not be the most appropriate for assessing the stimulus control by isolated elements. For example, after the AB+ vs. CD- task, more selections of A than selections of B in the test phase could result not from stronger control by A than by B, but rather from stronger avoidance of C than of D (due to the possibly negative role acquired by those elements under the extinction contingency throughout training).

A permutation test was used to assess the statistical significance of the mean difference between the choices of one positive element ( $S+_1$ ) and choices of the alternate positive element ( $S+_2$ ). One might expect a similar analysis involving the mean difference between the number of selection responses to one negative element and the number of selection responses to the alternate negative element. Such analysis might indicate, for example, stronger rejection of one negative element than rejection of the other one. However, because every negative element was always paired with the same positive element, both analyses (one comparing responses to positive elements; the other one comparing responses to negative elements) would produce the

same results. That is, it is possible, for example, that the observed RSC in Experiment 1 was due mainly to control by rejection instead of control by selection. Future studies should consider designing probe trials with all combinations of elements so that differences in positive and negative control can be identified (differences in stimulus control topographies; e.g., Dube & McIlvane, 1996; McIlvane & Dube, 2003).

Most previous studies on RSC reported this pattern of behavior in participants with autism and developmental disabilities, suggesting that this phenomenon is inherent to specific populations. However, more recent studies with normally capable humans (Critchfield & Perone, 1993; Dube, Balsamo, Fowler, Dickson, Lombard, & Tomanari, 2006; McHugh & Reed, 2007) indicated that RSC may develop in accordance with how the contingencies of reinforcement are arranged, especially when complex tasks are involved (Reed & Gibson, 2005; Reed, Petrina, & McHugh, 2011).

Consistent with such an approach, the present study appears to be relevant because it showed that RSC could also be observed in typically developing populations. If the phenomenon is widespread also in typically developing populations, then further replications of the present study should focus on the role of learning experience in the development of RSC. Such an approach is necessary to identify the variables related to RSC and understand this phenomenon as a basic behavioral process. For this purpose, knowing that an animal with a much simpler nervous system, the bee, did not present RSC is relevant.

## Acknowledgements

We thank Marília Pinheiro de Carvalho and Armando Machado for a critical review that greatly improved the manuscript and for suggesting and conducting the permutation tests. The first four researchers were supported by doctoral scholarships from the Sao Paulo Research Foundation (FAPESP: grants no. 08/50576-8, 07/52578-5, 2010/06422-6, and 07/00119-7, respectively). The work was conducted as part of the scientific program of the National Institute on Science and Technology on Behavior, Cognition and Teaching (INCT-ECCE), supported the National Council for Scientific and Technological Development - CNPq (grant no.573972/2008-7) and by the São Paulo Research Foundation - FAPESP (grant no. 08/57705-8).

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## Appendix

In the RSC test in Experiment 1, responses to positive elements represented by the value 1 and responses to negative elements represented by the value 0 were put in a  $7 \times 16$  table. Each column contains data for one test trial. Each line contains data for one participant. After a baseline task (e.g., AB+ vs. CD-, in which A and B are elements of a positive compound, whereas C and D are elements of a negative compound), each participant received eight probe trials of the type A vs. C and eight probe trials of the type B vs. D. Therefore, the first eight columns represent trials of the type A vs. C, whereas the last eight columns represent trials of the type B vs. D. For example, the sequence of entries 1, 0, 0, 1, 1, 1, 1, 0 and 1, 1, 1, 1, 1, 1, 1, 1 would indicate five responses to the positive element A and eight responses to the other positive element B. Differences between the sum of responses to A and sum of the responses to B were then averaged. A second table, similar to this one, was created and filled with the entries 1 and 0 randomly distributed. Each line of this second table contained the same number of 1 and 0 entries of the corresponding line of the first table. Considering the last example, the second table would have thirteen 1 values and five 0 values randomly distributed among its columns. Differences between the sum of values of the first eight columns and the sum of values of the last eight columns were averaged, as well as for the table with empirical values. The logic of this analysis is to examine the probability of obtaining a mean difference from the second table that is equal to or greater than the mean difference from the first table.

The same permutation test was performed with data from the RSC test in Experiment 2. The only difference was that the values were put in  $4 \times 8$  tables according to the number of subjects (four) and number of probe trials (eight).