SAME/DIFFERENT CONCEPT LEARNING AND CATEGORY DISCRIMINATION IN HONEYBEES

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAIʻI AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

PSYCHOLOGY

MARCH 2018

By

Nicole M. Muszynski

Dissertation Committee:

Patricia Couvillon, Chairperson
Paul Nachtigall
Scott Sinnett
Lorey Takahashi
Tim Tricas

Keywords: honeybees, learning, concept learning, category learning, same/different discrimination

ACKNOWLEDGEMENTS

I am eternally grateful to all of those who have helped me write, discuss, and complete my dissertation. First and foremost, without the tremendous help of my advisor, Dr. Patricia Couvillon, this dissertation would still be in its infancy. I owe many thanks to her for allowing me the chance to learn from and work with her. She not only has provided me with academic support, but she has motivated me to accomplish my goals, and she has inspired and taught me how to become a better experimenter, writer, and lifetime learner. I would like to give many thanks to my committee members, Dr. Paul Nachtigall, Dr. Scott Sinnett, Dr. Lorey Takahashi, and Dr. Tim Tricas, for their time, input, and encouragement. I have been fortunate to have had the opportunity to conduct my studies in such a wonderful environment as the Békésy Laboratory of Neurobiology. I greatly appreciate the many students, staff, and faculty members in the laboratory for having created such a warm and welcoming place to work and study. In particular, I would like to acknowledge Dr. Brad Jones, Mr. Stanford Togashi, and Mr. Scott Niimoto, who have all provided me with much needed technological assistance and support. I would also like to acknowledge Ms. Lynn Hata for her administrative assistance as well as her enjoyable baked goods. I have encountered many individuals throughout my time in Graduate school that have helped me grow and learn, and who have challenged me to think critically and deeply. For all those individuals I say, thank you.

ABSTRACT

Honeybees show a variety of associative learning phenomena with performance resembling that of vertebrates. This research explores the cognitively-complex phenomena of same/different concept learning and category discrimination. The free-flying procedure was used in all of the experiments. Foraging honeybees were trained individually to fly between the hive and the laboratory for sucrose reward. In Experiment 1, honeybees were trained in a four-stimulus oddity problem with two-color pattern stimuli that were unique on every trial. They were rewarded for choosing the odd stimulus on every trial and learned to choose correctly. In Experiment 2, four groups of honeybees were trained in a simultaneous same/different discrimination with a pair of identical patterns (same) and a pair of nonidentical patterns (different), again, with trial-unique training. Two groups were rewarded for choosing same, and two for choosing different. The results are the first to demonstrate same/different discrimination learning in honeybees. In Experiments 3 and 4, bees were trained to discriminate a pair of patterns from a pair of solid colors, with unique sets on every trial. In Experiment 3, the stimuli in the pairs were identical, and in Experiment 4, the stimuli in the pairs were nonidentical. Half the bees were rewarded for choosing solids, and half for choosing patterns. The bees easily discriminated the pairs, suggesting they formed categories of solids and patterns. Experiment 5 was like Experiment 1 but with a category dimension added to the four-stimulus oddity. On half the trials, the stimuli were a pattern and three identical solids, and on the others, a solid and three identical patterns. The bees choose correctly and the category dimension enhanced performance. Experiment 6 was like Experiment 2 but with a category dimension added to the same/different discrimination. On half the trials, the same pair was two solids and the different pair two patterns, and on the others, the same pair was two patterns and the different pair two solids. One group was rewarded for choosing same, and the other for choosing different. Both groups solved the discrimination. This set of experiments provides evidence of same/different concept learning and category discrimination in honeybees.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Invertebrates in Comparative Psychology

In the early history of comparative psychology, research was conducted on a wide variety of vertebrate species and a surprising number of invertebrate species (Corning, Dyal, & Willows, 1973) using both instrumental and Pavlovian conditioning techniques. This broad survey of learning established that all species likely could learn. Subsequently, research on learning began to focus intensely on a few model animal subjects, such as monkeys, rats, and pigeons. The primate work was somewhat expensive but rats and pigeons were abundant, hardy, inexpensive, and easy to maintain under laboratory conditions making them ideal research subjects. The results of experiments with these few species facilitated the discovery of the majority of the basic associative learning principles widely accepted in contemporary psychology.

With technological advances in cellular neuroscience came a strong interest in understanding the biological basis of learning. Most of the work focused on invertebrate species because of their simpler and more accessible nervous systems, often with large and fast neurons. The *Aplysia*, a gastropod mollusk, and the crayfish, a decapod crustacean, emerged as the model organisms (see reviews by Edwards, Heitler, & Krasne, 1999; Kandel, 2001), although they had not been well studied in learning experiments because of limitations in sensory, motor, or motivational capabilities. For studies of learning in invertebrates, octopus and honeybees emerged as the model organisms, although ironically they are not as suitable for studies on the biological basis of learning.

The results of experiments with honeybees and octopus suggest that the basic associative principles of learning are much the same as those in vertebrate species (Avarguès-Weber &

Giurfa, 2013; Bitterman, 1988; Bitterman, 1996). In fact, the recent work with honeybees has progressed to encompass a broader range of learning phenomena and has been extended to include questions about their cognitive capacities beyond associative learning. One cognitive phenomenon of great interest in contemporary research with vertebrate species is concept formation because the ability to form abstract or relational concepts cannot be explained with basic associative principles. The experiments reported here were designed to examine concept formation in honeybees.

Experimental Techniques for Honeybees

The techniques that have been developed for the study of learning in honeybees are a result of prior studies on the biology and ecology of honeybees. The earliest formal experiments with honeybees were conducted by Karl von Frisch (1915) primarily to determine their sensory capacities, their foraging activities, and their communication abilities. The sensory capacities of honeybees are extraordinary for an invertebrate species. They have color vision similar to that of humans, although they do not detect longer wavelengths (red), and they do detect the shorter wavelengths (ultraviolet) that humans do not. Honeybees have chemoreceptors on their antennae and tarsi (von Frisch, 1950; Scheiner, Page, & Erber, 2001) for kin recognition, discrimination of floral odors, sugar detection, and perception of pheromones (Robertson & Wanner, 2006). Honeybees also detect magnetic fields (Gould, Kirschvink, & Deffeyes, 1978; Walker & Bitterman, 1985) as well as vibration and touch (Kirchner, 1993; Nieh & Tautz, 2000; Rohrseitz & Tautz, 1999; Tautz & Rohrseitz, 1998). Clearly the honeybee has extensive sensory capabilities, and, in addition, their motor capabilities also are extensive and include flying, walking, moving their antennae, extension of the stinger, and extension of the proboscis, all of which provide a number of possible responses that can be measured in learning experiments.

Another important consideration for learning experiments is motivation. Learning experiments with vertebrate species may use motivation for food or motivation to avoid and escape noxious stimuli, but most of the learning studies with honeybees have capitalized on their motivation for nectar or sugar water.

There are two main techniques for studying learning in bees, the proboscis extension reflex technique, or PER, and the free-flying technique. The proboscis extension reflex technique was developed for working with restrained bees and has primarily been used to explore basic Pavlovian conditioning phenomena. The procedure is analogous to the salivary conditioning used by Pavlov with dogs. In PER, foraging honeybees are captured and then restrained, allowing only minimal head movement. When a bee's antennae are touched with sucrose (unconditioned stimulus), the unconditioned response is the extension of the proboscis, and the bee then is fed a small quantity of the sucrose. On conditioning trials, a novel stimulus, such as odor is paired with the sucrose, and after a few pairings, the novel stimulus (conditioned stimulus) comes to elicit the extension of the proboscis, now as a conditioned response. The PER technique has provided much evidence that the learning of honeybees is similar to that of vertebrates (Batson, Hoban, & Bitterman, 1992; Bitterman, Menzel, Fietz, & Schäfer, 1983; Couvillon, Hsiung, Cooke, & Bitterman, 2005).

Although the proboscis extension technique is useful for the study of Pavlovian conditioning, it does have limitations. The experimental bees must be restrained and so cannot unload the sucrose consumed, as would be the case for a foraging bee. Note that the foraging bee can hold about 50-60-µl. The possibility that the bees will satiate on the sucrose and stop extending the proboscis in the PER training means that there is a limit to how many trials can be conducted even with very small amounts of sucrose on each trial. Visual stimuli are not easily conditioned with the PER technique, although odors or touch applied to the antennae can easily serve as conditioned stimuli. Due to these limitations inherent in the PER technique, the free-flying technique is generally regarded as more powerful and flexible for conducting a broader range of learning experiments with honeybees.

The free-flying technique was originally developed by Karl von Frisch (1915) to study the honeybees' sensory capabilities (*e.g.*, color vision, odor, and landmark detection), and it was historically used as a learning assay to assess discrimination. Unlike the PER technique, the free-flying technique uses unrestrained foraging honeybees trained to visit a laboratory window or table to feed on artificial flowers. While in that experimental situation, the bee learns to associate a stimulus with a sucrose reward. Typically, the sucrose solution that is used to motivate the bees to return to the experimental situation is a highly concentrated 50% solution which is a much higher concentration than can be found at nearby flowers. When a foraging honeybee has been trained to visit an experimental situation, it can be presented with a variety of stimuli placed on the horizontal surface of a table or window box. For researchers whose main interest is to explore basic learning of bees, the free-flying technique is suitable for designing analogs of many of the basic vertebrate learning experiments, such as instrumental tasks.

In a basic color discrimination problem, a bee is presented with two stimuli, say a blue and an orange color. There is a drop of sucrose on one color and a drop of an aversive solution on the other color. On each visit to the situation, the bee is allowed to choose until it finds the sucrose on the "correct" color, then drinks the drop of sucrose, and returns to the hive when full. The bee unloads the sucrose at the hive and flies back to the situation for another trial, usually within three to five minutes. This free-flying technique has been used to study an extensive range of learning phenomenon in honeybees: acquisition, extinction, and choice discrimination, as well as the effect of reward parameters on learning such as amount, concentration, frequency, and delay (Bitterman, 1996; Couvillon & Bitterman, 1985; Couvillon & Bitterman, 1988; Couvillon, Lee, & Bitterman, 1991). As with the proboscis extension technique, the results are quite similar to those of vertebrates.

Although the free-flying method is widely used and has produced an enormous amount of data on honeybee learning, it has some limitations. First, the time between trials, the intertrial interval, is determined by the honeybee. This interval is the amount of time it takes an individual honeybee to leave the experimental situation, fly to the hive to unload, and then return to the experimental situation for another trial. The experimenter has no control over the intertrial interval and therefore the time can vary from trial to trial, typically three to five minutes if the training situation is close to the hives. Second, the duration of exposure to the stimuli cannot be controlled because bees are allowed to fly freely around the stimuli leaving the experimenter no control over the bees' orientation to each stimulus. Third, bees need to be rewarded on every visit to ensure that they continue to return to the experimental situation, so an unrewarded experience with a stimulus must be followed by reward on that stimulus or another stimulus. Despite these limitations, the greatest advantage of the free-flying technique is that it can be used with a variety of stimuli including colors, magnetic fields, landmarks, shapes, sizes, textures, *et cetera*. Furthermore, it is the main technique used in this laboratory and other laboratories for studying both basic learning phenomena and, now, more cognitively complex phenomena, such as concept learning.

Introduction to Concept Learning Studies

The ability of subjects to learn about the relationship between or among stimuli has been the primary focus of concept learning studies in nonhuman animals. This line of research is quite interesting because some animal researchers and most human researchers (see Kuczaj & Hendry, 2003; Penn, Holyoak, & Povinelli, 2008; Premack, 1983) have proposed that language may be an important or necessary component underlying the ability to learn concepts. Nonetheless, there is evidence of concept learning in a variety of vertebrate species. Most of those studies focus on whether the animals are capable of learning about "sameness" and "differentness." Recently, same/different concept learning has begun to be a focus of research with bees (Giurfa, 2015).

The term concept learning will be used throughout this paper but it is by no means standard. Other terms, often used interchangeably in the literature, include relational learning, relational discrimination, stimulus relations, relational concepts, abstract or relational category, abstract concepts, higher-order relations, generalized concepts, and more recently abstract thought (Cook and Wasserman, 2012; Wasserman, 2016). The conflicting definitions and terminology for "concept" may not just be a difference of style or phrasing. The different terminology may in fact be describing different learning phenomena that rely on different mechanisms. Furthermore, the variety and lack of "common currency of terminology" among researchers who study concept learning creates what Zentall, Wasserman, Lazareva, Thompson, and Rattermann (2008) describe as the "murky" topic of concepts. Regardless, the aim here is not to try to disambiguate the incoherent terminology, but instead, the aim is to determine if the cognitive abilities of honeybees require a conceptual explanation beyond associative learning principles. For example, although some might consider category learning to be a type of concept learning, in most cases associative principles can explain it in terms of learning about common stimulus features. (See Chapter 3 for a fuller discussion of concept vs. category learning.)

Methods for Studying Same/Different Concept Learning

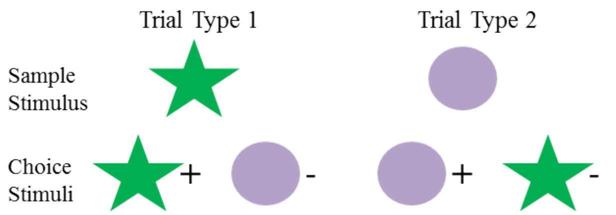
The main experimental designs and procedures that have been used to study same/different concept learning in vertebrates include matching-to-sample, nonmatching-to-sample, oddity discrimination, and same/different discrimination. There are a number of variations in the details of the procedures, but all of them require the subject to learn about the relationship among stimuli in order to respond appropriately to obtain reward. Four of the common procedures are diagrammed in Table 1. In the matching-to-sample procedure, shown in panel A of Table 1, a subject is first presented with a sample stimulus (*e.g.*, a green star). Following the presentation of the sample stimulus, the subject is presented with two choice stimuli, one that is identical to the sample and one that is different from the sample (*e.g.*, a green star vs. a purple circle). The subject is rewarded for choosing the stimulus that matches the original sample. In the classic matching-to-sample procedure, training involves only two stimuli, and on half of the trials, one of the stimuli serves as the sample and on the other half of the trials, the other stimulus serves as the sample. In order to rule out the possibility that the subjects learned specific responses on each of the two types of training trials, a transfer test with novel stimuli is necessary. Successful performance in the transfer test is taken as evidence for the learning of concept, in this case “sameness.”

The second procedure is the nonmatching-to-sample or oddity-from-sample procedure, that is identical to the matching procedure except the subject is rewarded for choosing the stimulus that does <u>not</u> match the sample. This procedure is shown in panel B of Table 1. Again, a transfer test to novel stimuli is necessary to rule out the possibility that the subjects learned specific responses on each of the two types of training trials. Successful performance in that test is taken as evidence for the learning of concept, in this case “differentness.” A common variation of the matching- and nonmatching-to-sample procedures is to impose a delay between exposure to the sample stimulus and the presentation of the comparison stimuli. This variation can be used to study short-term memory for the sample stimulus and also has been used to study concept learning.

The third procedure that often is used to study concept learning is the oddity discrimination procedure shown in panel C of Table 1. Typically, oddity problems involve three stimuli (although more stimuli can be used), one that is different or “odd” and two that are identical or “nonodd.” Only choice of the odd stimulus is rewarded. The traditional oddity problem is conducted with two stimuli, A and B. There are two basic types of training trials, ABB with A odd and BAA with B odd. (With the position, left, middle, and right, of the odd stimulus balanced over trials, there are six possible configurations of the stimuli: ABB, BAB, BBA, BAA, ABA, AAB.) Choice of the odd stimulus on each trial is rewarded. Again, in order to rule out the possibility that the subjects learned specific responses for the configurations of the training trials, a transfer test with novel stimuli is necessary. Successful performance on the transfer test is taken as evidence of concept learning, in this case “oddity.”

Table 1. Diagram of the four common procedures used to study same/different concept learning.

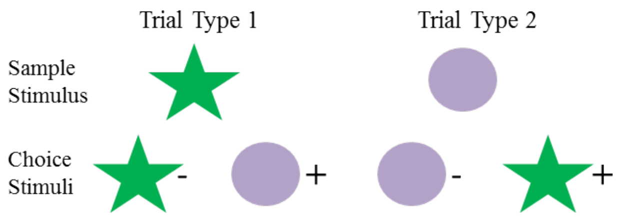
A. Matching-to-sample



Single-group Design

- Given a sample stimulus.
- Either simultaneous or successive presentation of two comparison stimuli.
- Rewarded for choice of the stimulus that matches the sample.

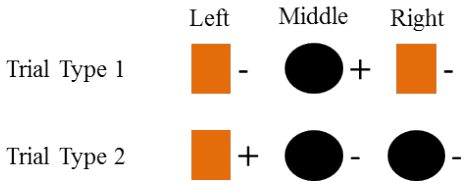
B. Nonmatching-to-sample



Single-group Design

- Given a sample stimulus.
- Either simultaneous or successive presentation of two comparison stimuli.
- Rewarded for choice of the stimulus that does not match the sample.

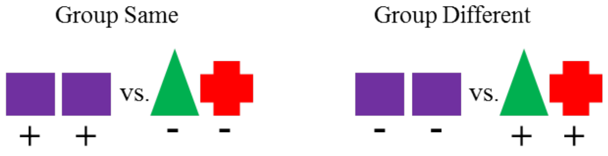
C. Oddity



Single-group Design

- Odd stimulus rewarded on every trial.
- In training, the odd stimulus appears equally often in each position (left, middle, and right), so there are six possible configurations of the two stimuli.

D. Simultaneous S/D



Two-group Design

- Group Same: rewarded for choice of the same pair.
- Group Different: rewarded for choice of the different pair.

The fourth procedure is the simultaneous same/different discrimination problem shown in panel D of Table 1. In this procedure, a subject is presented with two pairs of stimuli. In one pair the stimuli are the same (*e.g.*, two squares) and in the other pair the stimuli are different (*e.g.*, a triangle and a vertical rectangle). One group of subjects is rewarded for choosing the same-stimulus pair, and one group of subjects is rewarded for choosing the different-stimulus pair. If only a limited set of stimuli is used in training, it is possible that the subjects can learn specific responses for each pair of training stimuli. Therefore, a transfer test with novel pairs of stimuli is necessary, and successful performance in the test is taken as evidence for the learning of concept, in this case, “sameness” or “differentness.”

As was noted for all four of the procedures described above, transfer tests with novel stimuli are necessary if there is a small set of stimuli used in training. If the subjects show better-than-chance performance in training, there are two possible explanations. One possibility is that they learned a same/different concept, but the second possibility is that the subjects learned the correct response to the training stimuli. The primary purpose of a transfer test is to rule out the second possibility. It is expected that if the subjects had learned a concept, they will be able to respond correctly when presented with novel stimuli in a transfer test. Alternatively, it is expected that if the subjects had learned the correct response to the training stimuli, they would <u>not</u> be able to respond correctly when presented with novel stimuli in a transfer test.

For example, if an oddity experiment was conducted with a set of two stimuli (say, red-R and green-G), then there are two trial types, RGG with red odd and GRR with green odd. Since there are three positions, left, middle, and right, there are six different stimulus combinations that can be used in the oddity training (RGG, GRG, GGR, GRR, RGR, RRG). Typically training is quite extensive; if there are, say, 1200 trials in training, then each combination is presented 200 times (typically randomized over trials). If training performance is better than chance, one possibility is that the subjects had learned the correct response for each configuration (choose red if presented with RGG, choose G if presented with GRR, etc.). If so, the subjects will not choose the odd color on a transfer trial with a new set of colors (*e.g.*, yellow, purple, purple) at a level better than chance. The second possibility is that the subjects had learned to choose the odd color on the basis of the relationship among the colors presented on each trial, that is, they learned the oddity concept. If so, the subjects should be able to choose the odd color on a transfer trial with a new set of colors at a level better than chance.

The problem with transfer tests, as they have been implemented in same/different problems is that there is usually more than one trial with the novel stimuli. If the subjects are rewarded on the transfer test trials, then it is possible that the subjects can also learn to respond correctly to the novel stimuli. In those cases, it is important to look at the performance on the first transfer trial (Thomas, 1996). However, a one-trial transfer test may not be sensitive enough to determine if the subjects were using a concept to solve the original training problem. There could be a performance decrement on the transfer trial simply due to the change in stimuli. To avoid this problem, there is another procedure that can be used to assess concept learning. In the trial-unique procedure, the sets of stimuli presented on each training trial are always unique, so essentially every trial is a transfer trial. Therefore, successful performance in training is taken as evidence of concept learning. However, the trial-unique procedure is not always practical. For example, if extensive training is required for successful performance then the set of stimuli must be extremely large to have unique stimuli on each training trial. In those cases, transfer tests may be necessary. One variation is to use trial-unique stimuli in the transfer test rather than in the original training.

There are experiments that have successfully used some variation of a trial-unique procedure. These include matching-to-sample experiments with chimpanzees (Hayes, Thompson, & Hayes, 1953), dolphins (Herman & Gordon, 1974), rhesus monkeys (Katner, Davis, Kirsten, & Taffe, 2004), macaque monkeys (Overman & Doty, 1980), honeybees (Shishimi, 2013), and pigeons (Wright, Cook, Rivera, Sands, & Delius, 1988). Also included are oddity experiments with honeybees (Muszynski & Couvillon, 2015) and a Californian sea lion (Hille, Dehnhardt, & Mauck, 2006), as well as a same/different discrimination experiment with a harbor seal (Scholtyssek, Kelber, Hanke, & Dehnhardt, 2013). It is interesting to note that when the trial-unique procedure has been used it is typically for delayed matching-to-sample experiments in order to reduce memory interference from trial-to-trial (Hayes, Thompson, & Hayes, 1953). The majority of vertebrate studies of same/different concept learning are not trial-unique, and, therefore, use transfer tests, likely because learning is slow and many trials are required. All of the experiments reported here with honeybees use the trial-unique procedure. Generally, discrimination learning is very fast for honeybees and extensive training is not required (Couvillon & Bitterman, 1991). Therefore, it is feasible to use a trial-unique procedure with honeybees as has been demonstrated in recent same/different experiments with the oddity procedure (Muszynski & Couvillon, 2015) and with the matching- and nonmatching-to-sample procedures (Shishimi, 2013).

Previous Same/Different Concept Learning Experiments with Bees

There have been a number of attempts to demonstrate same/different concept learning in bees, including matching-to-sample, nonmatching-to-sample, same/different discrimination, and oddity learning. These early studies follow the tradition in comparative psychology, specifically in the comparative analysis of learning, of analyzing the performance of different species in similar learning problems (Bitterman, 1975). The aim is to understand the evolutionary trajectory, convergence and divergence, of learning capabilities. The emphasis of comparative psychology has been first and foremost to assess what various species are capable of learning rather than to assess why they have the capability or how it might be adaptive. Nonetheless, it is easy to conceive of reasons why learning a concept of same and different might be adaptive for most species including honeybees.

An average colony of honeybees contains about 20,000 bees. There is one queen bee (fertile female), several hundred or more drones (males), and the rest are worker bees (sterile females). The queen bee is responsible for laying eggs, the drones are responsible for mating with queens, and the workers are responsible for all of the hive functions both inside and outside. The life span of a worker bee is typically five to seven weeks. Newly hatched workers begin their lives maintaining the inside of the hive; such tasks might involve feeding the queen and other larvae, cleaning debris from the hive, packing pollen, processing and storing honey, and guarding the hive from intruders. The last few weeks of a worker's life involves gathering nectar, pollen, water, and propolis (plant sap).

Worker honeybees can forage up to a five-mile radius from their hive (*c.f.*, Greenleaf, Williams, Winfree, & Kremen, 2007, for a review of foraging range in multiple bee species). During their foraging bouts, it is imperative that the bees learn cues in order to navigate to and from the hive and the floral source; cues that they use include, odor, magnetic fields, flower color, landmarks, polarized light, and the location of the sun. While locating food sources it would be advantageous for bees to learn when to stay on a flower type and when to switch to new flower types. Even though honeybees are likely to sample different flower types, they have a tendency to stay with one at a time (Townsend-Mehler, Dyer, & Maida, 2011). The bee must be able to make a choice between flower types and to recognize same vs. different flower types. Furthermore, the bees may well need to discriminate nectar-containing flowers and empty flowers of the same flower type. In any case, it is clear that the ability to discriminate sameness and differentness could very well facilitate foraging efficiency.

The first formal study to look explicitly for same/different concept learning in the honeybee was by Giurfa, Zhang, Jenett, Menzel, and Srinivasan (2001). They conducted a series of delayed matching- and nonmatching-to-sample experiments with honeybees in a Y-maze with a single entrance or stem and two arms that create a Y-shape. Each bee was trained to fly through a “sample” stimulus card in the stem of the maze to reach the arms where two comparison stimulus cards were presented. For example, on a training trial with a blue sample card in the stem, the bees had to choose between a yellow and a blue stimulus card in the arms. Choice was defined as flying into a hole in the center of one of the two stimulus cards; correct choice was rewarded with access to sucrose in the feeding station behind the stimulus card. The stimuli could be colors, odors, horizontal and vertical lines, or circles and radial patterns. Bees in the matching group were rewarded for choice of the stimulus card that matched the sample card and bees in the nonmatching group were rewarded for choice of the stimulus card that did not match the sample card. After training had ended, the bees in each of the experiments had unrewarded transfer test trials with novel stimuli that were intermixed with additional training trials. The overall correct choice for all bees in both delayed matching and nonmatching-to-sample problems was significantly greater than chance for training and transfer tests. The authors interpreted the results as evidence of same/different concept learning. However, the repeated transfer tests used the same two “novel” stimuli, and the initial transfer performance was not reported. Arguably, the performance on the first transfer test is the best indicator of concept learning. Furthermore, the number of choices during transfer tests was summed for all bees, possibly giving disproportionate weight to the performance of high responders. Nonetheless, the results hinted at the possibility that honeybees can indeed solve same/different concept problems.

Brown and Sayde (2013) trained four colonies of bumblebees in a same/different problem. Only one colony (about 150-200 bees) was used at a time and each colony was trained and tested sequentially. The colony was placed flush against a small entrance to a wooden apparatus that consisted of a flight chamber, an LCD display mounted such that the screen was part of the floor of the apparatus, two chamber entrances (left and right) that were opened and closed by a moving door, and a reward chamber. Multiple bees were allowed to enter the apparatus at the same time. Bees then flew through the flight chamber over the LCD monitor that displayed two stimuli on the horizontal surface of the apparatus. The stimuli used in the experiment were either colors (blue and yellow) or black and white line patterns (45 and 135 degree orientations). If the two stimuli were the same, the bees had to choose one of the chamber doors, and if the two stimuli were different, the bees had to choose the other chamber door. Choice was defined as hovering in front of the chamber door. If a bee made a correct choice, it was allowed to feed freely at a feeding station in the reward chamber. Bees in each colony had 24-hour access to the experimental apparatus and training lasted several weeks. Following training, each hive had a transfer test with novel stimuli; the bees trained with color were transferred to line orientations, and the bees trained with line orientations were transferred to colors. The procedure for the transfer test was identical to the training procedure except that nondifferential reward was used, that is, choice of either chamber was equally likely to be rewarded. The cumulative responding for correct and incorrect choices for the bees in each colony was measured throughout training and transfer tests. In training, for all colonies expect one, the bees made the correct response more frequently than the incorrect response. In the transfer tests, all colonies made the correct response more frequently than the incorrect response.

Although the results are intriguing, they are difficult to interpret. The authors mention that the bees could have learned to choose the correct chamber by choosing the side that had an opened door, rather than the choosing the door that represented the correct relationship of the displayed stimuli. The authors also note that the unmarked bees from a colony were able to freely access the apparatus and that it is possible that only the performance from a relatively small number of bees accounted for most of the choices made throughout training and transfer tests.

Unlike Giurfa et al. (2001) and Brown and Sayde (2013) who conducted transfer tests with novel stimuli to evaluate the same/different concept learning abilities of bees, both Shishimi (2013) and Muszynski and Couvillon (2015) used the trial-unique procedure. Shishimi (2013) found better-than-chance performance in a series of matching- and nonmatching-to-sample experiments using a completely trial-unique procedure, that is, with different stimuli on each trial. Free-flying foragers were trained to visit a laboratory window for sucrose solution. The series of experiments included variation in the presentation of the sample stimulus and the delay between the sample stimulus and the choice stimuli. For example, in one of the delayed matching-to-sample experiments, a bee was rewarded for landing on a sample stimulus where it fed to repletion and then flew to the hive to unload. When the bee returned to the laboratory window, the bee had a choice trial with two stimuli, one identical to the original sample stimulus of the previous visit and one different from the sample stimulus. Choice of the matching stimulus was rewarded with sucrose, and, again, the bee returned to the hive to unload. On the next visit to the window, the bee was rewarded for landing on a new sample stimulus, flew back to the hive to unload and returned again for a choice trial with a stimulus that matched the most recent sample and one that was different. The bees had 16 choice trials, and the stimuli used on each trial were unique. The stimulus set included a variety of colors, shapes, and line patterns. Despite the very long delay between presentation of the sample and the choice trial (average three minutes), the bees learned to choose the stimulus that matched the sample on the previous visit. The results of this series of trial-unique experiments are the first to provide strong evidence for same/different concept learning in honeybees.

To continue this line of research on the conceptual learning abilities of honeybees, Muszynski and Couvillon (2015) conducted a series of trial-unique three-stimulus oddity and nonoddity experiments. Again, free-flying forager bees were trained to visit a laboratory window for sucrose solution. In the oddity experiments, bees were presented with three stimuli, one odd and two identical nonodd stimuli on each training trial. Choice of the odd stimulus was rewarded with sucrose, and choice of either of the nonodd stimuli was punished with an aversive stevia solution. In the nonoddity experiments, bees were rewarded for choice of either of the nonodd stimuli and punished for choice of the odd stimulus. In all of the experiments, there were 15 training trials, each with novel combinations of stimuli, that is, trial-unique. The stimuli were two-color patterns created with green, yellow, orange, and blue wedges arranged in a pinwheel shape. Performance in both the oddity and nonoddity experiments was significantly better than chance. The results provide additional evidence that honeybees are able to learn about concepts, here, an oddity concept.

In summary, the results of the four bee experiments reviewed above do suggest that bees can learn about sameness and differentness. Bees are the only invertebrates for which there is any evidence for the ability to learn about relationships among stimuli. There has been one attempt with octopus to demonstrate oddity learning (Boal, 1991) but it was unsuccessful. There is still much work that needs to be done in order to determine the extent and robustness of concept learning in bees and other invertebrates.

New Experiments with Honeybees

Overall, the purpose of the experiments presented in this dissertation is to build on the initial results for honeybees that suggest they are, in fact, using same/different concepts. The experiments are presented in four separate chapters, which are followed by a general discussion. The specific aims of the research were: **1)** to determine if the performance of honeybees in oddity problems can be improved by adding more nonodd stimuli; **2)** to determine if honeybees can solve a simultaneous same/different problem with the same two-color patterns used in the oddity studies; **3)** to demonstrate that honeybees can discriminate solid and two-color pattern stimuli as categories; and **4)** to determine if performance in same/different concept problems is facilitated by a category difference among the stimuli.

CHAPTER 1. CONCEPT LEARNING: FOUR-STIMULUS ODDITY LEARNING

Background Literature on Oddity Learning

Traditional Oddity

Oddity learning is arguably the simplest of the same/different concept problems and is a good place to begin with an invertebrate. The first formal experiment conducted by Robinson (1933) used a single macaque monkey who was presented with three objects, one odd colored object and two identical nonodd colored objects. The objects were placed on the floor about an arm's length away from the front of the monkey's cage. Attached to each of the objects was a heavy cord with a brass ring at the end. Only the odd object was moveable and if the monkey chose that object, it was allowed to eat the food that had been placed under the odd object. There were two trial types; on half the trials, the stimuli were presented as A+ B- B-, and on the other half, the stimuli were presented as B+ A- A-, with position (left, middle, right) balanced across trials. The subject did solve the problem, albeit after more than 400 training trials, and Robinson (1933) suggested that the monkey had learned a concept, here, "the abstraction of oddity." An associative explanation, however, is possible since there were only six combinations of stimuli (*e.g.*, ABB, BAB, BBA, BAA, ABA, AAB) and, with such extensive training, the monkey may simply have learned the consequences for response to each of the combinations.

The archetypal traditional oddity problem as conducted by Robinson (1933) is characterized by having a limited stimulus set consisting of only two stimuli that could form two basic trial types (ABB and BAA) with position balanced over trials. Interestingly, subsequent work using the traditional oddity problem has been limited, most likely due to the concern that subjects may simply learn the correct response to each of the stimulus combinations rather than a concept. Pastore (1954) increased the number of nonodd stimuli in a traditional oddity experiment. Three canaries were presented with one odd object and eight identical nonodd objects simultaneously. The canaries were trained to choose by jumping to a perch in front of an object, and choice of the odd object was rewarded with food. The subjects received two stimulus combinations (ABBBBBBBB and BAAAAAAAA) in alternation across trials, with the position of the odd target varied over trials. All three canaries showed better-than-chance performance after extensive training. Interestingly, the author characterized the traditional oddity problem as a series of reversals and suggests that an abstract concept of oddity is not necessary to explain the successful performance.

Zentall, Hogan, Edwards, and Hearst (1980) conducted a series of traditional oddity experiments with pigeons in which the number of nonodd stimuli on each trial varied from two to 24. The pigeons were presented with colored key lights and were rewarded with food for choosing the odd color whose position was balanced over trials. The pigeons' performance was facilitated by the increase in the number of nonodd alternatives. The authors suggest that the facilitation is due to a perceptual process, such as the Gestalt figure-ground principle, or, in contemporary terms, a "pop-out" effect (Blough, 2001).

Robinson (1933) attributed the success of her monkey in the traditional oddity problem to concept learning, but Pastore (1954) and Zentall et al. (1980) did not interpret their results as concept learning, but, instead, they suggested that a perceptual learning explanation was possible. It is worth noting, however, that the distinction between concept and percept is not clear (Bromer, 1940), and neither Pastore (1954) nor Zentall et al. (1980) offer any discussion as to what perceptual learning entails. Nonetheless, the traditional oddity problem is not the best procedure to study same/different concept learning in animals since its solution may be explained by simple associative learning.

Multi-stimulus-set Oddity

This limitation of the traditional oddity problem was readily recognized and there was still considerable interest in the concept learning capabilities of animals, and in subsequent research, there were several procedural variations of the oddity problem. The variations can be characterized as multiple-stimulus-sets, multiple oddity problems, transfer tests with novel stimuli, or some combination of these. Muszynski and Couvillon (2015) classified all of these variations as multiple-stimulus-set oddity problems.

The earliest multiple-stimulus-set oddity experiments were conducted with monkeys using the Wisconsin General Test Apparatus (WGTA: Harlow & Bromer, 1938). The WGTA consisted of an enclosure for the experimental subject with a wall that had a moveable tray. In the oddity experiment, the tray would contain three simultaneously placed objects, one odd and two nonodd, and food was placed underneath the odd object. The general procedure, if the subjects learned the original training problem, was to present them with a series of transfer problems, each with different objects. In experiments with monkeys by Bromer (1940), Meyer and Harlow (1949), and Moon and Harlow (1955), the monkeys were successful on the transfer problems.

Instead of using transfer tests, Levine and Harlow (1959) trained two groups of monkeys on a series of successive oddity problems, each with different objects. Monkeys in one group were presented with daily sessions of three different 12-trial oddity problems. Monkeys in the other group were presented with daily sessions of one-trial oddity problems that were created from a pool of 36 different objects, essentially making every trial unique in each daily session. Although both groups learned the oddity problems, the monkeys trained in the one-trial oddity problems had higher levels of performance compared to the monkeys trained in the 12-trial oddity problems. Subsequent studies with monkeys used this multiple-problem procedure to explore the effects of variations in training parameters on oddity learning. For example, Draper (1967) varied stimulus parameters including the size, color, and form of the odd objects to determine whether oddity problems were more likely to be solved with some dimensions than others. Davis, Leary, Stevens, and Thompson (1967) varied the training procedures in oddity problems, using specialized “guided” training and additional cues in order to facilitate learning.

The first multiple-stimulus-set oddity experiment with a species other than monkeys was conducted by Wodinsky and Bitterman (1953). Rats were trained in the jumping stand developed by Lashley (1938) to jump to the location of the odd stimulus of a set of three stimulus cards to obtain reward. The rats mastered the original oddity problem and successfully transferred to new problems. The results of later studies under different conditions, however, suggested that rats may have difficulty with oddity problems. In a study by Koronakos and Arnold (1957), rats were trained in a multiple-choice runway apparatus (Fields, 1953) to choose the odd stimulus of a set of five stimulus cards to obtain reward. The rats solved the original training problem but had difficulty in transfer tests. In more recent work, rats were presented with a tray of three objects and were rewarded for choosing the odd object based on either odor or visual cues (Bailey & Thomas, 1998; Thomas & Noble, 1988). Again, the rats solved a series of training problems but the first trial performance on each successive problem was not better than chance, in other words there appeared to be no transfer. The mixed results with rats are puzzling given that there is evidence that rats prefer novel objects or oddity (Forwood, Bartko, Saksida, & Bussey, 2007).

Research on multiple-stimulus-set oddity learning has since been conducted with a wide variety of vertebrate species. As was the case with rats, studies with cats produced mixed results. Cats trained in the WGTA to choose the odd object of a set of three stimulus objects to obtain reward successfully transferred only with specialized training (Warren, 1960; Boyd & Warren, 1957). In a broader comparative study with chimpanzees, monkeys, cats, and raccoons, again with the WGTA, both cats and raccoons solved the original oddity problem but were not given transfer tests. The chimpanzees and monkeys not only solved the original oddity problem but were successful on transfer tests (Strong & Hedges, 1966).

Ravens and gulls trained to peck the odd object out of a set of six three-dimensional objects arranged horizontally on a tray successfully solved the oddity problem as well as the transfer tests (Benjamini, 1983). Goats trained to choose the odd stimulus of a set of four stimuli projected in equal sectors on a computer monitor performed better than chance on a series of oddity problems. They were then shifted to a trial-unique transfer test with 48 unique sets of stimuli. Only a single goat was able to successfully transfer (Roitberg & Franz, 2004). A sea lion was first trained to press the odd stimulus card of a set of three stimulus cards in a series of simple discriminations problems of the form A+ B- B-. Then, the sea lion was transferred to a series of oddity problems. In the final transfer test, the stimuli were all novel and the procedure was trial-unique, and the sea lion performed better than chance (Hille, Dehnhardt, & Mauck, 2006). Archerfish were trained to spray a jet of water at the odd stimulus shape of a set of four stimuli in a traditional oddity problem, but only half of the four subjects were able to solve the oddity problem. In a transfer test with novel stimuli, none of the subjects solved the problem (Newport, Wallis, & Siebeck, 2014).

Besides the comparative study by Strong and Hedges (1966) that found successful oddity transfer in chimpanzees, there are surprisingly few studies of oddity in chimpanzees. The reason may be that chimpanzees have a small innate tendency to choose novel stimuli (Nissen & McCulloch, 1937). In a formal study to assess that tendency, Davenport and Menzel (1960) presented chimpanzees with what was essentially a trial-unique oddity problem. However, choice of the odd stimulus was never rewarded. The chimpanzees showed a tendency to choose the odd object from a tray of objects with a probability greater than expected by chance. However, a later study showed that chimpanzees trained in an instrumental chamber with images projected on response keys were able to successfully solve a trial-unique oddity problem with a probability of correct choice too high to be explained by the small innate tendency to choose novel stimuli (Devine & Ivens, 1969). Similarly, monkeys (Bromer, 1940), rats (Forwood, Bartko, Saksida, & Bussey, 2007), and gray jays (Waite, 2008) also have shown a small initial preference for the odd stimulus.

Summary

Multiple-stimulus set oddity experiments have been conducted with a variety of vertebrate species, most of which have shown reasonably good performance. In contrast, there has been almost no work on oddity learning in invertebrate species; there has been one oddity study conducted with octopuses (Boal, 1991) and one with honeybees (Muszynski & Couvillon, 2015). In a series of experiments, wild caught octopuses were trained inside laboratory tanks to grab the odd shell of a set of three mollusk shells to obtain frozen squid reward. Then, the octopuses were trained in a three-stimulus oddity problem with novel sets of stimuli on every trial, but they did not successfully solve the problem (Boal, 1991). As noted above, honeybees trained to choose the odd pattern from a set of three patterns were successful even with different stimuli on every training trial. In addition, honeybees trained <u>not</u> to choose the odd pattern from a set of three patterns also were successful (Muszynski & Couvillon, 2015). More research needs to be conducted with honeybees in order to explore whether oddity learning is a robust phenomenon and to determine the conditions and parameters that facilitate learning of the oddity concept. In the study presented here, the parameter of interest is set size, that is, the number of stimuli presented in an oddity problem.

Experiment 1: Oddity with Pattern Stimuli

Introduction

In Muszynski and Couvillon (2015), free-flying forager honeybees were trained in both oddity and nonoddity problems with three two-color pattern stimuli. In the oddity problems, bees were rewarded for choosing the odd pattern in a set of three patterns. In the nonoddity problems, bees were rewarded for choosing either of the two “nonodd” patterns from a set of three patterns. It is remarkable that honeybees were able to solve both problems, reaching a level of correct choice greater than chance. In fact, the honeybee is the first invertebrate species to learn an oddity problem. However, there was some variability and room for improvement in the level of correct choice. Typically, oddity problems have proved difficult for vertebrate species as well. In fact, vertebrates such as pigeons may require hundreds to thousands of trials to reach better-than-chance performance. Furthermore, there usually is a fair amount of variability, and asymptotic performance is not usually 100% correct. Although the bees reached asymptotic better-than-chance performance in only 15 trials, discrimination learning generally is very fast for honeybees and extensive training is not required (Couvillon & Bitterman, 1991).

One of the parameters that has been found to improve performance in oddity problems with vertebrate species is the number of incorrect stimuli presented on each training trial (Pastore, 1954 with canaries; Zentall et al., 1980 with pigeons; see also Nissen & McCulloch, 1937 with primates; Williams, 1967 with pigeons). One of the proposed mechanisms for this facilitation is a perceptual “pop-out effect,” which may increase attention to the odd stimulus (Blough, 2001).

In this experiment, the purpose was two-fold: to replicate the results of the previous oddity work and to determine if an increase in the number of incorrect exemplars would improve performance. The hypothesis is that the addition of another incorrect pattern stimulus will increase the level of correct choice in the honeybees' oddity performance, that is, that a four-stimulus oddity problem will be easier than a three-stimulus oddity problem.

Method

Subjects: The subjects were 8 honeybees (*Apis mellifera*) never used in prior experiments. They were captured in matchboxes at feeders containing 10-20% sucrose solution which were located near the hives behind the Békésy Laboratory at the University of Hawai‘i at Mānoa. Each subject was trained individually in a single daily session lasting from two to three hours.

Apparatus: The bees were trained in a wooden enclosure that was 61 cm wide, 61 cm high, and 61 cm deep, and recessed in a window on the exterior wall of the laboratory. The enclosure was open to the outside and on the inside was fitted with two sliding Plexiglas panels. The transparent panels allowed the experimenter access to the enclosure and permitted observation of the bee during training trials. They also served to prevent unwanted entrance of the bee into the laboratory. The training situation is shown in Figure 1.

Figure 1. The training situation used in all of the experiments. Shown in this picture is one of the trials in the four-stimulus oddity problem of Experiment 1.

Stimuli: The stimuli used were two-color patterns and consisted of six equal segments arranged in a pinwheel shape and mounted on the surface of a Petri dish, 5.5 cm in diameter. The patterns were constructed with two of the following colors in alternating sequence: blue, yellow, orange, and green. The four colors could be combined to make six different patterns: yellow and green, yellow and blue, yellow and orange, blue and orange, green and orange, and green and blue. From the six pattern stimuli, 15 unique sets of training stimuli could be created. (Note that the colors used to make the pattern stimuli were chosen because they have been used in previous experiments in this laboratory and were found to be highly discriminable and equally preferred.)

Pretraining: Each subject in the experiment was collected in a matchbox at the feeder and brought to the laboratory for pretraining. In order to ensure exposure to all of the colors to be used in training, the pretraining stimulus was constructed of four equally divided segments of the four colors (blue, green, yellow, orange). This pretraining stimulus was placed in the middle of the floor of the wooden enclosure and contained a 100-µl drop of 50% sucrose measured with a 1-ml syringe. (A foraging honeybee will typically drink between 40 and 60-µl before returning to the hive.) The captured bee inside of the matchbox was released at the drop on the surface of the pretraining stimulus, and as the bee began to drink, it was marked on the thorax with colored enamel for identification purposes. The bee drank until replete and then flew to the hive to unload the sucrose. If the bee returned to the enclosure on its own (usually in three to five minutes), it again found the pretraining stimulus with a drop of 50% sucrose. The bee then landed and drank the sucrose until replete and returned to the hive to unload. In the event that a marked bee did not return to the enclosure, it was recaptured at the feeder and re-placed on the pretraining stimulus. If the marked bee still did not return on its own, another bee was selected from the feeder. Pretraining ended after a bee had returned on its own to drink on the pretraining stimulus.

Training: Each bee was trained individually and given 15 training trials in a session that lasted two to three hours. As described above, it was only possible to generate 15 unique sets of the stimuli. An example of one of the training sequences used in Experiment 1 is shown in Figure 2. On each trial, there were four two-color patterns, three identical nonodd stimuli and one odd stimulus. A 100-µl drop of 50% sucrose (+) was placed in the middle of the odd stimulus and a 100-µl drop of 10% stevia (-) was placed in the middle of all the nonodd stimuli. Stevia solution was used because it is highly aversive to bees (see note at the end of this section), and it is not visually discriminable from sucrose. Note that stevia and sucrose are not visually discriminable. See Appendix C.

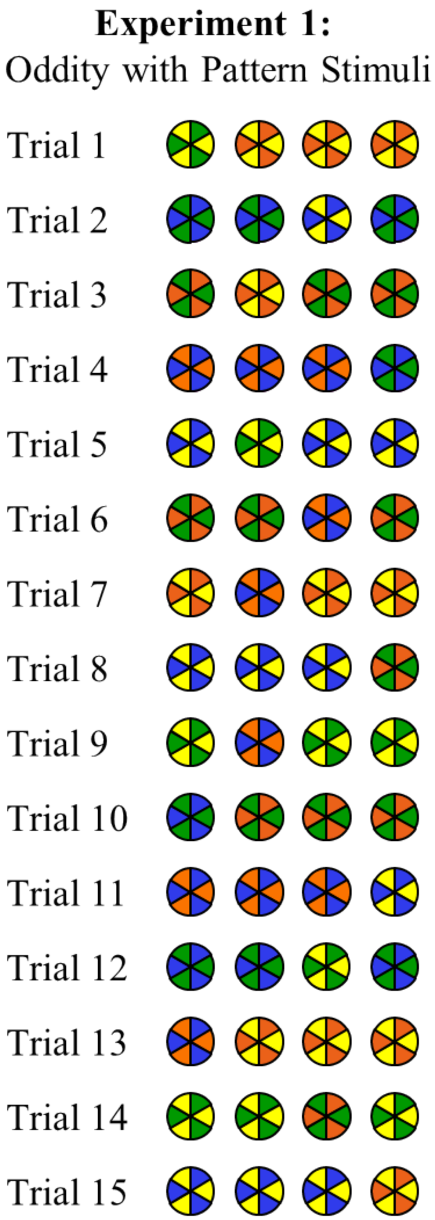


Figure 2. A sample training sequence for the four-stimulus oddity problem of Experiment 1.

Four training sequences were created from the 15 unique combinations of stimuli and two subjects were run with each sequence. The position of the odd stimulus was balanced so that throughout the 15 training trials the odd stimulus appeared three to four times in each of the four positions in a quasi-random sequence (far left, middle left, middle right, and far right). Each of the four sequences was created so that neither the same position nor the same two-color pattern stimulus was ever rewarded on two trials in a row.

All choices were recorded on each of the 15 training trials. A correct initial choice was defined as landing on the odd stimulus and making contact with the drop of sucrose. An incorrect initial choice was defined as landing on any of the nonodd stimuli and making contact with the drop of stevia solution. A correction procedure was used so that if the bee chose incorrectly it was allowed to choose again until it made contact with the drop of sucrose on the odd stimulus. The bee drank the sucrose, flew to the hive to unload, and then returned to the window (usually within three to five minutes) for the next trial. At the end of the session, the bee was captured and sacrificed to ensure that it was not used again in any experiments.

Results & Discussion

The performance of the bees is plotted in Figure 3 as the proportion of bees with an initial correct choice on each trial. Overall, the bees' performance reached a level above chance which is .25 for a four-stimulus oddity problem. Although the first trial was better than chance, there was no obvious reason, and the assumption is that it is just sampling error. For analysis of the data, the proportion of correct choice for all 15 trials was computed for each bee. The overall mean proportion of correct choice was .43, which is significantly greater than the chance value of .25, $t(7) = 3.6$, $p = .008$ with a standard error of the mean of .03. The results suggest that the bees had learned to choose the odd stimulus. The terminal performance reached about .5 which is greater than chance, but still relatively low.

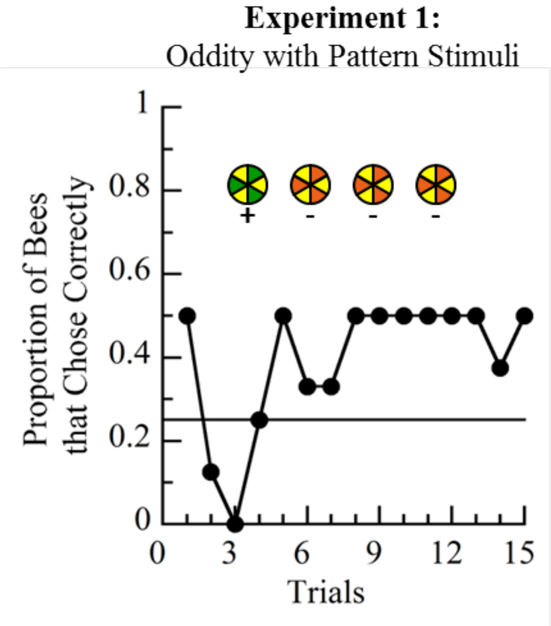

Experiment 1:
Oddity with Pattern Stimuli

Figure 3. Results for the four-stimulus oddity problem of Experiment 1. The horizontal line at .25 represents chance.

It is common in vertebrate studies of choice discrimination learning in difficult problems to further analyze the results to determine if the subject's performance could have been influenced by a position or stimulus preference (Moon & Harlow, 1955). If the bees did not have a position preference, it is expected that they will be equally likely to choose the odd stimulus in each of the four positions. Each of the four positions was odd on three or four trials. An analysis of variance of the proportion of correct choice for each position did not show any significant effect $F(3, 21) = 4.2$, $p = .74$. Similarly, if the bees did not have a stimulus preference, it is expected that they will be equally likely to choose the any of the six stimulus patterns when each of them was odd. Each of the six stimulus patterns was odd on two or three trials. An analysis of variance of the proportion of correct choice for each of the six stimulus patterns did not show any significant preference for any of the six two-color pattern stimuli $F(5, 35) = 1.28$, $p = .29$.

Another analysis, whether the bees showed a tendency to follow the previously rewarded position was analyzed. Position reward following is when a subject chooses the same position on trial $n+1$ that had been rewarded on trial n. For example, if on trial n the left position was rewarded, position reward following on trial $n+1$ would be indicated by initial choice of the left position. The mean proportion of trials on which the bees choose the position rewarded on the previous trial was .18 which was somewhat less than chance (.25, since there were four positions), $t(7) = -2.25$, $p = .059$. If anything, the bees had a tendency to not follow the previously rewarded position. That is, the bees had a tendency to switch from one position to another position from trial-to-trial. This is not surprising given that the training sequences were created such that the same position was never rewarded on two trials in a row.

None of the analyses give evidence that the bees used any systematic preferences or choice strategies to solve the oddity problem. Therefore, the honeybees’ performance suggests that they were able to learn a trial-unique oddity problem with four two-color pattern stimuli presented on every trial. Performance reached better than chance levels, but clearly, the problem was difficult (*c.f.,* Figure 3). It is not possible to compare directly the results of Muszynski and Couvillon’s (2015) three-stimulus oddity problem with the results of this four-stimulus oddity problem. However, the performance in the four-stimulus problem is certainly not better than that in the three-stimulus problem. It was hypothesized that the addition of the fourth stimulus may facilitate the honeybees’ performance in the oddity problem, but that did not appear to be the case.

There is some evidence in the vertebrate literature that using more than the two incorrect exemplars typical of the three-stimulus traditional oddity problem (ABB and BAA) improves performance. Pastore (1954) working with canaries failed to find oddity learning in the traditional oddity problem but found good performance when the number of incorrect exemplars was eight (ABBBBBBBB and BAAAAAAAA) in a nine-stimulus oddity problem. Zentall et al. (1980) trained pigeons with the number of incorrect exemplars varied from two to 24 and found the best performance with more exemplars. There may be several reasons that there was no qualitative improvement between the honeybees' performance in the previous three-stimulus oddity problems and their performance in the four-stimulus oddity problem presented here.

One reason may be that there was not a great enough perceptual difference between three and four stimuli. It may be worthwhile to increase the number of incorrect exemplars in future experiments. A second reason may be that the stimuli used were too similar, since the same two-color patterns were used throughout training but in unique sets on each trial. Pastore (1954) used household items that were different on more than one dimension, such as shape, size, texture, and color. An expanded set of stimuli, varying in multiple dimensions, might be useful for future studies of oddity in honeybees. A third reason may be that the mechanism underlying the oddity performance of honeybees is different from the mechanism underlying the oddity performance of vertebrate species. Oddity is only one of the same/different problems that have been studied in vertebrates, therefore, it may be instructive to look at the performance of honeybees in other same/different concept problems.

Although the addition of an incorrect exemplar did not appear to improve performance on the four-stimulus oddity experiment, the results of this experiment do add to the growing body of evidence that honeybees are able to solve oddity problems. The question now is how robust are these problem-solving abilities. A logical next step with honeybees would be to look at simultaneous same/different discrimination. There are no such studies with honeybees and the one study with bumblebees (Brown & Sayde, 2013), as discussed above, is difficult to interpret.

CHAPTER 2. CONCEPT LEARNING: SAME/DIFFERENT DISCRIMINATION

Background Literature on Same/Different Discrimination

Like the oddity procedure, the same/different discrimination procedure is used to assess concept learning in animals, that is, the ability to learn about the relationship among stimuli. The relationship that is learned in a same/different problem is whether the items in a pair of stimuli or in an array of stimuli are all the same or are different from one another. The most common variations of the same/different procedure include: simultaneous, delayed, array, and successive discriminations. See Table 2 for a depiction of each of the variations of the same/different procedure as they have been used with pigeons.

Early Studies: Rats & Primates

The earliest work on same/different discrimination learning utilized an array procedure (Wodinksy, 1954). A group of rats was presented with three stimulus cards in the Lashley jumping stand apparatus. The center window of the stand had a gray card. The left and right stimulus cards were both white (WW), or both black (BB), or one white and one black (WB and BW), balanced across trials. The rats were rewarded for jumping to the left card if there were two black or two white cards, and the rats were rewarded for jumping to the right card if there was one black and one white card. (The design is a two-item array version of the array procedure in panel C of Table 2.) The rats were able to discriminate and reached an asymptote of about 75% correct. Although the results suggest a same/different relational discrimination, as Wodinsky (1954) acknowledges, there was no transfer test to novel stimuli to rule out the possibility that the rats had simply learned the correct response to each array (BB, WW, BW, and WB).

Robinson (1955) conducted the first simultaneous same/different experiment with chimpanzees. (See panel D of Table and panel A of Table 2 for examples of simultaneous same/different discrimination.)They were presented with two pairs of three-dimensional objects. The objects in one pair were the same, and the objects in the other pair were different from one another. In order to receive a reward, chimpanzees had to choose one of the wooden objects in the same pair. After training, the chimpanzees were presented with novel household items and were successfully able to discriminate the same pairs of items from the different pairs. However, the study did not include a group trained with the different pair rewarded which is necessary to rule out the possibility that the chimps have a preference for “sameness” or are choosing on the basis of “more” or “less” of a stimulus.

In subsequent work, Robinson (1960) set out to determine what cues might be important in same/different discriminations. Chimpanzees again were trained on the simultaneous same/different problem of the earlier study in which reward was only given on the same pair. Then, the chimpanzees were presented with a series of unrewarded discriminations to determine what cue they might be using to solve the problem. The dimensions of the test stimuli varied in degree of sameness and number of objects. For example, the primates might be given a choice of a triad of same objects (AAA) and a pair of same objects (AA) or a choice of a triad of same objects (AAA) and a triad that had two same objects and one different object (AAB). The results of both unrewarded preference tests indicated that the primates preferred the set of objects that contained the higher number of same objects (AAA > AA and AAA > AAB).

Further research with primates, chimpanzees and orangutans, on same/different discrimination was conducted by King (1973). The stimuli in the experiment had two dimensions, color and shape. Two groups of subjects were presented on each trial with a pair of stimuli that were the same in shape but different in color (AX and BX) and a pair of stimuli that were the same in color but different in shape (CY and CZ). Both pairs had a same dimension, and the purpose of this study was to determine if the primates could learn to choose same on the basis of only one dimension despite the sameness in the other dimension. Group Same-Shape was rewarded for choosing the pair of shapes that was the same (blue circle and green circle or AX and BX) but not for choosing the pair of shapes that were different (yellow triangle and yellow square or CY and CZ). Group Same-Color was rewarded for choosing the pair of colors that were the same (yellow triangle and yellow square or CY and CZ) but not for choosing the pair of colors that were different (blue circle and green circle or AX and BX). Group Same-Color had an easier time than Group Same-Shape, but both groups solved the problem and were able to successfully transfer to novel sets of colors and shapes. There also were no species differences between the chimpanzees and the orangutans, and King (1973) noted that this kind of same/different problem could be used in future studies of "multi-cue learning."

Following these early rat and primate studies, there was very little additional work on same/different concept learning with rats. The reason may have been the lack of a good simultaneous choice procedure for rats because the Lashley jumping stand fell out of favor. However, the work with primates did continue and expanded to other concept or relational problems. The general conclusion from early studies seems to be that concept learning may be a cognitive domain reserved for humans and nonhuman primates. Such a view was pervasive and may be the reason that there was very little early work on any other species.

Matching-to-sample: Pigeons

Although there were few studies of concept learning in nonprimate species, there were a number of investigations of the general discriminative abilities of pigeons. Demonstrations of category learning in pigeons (Cumming & Berryman, 1961) for instance further ignited interest in the pigeons' ability to learn concepts. What followed was a series of experiments on matching-to-sample and nonmatching-to-sample to assess same/different concept learning in pigeons.

In a typical matching-to-sample problem, a subject is presented on some trials with a sample stimulus (red) followed by the presentation of two choice stimuli (red and green) and choice of red is rewarded; on other trials, the sample stimulus is green and the two choice stimuli again are red and green but now with choice of green rewarded. In a nonmatching problem, if the sample stimulus is red then choice of the green stimulus is rewarded, and if the sample stimulus is green then choice of the red stimulus is rewarded. The early work with pigeons produced ambiguous results. For instance, in Cumming and Berryman (1961), pigeons were trained in a typical matching-to-sample task using color stimuli. When transferred to novel colored stimuli, the pigeons did not show transfer.

Zentall and Hogan (1974) conducted both matching- and non-matching-to-sample with pigeons with a very small set of colored stimuli. Following training, transfer trials to novel colored stimuli were presented to all pigeons. Half of the pigeons in the matching group were given novel transfer trials to another matching problem, and the other half of the pigeons in the matching group were given novel transfer trials to a nonmatching problem. Likewise, half of the pigeons in the nonmatching group were given novel transfer trials in another nonmatching problem, and the other half of the pigeons were given novel transfer trials in a matching problem. It was expected that if the pigeons had learned a concept, the pigeons that were shifted to the same problem type would perform better on transfer trials than the pigeons that had been shifted to the different problem type. For example, pigeons that had been trained on a matching problem should have better performance in a new matching problem. The pigeons in both groups performed better on transfer trials when given the same problem type. The authors interpret their results as evidence of concept learning. However, the pigeons in all of the transfer conditions began the transfer problem with correct choice at about 50% which was chance. Although all of the groups learned the transfer problem faster than the original training problem, there was no evidence of better-than-chance performance even in the first session of the transfer problem. The data do not support a relational or conceptual interpretation.

Wilson, Mackintosh, and Boakes (1985) conducted a similar set of experiments with pigeons using colored stimuli with similar results. However, unlike Zentall and Hogan (1974), they included control groups trained on a conditional discrimination and then transferred to a matching-to-sample problem. The conditional discrimination does not require a relational explanation; for example, if the "sample" was brown, then pigeons had to choose blue when presented with a choice of blue and green, and if the "sample" was mauve, the pigeons had to choose green when presented with a choice of blue and green. The transfer from a conditional discrimination to a matching-to-sample problem should have been more difficult than the transfer of the matching- to-sample group to another matching-to-sample problem. In fact, there was no difference in the performance of the control and the matching group on the matching problem in the transfer test. Furthermore, like Zentall and Hogan (1974), there was no evidence of better-than-chance performance at the start of the transfer training. In both sets of experiments, the pigeons learned the transfer problems faster than the original training problems which is simply evidence of "learning to learn." There is no need to invoke a relational interpretation for the results of either set of experiments (Wilson et al., 1985; Zentall & Hogan, 1974).

Same/Different: Primates vs. Pigeons

The negative results with pigeons led Premack (1983) to argue that same/different concept learning is an ability that only primates can acquire. Essentially, he suggested that good performance in simple matching- and nonmatching-to-sample experiments does not demonstrate concept learning abilities. His view was that without variation in the training stimuli, matching- and nonmatching-to-sample reduce to conditional discrimination problems. Since the solution of conditional problems can be explained with associative principles, there is no need to invoke relational concepts like same/different to explain successful matching- and nonmatching-to-sample problems. On the basis of his work with monkeys and apes, Premack suggested that a better test for same/different concept learning is the simultaneous same/different discrimination problem (AA vs. CD, BB vs. EF, etc.).

However, Premack (1983) did conduct a simultaneous matching-to-sample experiment with language- and nonlanguage-trained chimpanzees. Instead of matching on the basis of single stimulus (*e.g.*, if red sample, choose the red stimulus), the chimpanzees were required to match the relationship, same or different. On each trial, a pair of items was presented as the sample (AA or AB). The chimpanzees were then presented with two more pairs of items (CC and ED), and if the sample was a pair of same items (AA) then they were rewarded for choice of the same pair (CC). If the sample was a pair of different items (AB) then they were rewarded for choice of the different pair (ED). Each trial was unique which made it unnecessary to conduct a transfer test with novel stimuli. Of all the chimpanzees, the only one that was successful at solving the problem was Sarah, who reportedly learned to solve the problem with 100% accuracy. Since

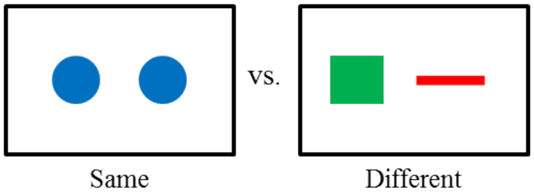
Sarah was a language-trained chimpanzee, Premack (1983) further suggested that language training might be a necessary component for learning a same/different concept.

Same/Different Problem Variations: Pigeon Researchers Answer Premack

Given the lack of evidence of same/different concept learning from the pigeon matching- and nonmatching-to-sample experiments, researchers began to design new same/different problems with the aim to find the conditions under which the pigeons might show learning about same/different concepts. Edwards, Jagielo, and Zentall (1983) turned to an array problem like that shown in panel C of Table 2 except using only two-item arrays. Pigeons were shown a single pair of shapes that were projected on a stimulus panel. If the two stimuli were the same, the pigeons were rewarded for making one response, and if the two stimuli were different, the pigeons were rewarded for making another response. The pigeons solved the discrimination and then were given transfer tests with novel colored key-lights. However, the performance began at chance which is not evidence for transfer of same/different learning.

Table 2. Diagram of the common same/different designs used with pigeons. (A. Blaisdell & Cook, 2005; B. Katz & Wright, 2006; C. Wasserman, Hugart, & Kirkpatrick-Steger, 1995; D. Young, Wasserman, & Dalrymple, 1997).

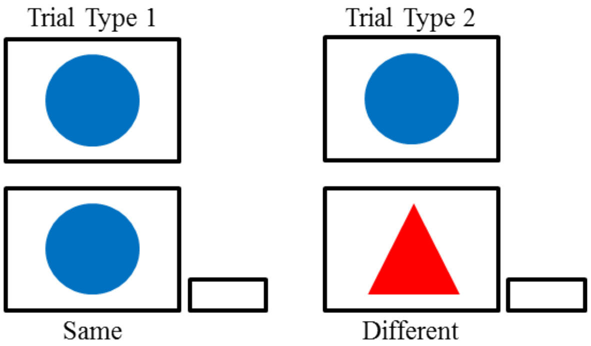
A. Simultaneous S/D



Two-group Design

- Group Same: rewarded for pecking the same pair.
- Group Different: rewarded for pecking the different pair.

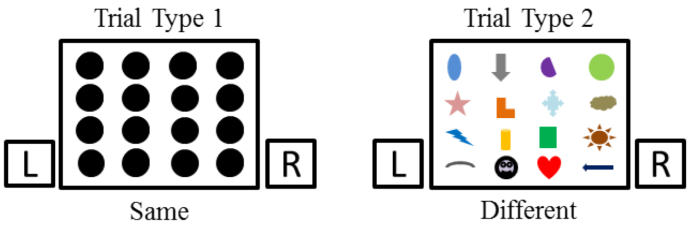
B. Delayed S/D



Single-group Design

- Top stimulus is presented first, followed by two bottom stimuli.
- If Trial Type 1 is presented, rewarded for pecks to the matching bottom stimulus.
- If Trial Type 2 is presented, rewarded for pecks to the white rectangle.

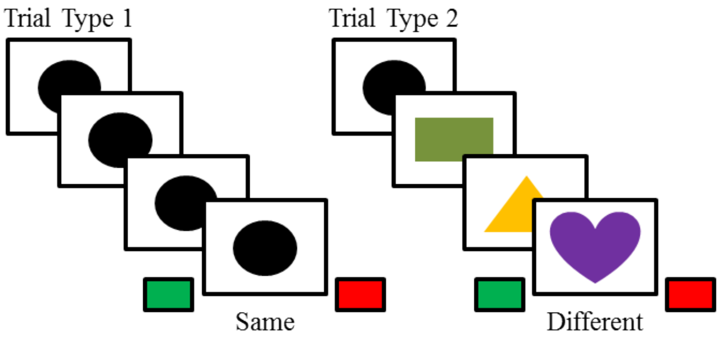
C. Array S/D (Two-item and Multi-item Arrays)



Single-group Design

- If Trial Type 1 is presented, rewarded for pecks to the left (L) response key.
- If Trial Type 2 is presented, rewarded for pecks to the right (R) response key.

D. Successive S/D



Single-group Design

- If Trial Type 1 is presented, rewarded for pecks to the green response key after 16 stimuli have been displayed.
- If Trial Type 2 is presented, rewarded for pecks to the red response key after 16 stimuli have been displayed.

Santiago and Wright (1984) also conducted a similar two-item array experiment with pigeons using a larger pool of stimuli which in this study were colored pictures. With this large stimulus pool, a large number of stimulus pairs could be used in training, approximating a trial-unique training procedure. If the two stimuli were the same, the pigeons had to make one response, and if the two stimuli were different, the pigeons had to make another response. The results suggest that the pigeons could solve the problem with extensive training. However, there were only two pigeons and the extensive training included repetition of the stimulus pairs so training was not actually trial-unique; therefore, it is difficult to interpret the results as same/different learning without a transfer test to novel stimuli.

Wasserman, Hugart, & Kirkpatrick-Steger (1995) used multi-item arrays instead of two-item arrays. Their arrays consisted of 4x4 grids of icons with the icons all the same on same trials and the icons all different on different trials like those shown in panel C of Table 2. On each trial, the pigeons were presented with a single array. If the icons in the array were all the same, the pigeons were required to make one response, and if the icons in the array were all different, the pigeons were required to make another response. After training, pigeons were given transfer trials to novel arrays, and performance was significantly better than chance. Furthermore, it was much better than the performance on two-item array discriminations. Wasserman et al. (1995) conclude that these array results argue, "…against Premack's (1983) earlier conclusion that, among nonhuman animals, only language-trained chimpanzees can show same/different conceptualization." (For a detailed review of more recent multi-item array procedures used with pigeons, see Cook and Wasserman, 2012.)

Young, Wasserman, and Dalrymple (1997) conducted another kind of same/different problem with pigeons using the successive same/different procedure shown in panel D of Table

2. In this procedure, pigeons were presented with a sequence of pictures and were required to make one response if the pictures had been all the same and were required to make a different response if the pictures had not been all the same. Although pigeons are able to solve this discrimination, the procedure has not been broadly used to test concept learning, most likely because there is a memory component in the problem that can complicate the interpretation of the results. In order to successfully solve this successive problem, the pigeon must remember every stimulus in the sequence that it had seen (typically 16 stimuli).

Katz and Wright (2006) used yet another kind of same/different problem with pigeons, the delayed procedure shown in panel B of Table 2. Pigeons were first presented with a photograph which was followed by the presentation of another photograph below the first and a white rectangle to the right of the two photographs. If the two photographs were the same, the pigeons were required to peck the bottom photograph, and if the two photographs were different, the pigeons were required to peck the white rectangle. Pigeons had extensive training with a large set of photographs and reached above-chance levels of performance. There were 90 unique transfer trials with novel stimuli, and the pigeons choose correctly on more than 80% of those trials. The results were clear evidence of same/different concept learning.

Contrary to Premack's (1983) argument that only primates were capable of same/different concept learning, there now is considerable evidence from different kinds of same/different problems that pigeons are capable of same/different concept learning. Premack (1983) also had argued that the simultaneous same/different problem is the best procedure for establishing the ability to learn same/different concepts. (See panel D of Table 1 and panel A of Table 2 for examples.) Given the success of the pigeons in several different types of same/different problems, Blaisdell and Cook (2005) argued that it was important to conduct a simultaneous same/different experiment with pigeons.

Blaisdell and Cook (2005) used the simultaneous same/different procedure with pigeons that had been used earlier with primates. They trained pigeons with two pairs of simultaneous shapes on a touch screen monitor in the design shown in panel A of Table 2. One group of pigeons was rewarded only for choosing the pair of stimuli that was the same and another group of pigeons was rewarded only for choosing the pair of stimuli that was different. Both groups of pigeons were successful on training and transfer tests. The results are the first clear evidence of same/different concept learning in pigeons using a procedure comparable to that used with primates. The authors suggest that the success on this simultaneous problem may have been facilitated by a large stimulus set and the use of the touch screen monitor.

In summary, Premack (1983) argued that only language-trained primates can learn concepts. His claim led many researchers to develop several new procedures for assessing same/different concept learning in pigeons. The results from these post-Premack experiments clearly indicate that pigeons can respond on the basis of same/different concepts. Interestingly, while the Premack-pigeon controversy inspired new experiments with pigeons, a number of studies appeared with other species. The results of these provide additional support for the idea that same/different learning might be a general ability in a variety of species, not just language-trained primates.

Comparative Same/Different Studies

In a separate line of investigations, researchers were interested in whether species other than pigeons and primates are able to learn same/different concepts. These comparative studies have used the traditional simultaneous choice procedure and the delayed procedure. The first such study was conducted by Chausseil (1991) using coatis as subjects. Coatis are moderately sized mammals that live in South America and are a member of the same family as raccoons. In this experiment, four coatis were presented with a simultaneous same/different task similar to that shown in panel A of Table 2. The stimuli were shapes created from black foil that was pasted on white cards. The coati had to climb up an elevated platform that bifurcated and had two boxes at the end. On one side of the platform, there was a box that had the same pair of stimuli displayed on the outside, and on the other side of the platform there was a box that had the different pair of stimuli displayed on the outside. Two of the coatis were rewarded for choosing the box that displayed the same pair and the other two were rewarded for choosing the box that displayed the different pair. Both groups were trained to 75% criterion of correct choice on the training pairs which required about 300 trials. All subjects had a series of 18 transfer tests with novel pairs of stimulus shapes and patterns, that were run for 50 trials each. All the subjects learned to respond correctly to all but one of the transfer tests. However, analysis of the first trial of each of the transfer tests showed performance at chance and therefore the performance in the transfer did not provide evidence for same/different concept learning.

Mercado, Killebrew, Pack, Mácha, and Herman (2000) conducted a study with dolphins on same/different concept learning using the two-item array procedure similar to that shown in panel C of Table 2. Two dolphins experienced with three-dimensional objects were given training with two objects held above the water by two different trainers. When given a command, the dolphins were required to touch each of the stimuli and then proceed to one of two paddles to make a response. If the two three-dimensional objects were the same, the dolphins were required to choose one response paddle (left), and if the two three-dimensional objects were different, the dolphins were required to choose a different response paddle (right). In the second stage of training, both dolphins were presented with the same two three-dimensional objects under the water. After performance on these new training trials reached a criterion of about 75% correct choice, the dolphins were then presented with a transfer test to novel two-dimensional objects. On the first exposure to the novel two-dimensional objects, one dolphin correctly classified the object pairs with 100% accuracy and the other dolphin with 80% accuracy. The authors interpret their results as evidence that dolphins perform similarly to primates and are capable of learning a same/different concept.

Scholtyssek, Kelber, Hanke, and Dehnhardt (2013) conducted an experiment with a harbor seal in a go/no go variation of the delayed same/different procedure shown in panel B of Table 2. All of the stimuli were presented on a touch screen monitor and consisted of a variety of different white shapes. The seal was trained to enter into a dark chamber that contained the touch screen monitor and was required to touch its muzzle to a target on the floor beneath the monitor to indicate it was properly positioned for a trial. Then, it was shown an object on its left followed by an object on its right. If the two objects were the same, the seal was required to remain still for several seconds (“no go”), and if the two objects were different, the seal was required to touch the monitor with its muzzle for several seconds (“go”). Correct responses were rewarded with fish, and incorrect responses were not rewarded and were followed by a brief “time-out.” The seal had extensive training culminating in trial-unique transfer tests with unfamiliar stimuli. The seal performed at a level significantly above chance, and the results are evidence of same/different concept learning.

In an attempt to expand the study of same/different concept learning to other avian species, Wright, Magnotti, Katz, Leonard, and Kelly (2015) studied the performance of Clark’s nutcrackers in a delayed same/different problem similar to that shown in panel B of Table 2. The same photographs used in the previous experiments with pigeons were used with the nutcrackers. The birds were first presented with a photograph which was followed by the presentation of another photograph below the first and a white rectangle to the right of the two photographs. If the two photographs were the same, the pigeons were required to peck the bottom photograph, and if the two photographs were different, the pigeons were required to peck the white rectangle. The nutcrackers had about 3300 training trials with a large set of photographs and then had transfer trials with novel stimuli. Only some of the birds (four out of seven) showed better-than-chance performance on the transfer tests, so it is difficult to interpret the results. In a nearly identical experiment, Magnotti, Wright, Leonard, Katz, and Kelly (2016) used black-billed magpies. Again, as with the nutcrackers, not all of the birds chose correctly on transfer trials: seven out of ten performed at levels above chance and three did not. Taken together, the results of the two studies suggest that under some conditions avian species other than pigeons can learn a same/different concept.

Newport, Wallis, and Siebeck (2015) studied archerfish in a simultaneous same/different experiment similar to that shown in panel A of Table 2. Archerfish are small triangular shaped fish that live in both fresh and brackish water. They disable prey, water-based and land-based insects, by spitting a highly accurate stream of water at them. In pretraining, all fish were presented with four successive problems, each with a same and a different pair of simple line drawings displayed simultaneously on a computer screen behind the Plexiglas wall of the tank. The choice response was spitting a jet of water at one of the pairs. For one group, choice of the same pair was rewarded, and for another group choice of the different pair was rewarded. Five out of the six fish discriminated the two pairs of stimuli in the four successive problems. Then, both groups were trained with combinations of six new line drawings again presented in same and different pairs on each trial. Only one fish from the different group was able to discriminate at a level above chance. Therefore, only that fish was subsequently tested in transfer trials with a new set of line drawings. Transfer performance was poor. The authors conclude that more research is needed to determine whether fish are capable of learning a same/different discrimination problem.

In a recent study, Martinho and Kacelnik (2016) conducted a simultaneous choice same/different experiment with mallard ducklings. The general design is like that shown in panel A of Table 2. The ducklings were hatched in a dark room and immediately put in a lighted area with other ducklings. The pretraining for all ducklings was exposure to a single pair of moving objects. The objects in the pair were either the same shape or different shapes (sphere/sphere or cone/cylinder). Then, the ducklings were returned to the dark room for a 30-minute retention interval. After that interval, each duckling was presented with two new pairs of moving objects. One pair of objects contained two novel shapes that were the same (pyramid/pyramid), and the other pair had two novel shapes that were different (cube/rectangular prism). The number of approaches to each pair of novel objects was recorded, and if more than half of their approaches was to one of the pairs of the moving objects, it was scored as a preference. Ducklings that were pretrained and imprinted with a same pair of objects were more likely to prefer the same pair of the novel set, and ducklings that were pretrained and imprinted with a different pair of objects were more likely to prefer the different pair of the novel set. The authors interpret their findings as evidence that ducklings can choose on the basis of a same/different concept, and furthermore, that they learned to do so without ever having received any explicit reinforcement.

Lastly, Russell and Burke (2016) conducted a series of simultaneous same/different problems using one echidna. Again, the general design is like that shown in panel A of Table 2.

The echidna is a mammalian species in the same monotreme subclass as the platypus. Both echidna and platypus lay eggs instead of giving birth to live young. The echidna was placed in a runway box where it encountered two hinged plywood doors that displayed a set of four shapes that were the same or a set of four shapes that were all different from each other. In training, if the background color was black, the echidna was rewarded for choosing the shapes that were all the same, and if the background color was white, the echidna was rewarded for choosing the shapes that were all different. The echidna reached about 75% correct on both same and different trials, and in the transfer training the echidna chose correctly on each trial with the new arrays. Although there was only one subject, the fact that the echidna could simultaneously choose same in one background condition and different in the other background condition is a strong argument for same/different concept learning.

In summary, the results of these same/different problems with a variety of species other than pigeons and primates provide strong evidence that the ability to learn a same/different discrimination may be a general capability of vertebrate species. It is generally agreed that good performance in a simultaneous same/different experiment provides the most unambiguous evidence of same/different concept learning (Blaisdell & Cook, 2005; Premack, 1983; Robinson 1955). Ironically, there are very few studies using the simultaneous same/different discrimination procedure. Most, if not all of them, are shown in Table 3. In spite of the fact that there are so few of these studies with vertebrate species, it still seemed reasonable to attempt such a study with honeybees. In Experiment 2, bees were presented with a simultaneous same/different problem in a design similar to that of Blaisdell and Cook (2005) as shown in panel A of Table 2. This experiment represents the first attempt to study simultaneous same/different discrimination in an invertebrate.

Table 3. Simultaneous same/different experiments. List of species that have been tested with a simultaneous same/different problem.

Experiments	Species	Design	Success
Robinson (1955/1960)	Chimpanzees	AA vs. CD	No
King (1973)	Chimpanzees & Orangutans	AX & BX vs. CY & CZ	Yes
Chausseil (1991)	Coati	AA vs. CD	Yes
Russel & Burke (2016)	Echidna	AAAA vs. CDFG	Yes
Blaisdell & Cook (2005)	Pigeons	AA vs. CD	Yes
Martinho & Kacelnik (2016)	Ducklings	AA vs. CD	Yes
Newport et al. (2015)	Archerfish	AA vs. CD	No

Experiment 2: Same/Different Discrimination with Pattern Stimuli

Introduction

Experiment 1 extended the oddity studies of Muszynski and Couvillon (2015) from a three-stimulus oddity problem to a four-stimulus oddity problem using the same set of two-color pinwheel stimuli. Honeybees were able to solve the oddity problem with four stimuli as they had with three stimuli. The finding that honeybees can solve oddity problems suggests that they are able to discriminate same from different stimuli. The question now is how general is their ability to discriminate same vs. different. As was discussed above in the literature review for this chapter, there are a number of procedures that have been developed for studying same/different discrimination in a variety of vertebrate species. Nonetheless, the general view is that the ability to solve a simultaneous same/different discrimination problem, like that shown in panel A of Table 2, can provide unambiguous evidence of same/different concept learning. There are no such studies with honeybees and indeed there are no such studies with an invertebrate species. In this experiment, honeybees were trained with the same set of two-color pinwheel stimuli as used in the three- and four-stimulus oddity problems. They were trained with two pairs of pinwheel stimuli presented simultaneously, and one pair had two identical stimuli and the other pair had two nonidentical stimuli. The training was trial-unique, that is the two pairs were unique on every training trial.

Interestingly, there are two possible kinds of trials for presenting a pair of same stimuli with a pair of different stimuli. For example, if A, B, and C each represent a two-color pinwheel, then the same pair of AA could be presented with a different pair of AB (AA vs. AB). In this case, the difference between the two pairs is accentuated by the fact that the B stimulus is the only one that is different from the others. In other words, the B stimulus might stand out as being odd. The other option is to present the same pair AA with an entirely different pair like CB (AA vs. CB). In that case, the B stimulus is not the odd one because both stimuli in the different pair are different than the stimuli in the same pair.

Given that the honeybees have successfully solved the oddity problems, it seemed reasonable to determine whether an oddity variation of the same/different problem would be easier than a nonoddity variation. Therefore, in this experiment, one group of bees, Oddity Type, was trained only with pairs of stimuli that shared a common stimulus, such as AA vs. AB. Another group of bees, Nonoddity Type, was trained only with pairs of stimuli that did not share a common stimulus, such as AA vs. CB. Examples of the actual training stimuli are shown in Figure 4. Visual inspection of the sequences suggests that in the Oddity Type training, the odd stimulus may “pop-out” and therefore make it easier to discriminate the same and different pairs.

Method

Subjects: The subjects were 32 honeybees (*Apis mellifera*) never used in prior experiments. They were captured at feeders containing 10-20% sucrose solution which were located near the hives in back of the Békésy Laboratory at the University of Hawaiʻi at Mānoa. Each subject was trained individually in a single daily session lasting from two to several hours.

Apparatus: The bees were trained in the same wooden enclosure used in Experiment 1 and shown in Figure 1. The dimensions of the wooden enclosure were 61 cm wide, 61 cm high, and 61 cm deep. The enclosure was recessed in a window on the exterior wall of the laboratory, open to the outside, and fitted with two sliding Plexiglas panels on the inside. The transparent panels allowed the experimenter access to the enclosure and permitted observation of the bee during training trials. They also served to prevent unwanted entrance of the bee into the laboratory.

Stimuli: The stimuli used were the same two-color pattern stimuli used in Experiment 1. Each stimulus consisted of six equal segments arranged in a pinwheel pattern and mounted on the surface of a Petri dish, 5.5 cm in diameter. The pinwheels consisted of two of the following colors in alternating sequence: blue, yellow, orange, and green. The four colors could be combined to make six different patterns: yellow and green, yellow and blue, yellow and orange, blue and orange, green and orange, and green and blue. These colors were chosen because they have been used in previous experiments from this laboratory and are highly discriminable and equally preferred.

Pretraining: Each subject in the experiment was collected in a matchbox at the feeder and brought to the laboratory for pretraining. In order to ensure exposure to all of the colors to be used in training, the pretraining stimulus was constructed of four equally divided segments of the four colors (blue, green, yellow, orange). This stimulus was placed in the middle of the floor of the wooden enclosure and contained a 100-μl drop of 50% sucrose measured with a 1-ml syringe. (A foraging honeybee will typically drink between 40 and 60-μl before returning to the hive.) The captured bee was released from the matchbox at the drop on the surface of the pretraining stimulus, and as the bee began to drink, it was marked on the thorax with colored enamel for identification purposes. The bee drank until replete and then flew to the hive to unload the sucrose. If the bee returned to the enclosure on its own (usually in three to five minutes), it again found the pretraining stimulus with a drop of 50% sucrose. The bee then landed and drank the sucrose until replete and returned to the hive to unload. In the event that a marked bee did not return to the enclosure, it was recaptured at the feeder and re-placed on the pretraining stimulus. If the marked bee still did not return on its own, another bee was selected from the feeder. Pretraining ended after a bee had returned on its own to the pretraining stimulus.

Training: In the simultaneous same/different discrimination problem used here there were two pairs on every trial. The stimuli in one of the pairs were the same and the stimuli in the other pair were different from each other. Of the possible pairs of two-color pattern stimuli, it was possible to create two variations of the problem. In the first variation, the same and different pairs of two-color patterns had no stimuli in common (Nonoddity Type) and in the second variation, the same and different pairs of two-color patterns had a shared stimulus (Oddity Type). In each of the variations (nonoddity and oddity), a group of bees was rewarded for choice of the same pair of stimuli and another group of bees was rewarded for choice of the different pair of stimuli. The four groups of bees were as follows: Nonoddity Type Same Group, Nonoddity Type Different Group, Oddity Type Same Group, and Oddity Type Different Group.

Each bee was trained with 15 unique pairs of stimuli. The pair of stimuli that was rewarded had a 100-µl drop of 50% sucrose (+) in the middle of each stimulus. The pair of stimuli that was not rewarded had a 100-µl drop of 10% stevia (-) in the middle of each stimulus. Note that stevia and sucrose are not visually discriminable. See Appendix C.

A wooden divider, approximately 25 cm in length, .5 cm in width, and 3 cm in height was used between the two pairs of stimuli in order to make the pairs more visually separable. Four training sequences were created for each of the four groups and two subjects in each group were run with each sequence. The position of the same pair and the different pair of stimuli were balanced throughout the 15 training trials, such that the same pair and different pair of stimuli appeared seven or eight times in each position in a quasi-random sequence. In addition, each of the four sequences was created so that a pair of two-color pattern stimuli never occurred more than once on successive trials, although individual two-color patterns could appear on successive trials. A sample training sequence for the two main groups of bees is shown in Figure 4.

All choices were recorded on each of the 15 training trials. For the Same Groups, a correct initial choice was defined as landing on either of the same stimuli and making contact with the sucrose drop. For the Different Groups, a correct initial choice was defined as landing on either of the different stimuli and making contact with the sucrose drop. An incorrect initial choice was defined as landing on either of the unrewarded stimuli (*i.e.*, different stimuli for the same subjects and same stimuli for the different subjects) and making contact with the stevia solution. A correction procedure was used so that if the bee chose incorrectly, it was allowed to choose again until it made contact with the drop of sucrose on one of the stimuli in the correct pair. The bee drank the sucrose, flew to the hive to unload, and then returned to the window (usually within three to five minutes) for the next trial. At the end of the session, the bee was captured and sacrificed to ensure that it was not used again in any experiments.

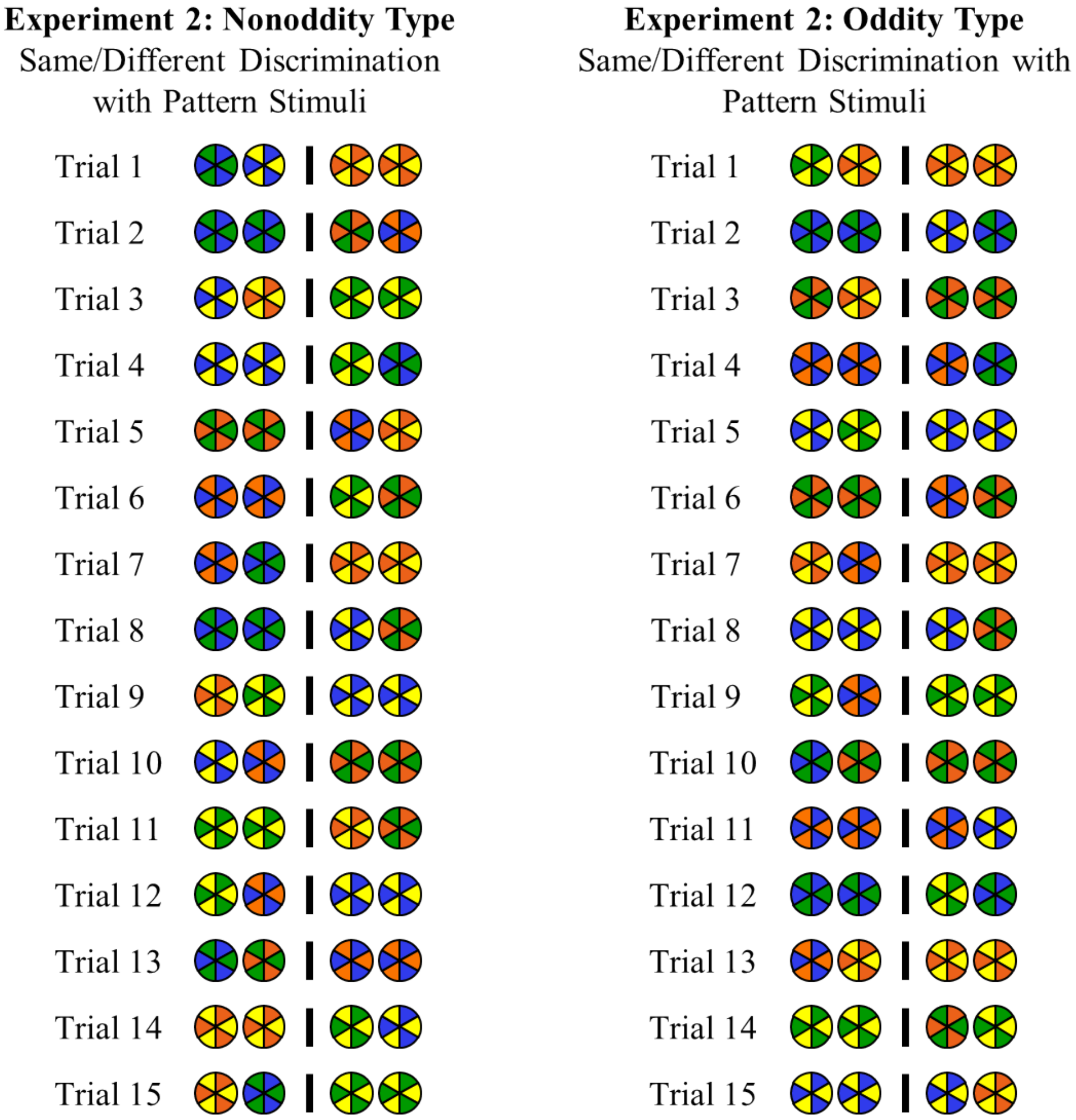


Figure 4. Sample training sequences for the same/different problem of Experiment 2. The black line between each pair represents a wooden divider.

Results & Discussion

The performance of the bees is plotted in Figure 5 as the proportion of bees with an initial correct choice on each trial. The bees in each group were able to solve the same/different problem with better-than-chance performance. For analysis of the data, the proportion of correct choice for all 15 trials was computed for each bee. For the bees in the Nonoddity Type Different Group, the overall mean proportion of correct choice was .67, which is significantly greater than the chance value of .5, $t(7) = 5.44$, $p = .001$ with a standard error of the mean of .03. For the bees in the Nonoddity Type Same Group, the overall mean proportion of correct choice was .59, which is significantly greater than the chance value of .5, $t(7) = 2.53$, $p = .04$ with a standard error of the mean of .03. For the bees in the Oddity Type Different Group, the overall mean proportion of correct choice was .66, which is significantly greater than the chance value of .5, $t(7) = 2.87$, $p = .02$ with a standard error of the mean of .05. For the bees in the Oddity Type Same Group, the overall mean proportion of correct choice was .72, which is significantly greater than the chance value of .5, $t(7) = 6.17$, $p < .001$ with a standard error of the mean of .03.

While all groups generally performed better than chance, the curves reflect a fair amount of variability both within and between groups suggesting that the discrimination is difficult. Furthermore, the choices on the initial trials for three of the groups is not at chance. Both of the same groups were initially likely to choose the different pair, and one of the different groups was initially likely to choose the same pair. There is no obvious explanation for the initial choices other than sampling error, and it may be worth exploring same/different preferences in future studies. Nonetheless, all of the groups reached levels above chance despite any initial preferences.

Although all groups performed at levels above chance, the Oddity Type Groups appears to perform better than the Nonoddity Type Groups. An overall ANOVA was conducted. The analysis was a 2 x 2 design with Type as one factor (Nonoddity or Oddity) and Group (Same or Different) as the other factor. There was no effect of the Type, *F*(1, 32) = 2.09, *p* = .16, a finding that suggests no difference in the performance between the Nonoddity and Oddity Type problems. There was no effect of Group, *F*(1, 32) = .07, *p* = .79, a result that suggests that there is no difference in the performance between the groups trained to choose the same pair or the groups trained to choose the different pair. In addition, there was no significant interaction between the Type of problem (Nonoddity vs. Oddity) and Group (Same vs. Different), *F*(1, 32) = 3.55, *p* = .07. The *p*-value is low which hints that the interaction may be real. Inspection of the data indicated that the Oddity Type Same Group performed marginally better than the Nonoddity Type Same Group. The variability within and between groups is relatively high as can be seen in the curves in Figure 5, so it is difficult to determine the significance of any interaction. Nonetheless, the results of the two same groups and two different groups were pooled and are shown in Figure 6. The pooled curves suggest that performance in the same and different problems was quite similar reaching asymptotic levels of about 75% correct choice.

The fact that there was no Type effect in the results of the ANOVA suggests that it did not matter whether the training trials were of the Oddity Type or Nonoddity Type. The expectation was that the oddity type of training might have enhanced the same/different discrimination. For the Oddity Type groups, on every trial, the same pair was AA and the different pair was AB. Although on every trial the actual stimuli were unique, the difference between the two pairs is accentuated by the fact that the B stimulus is the only one that is different from the others. In other words, the B stimulus might stand out as being odd. Inspection of the choices of the two Oddity Type groups did not reveal a tendency to choose the odd stimulus any more than the nonodd stimulus. In the Oddity Type Different Group, choice of either pattern, A or B, in the different pair (AB) was correct and rewarded. Of those correct choices, the mean proportion of choice of pattern B, the odd pattern, was .535, which is not significantly greater than chance, $t(7) = .72$, $p = .49$. In the Oddity Type Same Group, choice of either pattern, A or B, in the different pair (AB) was not correct and was punished with stevia. Nonetheless, it seemed reasonable to look at their choices when they made an incorrect choice by choosing the different pair. Of those incorrect choices, the mean proportion of choice of pattern B, the odd pattern, was .538, which was not significantly greater than chance $t(7) = .49$, $p = .64$. Therefore, both Oddity Type groups showed no preference for the odd pattern.

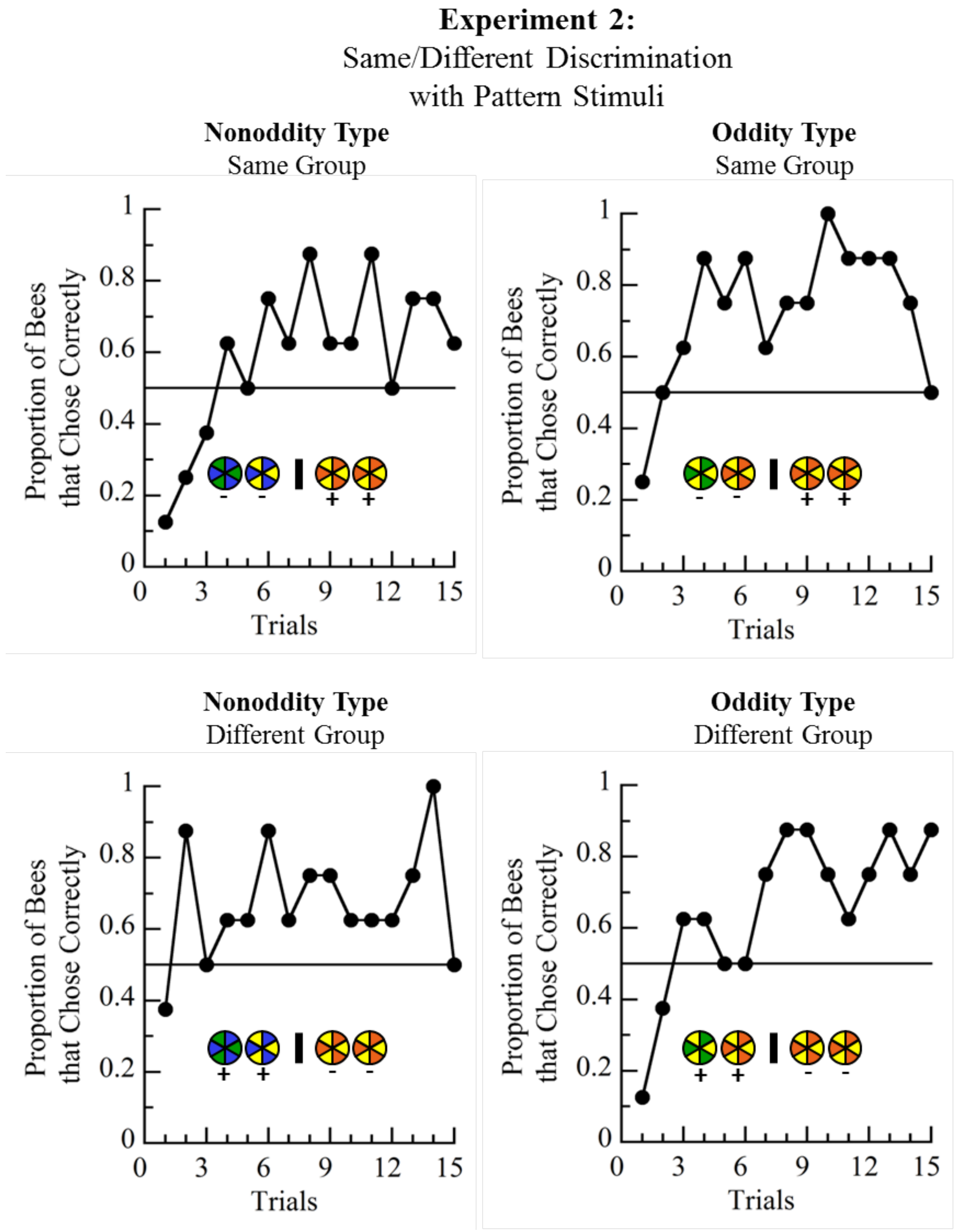


Figure 5. Results for the four same and different groups of Experiment 2. The horizontal line at .5 represents chance.

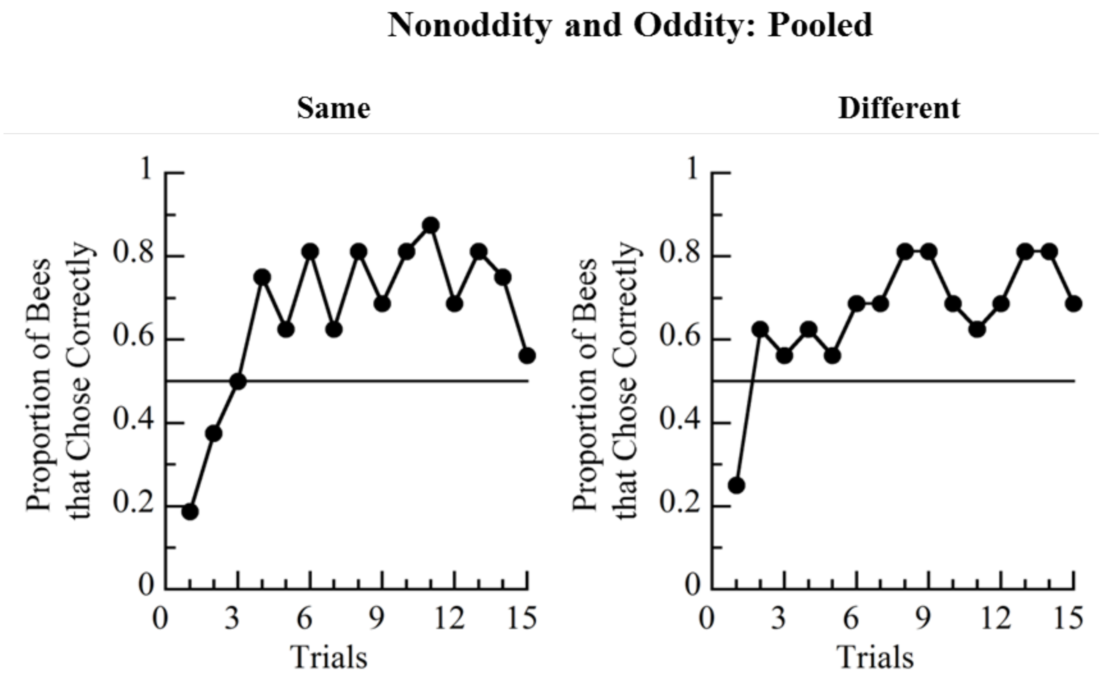


Figure 6. The pooled results for the Nonoddity Type and the Oddity Type groups of the same/different discrimination problem of Experiment 2. The horizontal line at .5 represents chance.

It is common in vertebrate studies of choice discrimination learning in difficult problems to further analyze the results to determine if the subject's performance could have been influenced by a position or stimulus preference (Moon & Harlow, 1955). In this case, the discrimination is reasonably good, but position and stimulus preferences might have contributed to some of the variability in the results. Position preference is the tendency to choose one position in training more than another position. In this experiment, the bees can choose either the left pair or the right pair of stimuli. If bees do not have a position preference, they should have equal frequencies of initial position choices across all training trials since the position of the rewarded and nonrewarded pairs was balanced. None of the four groups had a position preference: Nonoddity Type Different Group, χ 2 (1) = .53, *p* = .46; Nonoddity Type Same

Group, χ 2 (1) = .13, *p* = .71; Oddity Type Different Group, χ 2 (1) = .13, *p* = .71; Oddity Type Same Group, χ 2 (1) = .53, *p* = .46.

Pattern stimulus preference, that is, the tendency to prefer one two-color pattern over the other, was also analyzed. If bees do not have a preference, they should be equally likely to choose any of the six two-color patterns. Analysis of the first choice on every trial for each of the four groups did not reveal any preference for any of the six patterns: Nonoddity Type Different Group, χ 2 (5) = 2.3, *p* = .80; Nonoddity Type Same Group, χ 2 (5) = 2.9, *p* = .71; Oddity Type Different Group, χ 2 (5) = 2.2, *p* = .82; Oddity Type Same Group, χ 2 (5) = 2.6, *p* = .76.

It is possible also that the bees had a tendency to follow the previously rewarded position. Position reward following is when a subject chooses the same position on trial $n+1$ that had been rewarded on trial n. For example, if on trial n the left pair was rewarded, position reward following on trial $n+1$ would be indicated by choice of the left pair. The mean proportion of trials on which the bees choose the position rewarded on the previous trial was .41 for the Nonoddity Type Different Group, .44 for the Nonoddity Type Same Group, .32 for the Oddity Type Different Group, and .36 for the Oddity Type Same Group. The means for all of the groups were less than chance (.5 since there were two positions) and the analyses are as follows: Nonoddity Type Different Group, *t*(7) = -2.12, *p* = .07; Nonoddity Type Same Group, *t*(7) = -1.05, *p* = .33; Oddity Type Different Group, *t*(7) = -3.59, *p* = .009; Oddity Type Same Group, *t*(7) = -2.89, *p* = .02. Not only are the means for all of the groups less than chance, but for three of the four groups the means were significantly less than chance. There is no evidence for position reward following.

In summary, none of the analyses give evidence that the bees used any systematic preferences or choice strategies to solve the same/different discrimination. Therefore, the better-than-chance performance of all four groups suggests that the bees were able to learn a trial-unique simultaneous same/different discrimination. This is the first clear evidence of simultaneous same/different discrimination learning in honeybees. The results of this experiment, taken together with the results of the previous oddity experiment conducted in Experiment 1 and the results of the set of oddity experiments conducted by Muszynski and Couvillon (2015) make a strong case that honeybees are able to use a same/different relational concept.

That the honeybees solved the simultaneous problem is remarkable. However, as is clear from the results of the groups in this experiment, there is room for improvement in the bees' performance. It is possible that same/different concept discriminations are as difficult for honeybees as they are for the vertebrate species studied. It is also possible that discrimination of the two-color pattern stimuli is difficult for honeybees because of generalization from stimulus to stimulus. To improve the bees' performance, it might be useful to reduce that generalization in the stimulus set.

In this experiment and in Experiment 1, there were only six two-color patterns which could be combined to make 15 unique sets of two-color patterns. There is evidence in the vertebrate literature that expanding the stimulus set, either in number or discriminability, enhances an animal's ability to solve same/different problems (*e.g.*, Blaisdell & Cook, 2005; Katz & Wright, 2006). It would be ideal to have a larger set of stimuli with more variability for training honeybees. As a first step, a set of four solid stimuli can be combined with the two-color pattern stimuli. The addition of those four stimuli, would increase the number of unique combinations of stimuli that could be used in a training sequence. Also, the combination solids with two-color patterns should increase discriminability in the stimulus sets.

The purpose of the experiments in the next chapter is to determine if the solid stimuli are in fact discriminable from the two-color pattern stimuli when the specific stimuli are changed from trial-to-trial. Therefore, the solid and pattern stimuli can be viewed as two categories of stimuli. If the bees can discriminate solids from patterns, then the category difference can be used in both oddity and same/different discrimination problems. There is very little research on category learning in honeybees so it will be instructive to conduct such categorization experiments as a prelude to additional same/different studies.

CHAPTER 3. CATEGORY DISCRIMINATION: PATTERNS VS. SOLIDS

Introduction

The results of the oddity experiment from Chapter 1 and the same/different discrimination experiment from Chapter 2 provide strong evidence for same/different concept learning in honeybees. In both cases, however, it is clear that the discriminations are difficult and that there is room for improvement. The same six two-color pattern stimuli were used in both experiments and were combined to form 15 unique combinations for use on each of the 15 training trials. Expanding the stimulus set to include “one-color” solid stimuli of the same colors used in the two-color patterns will expand the number of unique combinations that can be used in both oddity and same/different discrimination experiments. The first step is to be sure that honeybees can discriminate two-color patterns from one-color stimuli when the specific stimuli are changed from trial-to-trial. Therefore, the discrimination of patterns and solids would reflect a category discrimination.

Category learning is defined as the ability to classify and organize stimuli or objects based on their shared physical or functional properties. Categories can consist of simple attributes, such as colors, shapes, and patterns, or complex attributes, such as pictures, paintings, and functional relationships (*e.g.*, tools vs. nontools). The early vertebrate studies of category learning were often discussed as examples of concept learning, and that view still persists (*c.f.*, Herrnstein, Loveland, & Cable, 1976; Zentall, Galizio, & Critchfield, 2002). Whether or not category learning is a type of concept learning, it is clear that category learning does not require a relational concept as is required to explain same/different discrimination. Furthermore, there are at least two associative theories that can account for successful categorization. The underlying assumption in Feature Theory is that there are common features in exemplars of a specific category. The underlying assumption in Exemplar Theory is that the features of an exemplar in a specific category become a stimulus configuration. Both theories rely on stimulus generalization and discrimination as the associative mechanisms to explain category formation (Pearce & Bouton, 2001). Interestingly, successful category learning can be explained by both theories because the predictions of the two theories tend to be identical.

There are two procedures used to study category learning in nonhuman animals, a simultaneous two-choice discrimination procedure and a successive discrimination procedure. In the simultaneous procedure, a subject is presented with two pictures and must respond to the stimulus that is in the category that is rewarded. For example, to determine if an animal can learn about the category of “tree” the subject might be presented with two simultaneous pictures, a picture containing a tree and a picture containing a flower, or a picture of a tree and a picture of a car. In order to receive a food reward, the animal must choose the picture that contains a tree. In the successive procedure, on the other hand, to determine if an animal can learn about the category of “tree,” the subject might be presented with a picture of a flower, followed by a picture of a car, followed by a picture of a tree, etc. In order to receive food reward, the animal must respond to the picture of the tree and inhibit response to the pictures of the flower and the car.

In both of these procedures used for category experiments, animals are typically given extensive training, often with the same stimuli repeated numerous times throughout training. It is possible that the subjects eventually learn the specific stimulus-response contingency for each picture. Therefore, it is necessary to conduct transfer test with novel stimuli from the same category after the original training. Poor performance on the transfer trials indicates that the subjects did not learn to discriminate on the basis of category but instead learned how to respond to each exemplar, that is, the subjects may have memorized the correct response for each exemplar. Clearly, although not likely concept learning, category discrimination is an example of complex discrimination learning. A wide variety of vertebrate species has shown successful category learning with a wide range of stimuli including visual and auditory stimuli (Porter & Neuringer, 1984). The specific interest here is whether or not honeybees can solve a category learning discrimination with one-color vs. two-color stimuli. There are, in fact, a few studies of category learning in honeybees. The results suggest that honeybees may be able to solve a variety of categorization problems.

Category Experiments with Honeybees

One of the first experiments to explicitly study category learning in bees was conducted by Giurfa, Eichmann, and Menzel (1996). They trained honeybees with a succession of different visual stimulus triads, either two symmetrical stimuli and one asymmetrical stimulus or two asymmetrical stimuli and one symmetrical stimulus. An unrewarded test trial with a novel set of stimuli was periodically interspersed among the training trials. The bees' performance on these repeated transfer tests was similar to that on the training trials, suggesting that the bees had learned to discriminate the categories of symmetry and asymmetry.

Zhang, Srinivasan, Zhu, and Wong (2004) used a matching-to-sample procedure to determine if honeybees could learn four categories of stimuli: flowers that were star-shaped, flowers that were circular, plant stems, and landscapes. Using a Y-maze, honeybees were presented with a sample stimulus at the entrance of the maze and then four choice stimuli. One of the four choice stimuli matched the category of the sample, and the other three were from the three other categories. The bees were trained with a limited set of stimuli and were rewarded for choosing the stimulus that matched the category of the sample stimulus. Honeybees were able to successfully categorize the stimuli in training and then were transferred to novel stimuli. Performance on transfer trials was better than chance but markedly worse than the performance on training trials. Nonetheless, Zhang et al. (2004) concluded that the honeybees were most likely processing the categories of star-shaped flowers, circular flowers, plants, and landscapes using features.

Avarguès-Weber, Portelli, Benard, Dyer, and Giurfa (2010) conducted a series of experiments on category learning in honeybees using two categories: schematic drawings that were face-like and drawings of nonface-like objects. In the first experiment, one group of bees was rewarded for choosing the face-like drawings, and another group of bees was rewarded for choosing the nonface-like drawings. Training was followed by unrewarded transfer tests with novel face-like and novel nonface-like stimuli. Both groups learned the discrimination and both groups showed successful transfer. In subsequent experiments, various aspects of the stimuli were manipulated (*e.g.*, superimposing the face-like stimuli over real photographs, using real photographs, removing the facial features from real photographs, etc.) to determine how the bees processed the stimuli. The aim was to determine whether honeybees were using individual features to process the stimuli or whether they were using more global configural cues.

Wu, Moreno, Tangen, and Reinhard (2013) conducted a series of experiments to determine whether honeybees could discriminate two different painting styles. (It is interesting to note that their study is very similar to one conducted by Watanabe, Sakamoto, and Wakita (1995) with pigeons.) The bees were trained in a chamber with two stimuli, a Monet painting and a Picasso painting, presented simultaneously on a vertical wall of the chamber. One group of bees was rewarded for choosing Monet paintings, and another group of bees was rewarded for choosing Picasso paintings. In one transfer test, the bees successfully discriminated grey-scale versions of the two kinds of paintings, and in another transfer test, the bees showed weak transfer to novel pairs of paintings. However, the authors suggest that the bees learned to discriminate the two "artistic styles."

Taken together the results of these few experiments do suggest that honeybees are able to sort stimuli into different classes or categories and respond appropriately. Evidence of category learning in honeybees as well as in vertebrate species indicates very well developed discrimination capabilities. In the two experiments presented here, honeybees were trained with a simultaneous procedure to discriminate pairs of pattern and solid stimuli. If the bees are successful then the categorical dimension of solid and pattern stimuli can be incorporated into same/different concept experiments, including oddity and simultaneous same/different discrimination.

Experiment 3: Discrimination of Pattern vs. Solid (No Within-pair Difference)

Introduction

The aim of this experiment was to determine if honeybees could discriminate two-color pattern stimuli and one-color solid stimuli. In training, a pair of identical two-color patterns was presented simultaneously with a pair of identical solids. The training was trial-unique, such that on every trial there was a unique set of pairs. One group of subjects was rewarded for choosing the pairs of patterns, and the other group of subjects was rewarded for choosing the pair of solids. It should be noted that this experiment could have been conducted with just two stimuli, one pattern and one solid. However, since pairs of stimuli would be used in future same/different problems, it seemed reasonable to present them as pairs in this category experiment.

Method

Subjects: The subjects were 8 honeybees (*Apis mellifera*) never used in prior experiments. They were captured at feeders containing 10-20% sucrose solution which were located near the hives in back of the Békésy Laboratory at the University of Hawaiʻi at Mānoa. Each subject was trained individually in a single daily session lasting from two to several hours.

Apparatus: The bees were trained in a wooden enclosure, shown in Figure 1, that was 61 cm wide, 61 cm high, and 61 cm deep, and recessed in a window on the exterior wall of the laboratory. The enclosure was open to the outside and on the inside was fitted with two sliding Plexiglas panels. The transparent panels allowed the experimenter access to the enclosure and permitted observation of the bee during training trials. They also served to prevent unwanted entrance of the bee into the laboratory.

Stimuli: The stimuli used were solid colored stimuli and two-color pattern stimuli. Both solid and pattern stimuli consisted of six equal segments arranged in a pinwheel pattern and were mounted on the surface of a Petri dish, 5.5 cm in diameter. For the solid stimuli, the six segments were all one color, either yellow, blue, orange, or green. The two-color pattern stimuli were the same as used in Experiments 1 and 2 and consisted of two of the following colors in alternating sequence: blue, yellow, orange, and green. Of the four solid colored stimuli and six pinwheel stimuli, 24 novel combinations of pattern and solid stimuli could be created. It is for this reason that 24 trials were used in this experiment.

Pretraining: Each subject in the experiment was collected in a matchbox at the feeder and brought to the laboratory for pretraining. In order to ensure exposure to all of the colors to be used in training, the pretraining stimulus was constructed of four equally divided segments of the four colors (blue, green, yellow, orange). This stimulus was placed in the middle of the floor of the wooden enclosure and contained a 100-μl drop of 50% sucrose measured with a 1-ml syringe. (A foraging honeybee will typically drink between 40 and 60-μl before returning to the hive.) The captured bee was released from a matchbox at the drop on the surface of the pretraining stimulus, and as the bee began to drink, it was marked on the thorax with colored enamel for identification purposes. The bee drank until replete and then flew to the hive to unload the sucrose. If the bee returned to the enclosure on its own (usually in three to five minutes), it again found the pretraining stimulus with a drop of 50% sucrose. The bee then landed and drank the sucrose until replete and returned to the hive to unload. In the event that a marked bee did not return to the enclosure, it was recaptured at the feeder and re-placed on the pretraining stimulus. If the marked bee still did not return on its own, another bee was selected from the feeder. Pretraining ended after a bee had returned on its own to the pretraining stimulus.

Training: Each bee was trained individually and given 24 unique training trials, as shown in Figure 7. On each trial, there were two pairs of stimuli. One pair consisted of two identical solids and the other pair consisted of two identical patterns. A wooden divider, approximately 25 cm in length, .5 cm in width, and 3 cm in height was used between the two pairs of stimuli in order to make the pairs more visually separable. For each set of stimuli, there was approximately 1 cm between the stimuli in the pairs. The two pairs were separated by approximately 6 cm. One group of bees (Group Solid) was rewarded for choosing the pair of solids, and another group of bees (Group Pattern) was rewarded for choosing the pair of patterns. A 100-μl drop of 50% sucrose was placed in the middle of each of the correct stimuli, and a 100-μl drop of 10% stevia was placed in the middle of each of the incorrect stimuli. Note that stevia and sucrose are not visually discriminable. See Appendix C.

Two training sequences were created from the 24 possible combinations of stimuli and two subjects in each group were run with each sequence. The left-right position of the pair of solids and the pair of patterns was balanced across the 24 training trials. Each sequence was designed so that a specific pair of solids or a specific pair of patterns was never presented on two trials in a row. An example of one of the training sequences is illustrated in Figure 7. All choices were recorded on each of the 24 training trials. A correct initial choice was defined as landing on either of the correct stimuli and making contact with the sucrose drop. For Group Solid, the pair of solids was correct, and for Group Pattern, the pair of patterns was correct. An incorrect initial choice was defined as landing on either of the incorrect stimuli and making contact with the stevia solution. A correction procedure was used so if the bee chose incorrectly, it was allowed to choose again until it made contact with the drop of sucrose on the correct pair of stimuli. The bee drank the sucrose, flew to the hive to unload, and then returned to the window (usually within three to five minutes) for the next trial. At the end of the session, the bee was captured and sacrificed to ensure that it was not used again in any experiments.

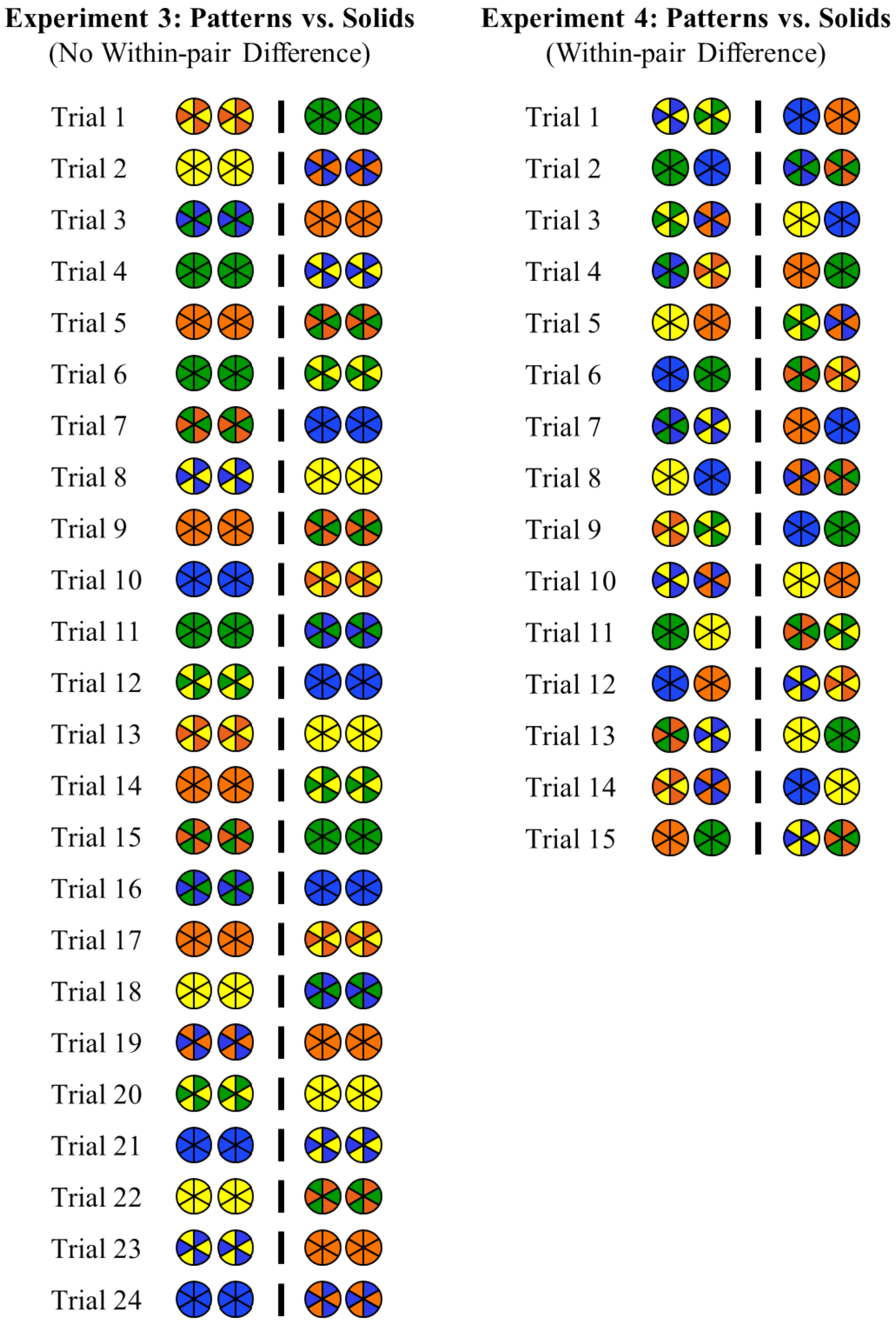


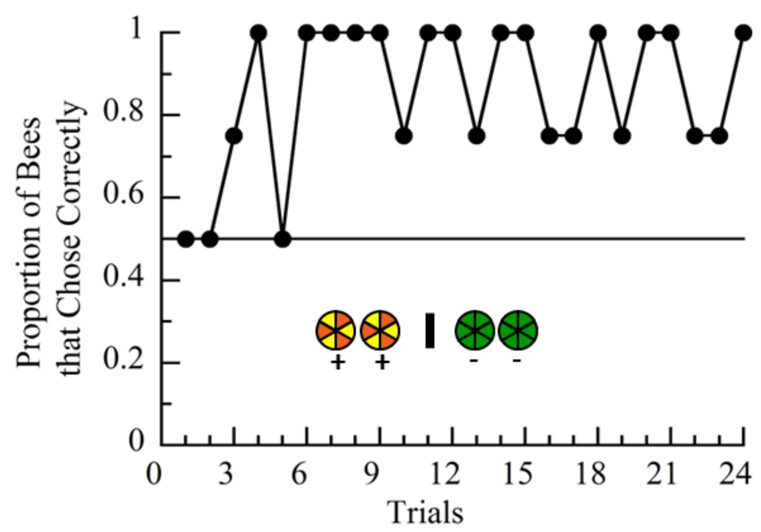
Figure 7. Sample training sequences for the category discrimination problems of Experiments 3 and 4.

Results & Discussion

The results for the two groups of bees is plotted in Figure 8 as the proportion of bees with an initial correct choice on each trial. The performance of the bees in both groups was remarkably good, and the honeybees appeared to easily solve this discrimination. Although the first trial performance of Group Solid was considerably higher than chance, their performance dropped dramatically on the second trial and then rose quickly to an asymptote of about .9 proportion correct choice. The first trial performance of Group Pattern was at chance, and within a few trials, their performance also rose quickly to an asymptote of about .9 proportion correct choice. For analysis of the data, the proportion of correct choice for all 24 trials was computed for each bee. For Group Pattern, the overall mean proportion of correct choice was .84, which is significantly greater than the chance value of .5, *t*(3) = 8.28, *p* = .004. For Group Solid, the overall mean proportion of correct choice was .89, which also is significantly greater than the chance value of .5, *t*(3) = 10.59, *p* = .002. A between group comparison did not reveal any differences between Group Pattern or Group Solid, *t*(6) = .86, *p* = .42, suggesting that there was no preference for either category.

Experiment 3: Patterns vs. Solids
(No Within-pair difference)

Group Pattern



Group Solid

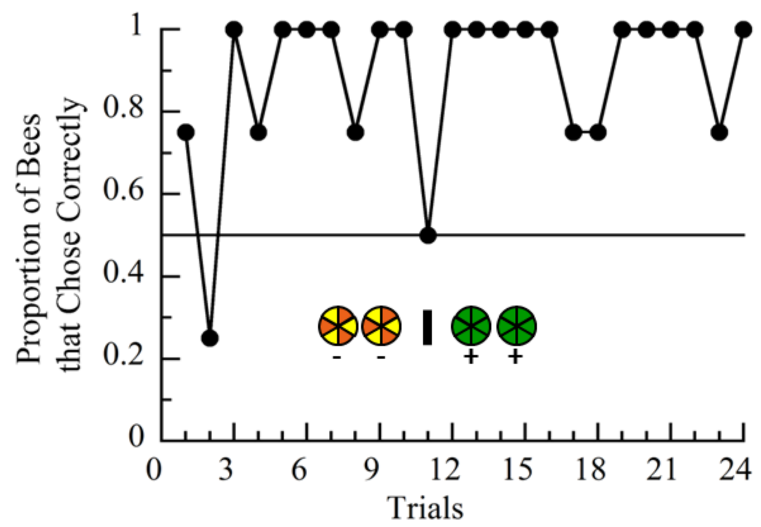


Figure 8. Results for the two groups in the category discrimination problems of Experiment 3. The top panel shows the results for the subjects in Group Pattern. The bottom panel shows the results for the subjects in Group Solid. The horizontal line at .5 represents chance.

All bees’ terminal performance reached about 90%. For this reason, it is unnecessary to further analyze the data for any systematic preferences. It is clear from the two graphs displayed in Figure 8 that the honeybees are able to easily discriminate a pair of identical solid stimuli from a pair of identical two-color pattern stimuli. Typically, in category discrimination problems, successful transfer to novel sets of stimuli that are similar to the training stimuli would indicate that the subjects had learned a category. Since the design of this experiment was trial-unique, successful performance suggests that the bees had learned to discriminate on the basis of category. Whether the bees were using the category “pattern” vs. “solid” or something akin to “more color” vs. “less color” is not possible to determine from the results of this experiment.

Although the training was trial-unique with unique sets of pairs on every trial, each solid pair was repeated six times throughout training, and each pattern pair was repeated four times. One possibility to consider is that the bees had not learned to discriminate on the basis of category, but had learned specific responses to specific stimuli. However, that possibility is unlikely because performance reached about 90% before the subjects were likely to encounter the pairs of stimuli more than once.

The goal of this experiment was to determine if honeybees can discriminate pattern and solid stimuli that change from trial-to-trial. The successful performance of the bees demonstrates the discriminability of the stimuli and also suggests that the bees may be categorizing the stimuli as patterns and solids. At this point, these results indicate that it will be possible to include both solid and pattern stimuli in future same/different concept experiments, including oddity and simultaneous same/different discrimination. Before proceeding to the same/different experiments, it seemed reasonable to further explore this category learning in honeybees. The next experiment used the same design except that the stimuli in the pairs were not identical.

Experiment 4: Discrimination of Patterns vs. Solids (Within-pair Difference)

Introduction

The results of Experiment 3 indicate that honeybees are able to easily discriminate pairs of identical pattern stimuli from pairs of identical solid stimuli. Since the procedure used was trial-unique, it is possible that the bees had formed categories such as “pattern” and “solid,” but it is also possible that they had formed categories such as “more” and “less” of a specific color on each trial. For example, on a trial with yellow-blue and yellow-blue vs. green and green, the bees may discriminate “more” of the green color vs. “less” of yellow and blue.

Here, in Experiment 4, bees were presented with a pair of pattern stimuli and a pair of solid stimuli, but unlike in Experiment 3, the stimuli in the pairs were not identical. For example, a trial could be yellow-blue and orange-green vs. blue and orange. In this case, successful performance would strongly suggest that the bees are discriminating pattern stimuli from solid stimuli rather than more or less of a specific color. Furthermore, success would also permit the pattern and solid stimuli to be incorporated in a number of different variations in future same/different concept experiments.

Method

Subjects: The subjects were 8 honeybees (*Apis mellifera*) never used in prior experiments. They were captured at feeders containing 10-20% sucrose solution which were located near the hives in back of the Békésy Laboratory at the University of Hawaiʻi at Mānoa. Each subject was trained individually in a single daily session lasting from two to several hours.

Apparatus: The bees were trained in the same wooden enclosure used in Experiment 1 and shown in Figure 1. The dimensions of the wooden enclosure were 61 cm wide, 61 cm high, and 61 cm deep. The enclosure was recessed in a window on the exterior wall of the laboratory, open to the outside, and fitted with two sliding Plexiglas panels on the inside. The transparent panels allowed the experimenter access to the enclosure and permitted observation of the bee during training trials. They also served to prevent unwanted entrance of the bee into the laboratory.

Stimuli: The stimuli used were the same solid and two-color pattern stimuli used in Experiment 3. Both solid and pattern stimuli consisted of six equal segments arranged in a pinwheel pattern and were mounted on the surface of a Petri dish, 5.5 cm in diameter. The solid stimuli could be one of the following colors: yellow, blue, orange, and green. The two-color pattern pinwheels were constructed of two of the following colors in alternating sequence: blue, yellow, orange, and green.

Pretraining: Each subject in the experiment was collected in a matchbox at the feeder and brought to the laboratory for pretraining. In order to ensure exposure to all of the colors to be used in training, the pretraining stimulus was constructed of four equally divided segments of the four colors (blue, green, yellow, orange). This pretraining stimulus was placed in the middle of the floor of the wooden enclosure and contained a 100-μl drop of 50% sucrose measured with a 1-ml syringe. (A foraging honeybee will typically drink between 40 and 60-μl before returning to the hive.) The captured bee was released from a matchbox at the drop on the surface of the pretraining stimulus, and as the bee began to drink, it was marked on the thorax with colored enamel for identification purposes. The bee drank until replete and then flew to the hive to unload the sucrose. If the bee returned to the enclosure on its own (usually in three to five minutes), it again found the pretraining stimulus with a drop of 50% sucrose. The bee then landed and drank the sucrose until replete and returned to the hive to unload. In the event that a marked bee did not return to the enclosure, it was recaptured at the feeder and re-placed on the pretraining stimulus. If the marked bee still did not return on its own, another bee was selected from the feeder. Pretraining ended after a bee had returned on its own to the pretraining stimulus.

Training: Each bee was trained individually and given only 15 training trials. The number of trials was determined by the number of two-color patterns. Since there were six two-color patterns, they could be combined into 15 unique pairs, and therefore, in 15 training trials, there was no repetition of the pairs of two-color patterns. Since there were four solid colors, they could be combined into six unique pairs of solids, and therefore, in 15 training trials, there was repetition of the pairs of solid colors, each appearing two to three times across training trials. See the sample sequence in the right panel of Figure 7. On each trial, there were two pairs of stimuli. One pair of stimuli consisted of two solid colors that were different from each other (*e.g.*, a yellow and a blue), and the other pair of stimuli consisted of two, two-color patterns that were also different from each other (*e.g.*, a yellow-orange and a green-orange). For each set of stimuli, there was approximately 1 cm between the stimuli in the pairs. The two pairs were separated by approximately 6 cm and a wooden divider was placed in between them. The wooden divider measured approximately 25 cm in length, .5 cm in width, and 3 cm in height and was used between the two pairs of stimuli in order to make the pairs more visually separable. One group of bees was rewarded for choosing the solid pair of stimuli (Group Solid) and another group of bees was rewarded for choosing the two-color pattern stimuli (Group Pattern). A 100-µl drop of 50% sucrose was placed in the middle of each of the correct stimuli and a 100-µl drop of 10% stevia was placed in the middle of each of the incorrect stimuli. Note that stevia and sucrose are not visually discriminable. See Appendix C.

Three training sequences were created from the set of pattern and solid stimuli. Two to four subjects were run with each sequence. The left-right position of the pair of solids and the pair of patterns was balanced quasi-randomly across the 15 training trials, such that solid pairs appeared seven or eight times in each position, and pattern pairs appeared seven or eight times in each position. Each sequence was designed so that a specific pair of solids or a specific pair of patterns was never presented twice in a row. An example of one of the training sequences is illustrated in Figure 7.

All choices were recorded on each of the 15 training trials. A correct initial choice was defined as landing on either of the correct stimuli and making contact with the sucrose drop. For Group Solid, the pair of solids was correct, and for Group Pattern, the pair of patterns was correct. An incorrect initial choice was defined as landing on either of the incorrect stimuli and making contact with the stevia solution. A correction procedure was used so if the bee chose incorrectly, it was allowed to choose again until it made contact with the drop of sucrose on the correct pair of stimuli. The bee drank the sucrose, flew to the hive to unload, and then returned to the window (usually within three to five minutes) for the next trial. At the end of the session, the bee was captured and sacrificed to ensure that it was not used again in any experiments.

Results & Discussion

The performance of the bees is plotted in Figure 9 as the proportion of bees with an initial correct choice on each trial. The honeybees were able easily to solve this discrimination. For analysis of the data, the proportion of correct choice for all 15 trials was computed for each bee. For Group Pattern, the overall mean proportion of correct choice was .77, which is significantly greater than the chance value of .5, $t(3) = 3.36$, $p = .04$. For Group Solid, the overall mean proportion of correct choice was .79, which is significantly greater than the chance value of .5, *t*(3) = 5.39, *p* = .01. There was no difference between the performance of Group Pattern and Group Solid, *t*(6) = .20, *p* = .84, suggesting that there was no preference for solids or patterns. These results indicate that honeybees can solve a trial-unique discrimination of pairs of solids and pattern stimuli when the stimuli in the pairs are not identical. The results of this experiment are similar to those of Experiment 3 where the stimuli in the pairs were identical.

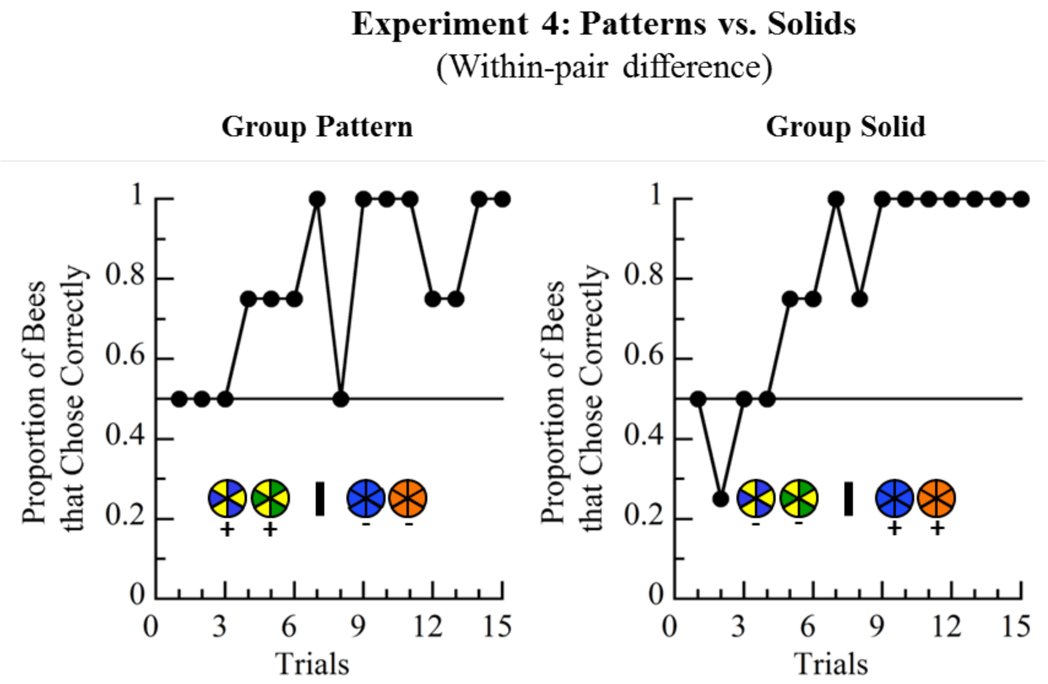


Figure 9. Results for the two groups in the category discrimination problem of Experiment 4. The left panel shows the results for subjects in Group Pattern. The right panel shows the results for the subjects in Group Solid. The horizontal line at .5 represents chance.

General Discussion of Experiments 3 & 4

The results of Experiment 3 indicate that bees can discriminate identical pairs of two-color pattern stimuli from identical pairs of solid color stimuli even when the stimuli change from trial-to-trial. Furthermore, there was no difference in performance for the group that had patterns rewarded and the group that had solids rewarded. The terminal performance for both groups reached 90-100% correct. As noted above, while the bees clearly could discriminate the pairs, they could do so on the basis of categories of pattern and solid, but it is also possible to do so on the basis of more or less of a specific color on each trial.

The results of Experiment 4 indicate that bees also can discriminate nonidentical pairs of two-color pattern stimuli from nonidentical pairs of solid color stimuli even when the stimuli change from trial-to-trial. Again, there was no difference in performance for the group that had patterns rewarded and the group that had solids rewarded. The terminal performance for both groups reached 90-100% correct, just as in Experiment 3. The fact that the bees could clearly discriminate the pairs when the stimuli in each pair were not identical strongly suggests that the bees had learned to discriminate on the basis of categories of pattern and solid.

The results of these experiments add to the growing body of evidence that honeybees can discriminate on the basis of category. As discussed above, category learning in honeybees has been studied with Monet and Picasso photos (Wu et al., 2013); face-like and nonface-like drawings (Avarguès-Weber et al., 2010); flower types, plants, and landscapes (Zhang et al., 2004); and symmetrical and asymmetrical shapes (Giurfa et al., 1996). As has been the case with research on category learning in vertebrate species, some of these studies have attempted to determine what features or configurations of the stimuli the honeybees are using to discriminate the categories. It would be interesting also with the two-color pattern stimuli and solid stimuli used here to experimentally determine what features or configurations the honeybees are using.

Nonetheless, the aim of these experiments was to establish that solids and patterns are discriminable in a trial-unique training situation in order to use them in additional studies of same/different concept learning. The use of solid colors along with the two-color pattern pinwheels that have been used in all of the previous experiments on same/different concept learning in honeybees will significantly increase the size of the stimulus set. The experiments presented in the next chapter incorporate both patterns and solids in an oddity problem (Experiment 5) and in a simultaneous same/different problem (Experiment 6).

CHAPTER 4. CONCEPT LEARNING WITH A CATEGORY DIMENSION

Introduction

In the following two experiments, both the two-color patterns and the solid colors were used in a four-stimulus oddity problem and in a simultaneous same/different discrimination problem. The use of the pattern and solid stimuli allows for a larger pool of stimuli and may possibly enhance the discriminability of the stimuli on each training trial. The patterns and solids were not incorporated as categories per se, but as an additional dimension for discrimination.

In the oddity problem (Experiment 5), there were two trial types; on half of the trials, a solid color was the odd stimulus and the two-color patterns were the nonodd stimuli, and on the other half of the trials, a two-color pattern was the odd stimulus and the solid colors were the nonodd stimuli. In order to choose correctly, subjects had to learn that the category dimension is irrelevant, and that the oddity problem can only be solved on the basis of the oddity relationship among the stimuli.

In the simultaneous same/different discrimination problem (Experiment 6), there were two trial types; on half of the trials, the same pair was two identical solid colors and the different pair was two nonidentical patterns, and on the other half of the trials, the same pair was two identical patterns and the different pair was two nonidentical solid colors. In order to choose correctly, subjects had to learn that the category dimension is irrelevant, and that the same/different discrimination can only be solved on the basis of sameness vs. differentness of the pairs.

There are no directly analogous experiments with vertebrates that incorporate an irrelevant category dimension in either an oddity or simultaneous same/different problem.

However, there is one seemingly similar vertebrate study with primates that incorporates an irrelevant <u>stimulus dimension</u> in a simultaneous same/different experiment. King (1973) trained both chimpanzees and orangutans to discriminate a pair of stimuli that was the same in shape but different in color (blue circle and green circle) from a pair of stimuli that was the same in color but different in shape (yellow triangle and yellow square). Group Same-Shape was rewarded for choosing the pair of shapes that was the same (blue circle and green circle) but not for choosing the pair of shapes that was different (yellow triangle and yellow square). Group Same-Color was rewarded for choosing the pair of colors that was the same (yellow triangle and yellow square) but not for choosing the pair of colors that was different (blue circle and green circle). Both groups solved the same/different discrimination problem and were successful in transfer tests to novel sets of objects. These results demonstrate that they were able to learn to attend to the relevant “same” dimension (*e.g.*, same-shape: blue circle and green circle) and ignore the other “same” dimension (*e.g.*, same-color: yellow triangle and yellow square). This experiment pitted two separable <u>stimulus dimensions</u> against each other, in order to determine which dimension was more salient. The honeybee experiments presented in this chapter include a <u>category dimension</u>, rather than two separable stimulus dimensions. The results of the category experiments in Chapter 3 indicate that the bees have no preference for either patterns or solids. The purpose of using the category difference for honeybees was to enhance the discrimination of the same/different relationship among the stimuli.

Young and Wasserman (1997) conducted an experiment with pigeons that is similar to that shown in panel C of Table 2. Pigeons were presented with arrays of icons that were all the same or arrays of icons that differed in a variety of categories. If the 4 x 4 grid used 16 different icons, discrimination was better than if the grid included some duplicate icons. For example, there were eight different icons with duplicates of each, the pigeons were less likely to identify that array as different. Although the study did not assess the effects of category difference on same/different discrimination, the different categories of icons may have served to enhance the discriminability of the same and different arrays. Perhaps the addition of the irrelevant categories of solids and patterns to the honeybee experiments also will serve to enhance stimulus discriminability in the oddity and same/different problems.

Experiment 5: Oddity with a Category Dimension

Introduction

In the oddity problem of Experiment 1, bees were presented with four two-color pattern stimuli on each trial, one odd pattern and three identical nonodd patterns. In the present experiment, bees again were presented with four stimuli on each trial. However, here there were two types of trials intermixed throughout training. On one trial type, there was one two-color pattern stimulus and three identical solid color stimuli. Choice of the odd pattern stimulus was rewarded. On the other trial type, there was one solid color stimulus and three identical two-color patterns. Choice of the odd solid color stimulus was rewarded. Therefore, the odd stimulus on half the trials was a solid color and on the other half was a two-color pattern. In order for the bees to learn this oddity problem, they would have to attend to the relationship among the stimuli on each trial regardless of the category dimension. It was expected that the addition of the irrelevant category dimension (solid and pattern) would facilitate the honeybees' performance on the oddity problem by increasing the discriminability of the odd stimulus.

Method

Subjects: The subjects were 8 honeybees (*Apis mellifera*) never used in prior experiments. They were captured at feeders containing 10-20% sucrose solution which were located near the hives in back of the Békésy Laboratory at the University of Hawaiʻi at Mānoa. Each subject was trained individually in a single daily session lasting from two to several hours.

Apparatus: The bees were trained in a wooden enclosure, shown in Figure 1, that was 61 cm wide, 61 cm high, and 61 cm deep, and recessed in a window on the exterior wall of the laboratory. The enclosure was open to the outside and on the inside was fitted with two sliding Plexiglas panels. The transparent panels allowed the experimenter access to the enclosure and permitted observation of the bee during training trials. They also served to prevent unwanted entrance of the bee into the laboratory.

Stimuli: The stimuli used were the same solid and two-color pattern stimuli used in Experiments 3 and 4. Both solid and pattern stimuli consisted of six equal segments arranged in a pinwheel pattern and were mounted on the surface of a Petri dish, 5.5 cm in diameter. The solid stimuli consisted of six equal segments of one of the following colors: yellow, blue, orange, and green. The two-color pattern pinwheels consisted of two of the following colors in alternating sequence: blue, yellow, orange, and green.

Pretraining: Each subject in the experiment was collected in a matchbox at the feeder and brought to the laboratory for pretraining. In order to ensure exposure to all of the colors to be used in training, the pretraining stimulus was constructed of four equally divided segments of the four colors (blue, green, yellow, orange). This stimulus was placed in the middle of the floor of the wooden enclosure and contained a 100-μl drop of 50% sucrose measured with a 1-ml syringe. (A foraging honeybee will typically drink between 40 and 60-μl before returning to the hive.) The captured bee was released from a matchbox at the drop on the surface of the pretraining stimulus, and as the bee began to drink, it was marked on the thorax with colored enamel for identification purposes. The bee drank until replete and then flew to the hive to unload the sucrose. If the bee returned to the enclosure on its own (usually in three to five minutes), it again found the pretraining stimulus with a drop of 50% sucrose. The bee then landed and drank the sucrose until replete and returned to the hive to unload. In the event that a marked bee did not return to the enclosure, it was recaptured at the feeder and re-placed on the pretraining stimulus. If the marked bee still did not return on its own, another bee was selected from the feeder. Pretraining ended after a bee had returned on its own to the pretraining stimulus.

Training: On each of the 16 training trials, there were four stimuli, one odd stimulus and three identical nonodd stimuli. On eight of the training trials, the odd stimulus was a solid color and the three identical nonodd stimuli were a two-color pattern, and for the other eight training trials, the odd stimulus was a two-color pattern and the three identical nonodd stimuli were a solid color. The two kinds of trials were intermixed quasi-randomly across the 16 training trials.

The six possible two-color patterns and the four solid colors that could be combined to create a total of 24 unique stimulus sets, with the restriction that the odd and nonodd stimuli shared a color in common. For example, if yellow-green was odd the three nonodd stimuli could either be yellow or green and if green was odd then the three nonodd stimuli could be yellow-green, blue-green, or orange-green. Four 16-trial training sequences were created from these possible sets, and two subjects were trained with each sequence. The odd stimulus occurred four times in each of the four positions in a quasi-random sequence over the training trials. The same stimulus could appear in two successive trials with the restriction that it could not be the odd stimulus in two successive trials. Each stimulus could serve as the odd stimulus one to two times over the training trials but never more than once in any position. An example of a training sequence is illustrated in Figure 10.

The four stimuli were presented simultaneously and positioned horizontally in the center of the floor of the wooden enclosure, 2.5 cm apart edge-to-edge. A 100-µl drop of 50% sucrose solution was placed on the top of the odd stimulus and served as reward. A 100-µl drop of 10% stevia solution was placed on the top of the nonodd stimuli and served as punishment. Note that stevia and sucrose are not visually discriminable. See Appendix C.

All choices were recorded on each of the 16 training trials. A correct initial choice was defined as landing on the odd stimulus and making contact with the sucrose drop. An incorrect initial choice was defined as landing on any of the nonodd stimuli and making contact with the stevia solution. A correction procedure was used so if the bee chose incorrectly, it was allowed to choose again until it made contact with the drop of sucrose on the correct odd stimulus. The bee drank the sucrose, flew to the hive to unload, and then returned to the window (usually within three to five minutes) for the next trial. At the end of the session, the bee was captured and sacrificed to ensure that it was not used again in any experiments.

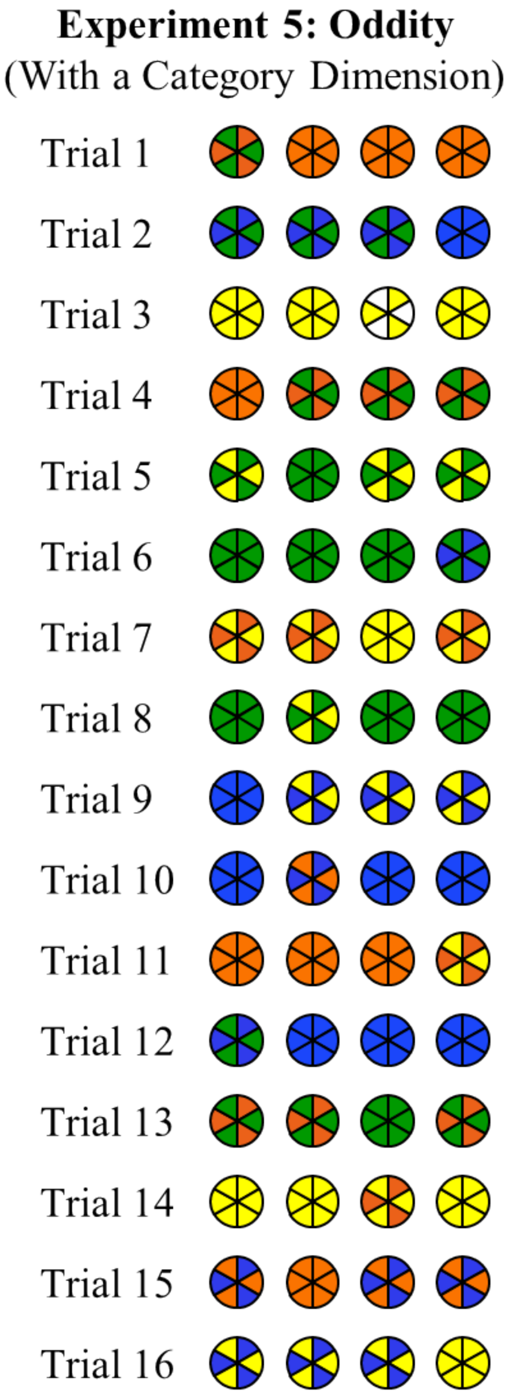


Figure 10. A sample training sequence for the four-stimulus oddity problem of Experiment 5.

Results & Discussion

The performance of the bees is plotted in the left hand panel of Figure 11 as the proportion of bees with an initial correct choice on each trial. Overall, the bees' performance reached a level above chance which is .25 for a four-stimulus oddity problem. Although the performance on the first trial was slightly better than chance, there was no obvious reason, and the assumption is that it is just sampling error. For analysis of the data, the proportion of correct choice for all 16 trials was computed for each bee. The overall mean proportion of correct choice was .63, which is significantly greater than the chance value of .25, $t(7) = 7.76$, $p = .0001$ with a standard error of the mean of .05.

In addition, an analysis was conducted on the performance of the bees on the two kinds of trials, that is, the trials on which the solid color was odd and the trials on which the two-color pattern was odd. The analysis indicated that the bees' performance was better on the trials with the pattern odd, $F(1,7) = 10.658$, $p = .01$, but performance on both kinds of trials was significantly greater than chance: for pattern odd, $t(7) = 13.13$, $p = .0001$ with a standard error of the mean of .04; for solid odd, $t(7) = 3.81$, $p = .006$ with a standard error of the mean of .07. The curves for the two kinds of trials are shown in the right panel of Figure 11. In fact, the terminal performance reached about .8 for the trials for which the solid was odd and .9 for the trials for which the pattern was odd. The results clearly indicate the bees learned to choose the odd stimulus on both kinds of trials.

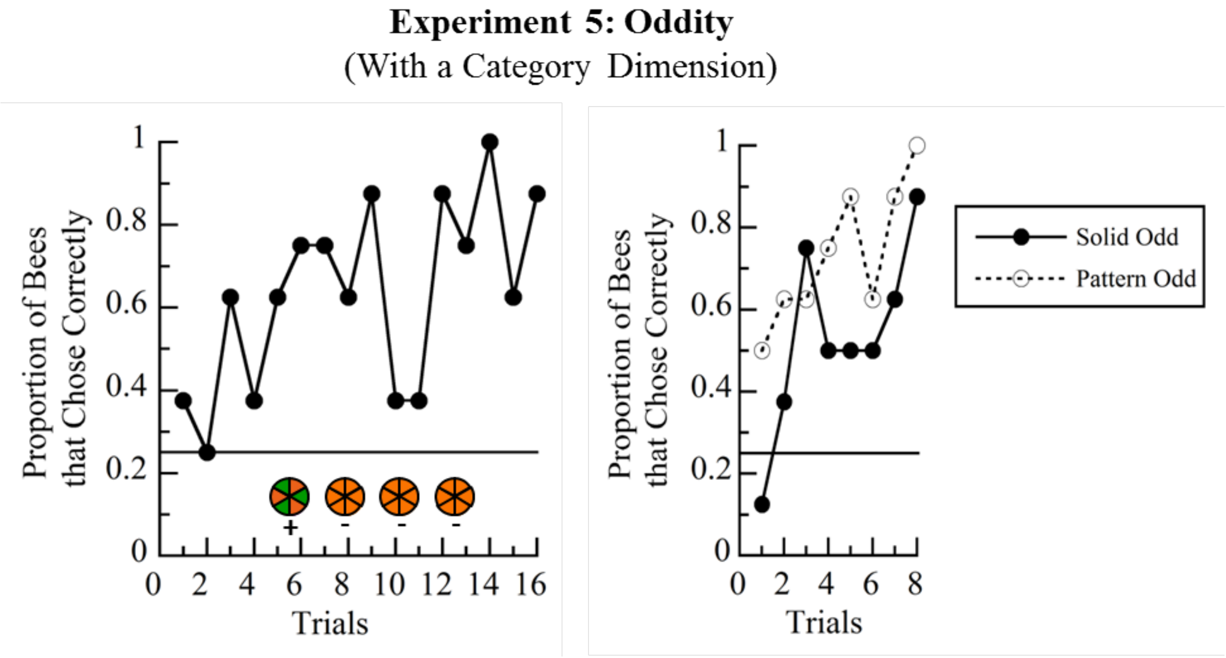


Figure 11. Results for the four-stimulus oddity problem of Experiment 5. The left panel shows the performance of the bees on each trial. The right panel shows the performance of the bees on each of the two trial types, pattern odd and solid odd. The horizontal line at .25 represents chance.

Unlike the performance in the oddity problem of Experiment 1, performance in this oddity problem was quite good. It was necessary in Experiment 1 to analyze position preferences, stimulus preferences, and position reward following in order to be sure that the small but significant oddity effect was not due to any systematic choice strategies. It is less of a concern here because performance reached such a high level. Nonetheless, the same analyses were conducted. There was no significant position preference, that is, the tendency to choose one of the four positions (far-left, middle-left, middle-right, and far-right) more than another position, χ 2 (3) = 2.62, *p* = .45. There was no significant stimulus preference, that is, the tendency to prefer one two-color pattern or solid stimulus over the others, χ 2 (9) = 4.03, *p* = .91. As was the case with in the analysis of the bees’ performance in Experiment 1, the performance in the current experiment also showed a tendency to not follow the previously rewarded position. The mean proportion of bees who followed the rewarded position was .14 and was significantly less than chance (.25, since there were four positions), *t*(7) = -4.07, *p* = .005. As in Experiment 1, the bees had a tendency to switch from one position to another position from trial-to-trial. Again, this is not surprising given that the training sequences were created such that the same position was rarely rewarded on two trials in a row.

The honeybees in Experiment 5 were able to successfully solve a four-stimulus oddity problem with the addition of a pattern/solid category difference. Qualitatively the performance is considerably better than that of the bees in Experiment 1 which were trained only with all pattern stimuli. The addition of the category may have increased the salience of the odd stimulus on each trial. It can be assumed that the pinwheel stimuli were similar enough to produce generalization that may have interfered with the oddity learning in Experiment 1. Future studies might well replicate Experiments 1 and 5 in a way that allows the effect of the category difference to be directly compared.

An interesting finding in this experiment is that performance was better on trials when a pattern was the odd stimulus than on trials when a solid was the odd stimulus. One possibility for the difference is the use of the shared color between the odd stimulus and the three nonodd stimuli (*e.g.*, for pattern odd: yellow-green, green, green, green and for solid odd: green, yellow-green, yellow-green, yellow-green). It is conceivable that on the trials in which the odd stimulus was a pattern, the pattern may have been perceptually easier for the bees to detect. In the next experiment, a category difference (patterns and solids) was incorporated into a simultaneous same/different design. The sequences for that experiment did not include the restriction of a shared color between the same and different pairs.

Experiment 6: Same/Different Discrimination with a Category Dimension

Introduction

In the previous experiment, a category dimension was incorporated into an oddity problem. The results were quite good in comparison those of Experiment 1which used only pattern stimuli. Although not formally comparable, the results of the two experiments suggest that the use of the category (patterns and solids) may have facilitated discrimination of the odd stimulus.

In the present experiment, a category dimension was incorporated into a simultaneous same/different discrimination problem. The previous simultaneous same/different experiment (Experiment 2) used only pattern stimuli and performance was better than chance, although there was room for improvement. The expectation here was that the addition of the category dimension might improve the discrimination of the same and different pairs.

Bees were presented with two pairs of stimuli on each trial. One pair consisted of two identical stimuli and the other pair consisted of two different stimuli. There were two types of trials that were intermixed across training. On one trial type, the same pair was two identical two-color pattern stimuli, and the different pair was two nonidentical solid color stimuli. On the other trial type, the same pair was two identical solid color stimuli and the different pair was two nonidentical two-color patterns. Bees in Group Same were rewarded for choice of the same pair (either solids or two-color patterns), and bees in Group Different were rewarded for choice of the different pair (either solids or two-color patterns). Successful solution of this problem requires the bees to attend to the relationship among the stimuli on each trial regardless of the category dimension.

Method

Subjects: The subjects were 16 honeybees (*Apis mellifera*) never used in prior experiments. They were captured at feeders containing 10-20% sucrose solution which were located near the hives in back of the Békésy Laboratory at the University of Hawaiʻi at Mānoa. Each subject was trained individually in a single daily session lasting from two to three hours.

Apparatus: The bees were trained in a wooden enclosure, shown in Figure 1, that was 61 cm wide, 61 cm high, and 61 cm deep, and recessed in a window on the exterior wall of the laboratory. The enclosure was open to the outside and on the inside was fitted with two sliding Plexiglas panels. The transparent panels allowed the experimenter access to the enclosure and permitted observation of the bee during training trials. They also served to prevent unwanted entrance of the bee into the laboratory.

Stimuli: The stimuli used were the same solid and two-color pattern stimuli used in Experiments 3, 4, and 5. Both solid and pattern stimuli consisted of six equal segments arranged in a pinwheel pattern and were mounted on the surface of a Petri dish, 5.5 cm in diameter. The solid stimuli consisted of six equal segments of one of the following colors: yellow, blue, orange, and green. The two-color pattern pinwheels consisted of two of the following colors in alternating sequence: blue, yellow, orange, and green.

Pretraining: Each subject in the experiment was collected in a matchbox at the feeder and brought to the laboratory for pretraining. In order to ensure exposure to all of the colors to be used in training, the pretraining stimulus was constructed of four equally divided segments of the four colors (blue, green, yellow, orange). This stimulus was placed in the middle of the floor of the wooden enclosure and contained a 100-µl drop of 50% sucrose measured with a 1-ml syringe. (A foraging honeybee will typically drink between 40 and 60-µl before returning to the hive.) The captured bee was released from a matchbox at the drop on the surface of the pretraining stimulus, and as the bee began to drink, it was marked on the thorax with colored enamel for identification purposes. The bee drank until replete and then flew to the hive to unload the sucrose. If the bee returned to the enclosure on its own (usually in three to five minutes), it again found the pretraining stimulus with a drop of 50% sucrose. The bee then landed and drank the sucrose until replete and returned to the hive to unload. In the event that a marked bee did not return to the enclosure, it was recaptured at the feeder and re-placed on the pretraining stimulus. If the marked bee still did not return on its own, another bee was selected from the feeder. Pretraining ended after a bee had returned on its own to the pretraining stimulus.

Training: In the simultaneous same/different discrimination problem used here there were two pairs of stimuli on every trial. The stimuli in one of the pairs were the same and the stimuli in the other pair were different. There were two trial-types intermixed across the 20 training trials, 10 with each. For one trial-type, the same pair was two identical solids and the different pair was two nonidentical patterns (*e.g.*, yellow and yellow vs. yellow-green and blue-orange). For the other trial-type, the same pair was two identical patterns and the different pair was two nonidentical solids (*e.g.*, yellow-orange and yellow-orange vs. blue and green). For each set of stimuli, there was approximately 1 cm between the stimuli in the pairs. The two pairs were separated by approximately 6 cm and a wooden divider was placed in between them. The wooden divider measured approximately 25 cm in length, .5 cm in width, and 3 cm in height and was used between the two pairs of stimuli in order to make the pairs more visually separable. The bees in Group Same were rewarded for choosing the same pair on every trial, whether it was two identical solids or two identical patterns. The bees in Group Different were rewarded for choosing the different pair on every trial, whether it was two different solids or two different patterns. A 100-μl drop of 50% sucrose was placed in the middle of each of the correct stimuli, and a 100-μl drop of 10% stevia was placed in the middle of each of the incorrect stimuli. Note that stevia and sucrose are not visually discriminable. See Appendix C.

Ten training sequences were created from the set of pattern and solid stimuli. There were eight bees in each group and one or two bees was trained with each sequence. There were 10 trials with solids rewarded and 10 with patterns rewarded. The left-right position of the pairs was balanced across trials. Each sequence was designed so that a specific pair of solids or a specific pair of patterns was never rewarded twice in a row. An example of one of the training sequences is illustrated in Figure 12.

All choices were recorded on each of the 20 training trials. A correct initial choice was defined as landing on either of the correct stimuli and making contact with the sucrose drop. For Group Same the pair of identical solids and the pair of identical patterns was correct and for Group Different the pair of nonidentical solids and the pair of nonidentical patterns was correct. An incorrect initial choice was defined as landing on either of the incorrect stimuli and making contact with the stevia solution. A correction procedure was used so if the bee chose incorrectly, it was allowed to choose again until it made contact with the drop of sucrose on the correct pair of stimuli. The bee drank the sucrose, flew to the hive to unload, and then returned to the window (usually within three to five minutes) for the next trial. At the end of the session, the bee was captured and sacrificed to ensure that it was not used again in any experiments.

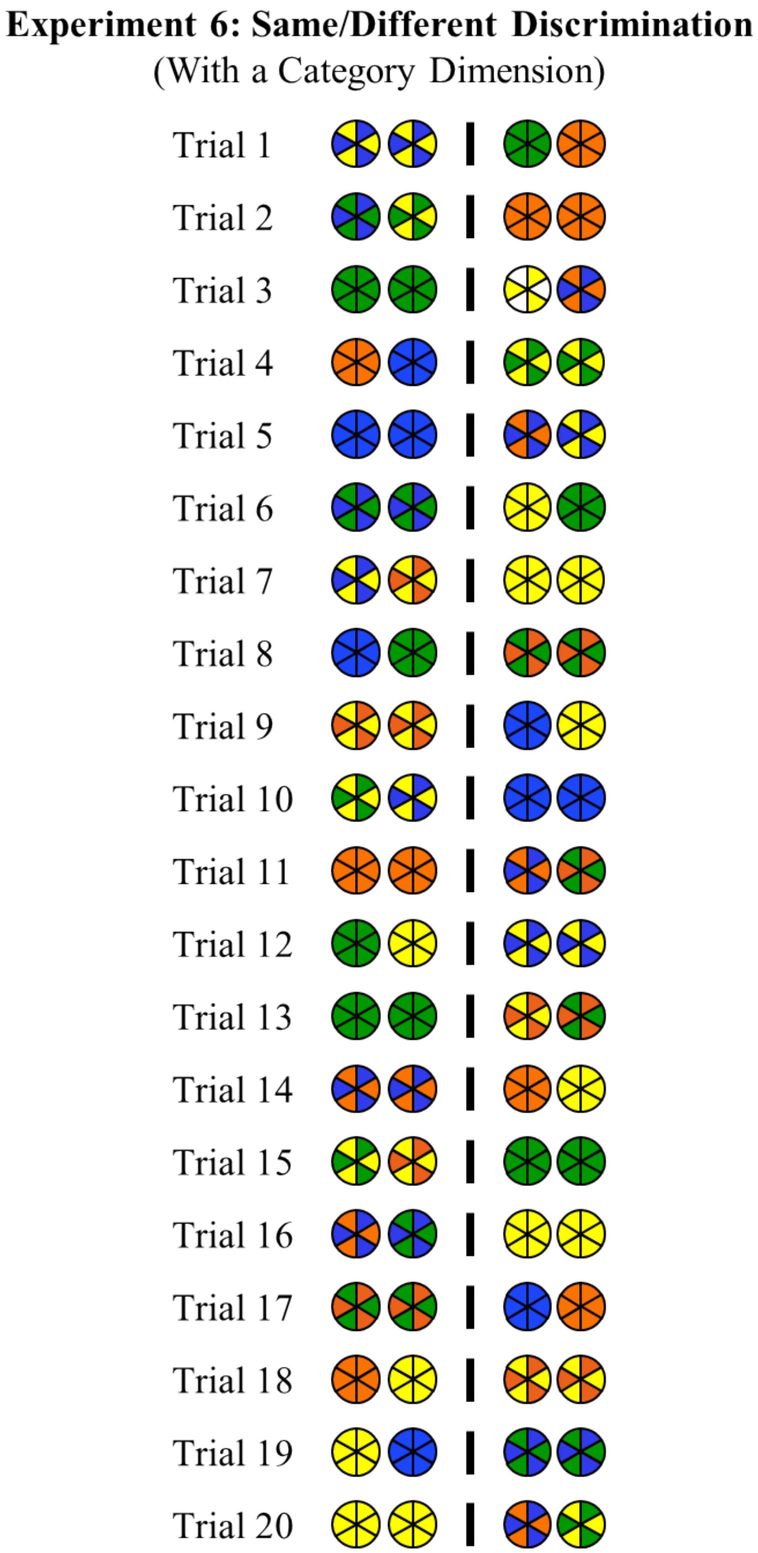


Figure 12. A sample training sequence for the same/different discrimination problem of Experiment 6.

Results & Discussion

The performance of the bees is plotted in Figure 13 as the proportion of bees with an initial correct choice on each trial. The bees in each group were able to solve the same/different problem with better-than-chance performance. For analysis of the data, the proportion of correct choice for all 20 trials was computed for each bee. For the bees in Group Same, the overall mean proportion of correct choice was .65, which is significantly greater than the chance value of .5, $t(7) = 7.94$, $p < .001$ with a standard error of the mean of .02. For the bees in Group Different, the overall mean proportion of correct choice was .69, which is significantly greater than the chance value of .5, $t(7) = 4.65$, $p = .002$ with a standard error of the mean of .04. There was no significant difference between the two groups, $t(14) = .96$, $p = .35$.

While both groups performed at levels better than chance, the curves reflect a fair amount of variability both within and between groups suggesting that the discrimination is difficult. This difficulty and variability in performance is particularly evident when looking at the graph for Group Same in Figure 13. Furthermore, the proportion of correct choice on the first trial was not at chance for either group, and the bees were more likely to initially choose the same pair. There is no obvious explanation for the high initial choice of same other than sampling error, since the performance of the bees on the next few trials was closer to chance before the proportion of correct choice gradually increased.

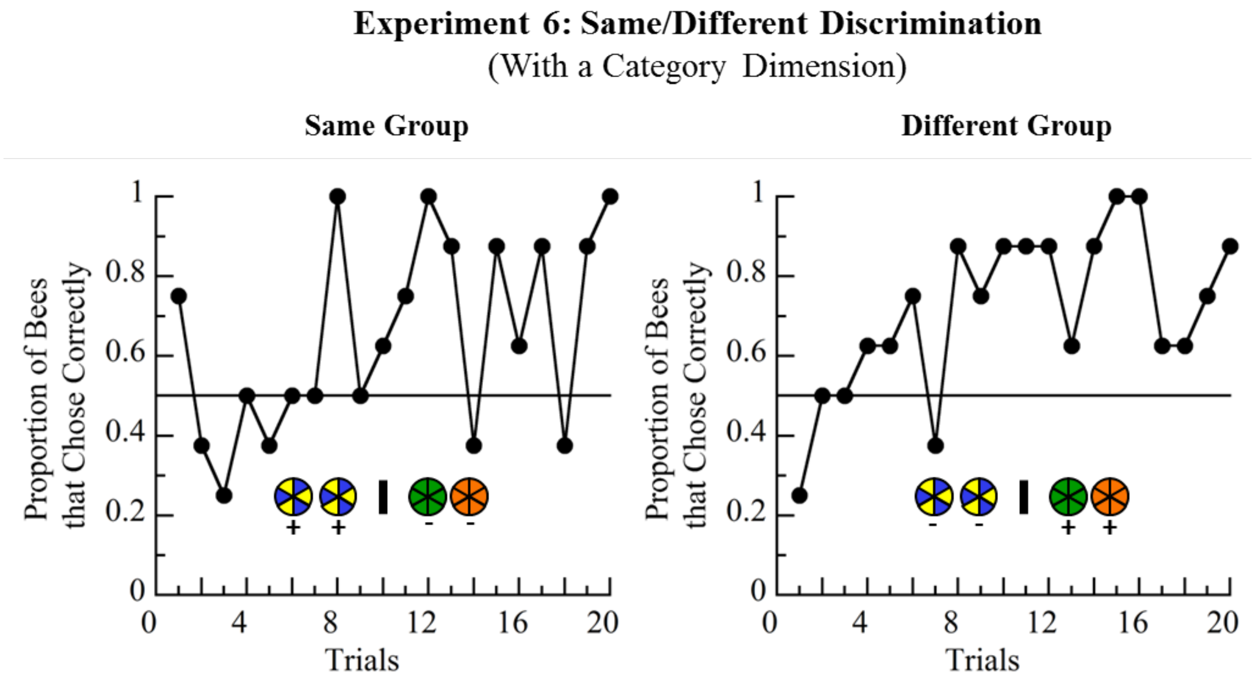


Figure 13. Results for the two groups in the same/different problem of Experiment 6. The horizontal line at .5 represents chance.

In addition, an analysis was conducted on the performance of the bees in both Group Same and Group Different on the two kinds of trials, that is, the trials on which the pair of solid colors was correct and the trials on which the pair of two-color patterns was correct. The analysis indicated that the bees' performance in both groups was not significantly impacted by trial type, that is, whether the correct pair was solids or patterns, *F*(1,14) = 3.31, *p* = .09. Furthermore, there was no significant interaction between trial type and group, *F*(1,14) = .743, *p* = .40. As is shown in Figure 14, the curves for the trials with solids rewarded and for patterns rewarded show similar levels of performance in both groups. The results clearly indicate the bees learned to choose the correct stimulus pair on both kinds of trials.

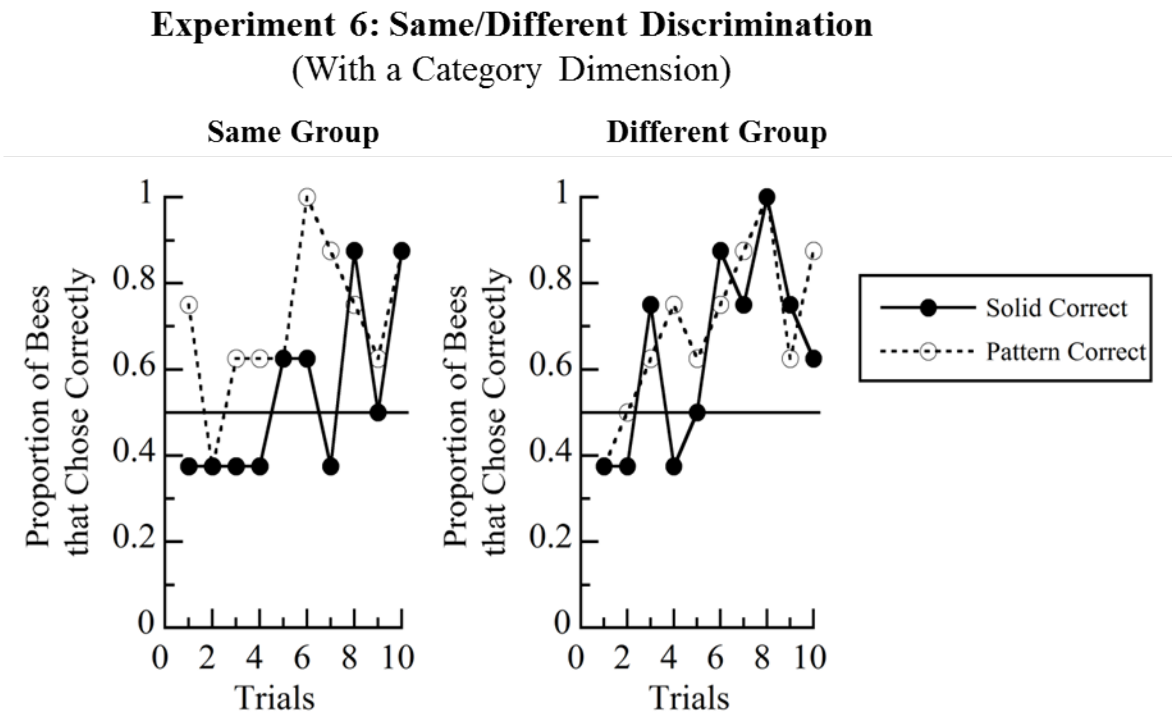


Figure 14. Results for the solid and pattern trial types of the same/different discrimination problem of Experiment 6. The line at .5 represents chance.

The performance of the bees in the simultaneous same/different problem of Experiment 2 with only pinwheel stimuli was better than chance but there was room for improvement. Performance in this simultaneous same/different problem was also better than chance and the performance reached a somewhat higher level. The results of Experiment 2 were analyzed for position preferences, stimulus preferences, and position reward following in order to be sure that the same/different learning was not due to any systematic choice strategies. The same analyses were conducted here. There was no significant position preference, that is, the tendency to choose one of the two positions (left or right) more than the other position, for Group Same, χ 2 (1) = .9, *p* = .34; for Group Different, χ 2 (1) = .1, *p* = .75. There was no significant stimulus preference, that is, the tendency to prefer one two-color pattern or one solid color stimulus over the others, for Group Same, χ 2 (9) = 2.5, *p* = .98; for Group Different, χ 2 (9) = 9.87, *p* = .36.

As was the case with the analysis of the bees' performance in Experiment 2, the performance in the current experiment also showed a tendency to not follow the previously rewarded position. The mean proportion of bees which choose the position rewarded on the previous trial was .46 for Group Same and .39 for Group Different. The analysis for each group was to compare the position reward following against chance which is .5 because there were two positions. The results for the two groups are as follows: Group Same, *t*(7) = -.79, *p* = .45 with a standard error of the mean of .04; Group Different, *t*(7) = -2.29, *p* = .05 with a standard error of the mean of .04. Again, this is not surprising given that the training sequences were created such that the same position was seldom rewarded on two trials in a row. The honeybees in Experiment 6 were able to successfully solve a simultaneous same/different problem with both pattern and solid color stimuli. Although not directly comparable, the bees' performance in Experiment 6 does seem to be somewhat better than the bees' performance in Experiment 2.

General Discussion of Experiments 5 & 6

The results of Experiment 5 provide additional evidence that bees can solve a trial-unique four-stimulus oddity problem. In this experiment, on some trials, the pattern stimulus was odd and the solid stimuli nonodd, and on other trials, the solid stimulus was odd and the pattern stimuli nonodd. Hence, there was an addition of a categorical dimension of pattern and solid. The terminal performance for the bees in this oddity experiment reached about 80% correct, considerably better than the 50% terminal performance reached in Experiment 1 with only pinwheels as the stimuli. It seems reasonable to conclude that the bees had formed a concept of oddity in both experiments, but that there was an enhancement in performance with the addition of the category dimension.

The results of Experiment 6 provide additional evidence that bees can solve a trial-unique simultaneous same/different discrimination problem. Throughout training, pairs of two-color patterns and pairs of solid were presented to the bees. For half the trials, the same pair was two-color patterns or and the different pair was solids, and for the other half of the trials, the same pair was solids and the different pair was two-color patterns. One group of bees was rewarded for choice of the same pair, regardless of whether it was patterns or solids, and the other group was rewarded for choice of the different pair, regardless of whether it was patterns or solids. The terminal performance for the bees in both groups reached about 75% correct, perhaps better than the terminal performance of 70% in the simultaneous same/different problem of Experiment 2 using all pinwheels. The bees in both Experiments 2 and 6 were able to solve the simultaneous same/different problem with trial-unique training. It is not clear that performance was enhanced with the addition of the category dimension of patterns and solids.

As discussed earlier, Young and Wasserman (1997) found that an increase in the variety of stimuli enhances performance on same/different problems in pigeons. Essentially, the addition of the categorical dimension in both the oddity and simultaneous same/different experiments reported here with honeybees served also to increase the variety of stimuli. The results for the oddity problem in Experiment 5 do appear to be considerably better than those for Experiment 1 which had no categorical dimension. It is tempting to conclude that the category dimension did in fact enhance performance. On the other hand, the results for the same/different problem in Experiment 6 may not be much better than those for Experiment 2 which had no categorical dimension. Further studies are needed to determine the conditions under which the performance of honeybees in same/different problem can be enhanced by the addition of a category dimension.

GENERAL DISCUSSION

The research on learning in honeybees has established that the basic associative learning principles are very similar to those for vertebrates (Bitterman, 1996). Recent research on learning in honeybees has focused on more cognitively complex problems, such as same/different concept learning. There are several procedures that have been used to study same/different concept learning in vertebrates including, matching- and non-matching-to-sample, oddity, and same/different discrimination. This dissertation describes research with honeybees using two of the procedures, oddity and simultaneous same/different discrimination.

Overall, the purpose of the six experiments presented in this dissertation was to build on the initial results for oddity learning in honeybees (Muszynski & Couvillon, 2015) that suggests they are, in fact, capable of forming same/different concepts. The experiments are presented in four separate chapters. The specific aims of the research in each chapter were: **1)** to determine if the performance of honeybees in oddity problems can be improved by adding more nonodd stimuli; **2)** to determine if honeybees can solve a simultaneous same/different problem with the same two-color pattern stimuli used in the oddity studies; **3)** to demonstrate that honeybees can discriminate solid and two-color pattern stimuli as categories in order to expand the stimulus set for same/different experiments; and **4)** to determine if performance in oddity and simultaneous same/different problems is facilitated by the addition of the category dimension.

Chapter Summaries

<u>Chapter 1. Concept Learning: Four-Stimulus Oddity.</u> The chapter begins with a detailed review of the historical development of studies of the oddity problem in a variety of species. Robinson (1933) was the first to conduct an oddity problem with a single monkey as her subject.

Since that initial study, the oddity problem has been studied in many vertebrate species, although most of the research has concentrated on primates. There was only one study with an invertebrate species, the octopus, whose results were difficult to interpret. The first clear evidence of oddity learning in an invertebrate was with honeybees (Muszynski & Couvillon , 2015). In that study, honeybees were trained in a three-stimulus oddity problem with sets of two-color pattern stimuli which were unique on every trial. The performance of the bees, like that of most of the vertebrates in oddity problems, indicated that the problem was difficult. The honeybees solved the problem, but there was much room for improvement in their performance.

Experiment 1 was a four-stimulus variant of Muszynski and Couvillon's (2015) three-stimulus oddity problem using the same stimuli. The aim was to determine whether the addition of a fourth stimulus on each training trial would improve the honeybees' choice of the odd stimulus. On each trial, a honeybee was presented with four two-color pattern stimuli one of which was different from the other three which were all identical. Choice of the odd stimulus was rewarded. The results showed that honeybees were able to perform at levels greater than chance. However, qualitative comparison of the performance with that of the previously-published three-stimulus oddity problem gave no indication that the performance was facilitated with the addition of another nonodd stimulus. In the next experiment, honeybees were trained in another kind of same/different problem.

<u>Chapter 2. Concept Learning: Same/Different Discrimination.</u> This chapter begins with a thorough review of the variety of procedures for studying same/different concept learning which were developed after Robinson (1933) conducted the first oddity experiment. The review of the procedures is organized historically and can be characterized in three major eras. The early studies were with primates and used variants of a simultaneous discrimination procedure. Then

there were studies with nonprimate species, primarily with pigeons. The pigeon work inspired the development of multiple procedures for studying same/different concept learning, including matching- and nonmatching-to-sample and a variety of discrimination procedures with either successive presentation of stimuli or with two-response tasks. The details of these variants are presented in Tables 1 and 2. Finally, the study of same/different concept learning spread to a variety of other vertebrate species. Despite the wide range of procedures developed for the study of same/different concept learning, it is the simultaneous discrimination procedure that is generally regarded as the best and most methodologically stringent procedure (Premack, 1983; Robinson, 1955). Interestingly, there are only eight published studies that have used this procedure. See Table 3 for a list of these experiments, including the species, design, and results.

The aim of Experiment 2 was to determine if honeybees could solve a simultaneous same/different discrimination using pairs of two-color pattern stimuli. There were two main groups of bees in this experiment, Group Same and Group Different. Both groups of bees were presented with two pairs of stimuli on each trial. One pair of stimuli was identical and the other pair had two nonidentical stimuli. The bees in Group Same were rewarded for choice of the same pair, and the bees in Group Different were rewarded for choice of the different pair. The results for both groups showed that honeybees were able to solve this simultaneous same/different discrimination, and there was no difference between the groups. These results are the first clear evidence of simultaneous same/different discrimination learning in honeybees.

<u>Chapter 3. Category Discrimination: Patterns vs. Solids.</u> This chapter begins with a discussion of how category discrimination problems differ from same/different problems. Essentially, a category discrimination can be solved on the basis of common features of the stimuli in the categories and does not require a relational or concept interpretation. There have

been a few recent studies of category discrimination in honeybees that suggest that honeybees can easily solve such problems. The purpose of the two experiments presented in this chapter was to determine if honeybees could discriminate pairs of solid colors from pairs of two-color pattern stimuli with novel pairs on every trial. Successful discrimination would be evidence that the bees could respond to solid colors and two-color patterns as categories. The plan was to incorporate the category difference into both oddity and simultaneous same/different problems.

In Experiment 3, bees were presented with a pair of identical two-color patterns and a pair of identical solid colors. There were two groups of bees, Group Pattern and Group Solid. The bees in Group Pattern were rewarded for choice of the identical pattern pairs, and the bees in Group Solid were rewarded for choice of the identical solid color pairs. The training was trial-unique with novel sets of stimuli on each trial. The results showed that the bees were able to easily discriminate the pairs of solids and the pairs of patterns.

In Experiment 4, bees were presented nonidentical pairs of two-color patterns and solids. Again, there were two groups of bees, Group Pattern and Group Solid. The bees in Group Pattern were rewarded for choice of the nonidentical pattern pair, and the bees in Group Solid were rewarded for choice of the nonidentical solid color pairs. The results showed that the bees were again easily able to discriminate the pairs. The training was trial-unique with novel sets of stimuli on each trial. The success of the bees in both Experiment 3 and Experiment 4 permitted the use of solid and pattern stimuli in the next experiments.

Chapter 4. Concept Learning with a Category Dimension. The purpose of the experiments in Chapter 4 was to determine if performance in an oddity problem and a simultaneous same/different problem could be improved with the use of a stimulus set that includes both solid colors and two-color patterns. The expectation was that the category dimension would increase the discriminability of the odd stimulus in the oddity problem and increase the discriminability of the pairs in the simultaneous same/different problem.

In Experiment 5, honeybees were trained in a four-stimulus oddity problem using both solid colors and two-color pattern stimuli. Each bee was presented with a unique set of stimuli on each trial. On half of the trials, the odd stimulus was a solid color and the three nonodd stimuli were identical two-color patterns. On the other half of the trials, the odd stimulus was a two-color pattern and the three nonodd stimuli were identical solid colors. Thus, the bees were required to learn to choose the odd stimulus, regardless of whether the odd stimulus was a pattern or a solid. The results showed that bees were able easily to solve this oddity problem. The performance of the bees in this experiment was qualitatively compared with the performance of the bees in Experiment 1 that had been trained only with two-color patterns. Note that the two experiments are not directly comparable because they were run at different times. Nonetheless, the performance of the bees with the category dimension was considerably better than the performance of the bees trained only with two-color patterns. This difference needs to be explored in future experiments in order to confirm the result and to determine the mechanism for the improved performance.

In Experiment 6, honeybees were trained in a simultaneous same/different discrimination using both solid colors and two-color pattern stimuli. There were two groups of bees, Group Same and Group Different. Both groups of bees were presented with two pairs of stimuli on each trial. On half of the trials, the same pair was two identical solid colors and the different pair was two nonidentical patterns. On the other half of the trials, the same pair was two identical patterns and the different pair was two nonidentical solid colors. The bees in Group Same were rewarded for choice of the same pair, regardless of whether the pair was two identical solids or patterns.

The bees in Group Different were rewarded for choice of the different pair, regardless of whether the pair was two nonidentical solids or two nonidentical patterns. The results for both groups showed that the bees were able to learn this simultaneous same/different discrimination, and there were no differences between the Group Same and Group Pattern. The performance of the bees in this experiment was qualitatively compared to the performance of the bees in Experiment 2 that had been trained only with two-color patterns. Note that the two experiments are not directly comparable because they were run at different times. There appears to be only a small improvement in the performance of the bees trained with the category dimension. It is not clear that performance was enhanced with the addition of the category dimension of patterns and solids. Given that the category dimension appears to have improved performance in the oddity problem, it is reasonable to further explore the effect of a category dimension in other same/different problems.

In summary, the results of the experiments reported here provide compelling evidence of both oddity and simultaneous same/different discrimination learning in honeybees. A simple associative explanation cannot account for the results because the training was trial-unique. Therefore, the bees had to have learned about the relationships among the stimuli in order to choose correctly. It should be noted that in the vertebrate literature, such results would be interpreted as evidence of concept learning.

Comparative Perspectives & Methodological Considerations

Despite the fact that vertebrates and honeybees shared a common ancestor about a half a billion years ago, there are many similarities between the performance of honeybees and vertebrates in a large number of analogous experiments. These remarkable similarities suggest that the biological mechanisms of learning, even complex learning, may occur at a basic cellular level. In other words, the mechanisms of learning may have been conserved throughout their evolutionary trajectory. However, it is also possible that convergent evolution occurred, that is, "different phenomena may be produced by the same processes, and what appear to be identical phenomena may be produced by different processes" (Bitterman, 1975).

It is important to note that the performance of vertebrates in same/different concept problems is often variable, sometimes successful and sometimes not (*c.f.*, Boyd & Warren, 1957; Warren, 1960; Strong & Hedges, 1966). Furthermore, extensive training is typically required in the vertebrate experiments, while the appearance of concept learning in these honeybee experiments did not require extensive training. In fact, the honeybees reached asymptotic performance within 15-24 trials, whereas vertebrates may require hundreds to thousands of trials to reach similar levels of performance. There are several methodological differences between the vertebrate experiments and the free-flying procedure used here with honeybees. These differences may contribute to the differences in performance on the oddity and same/different discrimination problems.

One methodological difference is that vertebrate experiments typically have massed trials, whereas the honeybees have spaced trials. For example, in matching- and nonmatching-to-sample experiments with pigeons, training is usually conducted in daily sessions with around 20-30 trials per session. The interval between the trials is typically extremely brief, from around 10 to 30 seconds. In contrast, all of the same/different problems reported here were conducted in a single daily session with 15-24 trials. The interval between the trials is the time it takes the bee to leave the window, deposit sucrose at the hive, and return to the window for a new trial. On averaged that intertrial interval was from three to five minutes, considerably longer than the 10-30 seconds between trials in the usual pigeon training. A short intertrial interval produces highly massed trials which can lead to interference in learning problems because one trial quickly bleeds into another trial. Spaced trials, on the other hand, reduce such interference and therefore may have contributed to the more rapid learning of the honeybees. Further research needs to be conducted to determine the extent to which spaced and massed trials affect performance on same/different concept learning problems with both honeybees and vertebrates.

A second methodological difference between the vertebrate and honeybee experiments is the use of punishment. Typically, in vertebrate experiments, subjects are given reward for choosing a correct stimulus and a “time-out” for choosing an incorrect stimulus. Very seldom do experiments using vertebrate species use an explicit punishment for an incorrect choice. In the honeybee experiments, subjects were given a reward of sucrose for choosing a correct stimulus but also were punished with the taste of an aversive solution for an incorrect choice. The use of punishment may facilitate learning in the honeybee by increasing the cost of making an incorrect choice, thereby increasing attention to the stimuli present on a trial prior to making a choice. Further research needs to be conducted to determine the extent to which the aversiveness of punishment of an incorrect choice affects performance on same/different concept learning problems with both honeybees and vertebrates.

A third methodological difference between the vertebrate and honeybee experiments is the stimulus-reward contingency. Typically, in the vertebrate experiments, reward is not provided directly on the correct stimulus. In pigeon experiments, for example, the reinforcement is provided in a food hopper that is located below the stimuli that is presented on a trial. In vertebrate experiments that use three-dimensional objects as stimuli, such as in the primate experiments with the Wisconsin General Test Apparatus, the reward is placed beneath or behind a correct object. In the honeybee experiments, the rewarding and punishing solutions are placed directly on top of the correct and incorrect stimuli. It is widely known in the long history of learning research that even a very short delay between a stimulus and reward can impair learning.

A fourth methodological difference between the vertebrate and honeybee experiments may be the value of the reward. In vertebrate experiments, typically small amounts of food are used as rewards in order to avoid satiation during training. In honeybee experiments, bees are rewarded with a highly concentrated sucrose solution and are allowed to drink until replete. There are no issues with satiation because the bee flies to the hive when replete and unloads the sucrose before returning for another training trial. The amount and quality of the reward may facilitate learning in the honeybee. Further research needs to be conducted to determine the extent to which reward value affects performance on same/different concept learning problems with both honeybees and vertebrates.

These methodological differences very likely contribute to the difference in performance between the vertebrate species and honeybees. It is interesting to note also that the lifespan of honeybees is considerably shorter than that of most, if not all, vertebrate species. For example, on average, a foraging bee’s lifespan is only about six weeks. The ability to learn about its environment, whether it be the location of a food source, what flowers to choose, or the ability to discriminate relationships among stimuli, may simply be compressed into a shorter developmental time-frame.

The differences in the performance of vertebrate species and honeybees, as well as the differences in performance among the vertebrate species, in same/different problems could also be due to variations in experimental design. As noted in the introductions to Chapters 1 and 2, there is a wide variety of procedures used to study oddity and same/different concept learning. These variations may not be superficial but may in fact result in tests of completely different cognitive processes. An obvious example is the successive same/different procedure shown in panel D of Table 2. Subjects are presented with a series of sequential stimuli that either are all the same or are different. Afterwards, subjects choose one response key if the series of stimuli were all the same or choose another response key if the series of stimuli were different. A case could be made that this procedure is not, in fact, testing the ability to discriminate same from different, but rather to remember a sequence of stimuli. One remedy that could be made to clarify this issue is for researchers studying same/different concept learning to clearly define what variation of problem they are using in their experiments. For instance, researchers often claim to be studying simultaneous same/different discrimination when, in fact, their procedure is very different than the original simultaneous problems used by Robinson (1955) with chimpanzees.

Future Experiments

A logical next step might be to begin exploring other types of conceptual problems in honeybees. Such concepts that have not been well studied, so far, in honeybees include number and time, as well as second order relational learning. For example, in a second order matching-to-sample problem, on each trial, subjects are presented with a sample that can depict either a “same” relationship or a “different” relationship. A same relationship might be two identical stimuli (AA), and a different relationship might be two different stimuli (BC). Subjects are then presented with a choice between, say BB and EF. In order to receive reward, subjects must choose the relationship that matches the sample. In other words, if the sample had been AA, then the subjects are rewarded for choosing BB, and if the sample had been BC, the subjects are rewarded for choosing EF. Second order relational learning is considered to be an extremely challenging problem, and has only been demonstrated by a language-trained chimpanzee named Sarah (Premack, 1983) and in hooded crows (Smirnova, Zorina, Obozova, & Wasserman, 2015).

In future studies with honeybees, the oddity and same/different problems could incorporate such a second order relationship. For example, in an oddity experiment, bees could be presented with three pairs of relationships on a trial. On one kind of trial, one of the pairs could be "same" and the other two pairs could be "different." On the other kind of trial, one of the pairs could be "different" and the other two pairs could be "same." On both kinds of trials, the bees would be rewarded for choosing the odd pair, that is, the pair that had the "odd relationship" among the three pairs. For example, on one trial, bees could be presented with the stimulus pairs AA vs. BC vs. EF and rewarded for choosing AA. On another trial, bees could be presented with the pairs DF vs. AA vs. BB and rewarded for choosing DF. Successful solution of second order problems would certainly solidify the evidence of concept learning in honeybees. Furthermore, such results would raise some very interesting questions about the cognitive abilities of honeybees and vertebrates.

Conclusion

Taken together, the experiments presented here provide strong evidence that honeybees are able to learn same/different concepts. Furthermore, the experiments on simultaneous same/different discrimination in honeybees are the first clear evidence that an invertebrate is capable of solving this type of problem. Wodinsky and Bitterman (1953) were the first to find evidence of same/different concept learning in rats and they concluded: "Perhaps we must think in terms of an abstract level of functioning which most of us have hitherto been reluctant to grant to the rat." Although they were writing about oddity concept learning in rats some 65 years ago, it seems reasonable to now think of invertebrates, such as the honeybee, as capable of "an abstract level of functioning."

APPENDIX A

Figure 1. The training situation used in all of the experiments. Shown in this picture is one of the trials in the four-stimulus oddity problem of Experiment 1.

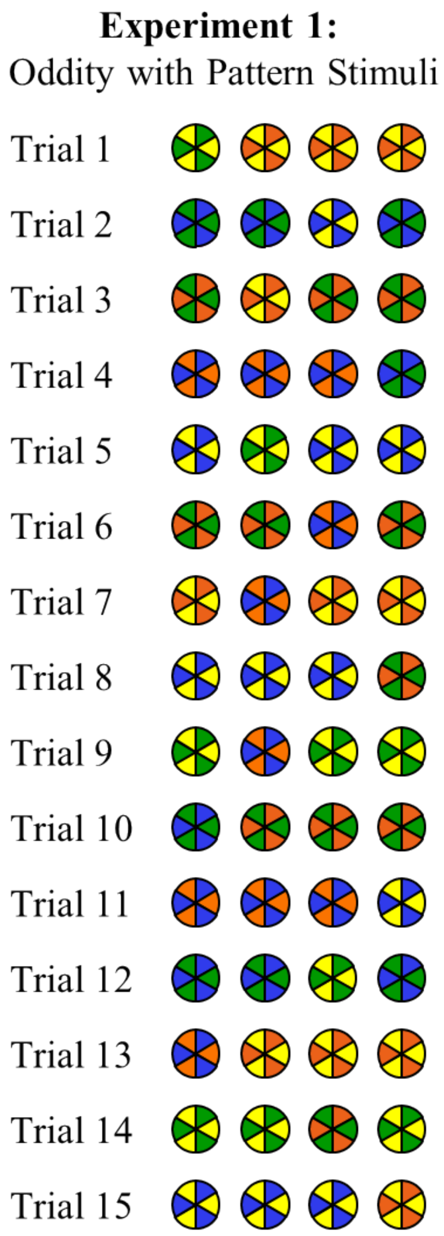


Figure 2. A sample training sequence for the four-stimulus oddity problem of Experiment 1.

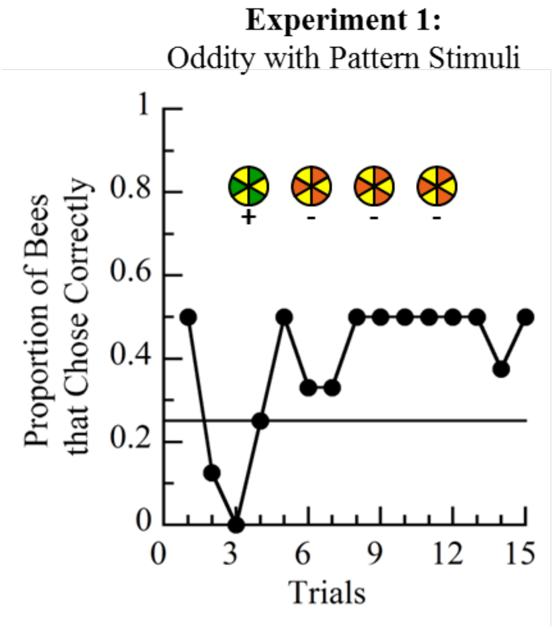


Figure 3. Results for the four-stimulus oddity problem of Experiment 1. The horizontal line at .25 represents chance.

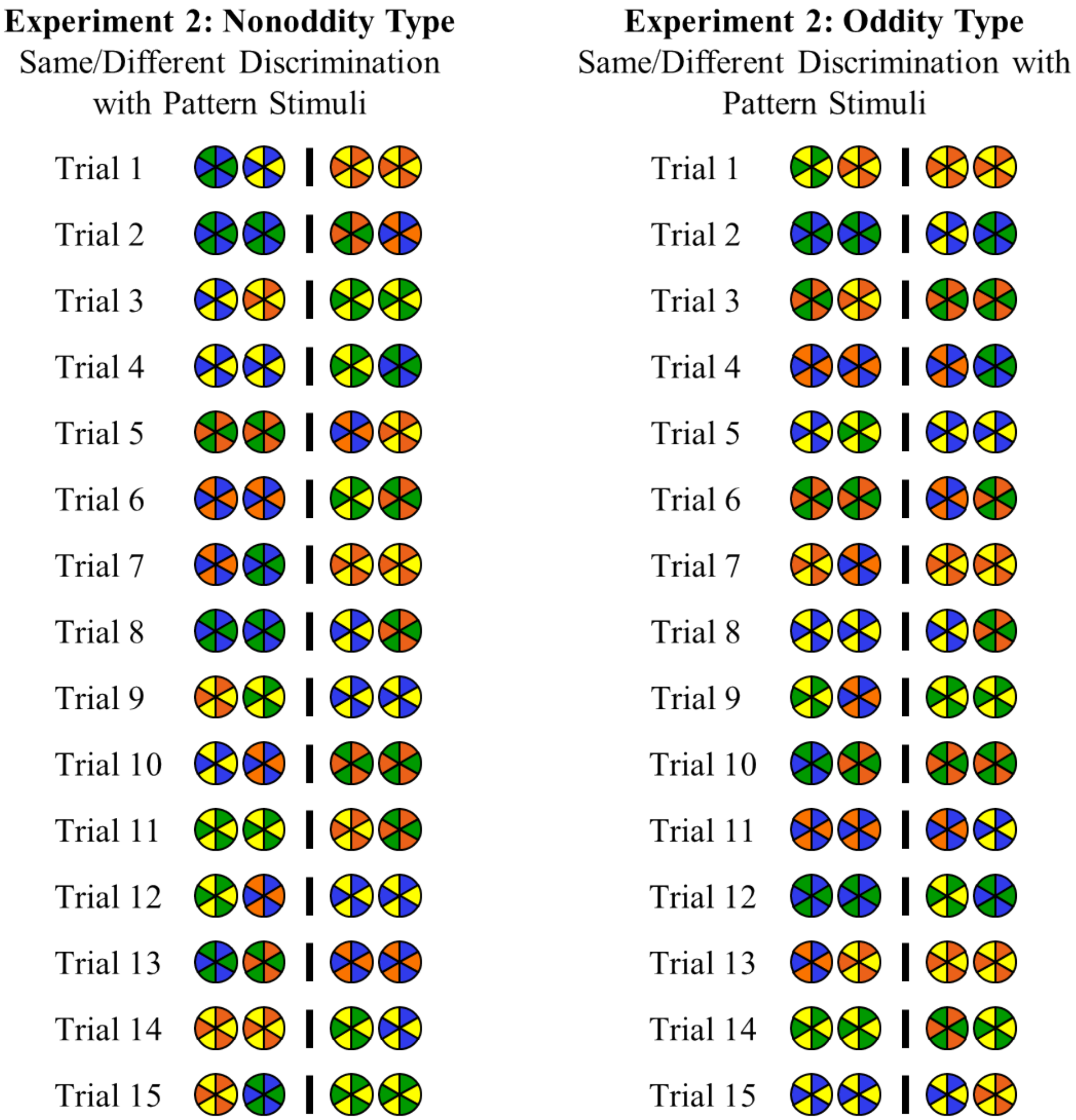


Figure 4. Sample training sequences for the same/different problem of Experiment 2. The black line between each pair represents a wooden divider.

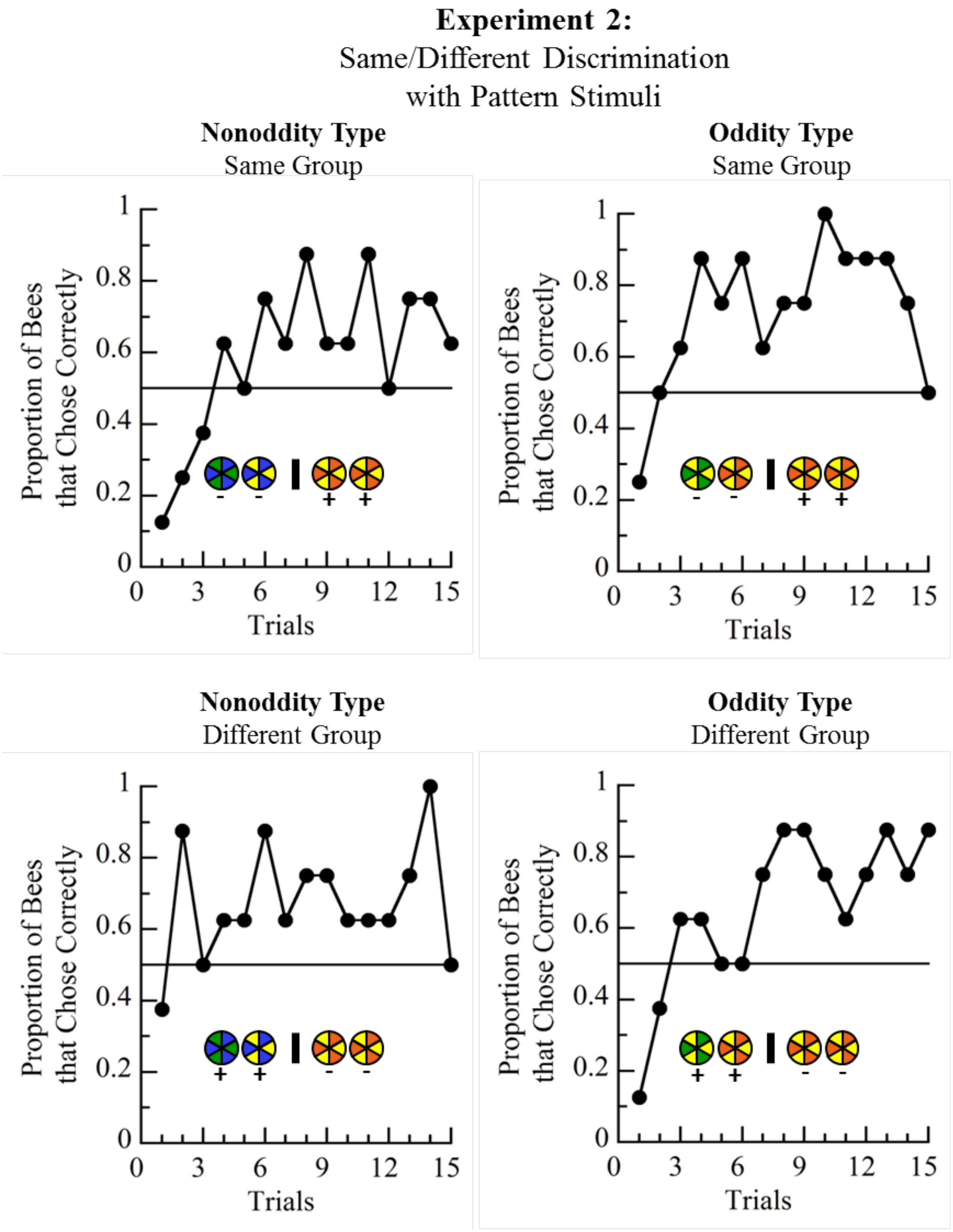


Figure 5. Results for the four same and different groups of Experiment 2. The horizontal line at .5 represents chance.

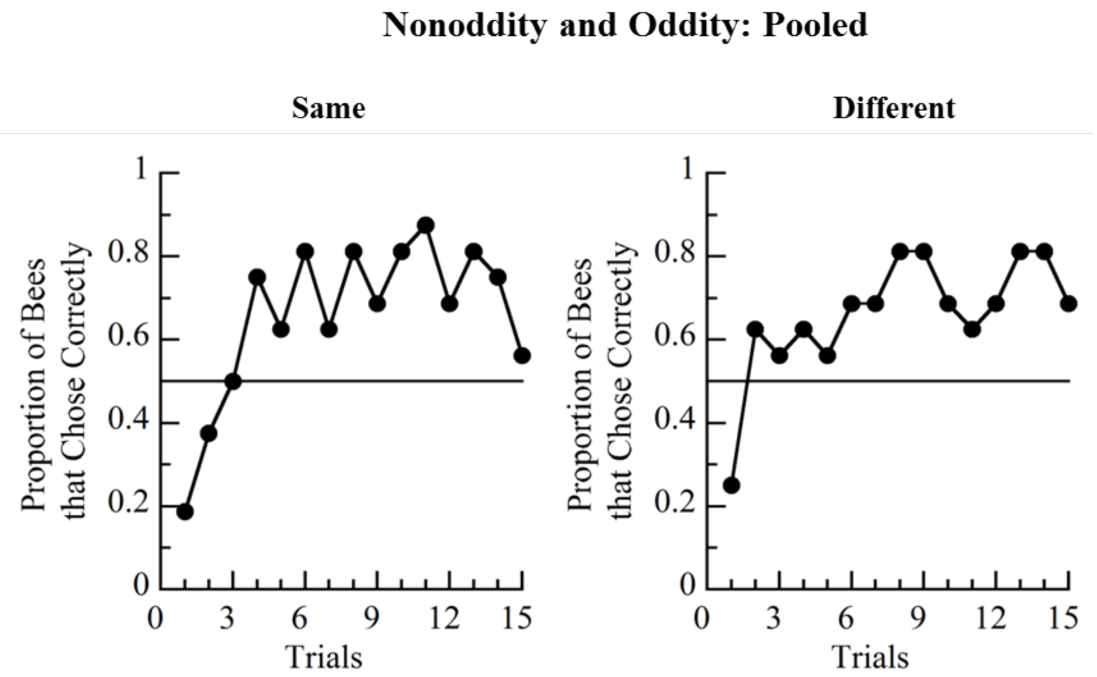


Figure 6. The pooled results for the Nonoddity Type and the Oddity Type groups of the same/different discrimination problem of Experiment 2. The horizontal line at .5 represents chance.

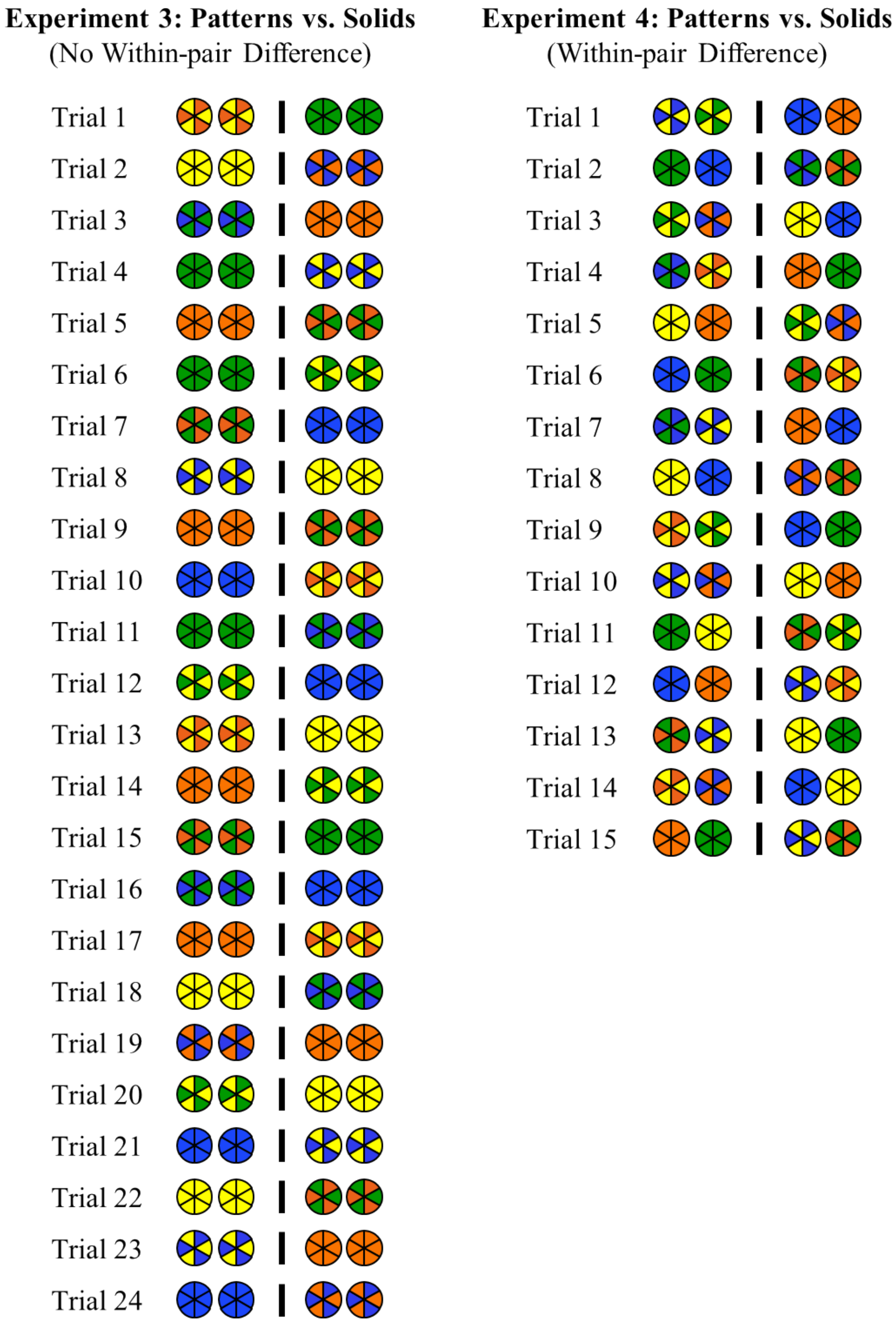
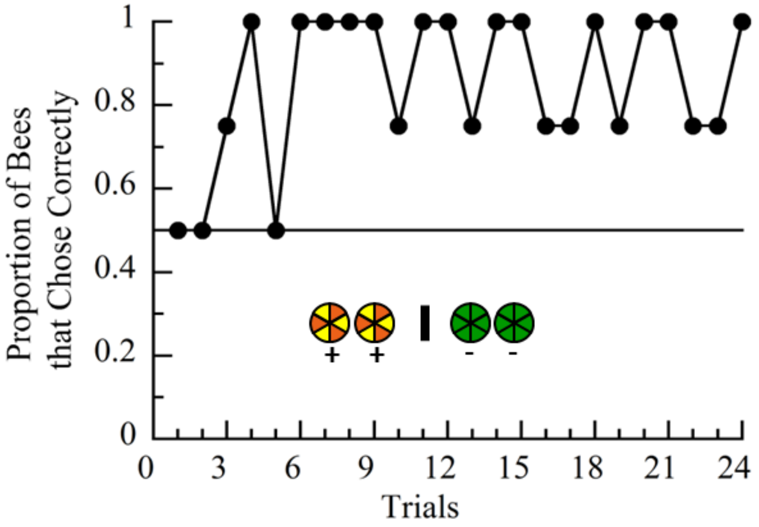


Figure 7. Sample training sequences for the category discrimination problems of Experiments 3 and 4.

Experiment 3: Patterns vs. Solids

(No Within-pair difference)

Group Pattern



Group Solid

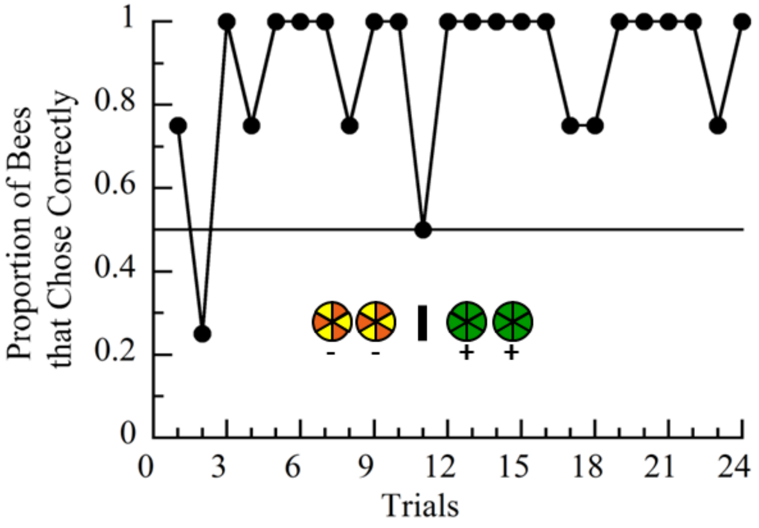


Figure 8. Results for the two groups in the category discrimination problems of Experiment 3. The top panel shows the results for the subjects in Group Pattern. The bottom panel shows the results for the subjects in Group Solid. The horizontal line at .5 represents chance.

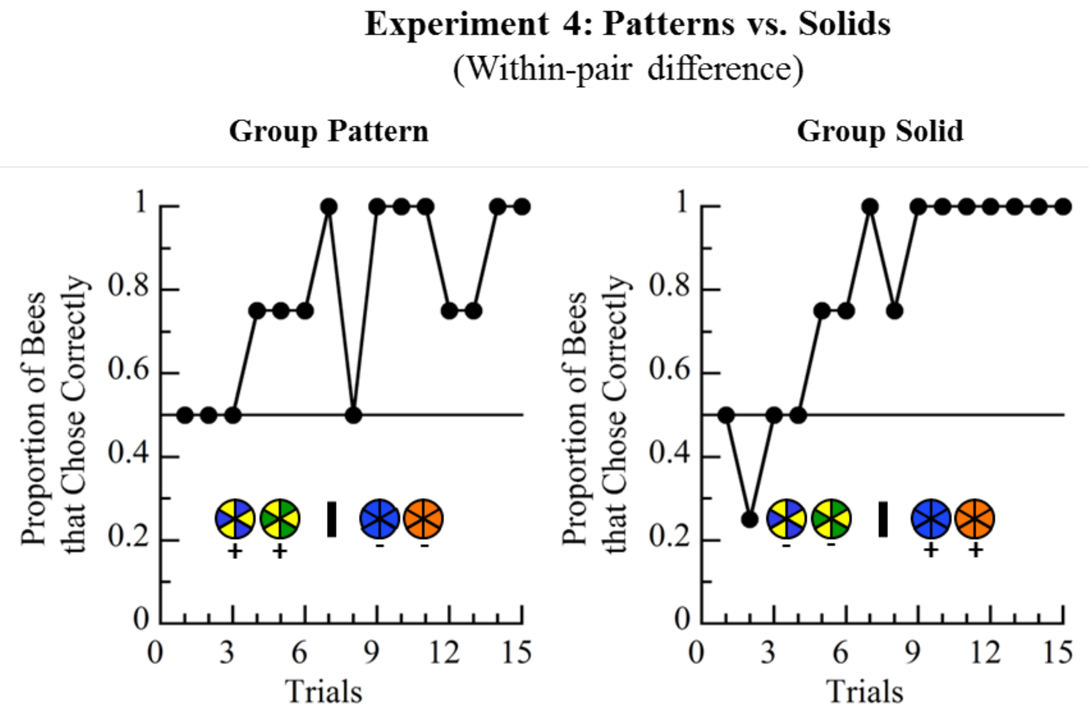


Figure 9. Results for the two groups in the category discrimination problem of Experiment 4. The left panel shows the results for subjects in Group Pattern. The right panel shows the results for the subjects in Group Solid. The horizontal line at .5 represents chance.

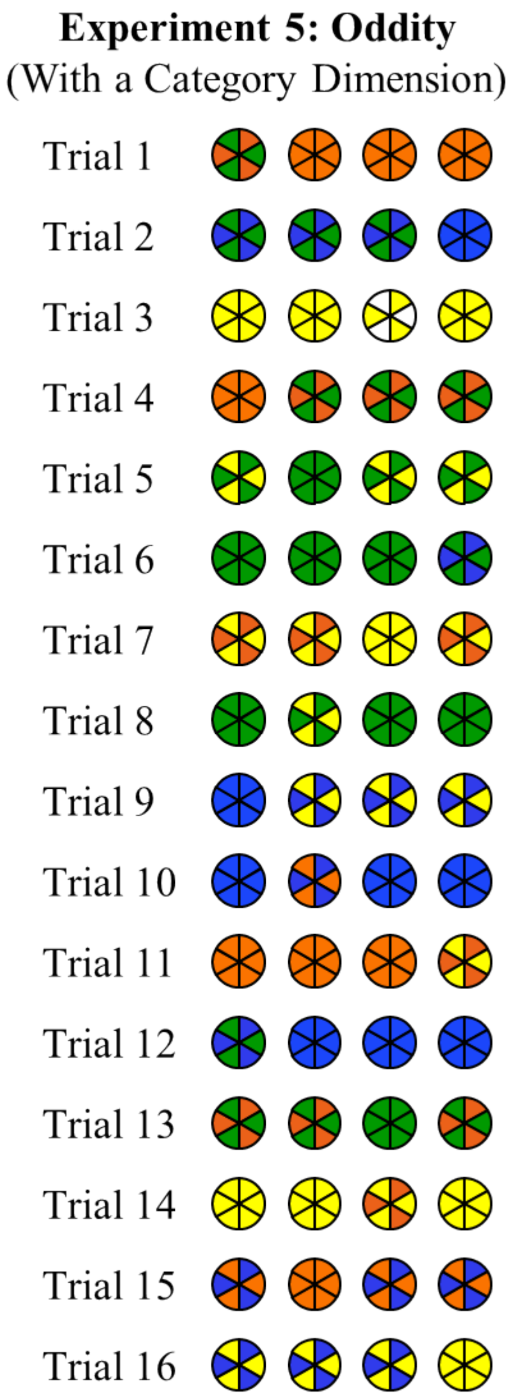


Figure 10. A sample training sequence for the four-stimulus oddity problem of Experiment 5.

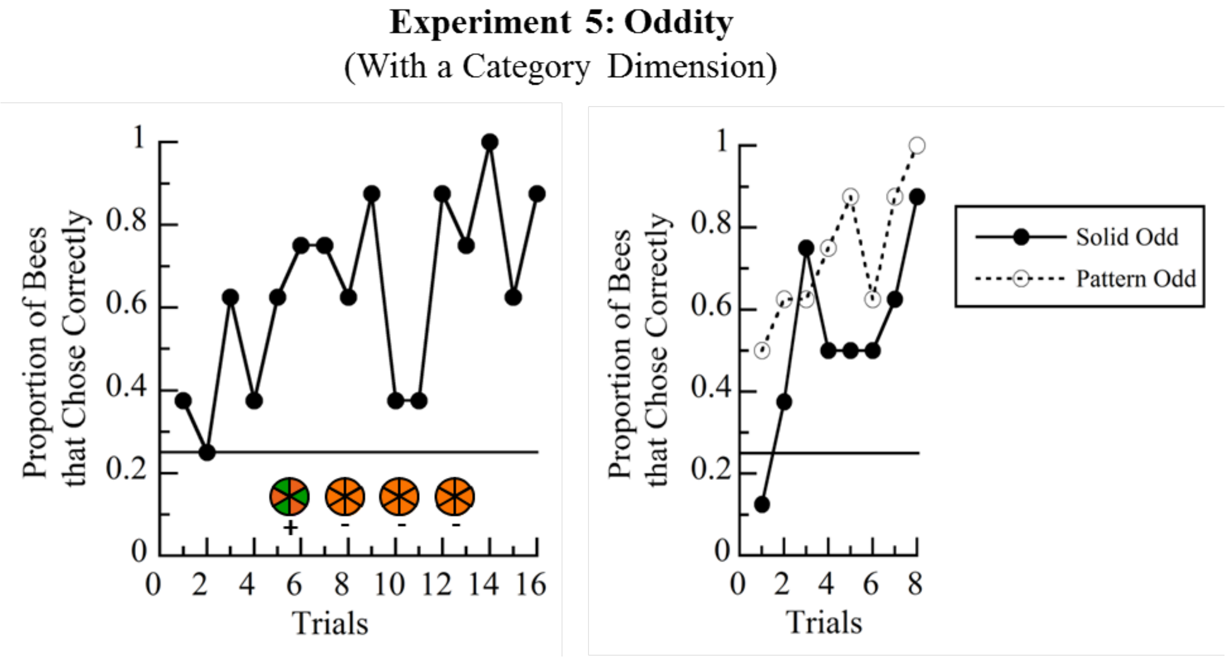


Figure 11. Results for the four-stimulus oddity problem of Experiment 5. The left panel shows the performance of the bees on each trial. The right panel shows the performance of the bees on each of the two trial types, solid odd and pattern odd. The horizontal line at .25 represents chance.

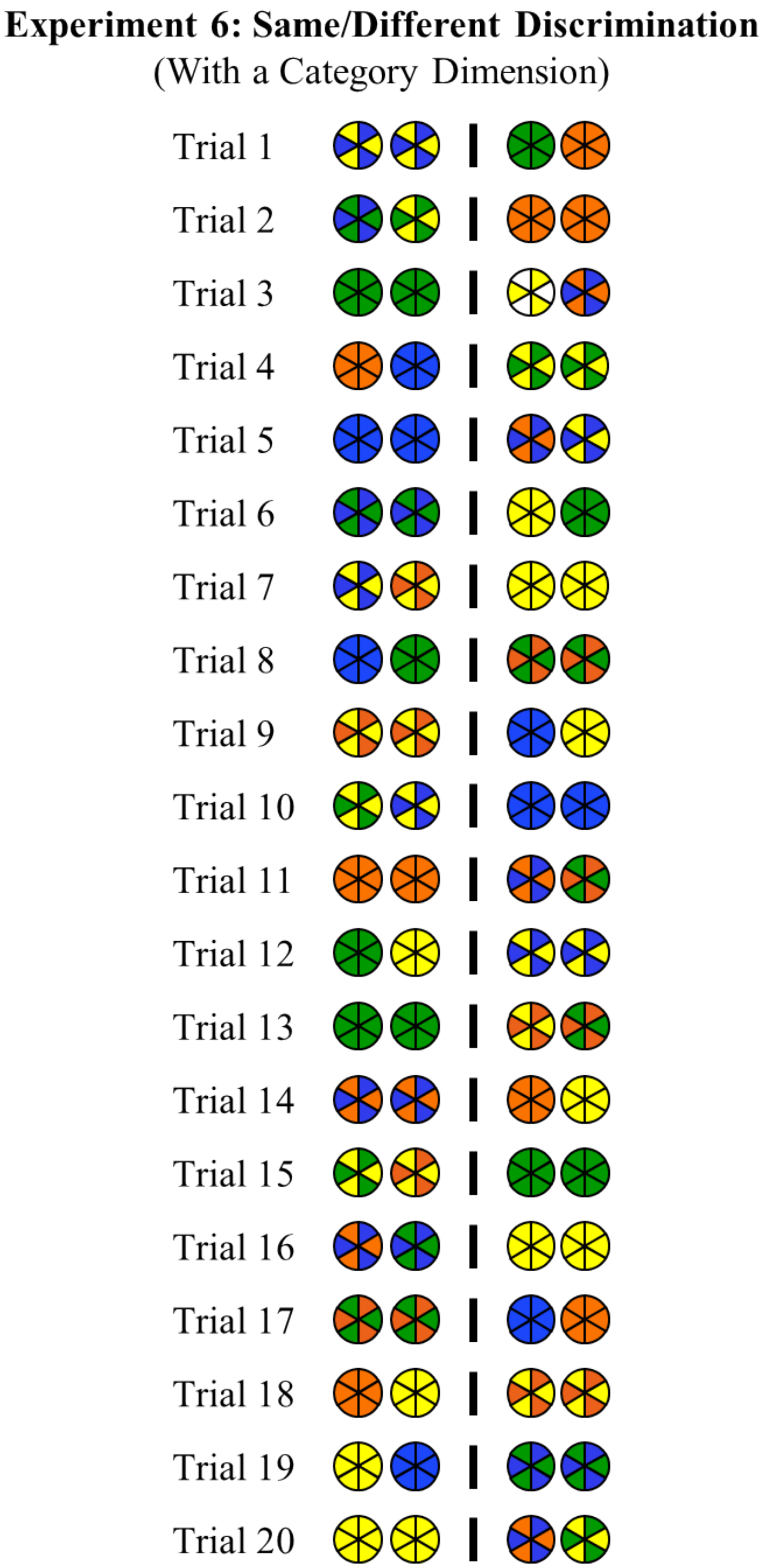


Figure 12. A sample training sequence for the same/different discrimination problem of Experiment 6.

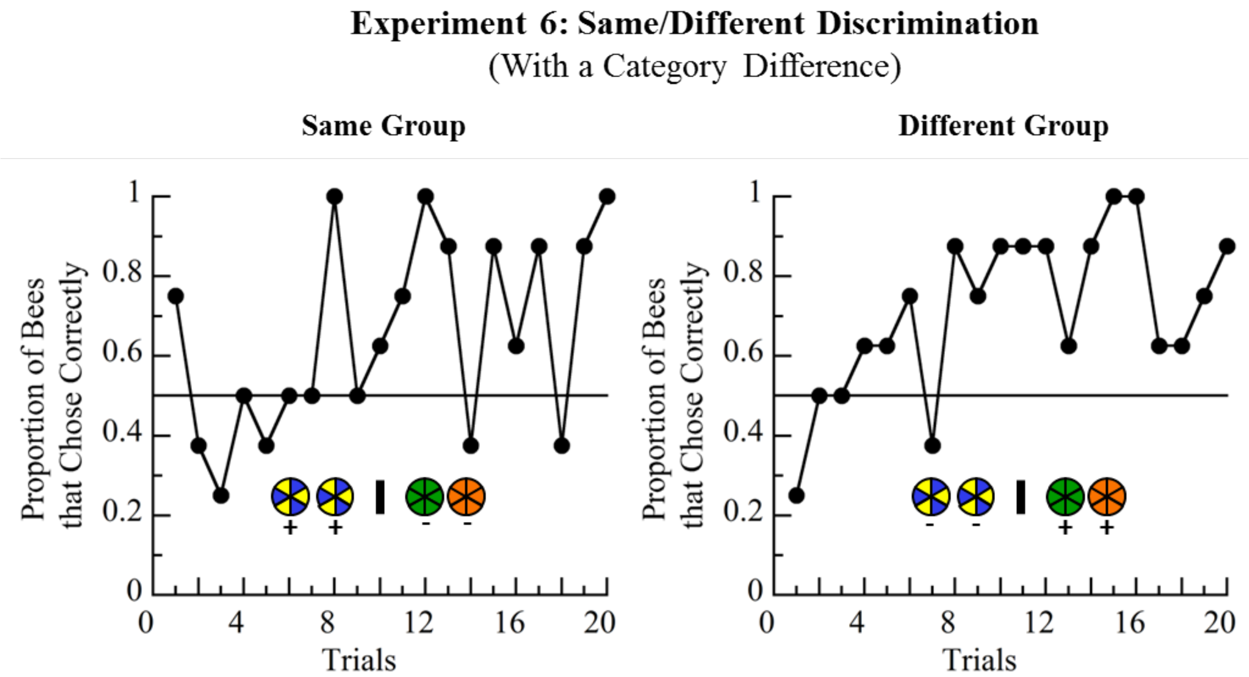


Figure 13. Results for the two groups in the same/different problem of Experiment 6. The horizontal line at .5 represents chance.

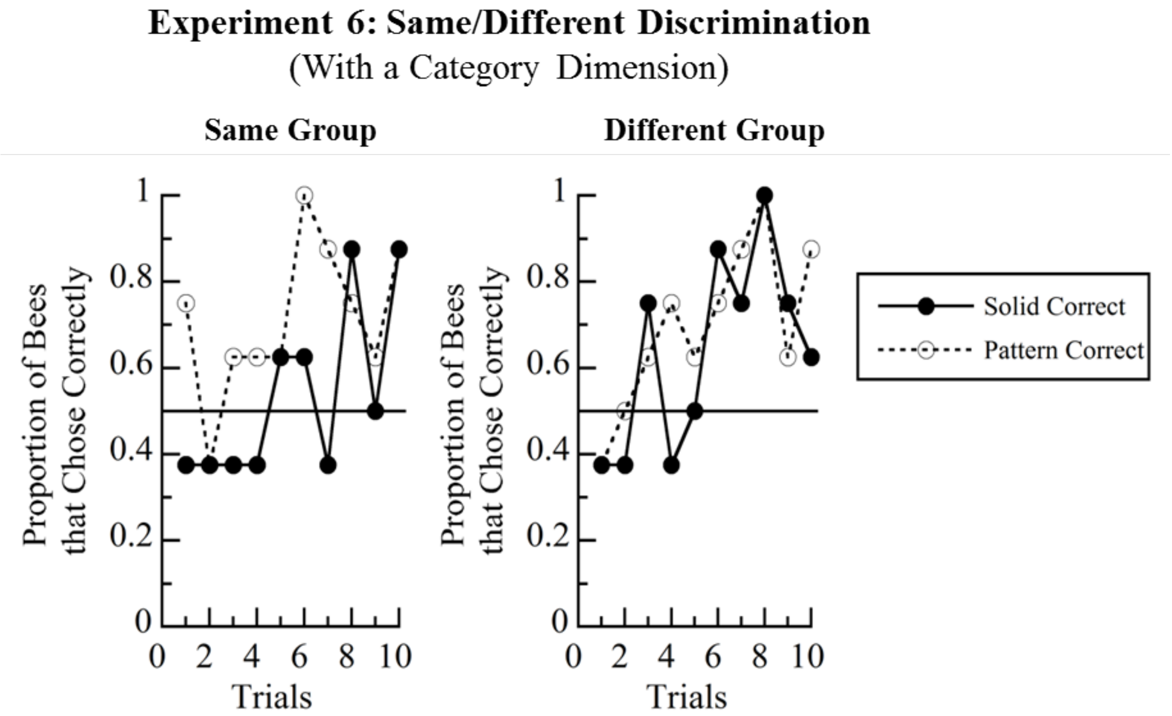
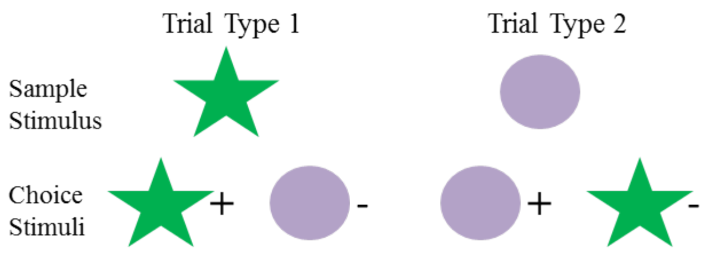


Figure 14. Results for the solid and pattern trial types of the same/different discrimination problem of Experiment 6. The line at .5 represents chance.

APPENDIX B

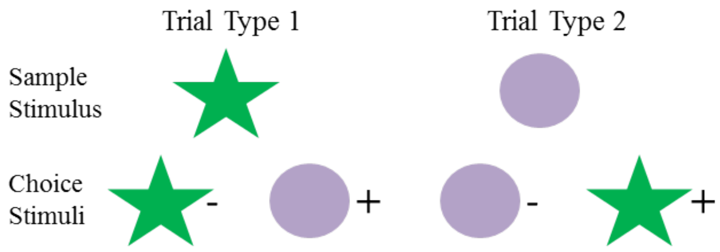
Table 1. Diagram of the four common procedures used to study same/different concept learning.

A. Matching-to-sample



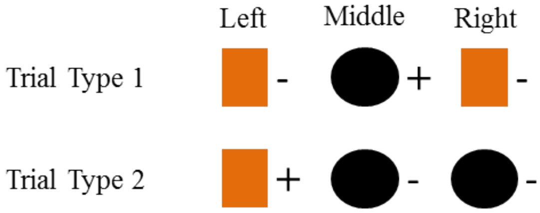
Single-group Design
- Given a sample stimulus.
- Either simultaneous or successive presentation of two comparison stimuli.
- Rewarded for choice of the stimulus that matches the sample.

B. Nonmatching-to-sample



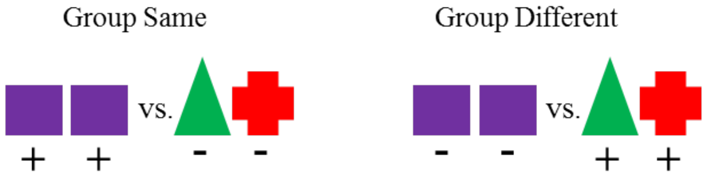
Single-group Design
- Given a sample stimulus.
- Either simultaneous or successive presentation of two comparison stimuli.
- Rewarded for choice of the stimulus that does not match the sample.

C. Oddity



Single-group Design
- Odd stimulus rewarded on every trial.
- In training, the odd stimulus appears equally often in each position (left, middle, and right), so there are six possible configurations of the two stimuli.

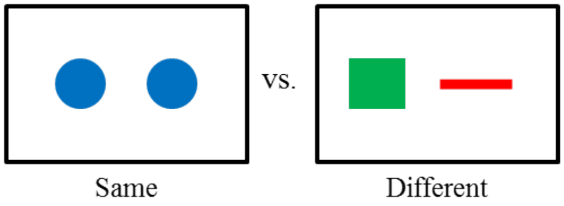
D. Simultaneous S/D



Two-group Design
- Group Same: rewarded for choice of the same pair.
- Group Different: rewarded for choice of the different pair.

Table 2. Diagram of the common same/different designs used with pigeons. (A. Blaisdell & Cook, 2005; B. Katz & Wright, 2006; C. Wasserman, Hugart, & Kirkpatrick-Steger, 1995; D. Young, Wasserman, & Dalrymple, 1997).

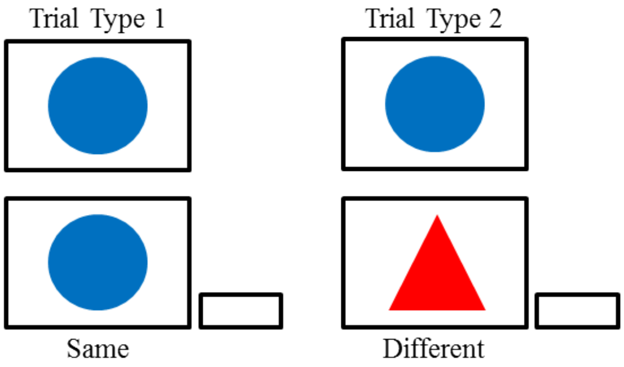
A. Simultaneous S/D



Two-group Design

- Group Same: rewarded for pecking the same pair.
- Group Different: rewarded for pecking the different pair.

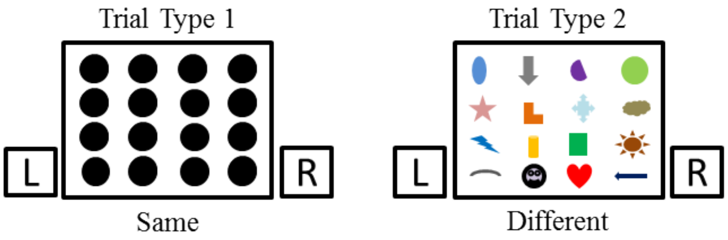
B. Delayed S/D



Single-group Design

- Top stimulus is presented first, followed by two bottom stimuli.
- If Trial Type 1 is presented, rewarded for pecks to the matching bottom stimulus.
- If Trial Type 2 is presented, rewarded for pecks to the white rectangle.

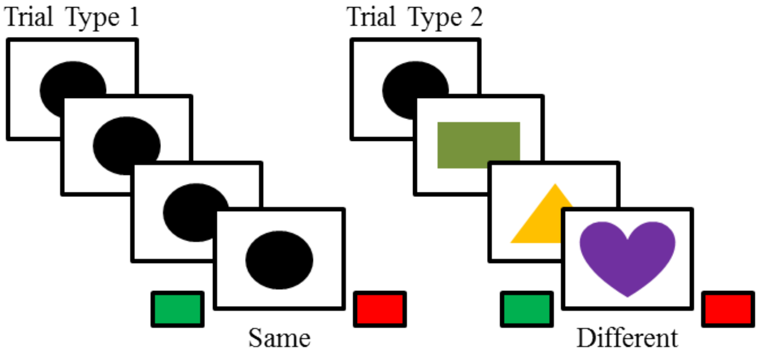
C. Array S/D (Two-item and Multi-item Arrays)



Single-group Design

- If Trial Type 1 is presented, rewarded for pecks to the left (L) response key.
- If Trial Type 2 is presented, rewarded for pecks to the right (R) response key.

D. Successive S/D



Single-group Design

- If Trial Type 1 is presented, rewarded for pecks to the green response key after 16 stimuli have been displayed.
- If Trial Type 2 is presented, rewarded for pecks to the red response key after 16 stimuli have been displayed.

Table 3. Simultaneous S/D Experiments. List of species tested with a simultaneous same/different problem.

Experiments	Species	Design	Success
Robinson (1955/1960)	Chimpanzees	AA vs. CD	Yes
King (1973)	Chimpanzees & Orangutans	AX & BX vs. CY & CZ	Yes
Chausseil (1991)	Coati	AA vs. CD	Yes
Russel & Burke (2016)	Echidnas	AAAA vs. CDFG	Yes
Blaisdell & Cook (2005)	Pigeons	AA vs. CD	Yes
Martinho & Kacelnik (2016)	Ducklings	AA vs. CD	Yes
Newport et al. (2015)	Archerfish	AA vs. CD	No

APPENDIX C

Tests were conducted to ensure that bees were unable to visually discriminate a drop of sucrose from a drop of stevia. In the control experiment, four subjects were presented with three identical pattern stimuli on every trial. One of the patterns had a sucrose drop placed on the middle and the other two patterns had a stevia drop placed on the middle. Each trial used a different set of pattern stimuli which were allowed to repeat two to three times across 15 training trials. It was expected that if the bees were able to discriminate sucrose from stevia, performance should have reached levels above chance, which was .33. Mean proportion of correct choice across the 15 training trials for all of the bees was .28, which was not significantly different than chance, $t(3) = -.68$, $p = .55$ with a standard error of the mean of .07. Therefore, stevia solution is used as punishment in all of the experiments reported here.

REFERENCES

Avarguès-Weber, A., & Giurfa, M. (2013). Conceptual learning by miniature brains. *Proceedings of the Royal Society of London B: Biological Sciences*, *280*, 1-9.

Avarguès-Weber, A., Portelli, G., Benard, J., Dyer, A., & Giurfa, M. (2010). Configural processing enables discrimination and categorization of face-like stimuli in honeybees. *Journal of Experimental Biology*, *213*, 593-601.

Bailey, A. M., & Thomas, R. K. (1998). An investigation of oddity concept learning by rats. *Psychological Record*, *48,* 447-456.

Batson, J. D., Hoban, J. S., & Bitterman, M. E. (1992). Simultaneous conditioning in honeybees (*Apis mellifera*). *Journal of Comparative Psychology, 106*, 114-119.

Benjamini, L. (1983). Studies in the Learning Abilities of Brown-Necked Ravens and Herring Gulls I. Oddity Learning. *Behaviour*, *84*, 173-194.

Bitterman, M. E. (1975). The comparative analysis of learning. *Science*, *188,* 699-709.

Bitterman, M. E. (1988). Vertebrate-invertebrate comparisons. In H. Jerison & I. Jerison (Eds.), *Intelligence and evolutionary biology: NATO ASI series.* Springer: Berlin-Heidelberg.

Bitterman, M. E. (1996). Comparative analysis of learning in honeybees. *Animal Learning & Behavior*, *24,* 123-141.

Bitterman, M. E., Menzel, R., Fietz, A., & Schäfer, S. (1983). Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). *Journal of Comparative Psychology*, *97,* 107-119.

Blaisdell, A. P., & Cook, R. G. (2005). Two-item same-different concept learning in pigeons. *Animal Learning & Behavior*, *33,* 67-77.

Blough, P. M. (2001). Cognitive strategies and foraging in pigeons. In R. G. Cook (Ed.), *Avian Visual Cognition.* [On-line] www.pigeon.psy.tufts.edu/avc/pblough.

Boal, J. (1991). Complex learning in *Octopus bimaculoides*. *American Malacological Bulletin, 9*, 75-80.

Boyd, B. O., & Warren, J. M. (1957). Solution of oddity problems by cats. *Journal of Comparative and Physiological Psychology*, *50*, 258-260.

Bromer, J. A. (1940). A genetic and physiological investigation of concept behavior in primates. (Unpublished doctoral dissertation). University of Wisconsin-Madison.

Brown, M. F., & Sayde, J. M. (2013). Same/different discrimination by bumblebee colonies. *Animal Cognition*, *16*, 117-125.

Chausseil, M. (1991). Visual same-different learning and transfer of the sameness concept by coatis (*Nasua Storr*, 1780). *Ethology*, *87*, 28-36.

Cook, R. G., & Wasserman, E. A. (2012). Relational discrimination learning in pigeons. In T. R. Zentall & E. A. Wasserman (Eds.), *The handbook of comparative cognition*. Oxford University Press, New York, NY.

Corning, W. C., Dyal, J. A., & Willows, A. O. D. (1973). *Invertebrate learning: Volume 2 arthropods and gastropods.* New York, NY: Plenum Press.

Couvillon, P. A., & Bitterman, M. E. (1985). Effect of experience with a preferred food on consummatory responding for a less-preferred food in goldfish. *Learning & Behavior*, *13*, 433-438.

Couvillon, P. A., & Bitterman, M. E. (1988). Compound-component and conditional discrimination of colors and odors by honeybees: Further tests of a continuity model. *Learning & Behavior*, *16*, 67-74.

Couvillon, P. A., Lee, Y., & Bitterman, M. E. (1991). Learning in honeybees as a function of amount of reward: Rejection of the equal-asymptote assumption. *Learning & Behavior*, *19*, 381-387.

Couvillon, P. A., Hsiung, R., Cooke, A. M., & Bitterman, M. E. (2005). The role of context in the inhibitory conditioning of honeybees. *The Quarterly Journal of Experimental Psychology Section B*, *58*, 59-67.

Cumming, W. W., & Berryman, R. (1961). Some data on matching behavior in the pigeon. *Journal of the Experimental Analysis of Behavior*, *4*, 281-284.

Davenport, R. K., Jr., & Menzel, E. W., Jr. (1960). Oddity preference in the chimpanzee. *Psychological Reports*, *7*, 523-526.

Davis, R. T., Leary, R. W., Stevens, D. A, & Thompson, R. F. (1967). Learning and perception of oddity problems by lemurs and seven species of monkey. *Primates*, *8*, 311-322.

Devine, J. V., & Ivens, J. R., Jr. (1969). Acquisition of the oddity concept in the chimpanzee. (No. 6571-ARL-TR-69-16). Aeromedical Research Lab (6571ST). Holloman AFB, New Mexico.

Draper, W. A. (1967). Cue dominance in oddity discriminations by rhesus monkeys. *Journal of Comparative and Physiological Psychology*, *60*, 140-141.

Edwards, C. A., Jagielo, J. A., & Zentall, T. R. (1983). “Same/different” symbol use by pigeons. *Learning & Behavior*, *11*, 349-355.

Edwards, D. H., Heitler, W. J., & Krasne, F. B. (1999). Fifty years of a command neuron: The neurobiology of escape behavior in the crayfish. *Trends in Neurosciences*, *22*, 153-161.

Fields, P. E. (1953). The efficiency of the serial multiple visual discrimination apparatus and method with white rats. *Journal of Comparative and Physiological Psychology*, *46*, 69-76.

Forwood, S. E., Bartko, S. J., Saksida, L. M., & Bussey, T. J. (2007). Rats spontaneously discriminate purely visual, two-dimensional stimuli in tests of recognition memory and perceptual oddity. *Behavioral Neuroscience*, *121*, 1032-1042.

Frisch K. V. (1915). Der farbensinn und formensinn der bienen. *Zoologische Jahrbucher: Abteilung fur Allgemeine Zoologie und Physiologie*, *35*, 1-182.

Frisch, K. V. (1950). *Bees: Their vision, chemical senses and language*. Cornell University Press; New York.

Giurfa, M. (2015). Learning and cognition in insects. *Wiley Interdisciplinary Reviews: Cognitive Science*, *6*, 383-395.

Giurfa, M., Eichmann, B., & Menzel, R. (1996). Symmetry perception in an insect. *Nature*, *382*, 458-461.

Giurfa, M., Zhang, S., Jenett, A., Menzel, R., & Srinivasan, M. V. (2001). The concepts of ‘sameness’ and ‘difference’ in an insect. *Nature*, *410*, 930-933.

Gould, J. L., Kirschvink, J. L., & Deffeyes, K. S. (1978). Bees have magnetic remanence. *Science*, *201*, 1026-1028.

Greenleaf, S. S., Williams, N. M., Winfree, R., & Kremen, C. (2007). Bee foraging ranges and their relationship to body size. *Oecologia*, *153*, 589-596.

Harlow, H. F., & Bromer, J. A. (1938). A test-apparatus for monkeys. *The Psychological Record*, *2*, 434-436.

Hayes, K. J., Thompson, R., & Hayes, C. (1953). Discrimination learning set in chimpanzees. *Journal of Comparative and Physiological Psychology*, *46*, 99-104.

Herman, L. M., & Gordon, J. A. (1974). Auditory delayed matching in the bottlenose dolphin. *Journal of the Experimental Analysis of Behavior*, *21*, 19-26.

Herrnstein, R. J., Loveland, D. H., & Cable, C. (1976). Natural concepts in pigeons. *Journal of Experimental Psychology: Animal Behavior Processes*, *2*, 285-302.

Hille, P., Dehnhardt, G., & Mauck, B. (2006). An analysis of visual oddity concept learning in a California sea lion (*Zalophus californianus*). *Learning & Behavior*, *2*, 144-153.

James, W. (1890). Reasoning. In *The principles of psychology* (pp. 325-371). New York: H. Holt and Company

Kandel, E. R. (2001). The molecular biology of memory storage: A dialogue between genes and synapses. *Science*, *294*, 1030-1038.

Katner, S. N., Davis, S. A., Kirsten, A. J., & Taffe, M. A. (2004). Effects of nicotine and mecamylamine on cognition in rhesus monkeys. *Psychopharmacology*, *175*, 225-240.

Katz, J. S., & Wright, A. A. (2006). Same/different abstract-concept learning by pigeons. *Journal of Experimental Psychology: Animal Behavior Processes, 32*, 80-86.

King, J. E. (1973). Learning and generalization of a two-dimensional sameness-difference concept by chimpanzees and orangutans. *Journal of Comparative Physiological Psychology*, *84*, 140-148.

Kirchner, W. H. (1993). Vibrational signals in the tremble dance of the honeybee, *Apis mellifera*. *Behavioral Ecology and Sociobiology*, *33*, 169-172.

Koronakos, C., & Arnold, W. J. (1957). The formation of learning sets in rats. *Journal of Comparative and Physiological Psychology*, *50*, 11-14.

Kuczaj, S. A., & Hendry, J. L. (2003). Does language help animals think? In D. Gentner & S. Goldin-Meadow (Eds.), *Language in mind*. Cambridge: MIT Press.

Lashley, K. S. (1938). The mechanism of vision: XV. Preliminary studies of the rat's capacity for detail vision. *The Journal of General Psychology*, *18*, 123-193.

Levine, M., & Harlow, H. F. (1959). Learning-sets with one- and twelve-trial oddity-problems. *American Journal of Psychology, 72*, 253-257.

Magnotti, J. F., Wright, A. A., Leonard, K., Katz, J. S., & Kelly, D. M. (2017). concept learning in Black-billed magpies (*Pica hudsonia*). *Psychonomic Bulletin & Review, 24*, 431-435.

Martinho, A., & Kacelnik, A. (2016). Ducklings imprint on the relational concept of “same or different”. *Science*, *353,* 286-288.

Mercado, E., Killebrew, D. A., Pack, A. A., Mácha, I. V., & Herman, L. M. (2000). Generalization of ‘same–different’ classification abilities in bottlenosed dolphins. *Behavioural Processes*, *50,* 79-94.

Meyer, D. R., & Harlow, H. F. (1949). The development of transfer of response to patterning by monkeys. *Journal of Comparative and Physiological Psychology, 42,* 454-462.

Moon, L. E., & Harlow, H. F. (1955). Analysis of oddity learning by rhesus monkeys. *Journal of Comparative and Physiological Psychology*, *48,* 188-194.

Muszynski, N. M., & Couvillon, P. A. (2015). Relational learning in honeybees (*Apis mellifera*): Oddity and nonoddity discrimination. *Behavioural Processes, 115*, 81-93.

Newport, C., Wallis, G., & Siebeck, U. E. (2014). Concept learning and the use of three common psychophysical paradigms in the archerfish (*Toxotes chatareus*). *Frontiers in Neural Circuits, 8,* 1-13.

Newport, C., Wallis, G., & Siebeck, U. E. (2015). Same/different abstract concept learning by archerfish (*Toxotes chatareus*). *PloS ONE, 10,* 1-14.

Nieh, J. C., & Tautz, J. (2000). Behaviour-locked signal analysis reveals weak 200–300 Hz comb vibrations during the honeybee waggle dance. *Journal of Experimental Biology, 203,* 1573-1579.

Nissen, H. W., & McCulloch, T. L. (1937). Equated and non-equated stimulus situations in discrimination learning by chimpanzees. I. Comparison with unlimited response. *Journal of Comparative Psychology*, *23,* 165-189.

Noble, C. S., & Thomas, R. K. (1970). Oddity learning in the squirrel monkey. *Psychonomic Science, 19*, 305-307.

Overman, W. H., & Doty, R. W. (1980). Prolonged visual memory in macaques and man. *Neuroscience*, *5*, 1825-1831.

Pastore, N. (1954). Discrimination learning in the canary. *Journal of Comparative and Physiological Psychology*, *47*, 389-390.

Pearce, J. M., & Bouton, M. E. (2001). Theories of associative learning in animals. *Annual Review of Psychology*, *52,* 111-139.

Penn, D. C., Holyoak, K. J., & Povinelli, D. J. (2008). Darwin's mistake: Explaining the discontinuity between human and nonhuman minds. *Behavioral and Brain Sciences*, *31,* 109-130.

Premack, D. (1983). The codes of man and beasts. *Behavioral and Brain Sciences*, *6,* 125-136.

Robertson, H. M., & Wanner, K. W. (2006). The chemoreceptor superfamily in the honey bee, *Apis mellifera*: expansion of the odorant, but not gustatory, receptor family. *Genome Research*, *16*, 1395-1403.

Robinson, E. W. (1933). A preliminary experiment on abstraction in a monkey. *Journal of Comparative Psychology, 16*, 231-236.

Robinson, J. S. (1955). The sameness-difference discrimination problem in chimpanzee. *Journal of Comparative and Physiological Psychology*, *48*, 195-197.

Rohrseitz, K., & Tautz, J. (1999). Honey bee dance communication: Waggle run direction coded in antennal contacts? *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *184*, 463-470.

Roitberg, E., & Franz, H. (2004). Oddity learning by African dwarf goats (*Capra hircus*). *Animal Cognition*, *7*, 61-67.

Russell, F., & Burke, D. (2016). Conditional same/different concept learning in the short-beaked echidna *(Tachyglossus aculeatus). Journal of the Experimental Analysis of Behavior, 105*, 133-154.

Scheiner, R., Page, R. E., & Erber, J. (2001). The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (*Apis mellifera L.*). *Neurobiology of Learning and Memory, 76*, 138-150.

Scholtyssek, C., Kelber, A., Hanke, F. D., & Dehnhardt, G. (2013). A harbor seal can transfer the same/different concept to new stimulus dimensions. *Animal Cognition*, *16*, 915-925.

Shishimi, G. (2013). Working memory and concept learning in honeybees. (Unpublished doctoral dissertation). University of Hawai‘i at Mānoa.

Smirnova, A., Zorina, Z., Obozova, T., & Wasserman, E. (2015). Crows spontaneously exhibit analogical reasoning. *Current Biology*, *25,* 256-260.

Strong, P. N. (1965). Learning and transfer of oddity as a function of apparatus and trials per problem. *Psychonomic Science*, *3*, 19-20.

Strong, P. N., & Hedges M. (1966). Comparative studies in simple oddity learning: I. Cats, raccoons, monkeys, and chimpanzees. *Psychonomic Science, 5*, 13-14.

Tautz, J., & Rohrseitz, K. (1998). What attracts honeybees to a waggle dancer? *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 183*, 661-667.

Thomas, R. K. (1996). Investigating cognitive abilities in animals: Unrealized potential. *Cognitive Brain Research*, *3*, 157-166.

Thomas, R. K., & Noble, L.M. (1988). Visual and olfactory oddity learning in rats: What evidence is necessary to show conceptual behavior? *Animal Learning & Behavior, 16*, 157-163.

Townsend-Mehler, J. M., Dyer, F. C., & Maida, K. (2011). Deciding when to explore and when to persist: A comparison of honeybees and bumblebees in their response to downshifts in reward. *Behavioral Ecology and Sociobiology*, *65*, 305-312.

Waite, T. A. (2008). Preference for oddity: Uniqueness heuristic or hierarchical choice process? *Animal Cognition*, *11*, 707-713.

Walker, M. M., & Bitterman, M. E. (1985). Conditioned responding to magnetic fields by honeybees. *Journal of Comparative Physiology A, 157,* 67-71.

Warren, J. M. (1960). Oddity learning set in a cat. *Journal of Comparative and Physiological Psychology*, *5*, 433-434.

Wasserman, E. A. (2016). Thinking abstractly like a duck (ling). *Science*, *353*, 222-223.

Wasserman, E. A., Hugart, J. A., & Kirkpatrick-Steger, K. (1995). Pigeons show same-different conceptualization after training with complex visual stimuli. *Journal of Experimental Psychology: Animal Behavior Processes, 21*, 248-252.

Watanabe, S., Sakamoto, J., & Wakita, M. (1995). Pigeons' discrimination of painting by Monet and Picasso. *Journal of the Experimental Analysis of Behavior*, *63*, 165-174.

Williams, D. I. (1967). Discrimination learning in the pigeon in relation to the number of negative stimuli. *Animal Behaviour*, *15*, 79-81.

Wilson, B., Mackintosh, N. J., & Boakes, R. A. (1985). Transfer of relational rules in matching and oddity learning by pigeons and corvids. *The Quarterly Journal of Experimental Psychology*, *37,* 313-332.

Wodinsky, J. (1954). The solution of oddity, matching-from-sample and similarity and dissimilarity problems. (Unpublished doctoral dissertation). University of Texas.

Wodinsky, J., & Bitterman, M.E. (1953). The solution of oddity-problems by the rat. *The American Journal of Psychology*, *66*, 137-140.

Wu, W., Moreno, A. M., Tangen, J. M., & Reinhard, J. (2013). Honeybees can discriminate between Monet and Picasso paintings. *Journal of Comparative Physiology A, 199,* 45-55.

Wright, A. A., Cook, R. G., Rivera, J. J., Sands, S. F., & Delius, J. D. (1988). Concept learning by pigeons: Matching-to-sample with trial-unique video picture stimuli. *Learning & Behavior, 16,* 436-444.

Wright, A. A., Magnotti, J. F., Katz, J. S., Leonard, K., & Kelly, D. M. (2016). Concept learning set-size functions for Clark's nutcrackers. *Journal of the Experimental Analysis of Behavior, 105,* 76-84.

Young, M. E., & Wasserman, E. A., & Dalyrmple, R. M. (1997). Memory-based same-different conceptualization by pigeons. *Psychonomic Bulletin & Review*, *4*, 552-558.

Zentall, T. R., Galizio, M., & Critchfield, T. S. (2002). Categorization, concept learning, and behavior analysis: An introduction. *Journal of the Experimental Analysis of Behavior*, *78*, 237-248.

Zentall, T., & Hogan, D. (1974). Abstract concept learning in the pigeon. *Journal of Experimental Psychology*, *102*, 393-398.

Zentall, T. R., Hogan, D. E., Edwards, C. A., & Hearst, E. (1980). Oddity learning in the pigeon as a function of the number of incorrect alternatives. *Journal of Experimental Psychology: Animal Behavior Processes*, *6*, 278-299.

Zentall, T. R., Wasserman, E. A., Lazareva, O. F., Thompson, R. K., & Rattermann, M. J. (2008). Concept learning in animals. *Comparative Cognition & Behavior Reviews*, 13-45.

Zhang, S., Srinivasan, M. V., Zhu, H., & Wong, J. (2004). Grouping of visual objects by honeybees. *Journal of Experimental Biology*, *207*, 3289-3298.