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Honey Bees (*Apis mellifera*) and the Solution of Practical Problems

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Experiments on the use of honey bees (*Apis mellifera*) to solve practical problems are described. These problems include studies of alcoholism, influence of consumer products on Africanized honey bees, the effect of pesticides considered "harmless," and the detection of adulterated hive products. The results of these experiments indicate that the study of invertebrate learning conducted within a comparative-psychological paradigm can provide much useful data to the solution of practical problems of significance to psychologists.

One might ask how can the comparative study of honey bees (*Apis mellifera*) help solve practical problems of interest to humans and honey bees alike. The results from experiments on honey bee learning provide ample experimental and theoretical issues to occupy any student of behavior. In the course of our investigations on standard problems in the comparative analysis of learning (Abramson, 1997) we had the opportunity to use proboscis extension and the proboscis conditioning paradigm for the study of practical problems.

Invertebrates have long been used for the study of practical problems. Many of these problems focus on such issues as pollination, vector control, pest control, aquaculture, and culture methods and are more of interest perhaps to the farmer, the extension entomologist, and the physician than to the psychologist (i.e., Buchsbaum et al., 1987; Damron, 2000; Lutz et al., 1937/1959; Pham-Delègue, Jouanin, & Sandoz, 2002).

Of the more fascinating uses of invertebrates are their applications as weapons in the military, as aids in criminal investigations, and as teaching tools in the psychology classroom. Honey bee hives have been catapulted over ramparts during the middle ages, and the mosquito olfactory system tuned to "sniff-out" enemy troops, for example during the Vietnam war (Lubow, 1977). More recently, the honey bee olfactory system has been used to detect landmines (Revkin, 2002) and insects are now commonly used in forensic investigations (Byrd & Castner, 2000). Invertebrates are also finding their way into the psychology teaching laboratory as a practical alternative to traditional vertebrate animals when demonstrating

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principles of learning (Abramson, 1990; Abramson et al., 1999; Katz, 1978; Owren & Scheuneman, 1993). We believe that the study of invertebrates for the solution of practical problems of interest to psychologists is an emerging area of scientific inquiry.

To illustrate the variety of ways in which invertebrates can help problems of interest to psychologists this paper will focus on research conducted in our laboratory. We will discuss several lines of research using honey bees. These lines include the use of honey bees to detect adulterated hive products, their importance as a potential animal model in the study of alcoholism, the use of the proboscis conditioning technique to test artificial diets in endangered honey bee species, and the influence of insecticides on honey bee learning. We will begin our discussion with a brief overview of the relationship between the performance of honey bees and vertebrates and a description of the technique used in our studies.

In the past 20 years there has been an explosion of research conducted within a comparative psychological framework in which the performance of European honey bees is compared to the performance of vertebrates. Similarities include overshadowing, potentiation, and within-compound association in compound conditioning, dimensional shift in choice problems; the overlearning-extinction effect and its dependence on magnitude of reinforcement; successive negative contrast, latent inhibition, unconditioned stimulus pre-exposure effect, and signaled avoidance. Similarities between European honey bees and vertebrates are also found in response to visual illusions (Abramson et al., 1996; Bitterman, 1996; Kartzev, 1996; Menzel & Müller, 1996; Smith & Abramson, 2003). Interestingly, there also appears to be differences in the learning of Africanized and European honey bees (for a review, see Abramson & Aquino, 2002a).

Of the techniques used to study learning in honey bees we used two that are the most common (for a review of invertebrate learning techniques, see Abramson, 1994). In the first, free-flying foragers are trained to shuttle back and forth from the hive to the laboratory, where they take sucrose solution from targets distinguished by color, odor, or position (e.g., Turner, 1911). We also employed a second technique in which the proboscis extension reflex is classically conditioned in harnessed foragers. The reflex is most easily studied by confining bees in small metal tubes. Once harnessed, bees readily extend their mouthparts (proboscises) to feed on a sucrose solution after the solution has been briefly applied to the antennae, on which sucrose sensitive contact sensillae are found (Minnich, 1932). One or more forward pairings of an odor with sucrose feeding increases the frequency of background proboscis extensions to odor. The proboscis extension reflex technique was first described by Frings (1944) introduced into the psychological literature by Bitterman et al., (1983), and refined most recently by Abramson and Boyd (2001). Detailed descriptions of how to use the free-flying technique is available in Abramson (1990). For those readers interested in viewing scanning electron microscope photographs of the proboscis and antennae of Africanized and European honey bees we urge you to consult Abramson and Aquino (2002b) and Erickson, Carlson, and Garment (1986).

The proboscis extension reflex technique has many advantages over the free-flying method, such as the ability to more effectively control training variables known to influence learning. Conditioned stimulus and unconditioned stimulus durations, interstimulus interval, and intertrial interval, for example, are all more successfully controlled with harnessed bees than with bees trained to return to the laboratory on their own accord. The proboscis extension reflex technique is also better suited for quantitative physiological and biochemical analysis (Menzel et al., 1991).

Influence of So-Called "Harmless Pesticides" on European Honey Bees

Honey bees contribute substantially to the pollination of various wild plants and food crops. The annual value of agricultural crops benefiting from honey bee pollination is estimated at as much as $20 billion/year in the United States alone (Southwick & Southwick, 1992). Evidence exists that sublethal doses of pesticides may be decreasing the number of honey bee colonies available for pollination and reducing the honey bees' effectiveness as pollinators. Sublethal doses of deltamethrin, for example, disrupt the homing flight of honey bees (Vandame et al., 1995), while parathion disrupts the communication dance of foragers (Schricker & Stephan, 1970). In addition to the disruption of natural behavior, it is known that sublethal exposure to permethrin, coumaphos, and diazinon retards learning as measured by the classical conditioning of proboscis extension (Mamood & Waller, 1990; Taylor, Waller, & Crowder, 1987; Weick & Thorn, 2002).

Studying the influence of sublethal amounts of pesticides on honey bee behavior is important for the survival of honey bees, public policy issues, honey bee population regulation, environmental degradation, and the use of biological controls. What have been neglected in these studies are the effects of pesticides specifically known not to "harm" honey bees. These compounds may include some of the new generation pyrethroids, insect growth regulators, and fermentation by-products, all of which are currently used in formulation of new products. Many of these new products are considered by the Environmental Protection Agency, and other regulatory bodies, as user-friendly, target-specific and environmentally safe. However, little is known about their effects, if any, on honey bee behavior, particularly learned behavior. In order to use these chemicals effectively and without injuring these important pollinators it is important to know what effects these agrochemicals have on honey bee behavior.

In addition to providing data on the effect of "harmless" chemicals on honey bee behavior, our work takes on added significance when we consider that it is possible to seek "fast track" approval for exemption labeling. Many of the compounds we are studying fall under this category. We consider this a potentially dangerous precedent because the effects of these fast track materials on honey bee behavior are unknown at this time.

Our first experiments on the study of chemicals considered "not harmful" to honey bees was an investigation of dicofol (Kelthane®). Dicofol is a chlorinated hydrocarbon insecticide and a chemical analog of DDT. It is considered nontoxic to most insects and is used primarily as an acaricide. We used the proboscis conditioning paradigm as our bioassay. Each bee received 12 acquisition trials followed by 12 extinction trials in which the unconditioned stimulus was omitted. To control for pseudoconditioning unpaired animals received an equal number of conditioned stimulus/unconditioned stimulus presentations in a pseudorandom sequence. Much to our surprise we discovered that honey bees pretreated with dicofol exhibited significantly lower levels of learning than honey bees not pretreated (Stone, Abramson, & Price, 1997).

Figure 1 shows a comparison between treated and untreated animals during acquisition and extinction. The untreated group begins to respond to the conditioned stimulus by the second trial, reaching an 80% probability of response by the third pairing. In contrast, the treated group reached only a 60% probability of response. The difference observed during acquisition carried over into the extinction phase as well. The dicofol data indicated, to us at least, that behavior studies need to be carried out on other pesticides considered "not harmful" to honey bees.

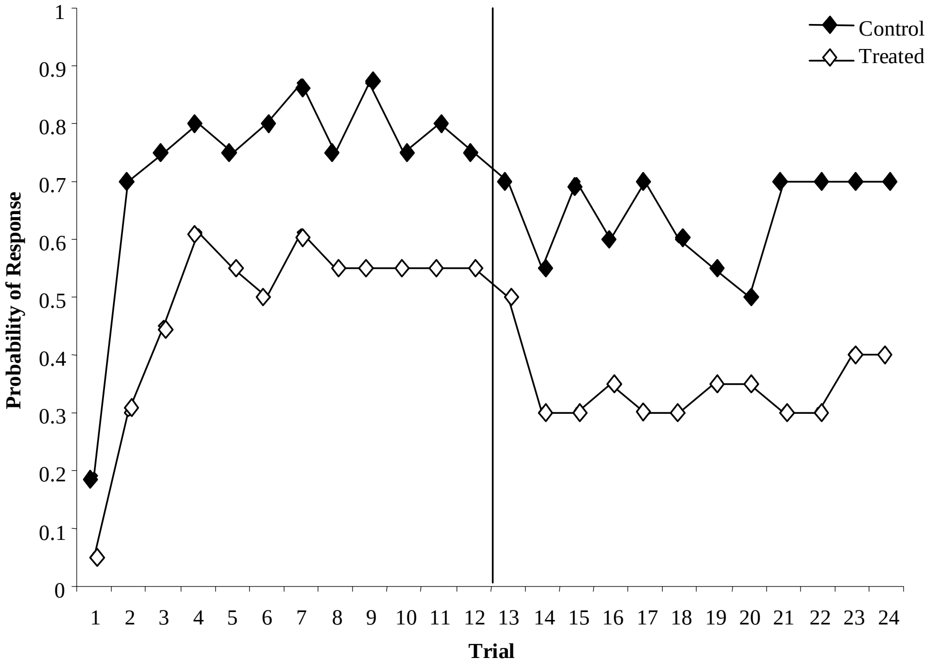

Figure 1. Performance of untreated and treated animals during acquisition and extinction of a conditioned proboscis extension response.

Presently we are engaged in a series of experiments investigating other agrochemicals including chinothionat (Eradex®), diflubenzuron (Dimlin®), propargite (Omite®) and sulfur. These studies are ongoing but it can be said that exposure to Dimlin® does significantly disrupt both simple and complex learning (manuscript in preparation). Again, such disruption in learning by exposure to "harmless" agrochemicals is an unanticipated result and has important implications to the agricultural industry.

Experiments on the Effect of Insecticides on Africanized Honey Bee Learning

The Africanized honey bee is important to the economy of Brazil in two main ways. Aside from the production of honey as a major agricultural product, bees serve as pollinators of the cotton crop as well as many others in the Brazilian economy.

Cotton (*Gossypium hirsutum*) is an important crop for the agrarian sector and development of the textile industry in Brazil. Brazil is a world leader in cotton production and is the major raw cotton producer in South America growing almost 50% of the continent's cotton (Cotton: World Statistics, 1997). Cotton production in Brazil was adversely affected soon after the appearance of the cotton boll weevil in 1983 (Sobrinho & Lukefahr, 1983) and has led, for example, to unemployment, depreciated land value, and the closing of cotton gins and oil mills (Ramalho & Santos, 1996). The major strategy to combat the boll weevil is the use of insecticides. The use of insecticides to control the boll weevil may have adverse effects on the honey bee population. Such effects, if present, may influence both honey and cotton production, as well as other crops. Our studies were designed to offer recommendations on what, insecticides, if any should be used.

The experiments in this series examine the effects of endosulfan, decis, baytroid, and sevin on the learning ability of AHBs. These insecticides were selected because they are recommended by the Brazilian government (Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA, and Centro Nacional de Pesquisa do Algodão - CNPA (EMBRAPA/CNPA) to combat the boll weevil (*Anthonomus grandis* Boheman) in northeast Brazil. Proboscis conditioning is a reliable and sensitive bioassay for determining the effects of toxicants on learning in European honey bees (e.g., Stone et al., 1997) and thus we had an interest in determining whether classical conditioning of proboscis extension can also be used as a bioassay in Africanized honey bees. This work is part of a larger project comparing the learning of Africanized and European honey bees (Abramson & Aquino, 2002a; Abramson et al., 1997a, 1997b; Abramson, Aquino, & Stone, 1999a).

The experiment under consideration differs from previous honey bee pesticide studies in four respects. First, we used insecticides recommended by government agencies (EMPRAPA/CNPA, Campina Grande, Paraíba State, Brazil). Our goal here was to determine whether any of the recommended insecticides interfere with learning in honey bees. Brazil has no formal mechanism for monitoring the effect of toxins on honey bee behavior. Gathering these data is important in the survival of honey bees in the northeast region of Brazil and for public policy issues such as insecticide use, honey bee population regulation, environmental degradation, and the use of biological controls.

Second, the effect of the insecticides was assessed on both acquisition and extinction trials. With the exception of the Stone et al. (1997) study, only acquisition measures are presented in assessment studies. A comprehensive analysis of the effect of an insecticide should, in our opinion, include both acquisition and extinction effects. An insecticide, for example, might exert its influence not on the acquisition of a learned response but on its persistence when the unconditioned stimulus is discontinued. Third, the insecticides are presented in a compound with the scent used as the conditioned stimulus. This manipulation was included to determine if any of the insecticides are repellent. If so, we would expect this to interfere with the development of a learned association. Fourth, the insecticides are presented as a compound with the sucrose solution used as the unconditioned stimulus. This is included to determine whether exposure to the insecticide during the course of learning influences the acquisition and/or stability of the learned response.

Results of our research (Abramson et al., 1999b) showed that: (1) bees readily consume each of the pesticides when placed in a sucrose solution; (2) the odors of the pesticides are not repellent to bees and such odors can serve as conditioned stimuli; (3) learning occurs to various degrees when the insecticides are combined with the sucrose solution and used as an unconditioned stimulus; and (4) feeding the insecticides to the bees one hour prior to conditioning leads to differing mortality.

Figure 2 shows the results of paired animals where the unconditioned stimulus consists of a compound of sucrose and insecticide. Bees receiving a US of decis and sucrose exhibit acquisition and extinction responses indistinguishable from animals given sucrose alone. Animals given a compound US of baytroid or sevin displayed only several conditioned responses during the first five trials. As conditioning progressed, no learning is evident. Because no bees learned in either of these two groups, extinction trials were not run. In contrast, bees receiving a US compound of endosulfan and sucrose acquired a conditioned response during the first eight trials similar to decis and the untreated controls. As training continues, however, the number of conditioned responses steadily declines. This decline continued during extinction trials.

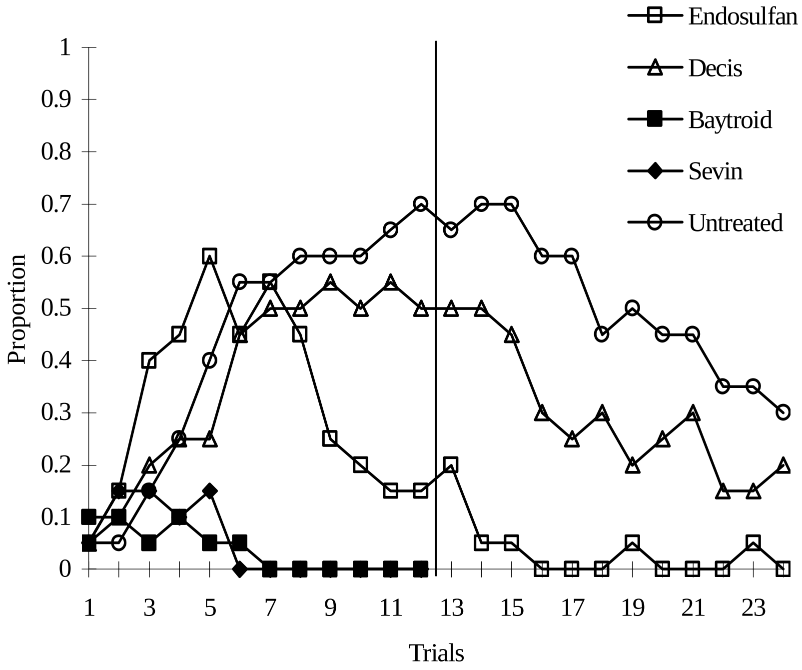

Figure 2. Mean proportion of bees responding to the CS over the course of 12 acquisition and 12 extinction trials in which the US was a compound of sucrose and one of four insecticides. The switch from acquisition to extinction occurred on Trial 13.

Study of Preferences of Africanized Honey Bees for Consumer Products

Studying the attractiveness of consumer products in honey bees is important from both a medical and psychological perspective. Approximately 0.4 percent of people in the United States have severe, or even fatal allergic reactions to bee stings (Reisman, 1992). Even among some nonallergic individuals, the thought of a bee sting is enough to produce a fear of bees, technically known in the psychological literature as Melissophobia (Halonen & Santrock, 1996; Matchett & Davey, 1991). Of the 12.5% of Americans who are stricken with some type of phobia (Regier et al., 1988), 5-15% of these individuals suffer from animal phobias - including a fear of insects (Zimbardo, 1992). In 1995 alone, 17,874 individuals required some type of medical treatment following attacks by bees, wasps, and/or hornets. This number increases to 72,582 when other invertebrates are considered (Litovitz et al., 1996). Anecdotal evidence suggests that the threat of a bee sting is reduced by wearing light colors, not wearing perfume, cologne, cosmetics, or hair spray, and avoiding quick movements when around bees (Free, 1961).

In addition to health care issues, studying the preferences of bees to consumer products such as soft drinks is interesting from a methodological perspective. The vast majority of what is known about proboscis conditioning in honey bees comes only from using sucrose solution as an unconditioned stimulus (Abramson, 1994; for an exception see Smith, Abramson, & Tobin, 1991). We have been unable to find any proboscis conditioning study in the literature that involves a naturally occurring unconditioned stimulus. The absence of research with naturally occurring unconditioned stimulus leaves open the possibility, however unlikely, that proboscis conditioning is only a laboratory phenomenon that depends heavily upon high concentrations of sucrose for successful conditioning.

Moreover, no studies have been reported investigating whether Africanized honey bees prefer some perfumes or cologne or demonstrate preferences to soft drinks, cosmetics, hair spray or other consumer products. Gathering these data provide health care professionals specializing in insect phobias and/or allergic reactions to insect venom empirically based research to identify and recommend perfumes, colognes, and other consumer products that are not attractive to bees.

As an initial step, the reaction of Africanized bees to perfumes and soft drinks was investigated (Abramson et al., 1997c). These stimuli were selected because they represent common consumer products with which bees come in contact. In addition, perfumes and soft drinks can be accommodated easily into the existing proboscis conditioning methodology as conditioned and unconditioned stimuli, respectively.

In the first experiment the ability of bees to discriminate two perfumes was assessed (Realm for Men® and Realm for Women®). The results indicated that these perfumes could function as conditioned stimuli in a discrimination paradigm where one odor (either Realm for Men® and Realm for Women®) was paired with a sucrose US and the other odor was not (the odors were counterbalanced). Each odor was presented 12 times for a total of 24 trials. Figure 3 shows the mean proportion of bees responding to both odors over the course of 24 training trials. The results clearly show that Africanized bees are able to use perfumes as conditioned stimuli.

In the second experiment harnessed bees were divided into individual groups and fed either Diet Pepsi, Spring water (control), Diet Guaraná (a popular Brazilian soft drink), Diet Coca-Cola, Coca-Cola, Sprite, Pepsi, Guaraná, Sukita Orange, Fanta Orange, Fanta Grape, or Sucrose (control). The dependent variable was the amount of time the proboscis was in contact with a strip of filter paper saturated with the substance. The results indicated that Africanized honey bees have soft drink preferences. There was little or no contact with the diet soft drinks and spring water, 45-65 s of contact to Coca-Cola, Sprite, Pepsi, Guaraná and Sukita Orange, 75-140 s of contact with Fanta Orange, Fanta Grape, and sucrose (sucrose elicited the most contact of the substances tested). When a sample of soft drinks were used as unconditioned stimuli with the odor of citral and geraniol as conditioned stimuli, conditioning was best to sucrose, Guaraná, and Fanta Orange. Little conditioning was observed with Pepsi and no conditioning was observed with Diet Pepsi. The results also demonstrated that perfumes could serve as cues in the same way as naturally occurring floral scents. These data have implications for individuals who suffer allergic reactions to bee stings and those engaged in managing public facilities such as schools, restaurants, and recycling centers.

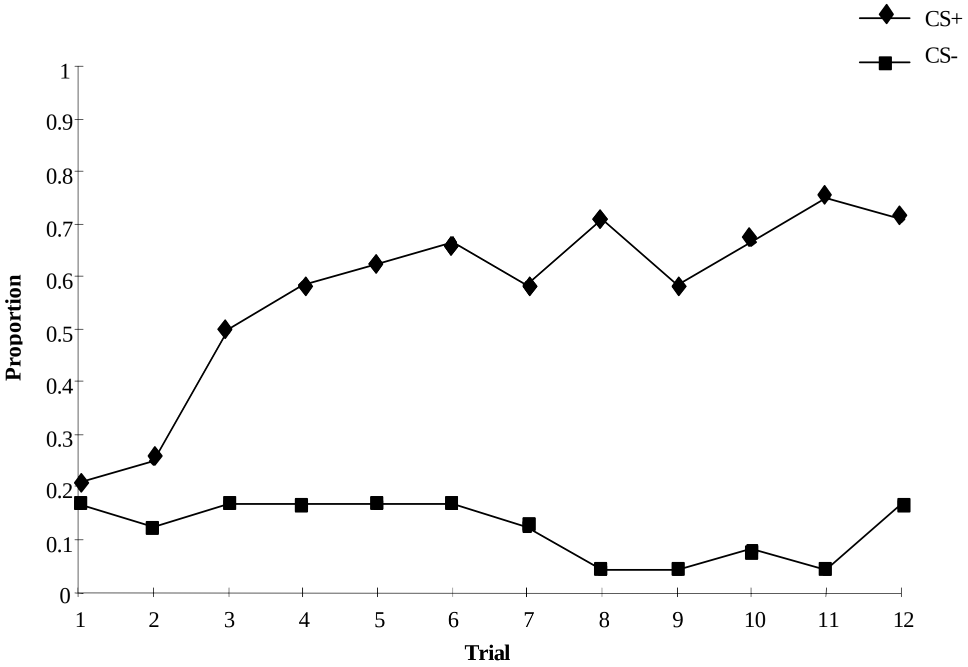

Figure 3. Mean proportion of bees responding to an odor followed by a sucrose feeding (CS+) and an odor not followed by a sucrose feeding (CS-) during a Pavlovian discrimination task.

One of the more interesting results from the present series of experiments is that, with the exception of diet soft drinks, the amount of contact time was not related to the efficacy of conditioning. Animals, for example, trained with a sucrose unconditioned stimulus performed as well as animals trained with a Fanta Orange unconditioned stimulus. Why this is so remains unclear although several possibilities can be suggested. The most straightforward explanation is that following food deprivation bees will associate a CS with any appetitive unconditioned stimulus. A second possibility is that soft drinks contain natural flavors and/or odors that the Africanized honey bees in our sample have experienced previously. Our colonies were located relatively near citrus groves and the vicinity of the hives was heavily populated with various flower types. An unconditioned stimulus containing familiar natural flavors and odors might be perceived by the Africanized honey bee as a potent unconditioned stimulus despite the low sugar content relative to the sweet sucrose solution.

A third possibility to explain the failure of Africanized honey bees to relate contact time with the efficacy of conditioning is that the effect might only be revealed when bees stop receiving the unconditioned stimulus. Extinction is one measure of persistence in conditioning experiments (Abramson, 1994) and previous work with European honey bees indicates that some conditioning effects are expressed only in extinction (Buckbee & Abramson, 1997). The role of extinction was not tested in these experiments because we focused on what attracts Africanized honey bees to a consumer. Once a consumer is stung the bee dies and extinction does not have the opportunity to develop.

Our results lead to several recommendations. Health care professionals counseling clients suffering from Melissophobia and/or allergic reactions to bee stings should recommend that the client's perfume and/or cologne be tested for its attractiveness to bees. If the scent is found to be attractive, the client may decide to use a scent that is not attractive to bees such as a spicy floral or its designer impostor. Clients who are afraid to wear perfume/cologne and cosmetics because it may attract bees, wasps, hornets, and fire ants can be given a list of scents that are "attraction safe." University laboratories specializing in insect behavior can collaborate with allergy clinics to create such an empirically based list of perfumes, cosmetics, and other consumer products that are Hymenoptera "safe."

We also recommend that bee traps be located near recycling centers and other public areas such as elementary schools to monitor the amount of honey bee activity. These traps can be maintained by local beekeeper associations and/or extension entomologists and provide an excellent training opportunity for students. Establishing these monitoring stations also provides a fine opportunity to educate the public on the importance of honey bees and other foraging insects.

Our results demonstrating the ability of bees to feed on soft drinks and to associate neutral stimuli with a soft drink feeding strongly suggest that a greater effort be made to remove appetitive unconditioned stimuli from these containers either by regularly washing out the containers or designing "bee proof" containers. Informal observation by the authors in the surrounding Stillwater, Oklahoma area suggested that bees regularly visit trash sites and recycling centers. We were especially concerned to see the large numbers of bees visiting garbage sites located near schools. Upon closer inspection it appeared that bees were attracted to these sites because of the residual sugar content of discarded soft drinks. A subsequent survey of 117 people revealed that only 44% of recyclers removed residual juice and other soft drinks prior to placing bottles within the receptacles (Aquino et al., 1997). We would predict that bees will learn to associate antecedent stimuli with the consumption of a soft drink in much the same way that a bee associates the odor of a flower with a nectar feeding. The negative publicity generated by a honey bee or "killer bee" attack at a recycling center, for instance, would certainly adversely influence the recycling effort.

Proboscis Conditioning as a Bioassay to Test Artificial Diets for Endangered Honey Bees

The success of the proboscis extension technique to measure learning in Africanized bees suggested that we extend the technique to the study of the stingless bee *Melipona*. The ability to use this technique on the stingless honey bee is important not only for what it may reveal about the learning process in this interesting honey bee but takes on an added significance because of the dramatic decline in the numbers of *Melipona* in Brazil due to the destruction of their natural habitats and food sources and destruction of colonies by humans raiding the colonies for honey (Lorenzon, 1996). Because the honey is produced in such small quantities (about 1 liter per hive each year) it is extremely expensive at almost $100 a liter and therefore profitable to sell.

The Uruçu bee is slightly larger than the Africanized bee, has an orange hairy thorax and orange tint to the antennae. In addition, it produces a very thin honey the color of which range from light green to dark yellow. The hives of Uruçu are smaller than *Apis* and consist of only a few hundred bees. They store their honey in honey pots and not in the familiar hexagonal cells of *Apis*. Because Uruçu is a member of the family of stingless bees their defensive mechanisms are restricted to strong mandibles, small hive entrance, sticky entrance tunnels and, honey, wax, and propolis that smell like "unwashed socks."

We designed our original experiments with the expectation that the proboscis conditioning technique could be used to study learning as it has in the Africanized and European honey bee. Much to our surprise no learning effects were discovered and no pseudo-conditioning effects were seen. There was a small central excitatory state effect. During the course of these experiments various conditioned stimuli were used including the usual floral scents, beeswax, and water stimulation. Our failure suggests that we must modify the procedure and/or change the training variables.

Central excitatory state (CES) refers to the temporary state of "excitement" generated in the nervous system of invertebrates following an unconditioned stimulus such as that provided by sucrose or honey. CES may serve as the basis of pseudoconditioning effects observed in invertebrates (Terry & Hirsch, 1997). CES was induced in both one-day old Africanized honey bees and in adult Uruçu by first stimulating them with sucrose and testing their responsiveness to water stimulation 3, 7, 15, 30, 60 and 120 seconds after sucrose stimulation.

Figure 4 shows the results of the CES experiment. In contrast to the negative results of the Pavlovian and pseudoconditioning experiments, there is a strong CES effect in one-day old Africanized bees. The CES effect, however, is weak in adult Uruçu. Over 70% of the Africanized honey bees responded to water stimulation at each of the intervals tested. About 50% of the Uruçu sample responded 3 seconds after sucrose stimulation and the number of bees responding rapidly declines as the intervals are extended.

Although no conditioning was evident, the proboscis conditioning situation could be used to rapidly test various artificial diets (Abramson, Aquino, & Stone, 1999a). In attempts to find artificial diets for *Melipona* the standard technique is to test the solution under field conditions in which foragers are observed to drink the solution and to form "honey pots." We believe that the proboscis extension technique is a more rapid method for testing suitable artificial diets. To examine this idea we used the proboscis extension technique to see how readily animals will drink two solutions known to be effective in field studies (Aidar, 1996a, 1996b; Alves, 1996). The results indicated that the harnessed bees readily consumed the solution in a matter of seconds. This is in contrast to the usual week or so needed for field-testing.

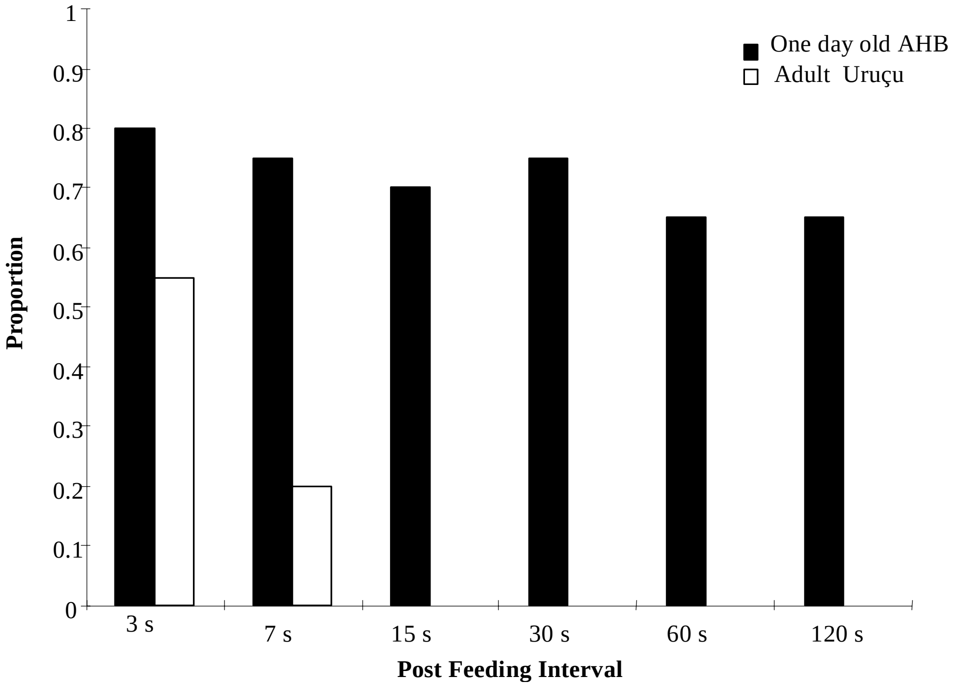

Figure 4. Mean proportion of one-day old Africanized honey bees (dark bars) and adult Uruçu honey bees (open bars) responding to water stimulation at each of the 6 post stimulation intervals.

Proboscis Conditioning as a Bioassay to Test Contaminated Hive Products

Beeswax is one of the most valuable hive products on the world market. It is widely used in both industry and medicine and can be found in products as diverse as cosmetics and shoe polish (Anam & Gathuru, 1985; Root, 1951). Beeswax can cost at least three times as much as vegetable waxes and about eight times as much as petroleum wax (*Chemical Marketing Reporter* 1979). The cost of beeswax is approximately $7.00 a kilogram in the U.S. (*American Bee Journal* 1997). In order to compete effectively in the world market for beeswax, the wax should be of the highest quality. As Anam and Gathuru (1985) note, quality can be reduced by the presence of contaminants, or by the addition of additives such as paraffin wax.

Both chemical and physical methods are available for detecting adulterated beeswax. Among the chemical methods for detecting adulteration are ester number, acid values, ratio number (ester number divided by the acid number), saponification cloud point, and chromatographic analysis are the most common. Of the physical methods, the most common include color, aroma, and melting point (Coggshall & Morse, 1984). There are no published records of experiments that attempt to use the honey bee in vivo in bioassays to detect adulterated beeswax.

The purpose of this experiment is to determine if the honey bee proboscis extension reflex can be used as a bioassay to detect adulterated beeswax. The idea for the bioassay came from an experiment investigating classical conditioning proboscis extension in Africanized honey bees (Abramson et al. 1997a). In that experiment it was noticed that the odor of beeswax comb foundation can serve as both a conditioned stimulus and unconditioned stimulus in classical conditioning. Because honey bees can be conditioned using 100% beeswax, the question naturally arises whether adulterated beeswax can also elicit a proboscis extension reflex.

The model adulterant chosen in the present study is carnauba wax. The Carnauba palm tree, *Copernicia cerifera* Arruda Camara, is native to northeastern Brazil. This wax was selected for our initial test of the proboscis extension reflex bioassay because, although it is a plant wax, carnauba can be added to beeswax in small amounts without being noticed by physical tests such as odor or color (Aquino, unpublished observations). Since the prices of both carnauba wax and beeswax fluctuate, there is the possibility that beeswax might be adulterated with carnauba when the price of carnauba is less then beeswax.

It is common practice to combine beeswax with carnauba for industrial purposes. For example, a carnauba wax and beeswax combination has been reported by Raghuvanshi et al. (1992) to be a suitable coating material for controlling in vitro release of the drug salbutanol sulphate. Carnauba wax, combined with beeswax, also has been reported to be safe for use in beauty aids such as cosmetics (CIREP, 1984). Because beeswax is an important commodity among Brazilian beekeepers, a quick and accurate method must be developed to insure the purity of beeswax before it is permitted to be sold on the market.

Subjects were exposed to either: 100% beeswax (honeycomb) (e.g., no carnauba wax), 100% beeswax (melted) (e.g., as commercial beeswax cake), 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% beeswax/carnauba mixtures, 0% beeswax (i.e., 100% carnauba wax), or unscented air. Maximum responding was observed in bees exposed to the scent of honey comb or melted beeswax cake. The addition of as little as 10% carnauba wax was readily detected and resulted in reduced proboscis extensions. Few proboscis extensions occurred to bees exposed to unscented air or 100% carnauba wax. The results indicate that the proboscis extension reflex can be used as a rapid, inexpensive, and reliable bioassay for the detection of adulterated beeswax. This bioassay is useful in developing countries where chemical and physical methods are unavailable for detecting adulterated beeswax and can serve as an initial component in a comprehensive program of adulteration detection.

Although our results illustrate the efficacy of the technique, we do not propose that the bioassay replace the quantitative methods now available to detect adulterated wax. Rather, we view the bioassay as an initial component in a comprehensive program of adulteration detection. For instance, the bioassay can be used initially to screen for potential adulterated wax. If the wax is suspect, quantitative testing is indicated. We believe that our bioassay will be particularly useful for developing countries that find the cost associated with quantitative methods prohibitive (Aquino, Abramson, & Payton, 1999).

The Honey Bee as an Animal Model for Alcoholism

The purpose of this line of research was to test the feasibility of creating an animal model of ethanol consumption using social insects. Honey bees were selected as the model social insect because much is known about their natural history, physiology, genetics, and behavior. They are also inexpensive to procure and maintain. Of special interest is their use of communication and social organization. *Drosophila melanogaster* is another insect used in ethanol studies. Mutations sensitive to ethanol-induced loss of postural control have been described (Moore et al., 1998). The sea hare *Aplysia californica* has also been used in ethanol research. Traynor et al. (1979) examined both tolerance and neurophysiological effects of ethanol exposure at the cellular level.

Using both between and within experimental designs, we conducted studies with harnessed foragers to determine if honey bees would consume ethanol mixed with sucrose (and in some cases water). Shuttle box and running wheel studies were conducted to examine the effect of ethanol on locomotion. The effect of ethanol on stinging behavior in harnessed foragers was investigated. The effect of ethanol on Pavlovian conditioning of proboscis extension was also investigated. Finally, in a self-administration study, foraging honey bees were trained to fly to an artificial flower containing ethanol.

Our results indicated that harnessed honey bees readily consume 1%, 5%, 10%, and 20% ethanol solutions; 95% ethanol will also be consumed as long as the antennae does not make contact with the solution; with the exception of 95% ethanol, consumption as measured by contact time, or amount consumed does not differ in animals that consume 1%, 5%, 10%, and 20% ethanol solutions; exposure to a lesser (or greater) concentration of ethanol does not influence consumption of a greater (or lesser) concentration; consumption of 10% and 20% ethanol solutions decrease locomotion when tested in both a shuttle box and running wheel situation; consumption of 1%, 5%, 10%, and 20% ethanol does not influence stinging behavior in harnessed foragers; ethanol solutions greater than 5% significantly impair Pavlovian conditioning of proboscis extension; and free-flying honey bee foragers will readily drink from an artificial flower containing 5% ethanol. The experiments on consumption, locomotion, and learning suggest that exposure to ethanol influence honey bee behavior similar to that observed in analogous vertebrate experiments.

Figure 5 shows the results of a proboscis conditioning experiment in which bees receive an ethanol solution as the unconditioned stimulus. Acquisition is rapid when sucrose only or a 1% solution serves as the unconditioned stimulus. In contrast bees receiving an unconditioned stimulus of 5%, 10% and 20% ethanol never acquired the proboscis extension response. Our results clearly demonstrate that as the ethanol concentration increases performance on a learning task decreases.

The development of ethanol model using honey bees, in our view, opens up some unique research opportunities that are not presently available in many vertebrate and invertebrate models. Honey bees offer a rich assortment of behavior including “language,” social interactions, non-associative learning (habituation and sensitization), associative learning (alpha conditioning, classical conditioning, and instrumental conditioning), and age related changes in performance in which to investigate the influence of ethanol. Of particular interest is the ability to study the effects of ethanol on the developing larvae. Ethanol, for instance, can be directly injected into a cell and with the aid of a video camera the influence of ethanol on development can be readily assessed. When the adult emerges from the cell it can be captured and given a battery of behavioral tests such as those described in this paper. Moreover, an observation hive can be used to study the effect of ethanol on social behavior and "language" in a natural situation (Abramson et al., 2000).

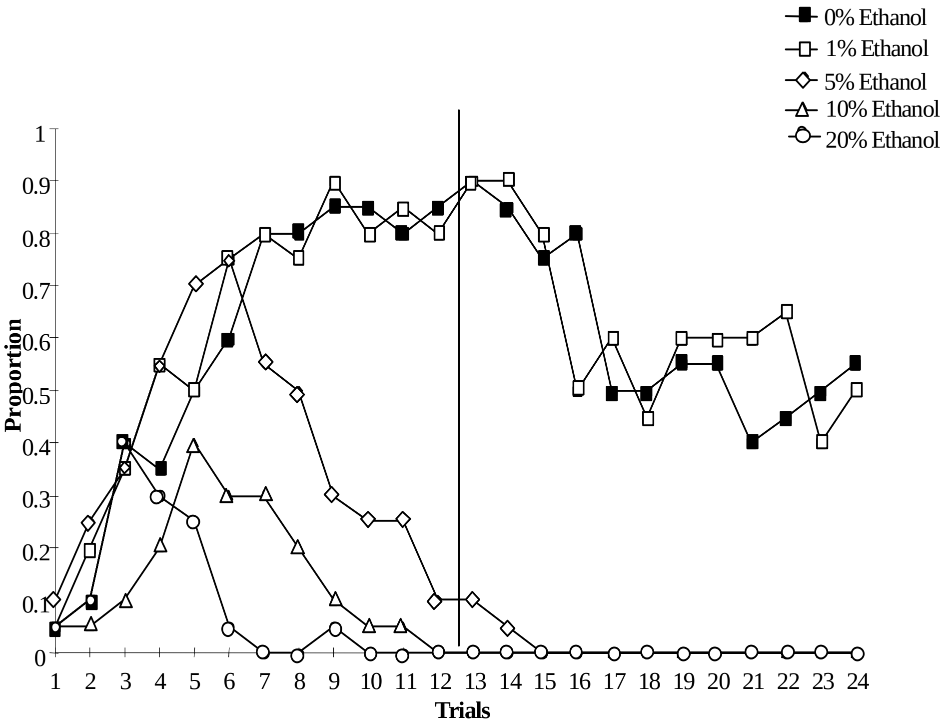

Figure 5. Mean proportion of bees responding to the solutions over the course of 12 acquisition and 12 extinction trials. The switch from acquisition to extinction occurred on Trial 13.

We now turn our attention to the effect of Antabuse® (disulfiram) on ethanol consumption in harnessed honey bees (Abramson et al., 2003). The goal of these experiments was to determine if honey bees can be used to test drugs that suppress drinking in humans. Antabuse® was selected as our target drug because of its wide use and record of at least partial success in ethanol consumption (Brewer, 1993; Garbutt et al., 1999). Antabuse® works by establishing a learned taste aversion between the consumption of ethanol and illness. Honey bees do not posses a liver but have an analogous structure known as fat bodies located predominantly in the abdomen. Fat bodies are a major center of metabolic activity and have direct contact with the hemolymph.

In the first series of experiments a factorial design was used with 5 levels of ethanol concentration (0%, 1%, 5%, 10%, 20%), 4 doses of Antabuse® (0, 37µg/g, 3.7 µg/g, .37µg/g), and 2 testing intervals (1 min, 10 min). Animals were fed a single 1µl dose of Antabuse® and contact time with an ethanol solution measured. A second series of experiments investigated the influence of Antabuse® on the formation of Pavlovian conditioning of the proboscis extension reflex. A factorial design was used with two levels of training (paired, unpaired), three levels of ethanol (0%, 1%, 5%), and 2 levels of pretreatment (distilled water, 3.7 µg/g). The results of the consumption experiments indicate that pretreatment with Antabuse® reduces ethanol intake although there was substantial variability. The findings of the Pavlovian experiments suggest that pretreatment with Antabuse® significantly reduced responding to a CS signaling the availability of ethanol. Figure 6 shows the mean proportion of proboscis extension to the CS for all paired groups. The bees show an initial acquisition phase but those given Antabuse® begin to respond less over the 12 training trials suggesting the operation of a learned taste aversion.

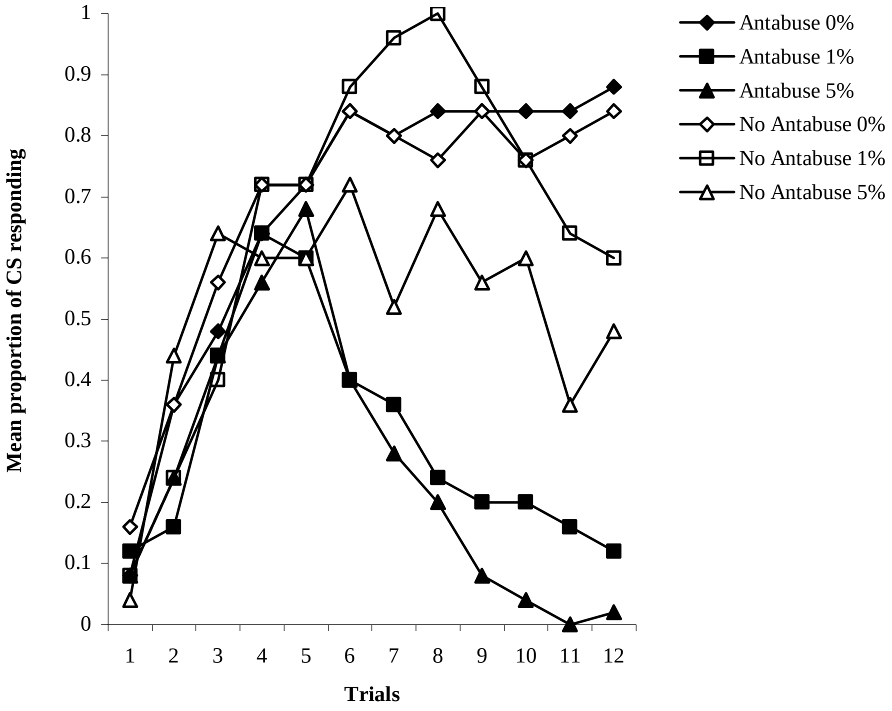

Figure 6. CS responding over the course of 12 acquisition trials in animals pretreated with Antabuse® and those pretreated with water only.

When considering the results of the consumption studies it is important to note that the bees are highly motivated to drink. They were food deprived for 24 hours and their antennae contain sucrose sensitive contact sensillae (Minnich, 1932). Highly motivated behavior and behavior under the control of reflexes are more difficult to modify than other forms of behavior (Abramson & Satterfield, 1999; Smith et al., 1991). Antabuse® is not effective for individuals that are highly motivated to drink (Brewer, 1993). One of the advantages of the honey bee is that motivation to drink is easily manipulated by prefeeding sucrose prior to any experimental manipulation.

Second, the usual Antabuse® regime for humans is to take daily doses over an extended period of time accompanied by therapy sessions (Brewer, 1993). In our experiments only a single dose was administered. This was done because little attention has been given to the development of a technique to keep harnessed bees experimentally viable for more then 30 hours. The fact that we obtain some effects after only a single dose and in highly motivated subjects suggests that more profound effects would be exhibited following standard administration protocols. Moreover, while it is ridiculous to administer "psychotherapy" to a honey bee, the ability to tease apart "cognitive processes" associated with vertebrate performance from the effect of the drug per se is not and is unique to invertebrate preparations.

The consumption and Pavlovian experiments provide further evidence that the honey bee is a potentially useful animal for ethanol studies. The purpose of these experiments was not to investigate Antabuse® per se but to determine if honey bees can be used to test pharmacological approaches to the treatment of alcoholism. In the case of Antabuse® the answer appears to be yes.

We do not suggest that vertebrates be replaced with honey bees. The usefulness of honey bees may be as a part of an integrated program of animal testing in which both invertebrates and vertebrates are used. Honey bees have much to recommend them for pharmacological studies including the ability to drink large quantities of ethanol, a social structure and language. Our present results suggest that honey bees can be used as a bioassay to test the efficacy of drugs used to reduce the consumption of ethanol.

Conclusions

The purpose of this article was to summarize some of our research on practical problems using the honey bee as a model organism. The study of "applied comparative psychology" might lack the glamour of theoretically driven research but, in our view, is equally important, and such practical research could in time converge with learning theory. The methods used to study learning in honey bees can be used to examine problems of alcoholism in humans, assist in the development of artificial diets for endangered animals, detect adulterated hive products, and study the impact of insecticides on non-target organisms. In regards to our experiments on practical issues, we believe that our insecticide work and the way we use the proboscis conditioning procedure as a bioassay to detect adulterated hive products and consumer products will serve as a model for similar studies.

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