KIN RECOGNITION BY ODOR DISCRIMINATION IN DWARF HAMSTERS (*Phodopus campbelli*) USING AN HABITUATION PARADIGM

by

JESSICA MAIE GODIN

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B.A., University of Southern Maine, 2003

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Major Professor
Dr. Jerome Frieman

Abstract

Male and female *Phodopus campbelli* were investigated for kin recognition abilities, assessed by kin discrimination of bedding, urine, fecal, or midventral glandular secretion odors. Using a habituation-discrimination paradigm, subjects were presented either the odors of a pair of their own brothers (C_1) or a pair of brothers that were unrelated to them (C_2). All subjects were then presented with a final test discrimination odor from a male donor unrelated to themselves or the first donors. All subjects differentiated between the bedding and glandular odors of two unrelated males, regardless of condition assignment. Subjects did not statistically differentiate between the odors of two brothers. These results suggest *Phodopus campbelli* are capable of recognizing kin based on bedding and midventral gland odors.

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In nature it may be in an animal’s best interest to determine the genetic similarity of conspecifics for reproduction and sociability. One proposed method by which individuals determine genetic similarity is kin recognition. Kin recognition is a cognitive process by which individuals are able to identify a conspecific as kin or non-kin, or to ascertain the degree of relation of that individual to themselves (Hepper, 1991; Tang-Martinez, 2001). Kin recognition is observed as a differential response, whether behavioral or physiological, toward conspecifics that correlates with genetic relatedness (Gamboa, Reeve, & Holmes, 1991).

Kin Recognition

In kin recognition a stimulus (better known as a signature) is used as a cue for recognition of an individual. Then, this signature is compared against acquired templates of known kin to assess kin recognition. Signatures of more closely related individuals will be more similar compared to those of less closely related individuals (Halpin, 1991). To be effective, then, signatures need to communicate unambiguous information about owner identity. A signature must represent a fixed and unique characteristic of the individual that possesses it (Hurst, Thom, Nevison, Humphries, & Beynon, 2005).

The strongest and most supported evolutionarily advantage of kin recognition is inbreeding avoidance (Shields, 1993). Inbreeding introduces a number of negative consequences into a population. Increased homozygosity resulting from inbreeding will result in an increase in the number deleterious alleles expressed within a population above chance levels (Werren, 1993). Inbreeding also can lead to decreased fecundity (capacity to produce surviving offspring; Riechert & Roeloffs, 1993) and a loss of ability to adapt to sudden environmental changes (Rowley, Russel, & Brooker, 1993).

Inbreeding does also, however, provide benefits that could be selectively advantageous. Genetic adaptation to local environmental conditions, coadapted gene complex maintenance, and reduced cost of non-dispersal are all benefits associated with inbreeding for certain populations (Waldman, 1991; Waldman & McKinnon, 1993).

Like inbreeding, its converse, outbreeding can be both advantageous as well as disadvantageous. Most researchers agree that an optimal balance must be achieved to maximize fitness (Barnard & Aldhous, 1991; Rowley, Russell, & Brooker, 1993; Waldman, 1991). Kin recognition could aptly be the tool for assessing the kinship of a potential mate to make this balance possible.

Kin recognition has been suggested to provide other benefits as well. In terms of parental care, it may reduce cannibalism, lessen fungal infestation susceptibility, or promote normal hatching and development. In terms of fitness, kin recognition might facilitate nepotism (favoritism shown to kin; Holmes & Sherman, 1982), cooperation, and altruism (Agrawal, 2001; Hamilton, 1964; Holmes & Sherman, 1982, 1983; Waldman, 1991).

Sherman and Holmes (1983; see also Hepper, 1991) review four basic mechanisms by which individuals recognize kinship signatures and thereby carry out kin recognition. The first mechanism is recognition by spatial location. This type of recognition associates certain territories or landmarks with kinship. Spatial proximity of conspecifics within this territory is the cue by which kinship is assessed (Tang-Martinez, 2001). Some species of bird provide good examples. Birds that utilize this mechanism accept presence in the home nest as viable evidence that chicks located there are their own offspring (Beecher, 1991). This type of recognition would help explain why certain bird species ‘allow’ brood parasitism. This mechanism is most suitable for highly philopatric animals (animals that remain at the natal site after sexual maturation; Drickamer, Vessey, & Jakob, 2002), whereby most individuals encountered within a natal area have a high probability of being kin. However, this mechanism also allows for greater error in accepting non-kin as kin as any individual within the correct location will be assumed to be kin (Tang-Martinez, 2001).

Another mechanism is recognition genes or recognition alleles (Tang-Martinez, 2001), also known as the green beard effect (Drickamer, Vessey, & Jakob, 2002). This type of recognition, though currently lacking substantial evidence (Tang-Martinez, 2001), suggests that kinship information is encoded into an individual’s genes and therefore requires no experience or learning to assess kinship. With this mechanism the same genes that code for the expression of a phenotypic label will also code for recognition of this label in others thereby causing preferential behavior towards those individuals that express this label (Tang-Martinez, 2001). Thus, this model relies on phenotypic assessment without decisive phenotypic matching (Holmes & Sherman, 1983).

The two mechanisms that have received copious attention are association (or familiarity) and phenotype matching. Although treated as separate mechanisms, many researchers agree that both often coexist (e.g., Heth, Todrank, & Johnston, 1998; Holmes & Sherman, 1982; Porter, 1988; Tang-Martinez, 2001). Both mechanisms require learning and early experience. The main differences between these two mechanisms are how and what cues are learned during early experiences with kin and how this early experience is utilized in future encounters with conspecifics.

Kin recognition by association, also called prior association, entails that cues are learned during a sensitive period of development from individuals that are most commonly encountered (Mateo, 2002). The cues learned during direct social contact are those that are individually distinctive cues of kin. These cues are used later in life to assess kinship. Individuals encountered later possessing these familiar cues are accepted as kin (Tang-Martinez, 2001). Cues can be acquired from the environment or be genetically influenced (Halpin, 1991). With this mechanism, if a conspecific does not possess the exact cues encountered and recognized during the sensitive period, it will not be recognized as kin when encountered in the future (Tang-Martinez, 2001).

Kin recognition by phenotype matching entails that cues are learned based on a referent's (whether self-referential or one of a known genetic relative) phenotypic information and are used to create a template. Holmes and Sherman (1983) point out that information from a non-self referent is learned through association. This template, based on the referent's expressed genetic information, is then used to compare and assess a new consepcific's degree of genetic relatedness. Individuals more similar in phenotype (i.e., more similar to the template) are considered more closely related. The degree of phenotype similarity is influenced by the number of genes that are shared between two individuals. The greater the number of shared copies of genes, the more related the individuals are proportionally; therefore, the more similar the phenotypes, the more similar the relatedness (Bee, in press). With this mechanism, individuals are able to recognize kin that have not been associated with before and judge the level of relation by the degree to which phenotypes are similar (Tang-Martinez, 2001). The following experiment focuses on phenotype matching as the most likely mechanism for kin recognition.

Recognition by any mechanism requires four components. First a signature needs to be identified by a receiver. Then the receiver must have the sensory capability to perceive the signature. Once perceived, the receiver must have the cognitive ability to reach a decision based on this signature. Finally, the receiver must possess the physical ability to respond actively to the signature based on that decision (Beecher, 1991). The active response is the only quantifiable element from which kin recognition can be inferred. One must, however, bear in mind that an absence of action does not necessarily infer an absence of the other three components. Motivational as well as environmental factors might change the action component in a different context (Barnard & Aldhous, 1991).

Kin recognition is inferred, experimentally, through a discrimination of kin (Hepper, 1991). This kin discrimination refers to differences in behavioral (or physiological) responses by an individual toward kin as compared to non-kin. This difference in response is based on conspecific cues that correlate with kinship (Tang-Martinez, 2001). Kinship can be conveyed by a number of cues including behavioral tendencies, vocalizations, chemical secretions, and visual displays (Waldman, 1991). The present experiment focused on the recognition of kin signatures based on discrimination of chemosensory signals, specifically smell.

Kinship discrimination has been noted in most mammals (Dewsbury, 1988; Halpin, 1986; Sánchez-Andrade, James, & Kendrick, 2005). Research has also shown that many rodents can make kinship discriminations on the basis of odor. Some examples are Ansell's mole rats (*Crypmys anselli*; Heth, Todrank, Begall, Wegner, & Burda, 2004), Arctic ground squirrels (*Spermophilus parryii*) and Belding's ground squirrels (*Spermophilus beldingi*; Holmes & Sherman, 1982), golden-mantled ground squirrels (*Spermophilus lateralis*; Mateo, 2002), mound-building mice (*Mus spicilegus*; Busquet & Baudoin, 2005), wood mice (*Apodemus sylvaticus*; Wolton, 1984), Mongolian gerbils (*Meriones unguiculatus*; Cheal, Klestzick, & Domesick, 1982; Halpin, 1974; O'Connell, 1984), prairie voles (*Microtus ochrogaster*; Newman & Halpin, 1988) rats (*Rattus norvegicus*; Hopp, Owren, & Marion, 1985), and golden hamsters (*Mesocricetus auratus*; Heth, Todrank, & Johnston, 1998; Mateo & Johnston, 2000; Tang-Martinez, Mueller, & Taylor, 1993; Todrank, Heth, & Johnston, 1999). Odor discrimination has been shown to start as early as four days of age in golden hamster pups (Devor & Schneider, 1974) and it has been suggested to be earlier in more precocial animals (Mateo & Johnston; 2000; Stavy, Goldblatt, & Trekel, 1985).

Evidence supports a connection between odor and the individual genetic makeup of its creator. In male mound-building mice (*Mus spicilegus*), the similarity between odor intensities reflects closer kinship, representing a graded relationship between genes and odors within kin groups (Busquet & Baudoin, 2005). Most research attributes this recognition to the major histocompatibility complex (MHC), which may mediate kin recognition at the cellular or molecular levels (Brown & Eklund, 1994). This immunological self-compatibility complex expresses a combination of alleles at just two loci (H-2K and H-2D) that can occur in 2,500 different combinations (Halpin, 1991). This vast amount of variation allows for numerous discriminable possibilities. Male behavioral responses to the perception of MHC odor types generally shows a preference for a mate of a different MHC type than their own (Boyse, Beauchamp, Yamazaki, & Bard, 1991). This complex has been proposed as a possible source of kin discrimination and is the best observed evidence for kin recognition as a mechanism.

In golden hamsters the assessment of kinship tends to be on the basis of their own odor rather than early social experience (Todrank, Heth, & Johnston, 1999), which suggests a genetic component over early socialization and supports a phenotype matching mechanism. Even rat pups have been shown to discriminate based on a genetic component of odor. Tel Aviv Strain Wistar rat pups had a preference for home bedding over neutral and novel odors but did not prefer a familiar artificial odor (Goldblatt, 1978). This suggests a genetically based aspect of odor partiality because it is not the familiarity of the substance that is important as much as the socially communicative properties that it offers.

In other research golden hamsters were unable to discriminate, in an habituation paradigm (explained below), between a pair of brothers that were unfamiliar to them (Todrank, Heth, & Johnston, 1999). This strongly suggests that related animals have very indistinguishable smells, at least from each other, leading to a high level of odor similarity in a kin-pair. Heth, Todrank, and Johnston (1998) showed that golden hamsters will scent mark in frequencies inversely related to the degree of relatedness to the scent’s originator. In other words, both male and female golden hamsters scent marked most in the presence of non-brother scent marks, intermediately in the presence of half-brother scent marks, and least in the presence of full-brother’s marks. This research clearly demonstrates an ability to assess degrees of genetic relatedness.

Most of the discrimination research concentrates on males. The small amount of research on females has been conflicting in comparison. Research suggests that female golden hamsters can differentiate between the odors of two male hamsters in an habituation paradigm (Tang-Martinez, Mueller, & Taylor, 1993), but most of the research suggests a sex difference in this ability or at least the sensory means used to do so. Removal of the vomeronasal organ in male golden hamsters resulted in an inability to discriminate between glandular and urinary odors in males but did not have this effect in females (Johnston & Peng, 2000). Some studies have even demonstrated that females can differentiate between two males' dominance status on the basis of odor (White, Fischer, & Meunier, 1986), which was not supported by research in the male golden hamster. Female golden hamsters can make subtle olfactory discriminations of males based on genotypic similarity (Fischer, Olsen, & Meunier, 1985). Reproductively, because the female decides which suitor to mate with, there may be a difference in how odors are sensed, which odors are important or are preferred, and how sensitive to the odor she will be in comparison to males.

Thus far the research has focused primarily on golden hamsters. The present experiment sought to apply kin recognition concepts to a different species of hamster: the dwarf hamster (*Phodopus campbelli)*. Lai, Vasilieva, and Johnston (1996) have demonstrated that male *Phodopus campbelli* show sexual preference for urine, midventral gland odor, and feces of females. Females show sexual preference for urine, and midventral gland odors of males, but not for feces. Though these tests were merely demonstrating a preference between odors of varying sex cycle stages, they demonstrate that *Phodopus campbelli* are able to make discriminations on the basis of odor. Given this evidence, *Phodopus campbelli* should have the sensory capabilities for kin recognition, much as has been demonstrated with golden hamsters and other species of rodents.

There are many reasons to believe olfactory discrimination differs between dwarf and golden hamsters based on social differences between the two (Reasner & Johnston, 1987). The dwarf hamster inhabits arid areas where there are sand dunes and plains of stiff grass (Nowak, 1999). They do not establish stable intrasexual dominance hierarchies and intrasexual aggression often results in death (Wynne-Edwards & Lisk, 1988), and males are dominant over females (Wynne-Edwards & Lisk, 1987). Dwarf hamsters are socially monogamous and biparentally participate in care of young to facilitate thermoregularity and water balance in the female during parturition and lactation, thereby enhancing pup survival and growth (Wynne-Edwards, 1998). In captivity dwarf hamsters exhibit a high degree of sociality (Wynne-Edwards & Lisk, 1984). Golden hamsters, on the other hand, inhabit northwestern Syria and live in dry, rocky steppes or brushy slopes. Adults generally live one to a burrow, females are typically dominant, and both sexes will fight each other readily (Nowak, 1999; Tang-Martinez, Mueller, & Taylor, 1993).

Social learning has been investigated in both dwarf hamsters and golden hamsters by Lupfer, Frieman, and Coonfield (2003). In their experiments a number of manipulations were conducted in both species to assess levels of socially acquired flavor preferences. First a subject was presented with a conspecific which had consumed a particularly flavored diet just prior to their introduction. The subject was then given a choice between the diet the conspecific had consumed and a novel diet. The authors found that dwarf hamsters would choose the same diet as the one they smelled on the conspecific more times than would be expected by chance alone, thus demonstrating a learned social prefernce. Golden hamsters, however, did not demonstrate this same preference. The only preference demonstrated by both dwarf and golden hamsters in this experiment was when the conspecific introduced was the subject’s mother or when presented with the hoard of a conspecific (without the conspecific present).

Their findings demonstrate an ability for golden hamsters to form preferences (as seen in the latter findings) based on socially learned odors, but a lack of motivation to do so in some situations. The most relevant finding was that dwarf hamsters would socially interact with an introduced conspecific, whereas golden hamsters showed little tolerance for an introduced conspecific.

In another study Lupfer (2003) found that golden hamsters would interact with a hamster-sized piece of wood that signaled the presentation of food while actively avoiding direct contact with a conspecific that signaled food. Dwarf hamsters, on the other hand, chewed on the cardboard or ignored it while behaving socially toward a conspecific that signaled food. These two studies not only exemplify that dwarf hamsters and golden hamsters respond behaviorally different in social situations, but also that they show different behavioral motivation in such situations.

Given that dwarf hamsters are more social in comparison to golden hamsters (Lupfer, 2003), there may be a difference in need or mechanism for kin recognition. For example, colonial swallows, such as bank swallows and cliff swallows, have well developed parent-offspring recognition whereas noncolonial swallows, such as rough-winged swallows and barn swallows, have a weak or altogether absent parent-offspring recognition ability (Beecher, 1991). This evidence based on two species which vary in sociality suggests that dwarf hamsters might have the capability for kin detection even stronger than that demonstrated by golden hamsters due to their more social nature.

Stronger evidence is suggested by a reproductive phenomenon found in dwarf hamsters that is not observed in golden hamsters. In dwarf hamsters the mere presence (indirect contact) with a male other than a female's mate can inhibit pregnancy (Wynne-Edwards & Lisk, 1984). Dwarf hamsters may also spontaneously abort a litter in the presence of another male's odor (Wynne-Edwards & Lisk, 1988). This effect would depend on the female's ability to distinguish between the odors of this new male and the stud male (Halpin, 1986). Although this may be a purely physiological reaction to the novel male's presence, it still necessitates some mechanism of kin discrimination and supports kin recognition.

The opposite might also be true. Living in predominantly monogamous (at least for a breeding season) social units might preclude an evolutionary need to assess kinship. In a given situation, relatives may share behavioral responses which lead to kin-biased behavior (Barnard & Aldhous, 1991), thus negating a need for phenotype matching or association. This bias would support a more spatially-mediated behavioral response such as recognition by spatial location, which Tang-Martinez (2001) would argue is actually an absence of recognition. Grafen (1990) also suggested such by stating that kin recognition would facilitate individual recognition and therefore preclude the need for kin recognition altogether. In this case a dwarf hamster would simply avoid mating with individuals it associates with most.

As mentioned previously, experimental assessment of kin recognition is inferred based on behavioral discrimination. The present experiment used an habituation paradigm to demonstrate kin recognition through discrimination of odors. This paradigm is one of the most widely used techniques for demonstrating individual differences in response to odors (Halpin, 1986). Odor discrimination is ideal because olfactory cues have been well established as the dominant cue utilized in rodent social recognition (Porter, 1988).

Habituation and Odor Discrimination

Habituation is an innate, pervasive phenomenon exhibited by most creatures in the animal kingdom (Leslie, 1996; Sanchez-Andrade, James, & Kendrick, 2005). There is evidence that even fetuses will habituate to odor cues *in utero* (Robinson & Smotherman, 1991). Habituation, as a process, refers to a decline or cessation in response magnitude as a result of repeated stimulus exposure. Biologically, this phenomenon serves as a cost reduction mechanism because it prevents unneeded responses to repeated and thus familiar events (Leslie, 1996). In this way an animal does not waste time or energy on unnecessary, inappropriate, or ineffective behaviors directed toward the stimulus (Shettleworth, 1998).

An habituation paradigm seeks to exploit this process. Habituation is an innate process that does not require decision making. Decision making is not necessary for habituation making it ideal for investigating recognition as motivation and context should not interfere with its elicitation. The methodology for habituation paradigms is most aptly described by Halpin (1986, 1974) as an habituation-discrimination procedure.

The habituation procedure consists of two phases: an habituation phase and a discrimination (test) phase. During the habituation phase, the subject is repeatedly presented with the same odor (A). Habituation is observed by a decrease in time investigating the initial odor (A) over a series of presentations. Once habituated to odor ‘A’ a novel odor (B) is introduced in conjunction with it or in its stead. This is the discrimination or test phase. The rationale is that if ‘A’ and ‘B’ are perceived as different, then during the discrimination phase odor ‘B’ should elicit stronger responses (assuming the subject has habituated to odor ‘A’). In other words, if the subject perceives a difference between the habituation odor (A) and the discrimination odor (B), it will spend more time investigating the discrimination odor in comparison to time investigating the habituation odor on its last presentation (Heth, Todrank, Begall, Wegner, & Burda, 2004).

The habituation-discrimination paradigm was used to investigate the olfactory capability of *Phodopus campbelli* hamsters to discriminate odors using a pair of genetically similar odors (pair of brothers) for the ‘related’ condition and a pair of genetically less similar odors (pair of brothers that were not brothers of the subject) for the ‘unrelated’ condition. If *Phodopus campbelli* hamsters are able to discriminate between odors in the same way golden hamsters do, they should show the same or a decreased level of responding to the test odor when the odors are genetically similar, and should show increased level of responding to the test odor when the odors are genetically dissimilar.

The types of odors investigated were urine, feces, bedding, and glandular odors, as these are the most common sources of odor used to investigate kin recognition (Halpin, 1986). Glandular odors refer to secretions excreted from a sexually dimorphic, sebaceous gland located on the midline of the ventral surface (Reasner & Johnston, 1987). This is an enlarged and specialized sebaceous gland that has been implicated in sexual communication (Lai, Vasilieva, & Johnston, 1996). These four odors were selected to investigate which odor, if any, is most motivationally pertinent to the dwarf hamster. In unpublished data Halpin and McCurdy found that only odors from flank glands (an anatomically different gland from the midventral gland in *Phodopus campbelli*, but thought to be utilized for the same purposes), urine, and soiled cage shavings were informative to golden hamsters, whereas feces were not (as cited in Halpin, 1986). The same was observed in Mongolian gerbils (Halpin, 1974). As mentioned earlier, preference tests have shown that urine and midventral glands are sexually relevant to *Phodopus campbelli* hamsters, with fecal odors being informative to males. Investigation of the four selected odors should demonstrate whether these odors are used for kinship identification as well as provide an adequate comparison between which cues are utilized by dwarf hamsters to those demonstrated to be pertinent for golden hamsters.

Urine odor collection followed the collection procedure utilized by White, Fischer, and Meunier (1986) in their assessment of urine odor discrimination by golden hamsters. Fecal boli and glandular secretion collection followed a slightly modified version of the collection procedure utilized by Johnston and Peng (2000) in their assessment of fecal and glandular odors discrimination in prairie voles. The habituation procedure followed that used by Todrank, Heth, and Johnston (1999) in their assessment of discrimination for and memory of odors of close relatives in golden hamsters.

Hypotheses

Based on the literature, three hypotheses were explored in the present experiment. First, it was hypothesized that dwarf hamsters would demonstrate habituation quicker when the habituation odor source donor was a brother of the subject (Condition 1) than when the habituation odor source donor was unrelated to the subject (Condition 2). Second, it was hypothesized that dwarf hamsters would be able to discriminate between the odors of a pair of cousins, but not between the odors of a pair of brothers. Third, it was hypothesized that there would not be a sex difference in response to kin odors.

Method

Participants

Twenty-four dwarf hamsters (*Phodopus Campbelli*), of known genetic relation served as subjects. Mature males (*n*=11) and females (*n*=13) were tested. Because the female estrus cycle is variable and cannot be controlled for, only males served as odor donors for all odor types. Each donor male served as an odor donor for two other subjects.

Housing

Animals were bred in-house to allow for documentation of genetic relation and raised to adulthood. Each was housed individually in an opaque, standard shoebox cage with ad lib access to food and water throughout experimentation. All animals were fed the same diet and maintained in the same colony room. Testing occurred in an annex off this colony room. No animals were present in this annex other than the subject during testing.

Apparatus

Wire mesh was used to line the cage of the odor donor for the collection of urine. Filter paper was also used for collection and presentation of urine odor. Glass specimen discs (5 cm in diameter) were used for all odor presentations. Disposable gloves were used between trials and for any sample and odor source donor handling. A video camera was used to videotape all odor presentations.

Videotaping Procedure

At one end of the test cage two circles were drawn centrally with a fine-tipped, black, permanent marker. One circle was the size of the glass specimen discs (5 cm in diameter) and was used as a guide for the placement of the disc for stimulus presentation. This ensured that the stimuli would be presented in the same place each time. A second circle was drawn circumferentially 0.5 cm around the stimuli circle. A video camera (Panasonic, PV-L352D) was suspended above the test cage, providing a bird's-eye record. Time investigating the odor sample was recorded whenever the subject's nose crossed into the outer circle within a 5 minute trial, excluding time spent chewing on the glass specimen disc or incidental crossing (for example, grooming over the area). Because the bedding sample was not adhered to the surface of the specimen disc and could be moved about, time was also recorded whenever the subject was in direct contact with a piece of bedding outside of these circles.

General Procedure

Odor Stimulus Collection. Procedures for collecting the various odor stimuli for habituation and testing are described below.

Habituation and Testing. In the habituation trials (1-4), the odor stimulus was placed on a glass specimen disc, which was then secured to the floor of an empty, clean shoebox cage by way of double sided tape. The subject was then placed in this cage. Each trial presentation lasted 5 minutes in duration, and four trials were conducted to establish familiarity and encourage habituation to the odor. There was an intertrial time of 5 minutes. The testing shoebox cage was cleaned between each trial with a disinfectant spray (A-33). The time each subject spent with its nose within 0.5 cm of the specimen disc was recorded. (Bedding was the one exception to this, outlined below.) Time spent chewing on or moving the specimen disc was not included. The fourth habituation trial (H_4) served as the baseline against which performance on the two test trials following it were compared. Subjects were returned to their home cage between each trial.

The fifth trial was the first discrimination test trial (T_1). On this trial, a glass specimen disc containing a novel sample was presented. This novel odor was from a male genetically related (a brother) to the habituation odor donor. Depending on condition assignment this male was either genetically related to the subject (Condition 1; C_1) or distantly genetically related to the subject (Condition 2; C_2). Time in contact with the specimen disc was measured. An increase in the time in contact with the novel odor in comparison to the time in contact during the last habituation odor presentation is indicative of discrimination of the odor stimuli sources.

The sixth trial was the final discrimination test trial (T_2). On this trial, a glass specimen disc containing a second novel odor was presented. This novel odor was from a male distantly genetically related (not a brother) to either of the previous odor donors (habituation and first discrimination test). This male was also distantly genetically related to the subject, regardless of condition assignment. Time in contact with the specimen disc was measured. An increase in time in contact with the novel odor in comparison to the two previous presentations is indicative of discrimination of the odors. See Table 1.

All glass specimen discs were cleaned between subjects. Each disc was first sprayed down with Windex and then cleaned with Dawn liquid soap (original scent). Discs were then dried and allow exposure to the air for at least an hour before being used again.

Odor Source 1: Bedding

Sample Collection. On testing day, bedding was collected from each odor donor's home cage to be used as the bedding odor stimulus for two other subjects. Bedding was chosen on the basis of apparent nesting site. Areas heavily contaminated by other substances were avoided as much as possible.

Amount of bedding selected was such that it was easily pinched between the thumb and forefinger without spillage. The habituation and first discrimination test odor donors were brothers, and the second discrimination test odor donor was a male unrelated (not a brother) to them. Half of the subjects were presented with habituation and first discrimination test odor donors that are related to them (C_1) and half the subjects were presented with habituation and the first discrimination test odor donors that are unrelated to them (C_2). The second discrimination test odor donor was always unrelated to the subject, the habituation odor donor, and the first discrimination test odor donor.

Habituation and Testing. In the habituation and test phases, bedding was placed on the specimen disc and presented as described in the general procedure, with one exception. Because bedding was the only odor source stimulus that could not be directly affixed to the surface of the disc, the subjects were fully capable of moving it off the disc. If bedding was moved off the disc, then time in direct physical contact with the sample was also recorded and added into the total time. Each trial consisted of a fresh bedding sample.

Odor Source 2: Urine

Sample Collection. On testing day, urine was collected from each odor donor to be used as the urine odor stimulus for two subjects. A clean shoebox cage was lined with filter paper to capture any urine. Wire mesh was placed atop this to allow space between the odor donor and the filter paper (preventing direct contact between the odor donor and the filter paper). Odor donor males were moved from their home cages to this cage for a period of 8 hours. After this period all filter paper was retrieved and any fecal material present was discarded. Any collected urine sections obviously contaminated by fecal material were also discarded. Sections of obvious urine deposit were cut into approximately equal sections (approximately 0.25-1 cm^2), sealed in plastic sandwich baggies, labeled, and stored at approximately 15ºF until testing. Testing was conducted within 24 hours of retrieval.

Habituation and Testing. In the habituation phase, a square section of urine suffused filter paper was placed on a glass specimen disc. The filter paper sample was then moistened with 3-5 drops of distilled water just prior to exposure to ‘refresh’ the urine odor as much as possible. Each trial consisted of a fresh sample on a clean glass specimen disc. Habituation and testing followed the general procedure described above.

Odor Source 3: Feces

Sample Collection. On testing day, feces were collected from each odor donor to be used as the fecal odor stimulus for two subjects. Odor donors were moved to individual, clean shoebox cages, without bedding or food an hour prior to the experiment (water was available throughout). Fresh looking fecal boli, not contaminated by other substances were collected just prior to sample preparation.

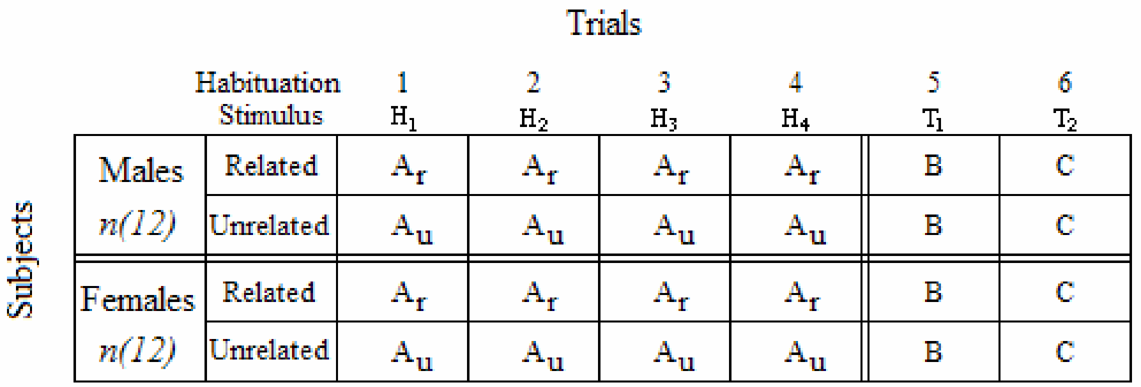
Habituation and Testing. In the habituation phase, a fecal bolus was moistened with 5 drops distilled water, stirred with a clean wooden stir stick, and spread evenly on a glass specimen disc. Each trial consisted of a fresh sample on a clean glass specimen disc. Habitation and testing followed the general procedure described above.

Odor Source 4: Glandular Secretion

Sample Collection. On testing day, glandular secretions were collected from each odor donor to be used as the glandular secretion odor stimulus for two subjects. Glandular secretion odors were collected just prior to presentation of the first habituation trial (H_1) by rubbing the odor donor's midventral area on a glass specimen disc, 10-15 times, avoiding the anus as much as possible. Given the size of the glass specimen disc in relation to the size of the odor donors, the odor sample is most likely more than just sebaceous gland secretions. Oral secretions, penile secretions and odors from the hair and skin are also possible residues left on the sample. No donor was collected more than twice on any given day. This means the subject was presented the same disc (i.e., the sample was not refreshed) for each habituation trial. The habituation sample and both test discrimination sample odors were collected at the same time.

Habituation and Testing. In the habituation phase, glandular secretions from the habituation odor donor were presented on a glass specimen disc. Habituation and testing followed the general procedure described above.

Table 1: General Experimental Procedure



			Trials					
		Habituation Stimulus	1 H_1	2 H_2	3 H_3	4 H_4	5 T_1	6 T_2
Subjects	Males *n(12)*	Related	A_r	A_r	A_r	A_r	B	C
		Unrelated	A_u	A_u	A_u	A_u	B	C
	Females *n(12)*	Related	A_r	A_r	A_r	A_r	B	C
		Unrelated	A_u	A_u	A_u	A_u	B	C

Stimulus: Relationship of habituation odor donor to Subject
A: Habituation odor source; r: related to subject, u: unrelated to subject
B: Genetic relative of habituation odor donor; first test discrimination trial
C: Distant genetic relative of habituation odor donor; second test discrimination trial

Data Analysis

The time each subject spent with its nose within 0.5 cm of the glass specimen disc in each trial was recorded (as well as time in direct physical contact in the case of bedding). A 2 x 2 x 4 mixed design ANOVA with habituation odor condition (related, unrelated) and sex as between subjects variables and habituation trials as the within subjects variable was used to analyze habituation trials (1-4) to assure habituation to the stimulus odor occurred. A 2 x 2 x 3 mixed design ANOVA with habituation odor condition and sex as between subjects variables and test stimuli (including habituation trial 4) as the within subject variable was used to analyze discrimination trials. The data of main interest are a comparison of time spent investigating the odor stimuli on the final habituation trial, first test trial (habituation stimulus was a brother), and second test trial (unrelated to the habituation odor donor source and first test odor donor source) trials. For each significant test discrimination effect a post-hoc Tukey HSD analysis was conducted at the 0.05 significance level to determine which trials were statistically significantly different.

Results

Odor Source 1: Bedding

Habituation. Habituation to the bedding odor stimulus is presented in Figure 1. There were statistically significant effects of trial, *F*(3,60) = 36.20, p < .001, and condition, *F*(1,20) = 5.21, p < .05. Subjects habituated to the bedding odor stimulus. Condition 1 subjects (siblings of the habituation odor source) demonstrated habituation quicker than Condition 2 subjects (unrelated to the habituation odor source) did. No other effects were statistically significant. The results for habituation to the bedding odor stimulus support the first and third hypotheses of the present experiment. (Hypothesis two is not relevant to habituation testing.)

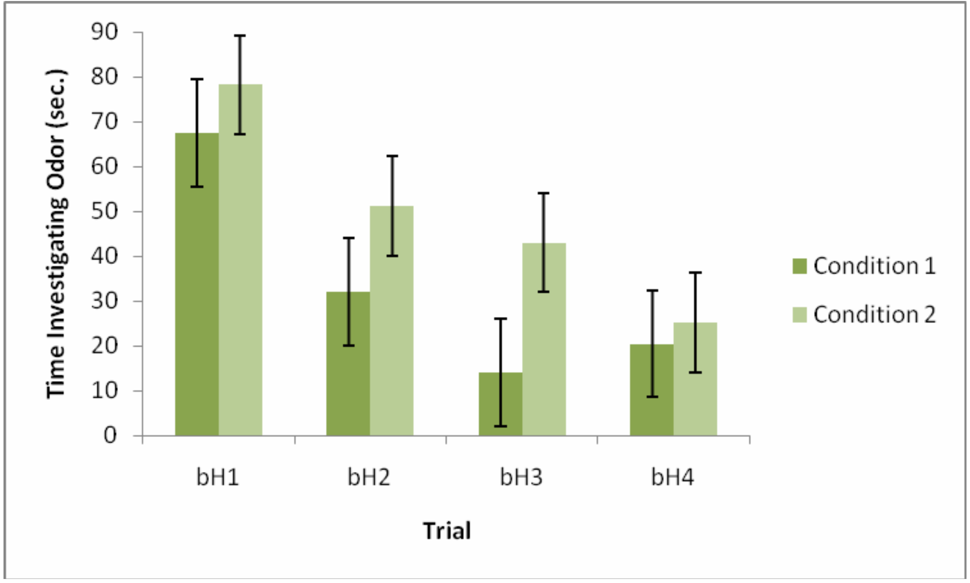


Figure 1. Habituation to the bedding odor source based on condition assignment. Condition 1 subjects were siblings of the habituation odor source donor. Condition 2 subjects were unrelated to all odor source donors. Bars represent standard errors.

Discrimination. Discrimination of the bedding odor stimulus is presented in Figure 2. There was a statistically significant effect of trial, *F*(2,40) = 7.46, p < .01. Subjects were able to discriminate between the bedding odors.

A post-hoc Tukey HSD test was conducted to assess which odors were differentiated between. The last habituation trial (bH_4) and the second discrimination test (bT_2; “unrelated” odor donor) were found to be significantly different from each other in terms of time spent investigating each at the 0.05 level. The difference in time spent investigating the first discrimination test (bT_1) in comparison to either of the other trials (bH_4 and bT_2) did not reach statistical significance. The post-hoc Tukey HSD result shows that subjects differentiated between the odors of a pair of unrelated individuals (bH_4 and bT_2), but did not differentiate between a pair of brothers. No other effects were statistically significant. The results of discrimination of the bedding stimulus support the second and third hypothesis. (Hypothesis one is not relevant to discrimination testing.)

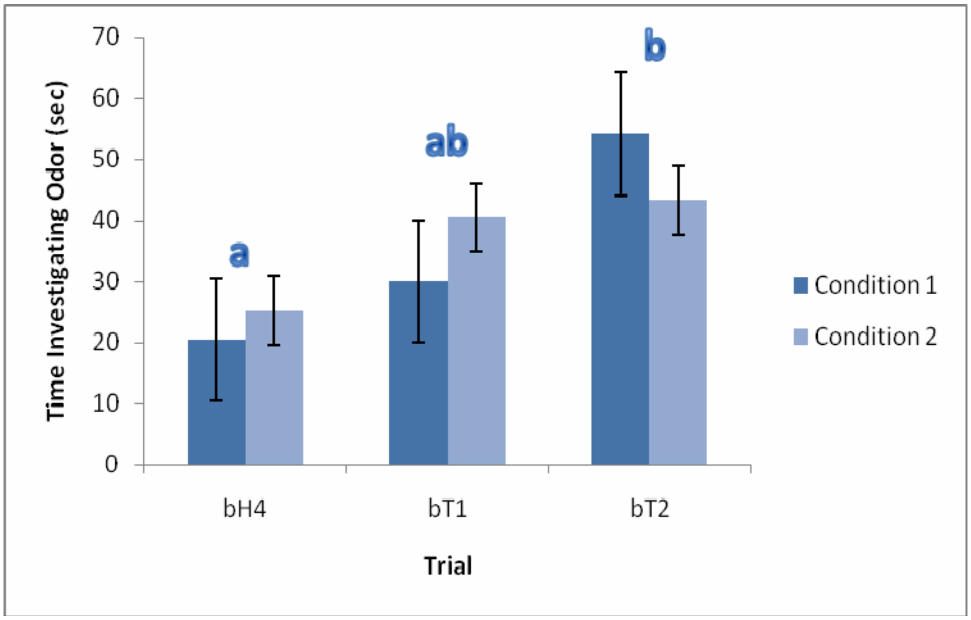


Figure 2. Discrimination of bedding odor sources based on condition assignment. Condition 1 subjects were siblings of both the habituation odor source donor and the first discrimination odor source donor. They were unrelated to the second discrimination test odor source donor. Condition 2 subjects were unrelated to all odor source donors. ‘a’, ‘ab’, ‘b’ indicate post hoc Tukey HSD test results, significant at $p < .05$. Bars represent standard errors.

Odor Source 2: Urine

Habituation. Habituation to the urine odor stimulus is presented Figure 3. There was a statistically significant effect of trial, *F*(3,60) = 10.80, p < .001. Subjects habituated to the urine odor stimulus. No other effects were statistically significant. The third hypothesis was the only one supported by these findings.

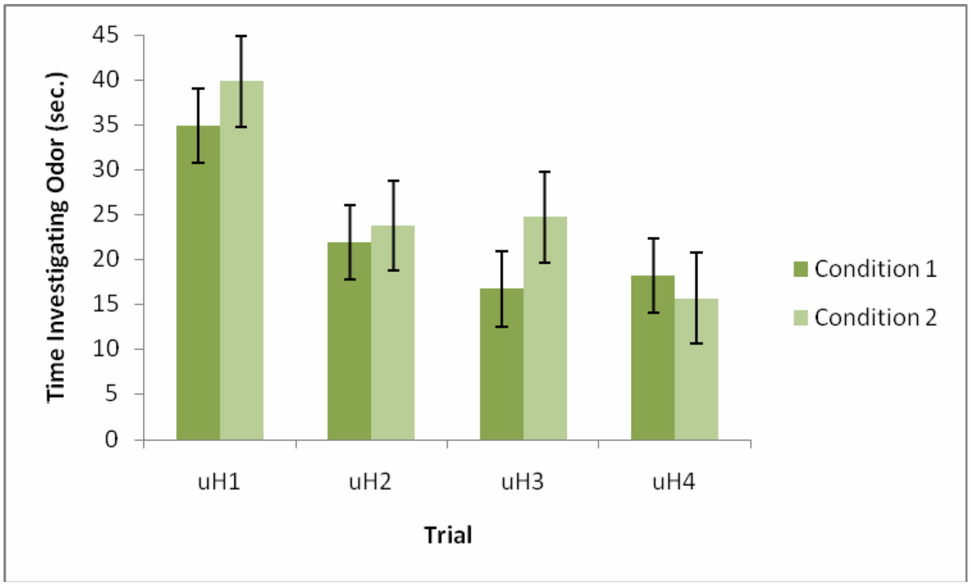


Figure 3. Habituation to the urine odor source based on condition assignment. Condition 1 subjects were siblings of the habituation odor source donor. Condition 2 subjects were unrelated to the habituation odor source donor. Bars represent standard errors.

Discrimination. There was not a statistically significant effect of trial, *F*(2,40) = 0.21, nor were there any other statistically significant effects. The result for the discrimination of the urine stimulus suggests that subjects did not respond differentially on the basis of kin in this experiment. The third hypothesis was the only one supported by these findings.

Odor Source 3: Feces

Habituation. Habituation to the fecal odor stimulus is presented in Figure 4. There was a statistically significant effect of trial, *F*(3,60) = 28.02, p < .001. Subjects habituated to the fecal odor stimulus. No other effects were statistically significant. The third hypothesis was the only one supported by these findings.

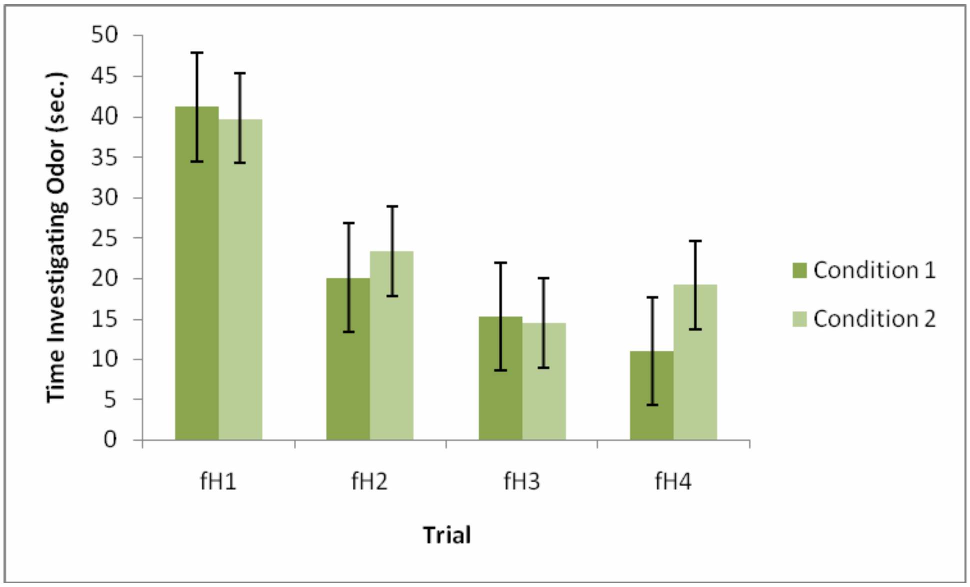


Figure 4. Habituation to the fecal odor source based on condition assignment. Condition 1 subjects were siblings of the habituation odor source donor. Condition 2 subjects were unrelated to the habituation odor source donor. Bars represent standard errors.

Discrimination. There was not a statistically significant effect of trial, *F*(2,40) = 0.21, nor were there any other statistically significant effects. Subjects did not respond differentially on the basis of kin.

There was a statistically significant Trials x Sex interaction, *F*(2,40) = 5.63, p < .01 (see Figure 5). Tukey HSD post-hoc analysis revealed males responded significantly less to the first test discrimination (gT_1) than to either the habituation odor source (gH_4) or the second test discrimination (gT_2) source at the 0.05 level. Females did not demonstrate a statistically significant difference in response to any trial. The findings for the fecal odor stimulus did not support any of the hypotheses.

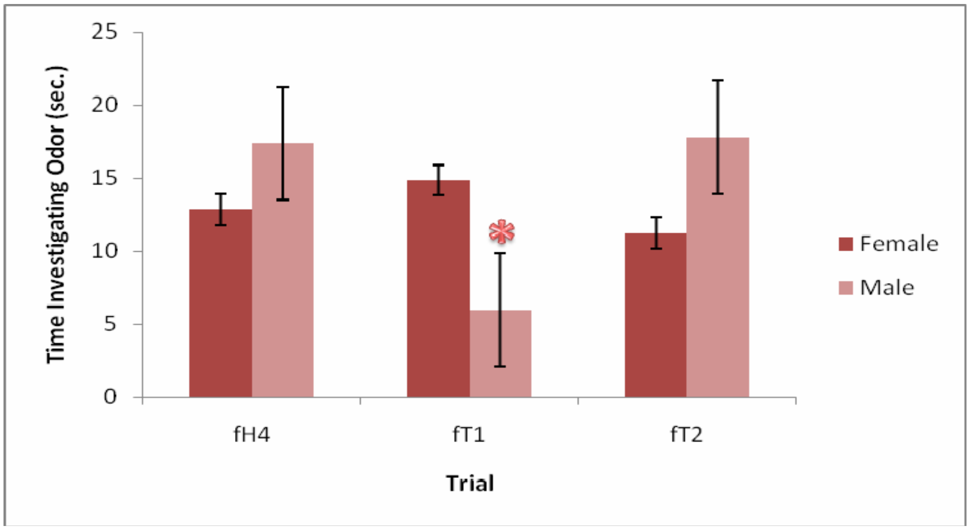


Figure 5. Trial x Sex interaction of fecal odor discrimination. * p < .05. Bars represent standard errors.

Odor Source 4: Glandular Secretion

Habituation. Habituation to the glandular secretion odor stimulus is presented in Figure 6. There was a statistically significant effect of trial, *F*(3,60) = 30.82, p < .001. No other effects were statistically significant. Subjects habituated to the glandular secretion odor stimulus. No other effects were statistically significant. The third hypothesis was the only one supported by these findings.

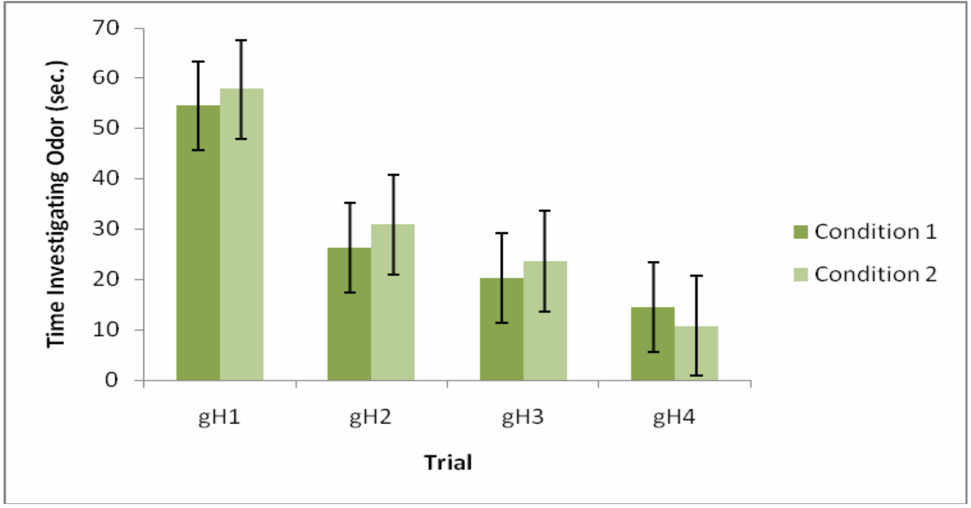


Figure 6. Habituation to the glandular secretion odor source based on condition assignment. Condition 1 subjects were siblings of the habituation odor source donor. Condition 2 subjects were unrelated to the habituation odor source donor. Bars represent standard errors.

Discrimination. Discrimination to the glandular secretion odor stimulus is presented in Figure 7. There was a statistically significant effect of trial, $F(2,40) = 8.91$, $p < .001$. Subjects were able to differentiate between the odors.

A post-hoc Tukey HSD test was conducted to assess which odors were differentiated. The last habituation trial (H_4) and the second discrimination test (T_2; "unrelated") were found to be significantly different from each other in terms of time spent investigating each at the 0.05 level. The difference in time spent investigating the first discrimination test (T_1) in comparison to either of the other trials (H_4 and T_2) did not reach statistical significance. No other effects were statistically significant. Post-hoc Tukey HSD results show that subjects differentiated between the odors of a pair of unrelated individuals, but did not differentiate between a pair of brothers. No other effects were statistically significant. The results of discrimination of the glandular secretion stimulus support the second and third hypotheses. (Hypothesis one is not relevant to discrimination testing.)

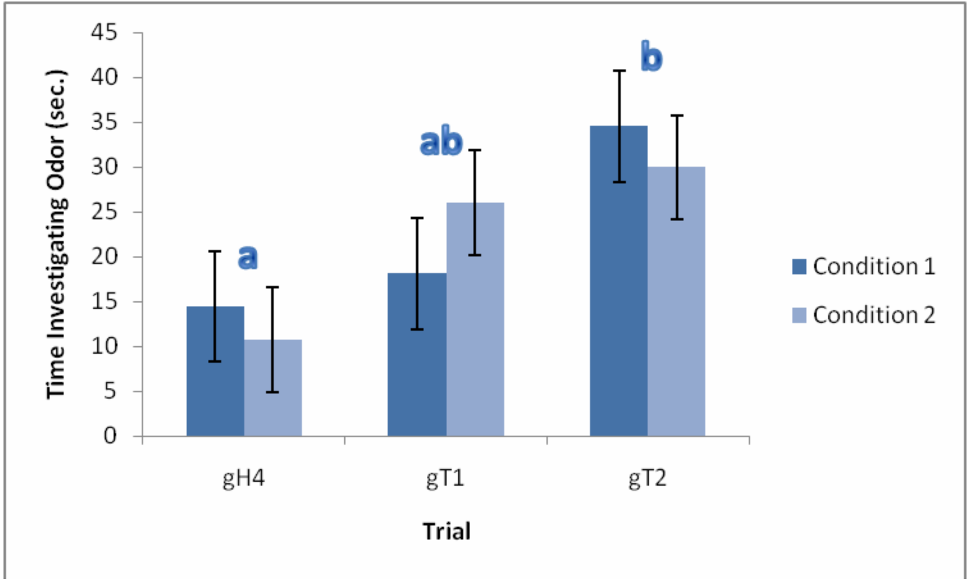


Figure 7. Discrimination of glandular secretion odor sources based on condition assignment. Condition 1 subjects were siblings of both the habituation odor source donor and the first discrimination odor source donor. They were unrelated to the second discrimination test odor source donor. Condition 2 subjects were unrelated to all odor source donors. 'a', 'ab', 'b' indicate post hoc Tukey HSD test results, significant at $p < .05$. Bars represent standard errors.

Discussion

Odor Source 1: Bedding

Habituation to the bedding odor source was demonstrated by subjects. Interestingly, there was an effect of condition. The habituation odor source donor for the subjects in Condition 2 was an individual “unrelated” to the subject, whereas in Condition 1 the habituation odor source donor was a brother of the subject. Subjects in Condition 2 spent more time investigating and habituating to the odor of the “unrelated” odor compared to subjects in Condition 1.

This finding agree with Heth et al.’s (2004) finding that Ansell’s mole-rats (*Cryptomys anselli)*, also known as the Zambian mole-rat, spend more time investigating an unrelated individual’s odor than that of their own sibling’s. Interestingly, like *Phodopus campbelli*, Ansell’s mole-rats are gregarious and breed monogamously (the comparison is not exact as *Phodopus campbelli* are monogamous only for a breeding season), suggesting that the social make-up of the animal might play a role in this finding.

Discrimination of the bedding odor stimulus was demonstrated when the odor source donors were unrelated to each other (H_4 and T_2), but not when the odor source donors were brothers. This result suggests that *Phodopus campbelli* are capable of discriminating between the odors of genetically dissimilar individuals, which agrees with previous kin discrimination research. Also in agreement with previous research, this result suggests that *Phodopus campbelli* are not able to distinguish between the odors of a pair of brothers. Todrank, Heth and Johnston (1999) found a similar result with golden hamsters. This was an expected result.

The absence of a statistically significant effect of condition for discrimination was not expected considering that subjects responded differently based on the genetic relationship status of the odor source to self during habituation. There is no suggestion in the literature why this might be the case.

Odor Source 2: Urine

Habituation to the urine odor source was demonstrated by subjects, as expected. Subjects did not, however, discriminate between individuals on the basis of odor, genetically dissimilar or similar. This was not an expected finding.

Urine is one of the most commonly used odor sources utilized for kin discrimination. Golden hamsters (Tang-Martinez, Mueller, & Taylor, 1993; White, & Meunier, 1986) and Mongolian gerbils (Halpin, 1974) have both demonstrated discrimination on the basis of urine (although findings were low in Mongolian gerbils).

The present finding seems to disagree with those found in other research. Urine has been implicated in other research as being an important cue to *Phodopus campbelli*. Lai, Vasilieva, and Johnston (1996) found that male *Phodopus campbelli* showed a preference for female urine in sexual contexts. Wynne-Edwards and Lisk (1987) contend that both males and females use urine to mark their environment.

The present finding also does not coincide with research conducted on the major histocompatibility complex (MHC). The MHC is an immune system function which is used by an individual to recognize self from non-self. Urine has been shown to include components of MHC which can then be utilized by other animals as a signature of the genomic make-up of the carrier (Brown & Eklund, 1994). The lack of statistical significance regarding the discrimination between urine odors would seem to suggest that subjects in this experiment did not behaviorally react to signatures if present in the odor stimulus, although the reaction to do so is pervasive elsewhere in the rodent literature.

Odor Source 3: Feces

Habituation to the fecal odor source was demonstrated by subjects, as expected. Subjects did not, however, discriminate between individuals on the basis of odor, genetically dissimilar or similar! This was not an expected finding.

Though it was an expectation of this experiment that *Phodopus campbelli* would be able to discriminate between two genetically dissimilar fecal odors, a review of the research suggests the obtained results might be expected. Mongolian gerbils do not discriminate between individual differences if the biological odor is feces (Halpin, 1974). Haplin and McCurdy (unpublished) found that male golden hamsters do not discriminate between individual differences based on fecal odors. This was later confirmed by Johnston (as cited in Halpin, 1986).

It was expected that subjects would respond differently based on condition assignment. This was not found; however considering subjects did not respond in a kin-biased way to test trials it makes sense that they did not do so based on condition either.

There was a statistically significant Trials x Sex interaction. This was surprising, as sex differences were not hypothesized for any of the odor sources in this experiment. Tukey HSD post-hoc analysis revealed an even more puzzling

result. Males responded significantly less to the first discrimination test (gT_1) than to either the habituation odor source (gH_4) or the second discrimination test (gT_2). The latter two trials did not differ significantly from each other. Females did not show this differential treatment of the odors. This finding in females agrees with Lai, Vasilieva, and

Johnston's (1996) finding that female *Phodopus campbelli* did not respond differentially to fecal odors from two genetically dissimilar males.

There appears to be sex differences regarding investigation of fecal odor sources in other species as well. As mentioned previously, male golden hamsters do not discriminate between individual differences based on fecal odors (Halpin, 1986); however, Tang-Martinez, Mueller, and Taylor (1993) showed that female golden hamsters do discriminate between male golden hamster feces.

Contrasting the differences in social structure might provide insight to the apparent sex difference in consideration of fecal odors. The female is dominant in golden hamster social hierarchies (Nowak, 1999; Tang-Martinez, Mueller, & Taylor, 1993), whereas the male is dominant in *Phodopus campbelli* social hierarchies (Wynne-Edwards & Lisk, 1987). This inverse of social dominance might explain the reversed findings regarding feces and sex in this experiment compared to the literature on golden hamsters.

Considering that subjects did not discriminate between fecal odors in a kin-biased way is suggestive that the sex difference observed is the result of feces relaying something other than kin-related information. It would seem that this finding, in addition to the findings in the more dominant female golden hamster, would suggest that it may be due to territoriality rather than kin-recognition.

As mentioned above, the male *Phodopus campbelli* is the dominant sex. Wynne-Edwards (1998) has demonstrated that *Phodopus campbelli* fail to establish stable intra-sexual hierarchies so that intrasexual aggression often results in death. Wynne-Edwards and Lisk (1988) also suggest that male-male aggression would probably keep two breeding males from occupying the same space. It would seem vital for *Phodopus* *campbelli* to have a cue that would communicate this information so that interactions would be infrequent. The sex difference seen in this experiment suggests that feces could be this cue.

Odor Source 4: Glandular Secretion

Habituation to the glandular secretion odor source was demonstrated by subjects. Discrimination of the glandular secretion odor stimulus was demonstrated when the odor source donors were unrelated to each other (H_4 and T_2) but not when the odor source donors were brothers (H_4 and T_1). These results suggest that *Phodopus campbelli* are capable of discriminating between glandular odors of genetically dissimilar individuals. These results also suggest that *Phodopus campbelli* are not able to distinguish between the odors of a pair of brothers. This agrees with Todrank, Heth, and Johnston’s (1999) research, which showed golden hamsters were unable to discriminate between a pair of brothers that were unfamiliar to them. This was an expected result of this experiment.

As mentioned earlier, the results for glandular secretions are possibly confounded with other odors, especially oral secretions. This is a possible limitation for the present experiment. Lai, Vasilieva, and Johnston (1996) found that oral secretions are important for *Phodopus campbelli* sexual communication. Given other research findings it seems highly likely that the result found in the present experiment for glandular secretions are valid, however the possibility of the presence of other secretions contributing to the findings cannot be ruled out.

General Discussion

Habituation to the habituation odor source was observed for all four odor sources.

Bedding (Odor Source 1) and glandular secretions (Odor Source 4) provided evidence that *Phodopus campbelli* are able to discriminate between conspecific odors that vary by degree of genetic relationship. This agrees with much of the research investigating kin-recognition in rodents (examples reviewed in the introduction of this thesis). The findings of the present experiment also agree with the research that bedding and glandular secretions seem to be the most biologically significant sources of odors in regards to kin recognition (of the sources routinely investigated).

Unlike bedding (Odor Source 1) and glandular secretions (Odor Source 4), urine (Odor Source 2) and feces (Odor Source 3) did not result in an obvious demonstration of kin discrimination ability by the subjects. Interpreting this lack of discrimination must be conducted with caution. Kin discrimination refers to the differential behaviors exhibited towards kin and non-kin. It is used to infer kin recognition, which is the mechanism used to identify degrees of relatedness (Hepper, 1991). Described in another way, kin discrimination is a differential response to a perceived stimulus whereas kin recognition is the cognitive mechanism that allows for knowing this difference (Tang-Martinez, 2001). The absence of discrimination does not, however, directly indicate a lack of recognition (Hepper, 1991; Tang-Martinez, 2001).

The ability to recognize another individual has four elements: identification, perception, decision, and action by the perceiver (Beecher, 1991). A lack of behavioral demonstration of recognition can result from a failure at any one of these elements. Habituation was demonstrated to each odor source type. This would suggest that each odor source was both identified and perceived by the subjects. Therefore, the lack of observable discrimination of urine (Odor Source 2) or feces (Odor Source 3) may have been due to a failure to decide what action to take based on the information gleaned, or a lack of motivation to do so.

The two most plausible reasons that subjects may have failed to decide are age and context. Carr, Yee, Gable, and Marasco (1976) found Long Evans rats' reactions to odor sources depends on the age of the odor donor, while the age of the subject seemed to have little effect on reaction. *Phodopus Campbelli* reach sexual maturity within the first two months of life (Nowak, 1999). It may be that the odor source donors were not yet mature enough for these stimuli to be socially relevant to the subject.

The most likely explanation for why subjects did not behaviorally demonstrate discrimination of the urine (Odor Source 2) and fecal (Odor Source 3) odors is due to contextual interference. This would explain a failure at either the decision or action requirements, or both. Kin recognition is highly context dependent (Waldman, Frumhoff & Sherman, 1988). It is possible that the cues presented in this experiment were irrelevant given the context they were presented in, because at the start of testing subjects were between 35 and 39 days of age. Dewsbury (1988) points out that kin-biased reproductive behavior may be based on active mate choice, suggesting that kin-biased behavioral displays may be dependent on the outcome of those behaviors (from a social interaction standpoint). Given that the odor sources in this experiment were presented in the absence of any social reinforcement, one could argue that the fecal and urinary odors presented were not biologically relevant given the context they were presented in.

A lack of kin discrimination in one recognition context does not limit recognition in all contexts (Gamboa, Reeve, & Holmes, 1991). It is possible that the current experiment did not provide a context in which utilization of urine or fecal odors would be biologically significant. Therefore, it cannot be concluded that Odor Sources 2 and 3 imply that *Phodopus campbelli* do not utilize urine or fecal odors for the purpose of kin recognition. The only inference that can be postulated is that the experimental context did not motivate the animal to respond in an overtly kin-biased manner to either.

Interestingly, for both bedding (Odor Source 1) and glandular secretions (Odor Source 4), the subjects spent more time investigating the first discrimination odor (odor source was a brother to the habituation odor source; T_1) in comparison to the last habituation odor (H_4). This would seem to suggest that *Phodopus campbelli* might display individual recognition. Although time spent investigating T_1 was not statistically significant from either the times spent investigating the last habituation stimulus (H_4) or the second test discrimination stimulus (T_2), subjects' times did suggest differential responding. The response to T_1 was intermediary between both H_4 and T_2 suggesting at least a partial discrimination between the brothers. Further experimentation is necessary to know whether individual recognition is a possibility for *Phodopus campbelli*.

The intermediary response of T_1 also lends support to the idea that relatedness is continuous rather than discrete, that is, composed of properties of the individual (Shields, 1993). Todrank and Heth (2003) contend that kin are more closely related genetically by an increase in the degree of similarity at the genome; "non-kin" does not necessarily denote *not* genetically related. Social behavior may also act across a spectrum of similarity rather than distinct classes such as kin or non-kin (Todrank & Heth, 2003). The differential response of T_1 hinted at with Odor Sources 1 and 4 might be an observable display of this continuum. The first discrimination odor source (T_1) was intermediate in degree of genetic relatedness to the habituation odor source. The behavioral response observed may reflect a grade in similar fashion to each of the stimuli presented.

Finally, the results of this experiment suggest that phenotype matching is this method. Animals were counterbalanced in a way that half the subjects were presented with a pair of brothers that were the subject's own brothers, and the other half were presented with a pair of brothers that were unrelated to the subject. The results of this experiment show that subjects in both conditions responded similarly. If *Phodopus campbelli* discriminated kin based on kin recognition by association, then animals in Condition 2 (unrelated to habituation and first discrimination test odor donor) should have responded differently than animals in Condition 1 (sibling of habituation and first discrimination test odor donor) because they had never associated with the odor donors before. This would suggest that the referent being utilized was most likely of self rather than of past association.

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