GENETICS, EXPERIENCE AND STRATEGY AS FACTORS IN THE FOOD HABITS OF PEROMYSCUS: USE OF OLFACTION

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ABSTRACT

GENETICS, EXPERIENCE AND STRATEGY AS FACTORS IN THE FOOD HABITS OF PEROMYSCUS: USE OF OLFACTION

By

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Feeding behavior in Peromyscus maniculatus bairdi and P. leucopus noveboracensis was studied by examining the extent to which food habits could be varied by genetic and experiential manipulation and by measuring the strategy mice use in locating and returning to food. Since these mice use olfaction to locate food, three essential oils were used as odor stimuli with laboratory chow to provide three different food-odor combinations.

Species (genetic) preferences of the mice were tested among three taxa, two stocks of different breeding histories (wild caught and domestic), and several age groups. When the mice were individually presented with all three food-odor combinations, the percentage of the diet consumed from each combination revealed no group differences. All experimental stocks showed a strong pine preference, which provided a baseline for interpretation of the experience and strategy studies.

A series of control experiments determined that the mice were not: (1) discriminating odor intensities; (2) using a dish preference; (3) discriminating soiled from unsoiled food; and (4) preferring pine because of pine shavings in their rearing cages. Also, there were no seasonal shifts in the preference pattern. Use of a position habit was precluded by rotating the positions of the dishes in all experiments.

Modifiability of the food-odor preferences was examined by providing young and adult mice with olfactory experience that would affect their subsequent preferences. Mice were conditioned for two weeks to associate one of the three odors with food. Control mice were conditioned with laboratory chow, but no odor stimuli. Later preferences were determined by: (1) an appetitive test which measured the number of sticks a mouse gnawed through to get its preferred food-odor combination, and (2) a consummatory test which measured the amount of food consumed at each of the three food-odor combinations. Mice were tested both immediately and one month after the conditioning. Throughout the experiment no mouse was ever tested twice. Young *P*. *m*. *bairdi* and young and adult *P*. *leucopus* were significantly affected by the olfactory conditioning, but adult *P*. *m*. *bairdi* were not. Groups that showed a significant experience effect immediately also showed a significant effect one month later.

Feeding strategies used by young and adult mice were tested for 26 days by using an apparatus which automatically monitored feeding activity at three food hoppers containing the three food-odor combinations. Reaction to a novel food-odor stimulus was tested by replacing the anise odored food with a sassafrass food-odor combination for days 21-26. Analyses of the strategy patterns showed that young mice adopted a more conservative strategy than adults during the first ten days (1-10), but all groups used the same strategy for days 11-20. Young *P. m. bairdi* avoided the novel food-odor combination, while the other test groups all consumed more than half of their diet from the new source. Both species shifted from a position habit (days 1-10) to a following response (days 11-20), using an odor cue to locate food, but the shift was more dramatic for *P. m. bairdi*. Adult *P. leucopus* switched feeding sites more frequently than any other group.

The principle conclusion was that *P. leucopus* showed more flexibility in feeding behavior than *P. m. bairdi*. Although young mice of both species showed a pine odor preference and both species were affected by early olfactory experience, the avoidance of a novel food stimulus by *P. m. bairdi* indicated that it was already restricted in its feeding behavior. Also, at maturity *P. leucopus* were affected by olfactory experience, but not *P. m. bairdi*, and the

P. *leucopus* switched feeding sites more frequently than the other test groups. Food habits were discussed in relation to the distributions of these two species of mice.

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INTRODUCTION

The thesis presented here is that variation in food habits among species of small mammals are attributable to differences in the inherited capacity of each species to be affected by ontogenetic experiences. Variations in food habits are the differences in dietary preferences of each species. Variation is also used here as the relative fixity of food preferences between species. Finally, variation refers to the degree to which food preferences can be modified by prior experience in each species. Ontogenetic experiences are in the form of the visual, gustatory and olfactory cues associated with foods encountered during development. The variety of food cues encountered may influence the initiation and maintenance of species differences in flexibility of food habits. For example, if an individual of a less variable species encounters a restricted range of food cues during development its food preferences will be less flexible than individuals of the same species encountering a broad range of food cues, or a different and more variable species encountering a restricted or broad range of food cues during development. This is a verbal statement of a cross-classified design with two species (variable and

non-variable) and two ranges of food cues (restricted and broad). This dissertation was primarily concerned with species differences using restricted sets of food-cues. I also tested the strategy used by small mammals to locate food as a measure of the flexibility of feeding behavior.

My eventual goal is to investigate the role of food habits in the distribution of small mammals. Animals are not distributed randomly in space. Among the factors which have been used to explain the departures from randomness are climatic parameters (Dice, 1922; Johnson, 1926), social influences (Sheppe, 1961, 1967), vegetation type (Wecker, 1963; Harris, 1952), and food habits (Drickamer, 1970). Species food preferences, or those modified by experience could limit small mammal distributions to the habitats where those foods occur. The flexibility in food habits exhibited by a species may also be a predictor of the number of types of habitats it will occupy.

The animals used in these studies were mice of the genus Peromyscus: _Peromyscus maniculatus bairdi_ and _P. leucopus noveboracensis_. These two species use primarily olfaction in finding food (Holling, 1955, 1958; Howard and Cole, 1967; Howard et al., 1968). The odor stimuli used in these studies were three essential oils placed on cotton in the bottom of food dishes filled with laboratory chow. The assumption being made here is that the three odors in combination with the

food are perceived by the mice as three different foods with identical nutritional values.

Genetics

Genetic bases for food habits have been claimed by Barnett and Spencer (1953), and Young (1933). These authors report that rats have specific affinities and aversions for various foods and flavors. Their conclusions are based on observational evidence and not on studies designed to investigate the specific role of genetics in determining food preferences. Experiments have shown that there is a genetic component to the saccharin preferences of the rat (Nachman, 1959), and that the alcohol preferences of laboratory *Mus* are inherited (McClearn and Rodgers, 1961). In pilot work for the current study, *P*. *m*. *bairdi* and *P*. *leucopus*, born in the laboratory to wild caught parents, showed the same preference for seeds from their native field or forest habitat as did their parents (Drickamer, 1970, unpublished). This suggests a genetic component in determining food habits in *Peromyscus*.

In the current study I used olfactory preferences in the place of natural food preferences since the diets comprised of a few natural foods resulted in death and lack of reproduction of the mice. It was necessary to establish a baseline for the olfactory preferences of each experimental stock. Since both young and adult mice of each species were

used, the development and stability of the odor preferences were tested by examining both cross-sectional and longitudinal groups (King, 1969). These groups will distinguish whether young mice develop their preferences as they mature, or whether the preferences exist from the time they first begin to eat food. No differences in food-odor preferences between cross-sectional and longitudinal test groups would indicate a genetic component.

In addition to species and age differences, other stocks were used to test the hypothesis concerning the genetic basis for the olfactory preferences. One of the factors contributing to domestication in *P. m. bairdi* has been relaxed selection pressure in the laboratory (Wecker, 1963; Price, 1967). Testing the odor preferences of wild caught and domestic stocks of *P. m. bairdi* and *P. m. gracilis* could point to any changes that have occurred in the laboratory due to relaxed selection pressures for the olfactory preferences.

The first experiment tested the following specific hypotheses:

(1) All of the different species, age, and genetic stocks will exhibit the same olfactory preferences.

(2) Longitudinal and cross-sectional developmental groups of young *P. m. bairdi* and *P. leucopus* will exhibit the same food-odor preferences.

Experience

The role of early experience in feeding habits is a controversial topic, and the evidence to date is equivocal.

On the one hand, early experience is claimed to be the sole determinant of feeding preferences (Kuo, 1967). Chow dogs, cats and mynah birds were fed on different early regimes and then tested for up to six months. Animals given restricted early feeding experience would not eat new foods, while animals given a variety of foods at an early age would accept new foods (Kuo, 1967). Pre-feeding rats just prior to testing their food preferences altered their choice of foods (Young, 1940). Positive early experience effects have also been reported for other groups of vertebrates including snakes, turtles, and birds (Burghardt, 1967a, 1967b; Burghardt and Hess, 1966; Rabinovitch, 1966, 1969).

In contrast, Bronson (1966) was unable to demonstrate any early experience effect in white rats. He fed young rats plain mynah bird pellets or the same pellets encased in a gelatin capsule. When the rats were tested later the early feeding experience had no effect on their food preference. In much of the work with early experience it has been assumed that young animals were more modifiable than adults (see Beach and Jaynes, 1954).

The specific hypothesis tested in this experiment was:

(1) Young _P_. _m_. _bairdi_ and _P_. _leucopus_ conditioned to eat at one of three food-odor combinations for two weeks will prefer that food-odor combination in subsequent tests, while adult mice of both species given similar olfactory conditioning will not show any experience effect.

Strategy

The strategy that a mouse adopts in locating and returning to feeding places can affect the types of foods eaten. Mice could adopt a completely random strategy. In fact, they do not (Zimmerman, 1965; Whitaker, 1966; Holling, 1965). What are the alternatives?

The mice could use a position habit. This would imply that the mouse returns repeatedly to the same feeding site. Use of this strategy would restrict the mouse to whatever food(s) were available at that site. Another strategy would be for the mouse to associate various odors (or other cues) with foods, enabling the mouse to locate other similar food items on the basis of the food-cue association. This type of strategy is analogous to the visual search images reported for birds preying on various insects (L. Tingergen, 1960; Mook et al., 1960; N. Tinbergen, 1967). In *Peromyscus* the search image would be olfactory rather than visual.

This experiment examined some of the possible strategies that *Peromyscus* could adopt in feeding at the three food-odor combinations. The following experimental hypotheses were tested:

(1) *P*. *m*. *bairdi* and *P*. *leucopus* will both use a following response as opposed to a position habit in locating and returning to preferred food sources.

(2) Young mice will adopt a different feeding strategy than adult mice.

LITERATURE REVIEW

The literature on food habits and factors affecting feeding behavior in _Peromyscus_ will be reviewed by examining: (1) food habits and distribution; (2) the sensory modalities used in the location and selection of food; (3) the genetic bases for food habits; (4) the effects of experience on food habits and preferences; and (5) strategies used in food location.

Food Habits and Distribution

Distribution

The study of factors affecting the distributions of small mammals has evolved from an examination of environmental parameters as constraints on the animal, to considerations of distributions as a function of the animal. The habitat segregation of two species of deermice, _Peromyscus maniculatus bairdi_ (grassland inhabitant) and _P. leucopus noveboracensis_ (woodland inhabitant) has been studied extensively.

One group of hypotheses tested have attempted to find relationships between environmental parameters such as climate, vegetation and soil type, and the distributions of small mammals (Dice, 1922; Johnson, 1926; Hardy, 1945; Verts, 1957).

This type of approach has been termed habitat correlation (Klopfer, 1969). These studies provided predictive associations for habitat characteristics and the presence of a given species of small mammal, but have not provided evidence that the animals are restricted to particular habitats. Both *P. m. bairdi* and *P. leucopus* are capable of living in either grassland or woodland habitat (Dice, 1922).

Methods of measurement of environmental parameters have permitted refined correlations of habitat characteristics and animal distributions, but the small rodent has been considered a machine receiving input from the environment. The systems of the animal, in a predictable machine-like manner, use the incoming sensations in directing the animal's course of behavior, and in controlling its distribution.

Although environmental factors may provide the limits for an animals distribution, a new concept emerged; the existence of psychological factors affecting its distribution within those limits (Lack, 1933, 1937; Klopfer, 1969). According to this hypothesis the animal receives incoming stimuli from the environment (sensation), interprets the stimuli (perception), and makes an active choice of habitat. Psychological factors affecting habitat selection in mice include: (1) vegetation type and form; (2) amount of cover; (3) nest sites; (4) social influences; and (5) food habits. In some species, such as *Microtus* (a herbivore) hunger may override a preference for a particular habitat location, leading to

movement to a new habitat with more food. In *Peromyscus* (an omnivore) foods appear to be so abundant in the habitats that hunger is probably not a factor driving the mice into new habitats in search of food. Only the general form and types of vegetation have been examined in experimental studies of habitat selection in small rodents. Subspecies of *Peromyscus* selected an artificial habitat which most nearly resembled the vegetation form in their native environment (Harris, 1952). In another study, *Microtus* selected an artificial habitat similar to their normal one, but *P. leucopus* failed to select between the two sets of grassland cues presented (Wirtz and Pearson, 1960). This raises questions concerning the validity of the two models of grassland cues used to test a forest species.

Psychological factors in distribution are in part determined by inheritance (Wecker, 1963). That this genetic base may be modified by experience is indicated by the strong preferences grass-reared *P. m. bairdi* showed for a grassland over a woodland habitat, whereas *P. m. bairdi* reared in a woodland enclosure did not prefer that habitat (Wecker, 1963). These results may be explained by a genetic predisposition (an inclination before the actual choice) to select a grassland habitat (Wecker, 1963, 1964). The early grassland experience reinforced this predisposition. Wecker also suggested an apparent relaxation of selection pressures in a stock of *P. m. bairdi*, maintained in the laboratory for 15-20

generations, as an explanation for their failure to select the grassland habitat over the forest. It appears that for vegetation type there is an interaction of a genetic predisposition to respond to certain cues and the early experience of the mouse. In a similar manner, the food habits of grassland and woodland _Peromyscus_ could have a genetic basis reinforced by experience.

Food

The correlation between the availability and abundance of foods in the habitat and the diets of small rodents has been determined by gut analyses (Hamilton, 1941; Whitaker, 1963, 1966; Thompson, 1965). Although predictions can be made from these correlations, experiments should: (1) examine the feeding behavior per se, or (2) study the relationship between diet and choice of habitat.

The diet of _Microtus_ was comprised of a very few foods selected from those that were abundant in the environment (Zimmerman, 1965). Whitaker (1963, 1966) found that _Peromyscus_ and _Mus_ were eating only a selected few of the foods available in their habitats. This suggests orderly processes underlying the feeding habits of small rodents. These selective feeding behaviors, or strategies, adopted by the mice, cannot be determined from gut analyses alone, but require systematic observation of the feeding behavior and pattern. Further evidence for an orderly feeding process comes from Holling (1965). _P_. _leucopus_ varied in their

selection of sawfly prepupae depending upon the alternate foods available and on the relative palatabilities of these foods.

Selective feeding behavior may explain, in part, the observed distributions of the mice. If mice learn to eat the foods available in their natal habitat through experience, they will remain in that habitat where those foods are available. Two hypotheses may be tested. One is that experience can affect the food habits of the mice. This I am testing in the present thesis. The other hypothesis is that adult wild caught mice from different habitats will select foods from their natural diets when given a choice among foods from a variety of habitats. This hypothesis has been tested (Drickamer, 1970). *P. m. bairdi* and *P. leucopus* preferred seeds from their own grassland and forest habitats respectively in a choice test.

Studies of food habits and distributions of small mammals have lead to the following conclusions: (1) distribution is not only a function of the environmental parameters, but also the animal actively selects its habitat; (2) studies of habitat selection indicate that inherited predispositions to respond to certain environmental cues may interact with experience in affecting the animals' choice of habitat; and (3) mice choose their diet selectively from the foods that are abundant in their environment.

Sensory Cues

Olfaction in deermice was tested by their ability to locate and dig up buried food under conditions of low light and total darkness (Howard et al., 1968). Mice were able to locate preferred foods and dig them up with equal facility under either lighting condition, indicating that olfaction could be used exclusively. *Peromyscus* also locate, differentiate and dig up prepupae of the European sawfly (*Neodiprion sestifer*) using olfaction (Holling, 1955, 1958). Healthy prepupae were clearly selected over dead or parasitized animals. Larger prepupae could also be differentiated from smaller ones. Holling concluded, through a series of elimination experiments, that the mice were using olfaction in locating the buried food and in digging up the healthy prepupae. The eating phase of the feeding sequence involved both the gustatory and olfactory modalities.

Genetics

Two approaches have been taken in the investigation of genetic factors and food habits: (1) measures of specific preferences, particularly in young animals, for foods that have not been previously encountered; and (2) genetic manipulation studies involving selection and breeding experiments.

Young rats with no prior experience exhibited preferences for special food items when presented with a variety of foods

(Harlow, 1932). In similar studies Barnett and Spencer (1953a, 1953b) and Young (1932) have reported preferences and aversions for a variety of foods and tastes in the rat. None of these studies have eliminated prior feeding or maternal influences as experiential factors contributing to the observed preferences. Genetic bases for food habits have also been demonstrated in other organisms. Newly hatched snakes showed preferences for foods from the natural environment of their parents (Dix, 1968). Species differences in the amount of tongue-flicking toward animal skin extracts by newborn snakes also correlated with adult food preferences for the species (Burghardt, 1967a). For *Peromyscus* it has been shown that mice born in the laboratory will select seeds from the natural habitat of their parents when given a choice among a variety of seeds (Drickamer, 1970, unpublished). Pilot work for the present studies showed that *P. m. bairdi* and *P. leucopus* preferred pine odored food to anise or wintergreen, and that experience with an odor other than pine would not completely eliminate the species preference for pine.

Genetic manipulation studies have been conducted with taste preference. A genetic basis for taste preference in rats has been demonstrated by successfully selecting two lines, one for saccharin preference and the other for a lack of preference (Nachman, 1959). McClearn and Rodgers (1961) demonstrated, through the use of inter-strain crosses, that

alcohol preference was inherited in laboratory Mus musculus. The genetic manipulation method is dependent upon observable behavioral differences which may then be selected or cross-bred to determine their genetic bases.

Strategy

The hypotheses proposed in my introduction suggest two areas for reviewing the literature on feeding strategies: (1) the alternative strategies which could be employed by the mice, and (2) the changes in the strategy that occur when a novel food source is introduced.

Data from field studies of food habits in Peromyscus suggest that the mice are selecting their diets from the food items that are abundant in the environment (Whitaker, 1963, 1966; Hamilton, 1941). Gut analyses show that the bulk of the diet of these mice is comprised of a very limited number of food items (non-random feeding). Thus the diet of the mouse reflects both the abundance of various food items in the habitat, and a degree of selectivity on the part of the mouse in choosing its diet. Zimmerman (1965) showed that Microtus exhibited selectivity, even among the very abundant food plants in their surroundings. What strategy(s) were the mice using to select their diet from all the foods available?

The use of a position habit is one alternative. Rats will return to the same location repeatedly for food and may

fail to locate the food if its location is shifted (Young, 1938, 1945; Barnett, 1963). The use of a position habit by a small mammal in nature would restrict its food sources to those food items that were available in the immediate area to which it repeatedly returned for food.

The mice could be using a mechanism to find food that is analogous to the visual search images reported for birds locating invertebrate prey (L. Tinbergen, 1960; Mook et al., 1960). The search image concept implies that the animal pays selective attention to certain cues characteristic of the prey species, or in other words there is some change in the stimulus filter, either peripheral, or central. This means that as the animal moves about randomly in its environment, it encounters an edible prey item, makes a cue-food association and then locates additional similar prey in the area (Holling, 1965; N. Tinbergen et al., 1967). Prey at very low densities have a low risk of being eaten in this system. Their numbers are too low for the formation of a search image by the predator. At very high densities the predator drops the search image in order to insure a varied diet, rather than a diet comprised all of one prey species (L. Tinbergen, 1960). Prey at moderate densities are most subject to the formation of search images in this system.

In a predation model Holling (1959) proposed that mice (P. leucopus) learn to locate and consume their prey. This learning process is characteristic of the formation of search images. As Peromyscus learns the olfactory cues associated with healthy prepupae, they develop a search image and find more prey in each localized area where they feed. The diet of Peromyscus (Whitaker, 1966) in the wild does not correlate with the list of all abundant foods in the habitat. The fact that the mice select only a few of the foods to eat suggests that they may use several search images at one time. The long list of items which show up irregularly in the mouse's diet might be present in the environment at densities too low for the formation of search images. Search images might also be used by the mice to locate other clumps of prey similar to the ones already consumed.

The reaction of the mouse to novel foods in the environment is an important part of its strategy. Wild strains of rats (Rattus norvegicus) will avoid novel stimuli, while domesticated white rats explore and sample new foods (Chitty and Shorten, 1946; Barnett, 1953, 1956; Richter, 1953). No comparisons have been made between young and adult rats for their reaction to novel, but natural, food stimuli. Wild rats will learn to avoid poisoned food through experience by consuming sublethal doses of the bait (Richter, 1953). This toxophobia is learned more rapidly by young rats than adults. The reaction to novel foods may wane with time (Thompson as cited in Barnett, 1963). Young chimpanzees avoided novel objects for a period of time after they were placed in their environment, while adults made contact with the new objects

soon after they were introduced (Welker, 1956). As the chimpanzees acquired more experience their fear of new objects disappeared. If young mice use a conservative feeding strategy when they first leave the nest, they will learn to eat only the foods that are in the area of the natal home site. Young mice should then show a strong avoidance to novel foods. As the young mice disperse, they encounter many new types of foods and the neophobia wanes.

Experience

The effect of early feeding experience on food preferences is a controversial subject. Positive effects of early feeding experience have been demonstrated in several vertebrate groups, including turtles (Burghardt and Hess, 1966; Burghardt, 1967b), birds (Rabinovitch, 1966, 1969; Kuo, 1967), and cats and dogs (Kuo, 1967), but in small rodents the evidence to date is equivocal. There are at least two schools of thought regarding early experience effects on food habits.

One school claims that the food habits of adult animals are entirely a function of experience (Kuo, 1967). Cats, dogs and mynah birds, given either a restricted or a varied early experience, showed entirely different behaviors when tested with a new food. Animals reared on a restricted regime avoided new foods, while animals reared on varied diets ate new foods readily (Kuo, 1967). Kuo's major failing was that he did not account for any inherited preferences or aversions for his various early feeding regimes and test foods, that is he does not provide any baseline data on the food preferences and reactions of naive animals. The most that can be said from Kuo's statements (no data are presented) is that the food habits of the animals tested were modified by experience.

The second school of thought hypothesizes that each species exhibits baseline preferences (inherited) which may be modified by experience. Zebra finches normally prefer red millet or white millet to canary seed (Rabinovitch, 1969). Birds reared on each of these different seeds exhibit different preferences according to their rearing regime. Those reared on either red or white millet select that seed in choice tests, but finches reared on canary seed selected both canary seed and millet in preference tests. The inherited predispositions of the birds to prefer the millet interacted with their early feeding experience with canary seed.

A number of studies have tested the effects of early feeding and taste experience in small mammals, without adopting either of these schools of thought. The results of these studies have been contradictory. Some authors (Harlow, 1932; Kuo, 1967) have reported positive early experience effects. Other investigators have shown no significant early experience effects (Bronson, 1966; Forgus and Hutchings, 1960). Still another group of authors (Warren and Pfaffman, 1959; Siqueland, 1965; Thios et al., 1962) have reported intermediate results, with positive effects right after the experience, but no lasting impact.

Rats given a choice between two completely adequate diets, one of which had been their normal food prior to the test, preferred the diet to which they were accustomed. This preference may have resulted from a combination of experience effects, knowledge that the normal food was quite adequate, and a neophobia for the new food. The work of Kuo (1967), outlined above, showed strong and lasting effects of early feeding experience.

Studies providing rats with early taste experience with bitter substances like sodium-octa-acetate (SOA) and hydrogen chloride (HCl) have demonstrated that the preferences of the rats were positively affected immediately after the experience (Warren and Pfaffman, 1959; Siqueland, 1965). These same rats retested after an intervening period showed no effects of the experience.

Thios et al. (1962), provided young rats with experience drinking water solutions of three different concentrations of sodium chloride (NaCl). When the rats were given their choice of all three solutions, each group preferred the concentration at which they had been given early experience. The results of these taste experiments all indicate that experience may be effective for a short period, but no long-term effects are recorded. This, I feel, may be the result of the aversive taste stimuli used in the experiments. The use of neutral or

pleasant stimuli in similar experiments will be needed to determine the true effect of early taste experience.

Rats given early drinking experience with kerosene tainted water, did not show a significant preference for the tainted water when tested immediately after their experience (Forgus and Hutchings, 1960). Lasting effects were not tested. Young rats reared with mynah bird pellets, either plain or in a gelatin capsule, showed no significant early feeding experience effects (Bronson, 1966). Bronson also tested the possibility that the earliest food might acquire special reward value that was lasting in effect. Again the results indicated no effect of the early feeding.

The question of the effects of early feeding and drinking experience on food habits or taste preferences remains unanswered. Several important criticisms pertinent to all of the above studies could, I think, indicate the reasons for the conflicting results which have been reported. These include: (1) lack of adequate sample sizes; (2) re-use of animals from one test to another, thus confounding the second test with the additional experience gained during the first test exposure; (3) failure to determine the baselines of the species preferences for the substances used; and (4) the use of aversive taste and/or food stimuli.

GENERAL METHODS

Experimental Subjects

The mice used in these studies were from three taxa: *Peromyscus maniculatus bairdi*, *P. m. gracilus*, and *P. leucopus noveboracensis*. Wild caught *P. m. bairdi* and *P. leucopus* were trapped at the Rose Lake Wildlife Experiment Station, Bath, Michigan. Wild caught *P. m. gracilis* were trapped at the Dunbar Forestry Station, Channel View, Michigan. First generation laboratory (F_1WC) stocks used in the studies were the offspring of the above wild caught mice. Domestic (dom.) stocks of *P. m. bairdi* and *P. m. gracilis* have been laboratory bred for nearly 20 years (see King, Price and Weber, 1968).

Maintenance

Four separate rooms were used to house the mice during these studies: (1) a breeding chamber maintained at 65-78° F; (2) a holding room maintained at 68-73°F; (3) an experimental test room maintained at 68-73°F; and (4) an experimental chamber used for conditioning the mice, maintained at 62-77°F. All chambers were fixed on a 14 hours light, 10 hours dark cycle throughout the experiments.

Relative humidity in all the chambers varied from 20 to 60 percent. All rooms and chambers contained ventilation fans to maintain the air circulation.

Mice were housed in plastic laboratory cages measuring 6" by 12" by 6" deep, with fitted wire mesh lids. Purina Laboratory Mouse Chow and water were provided *ad libitum* throughout the studies. Prior to the experimental procedures mice were provided with pine shavings bedding and cotton nesting material in their cages.

Young mice were housed with their parents until weaning at 21 days of age. Mice used as adults (90-150 days of age) were housed as litter mates (bisexual groups) until the experimental procedures were begun. All experimental tests were made on mice housed individually without pine shavings.

Odor Stimuli

The odor stimuli for all of the studies were three essential oils: pine, wintergreen, and anise, obtained from Magnus, Maybee and Rhynard Inc. (Paramus, New Jersey). Three separate food-odor combinations were obtained by placing three to five drops of oil on cotton in the bottom of a round metal food dish (one inch deep and three inches in diameter) filled with laboratory chow. The food was covered with a metal retainer with large holes which enabled the mice to gnaw the food without removing it.

Dependent Variable

Food consumption has been used previously as a dependent variable in studies of early experience effects (Rabinovitch, 1969; Burghardt, 1967b; Bronson, 1966). Some authors have used absolute weights of food eaten as a dependent variable in comparing animals and treatment groups. This measure totally ignores the weight of the animal which may vary widely. Thus, a larger animal, consuming more food, will contribute more to an overall measure of preference than a small animal eating lesser quantities of food. In order to avoid this error, the total weight of food consumed must be divided into the consumption from each food container for each mouse. This produces a dependent variable which is the percentage of the diet taken from each alternative food choice. A day by day computation provided the dependent variable used throughout these studies. Weights were taken to the nearest 0.1 gram on one of two scales; a Shadowgraph Scale (Exact Weight Scale Company, Columbus, Ohio), or an Autogram 1000 Scale (O'Haus Corporation, Union, New Jersey).

Use of the percentages of the diet eaten from each of the three food-odor combinations introduces the problem of dependence of each percentage. Since an animal has a limit to the amount of food it can consume during any given time period, consumption at one dish early in the activity period limits the amount of food that can be consumed from the other

dishes during the same day. Thus, all of the analyses conducted were done separately for each food-odor combination. This method of analysis technically permits the use of only two of the three food-odor combinations as there are only two degrees of freedom. To obtain a clear and complete picture of the odor preference patterns, I have analyzed all three combinations.

GENETIC FACTORS

Purposes

The purposes of this first experiment were: (1) to determine the species specific odor preferences among the three food-odor combinations; (2) to test for species and age differences in odor preferences; (3) to determine whether domestication has altered the odor preferences of the mice; and (4) to test the hypothesis that young mice tested repeatedly (longitudinal), or only once (cross-sectional), at various ages, have the same odor preferences.

Test Subjects

Eight groups of 10 adult mice each were tested. These eight stocks were: (1) wild caught _P_. _m_. _bairdi_; (2) F_1WC _P_. _m_. _bairdi_; (3) domestic _P_. _m_. _bairdi_; (4) wild caught _P_. _m_. _gracilis_; (5) F_1WC _P_. _m_. _gracilis_; (6) domestic _P_. _m_. _gracilis_; (7) wild caught _P_. _leucopus_; and (8) F_1WC _P_. _leucopus_. Young mice used were all from F_1WC stocks of _P_. _m_. _bairdi_ and _P_. _leucopus_. One test group of eight mice of each species was tested longitudinally and 10 groups of eight mice each, from each species, of various ages were tested cross-sectionally. Throughout the experiment no two mice from the

same litter were used in any of the test groups and no mouse was used more than once.

Procedure

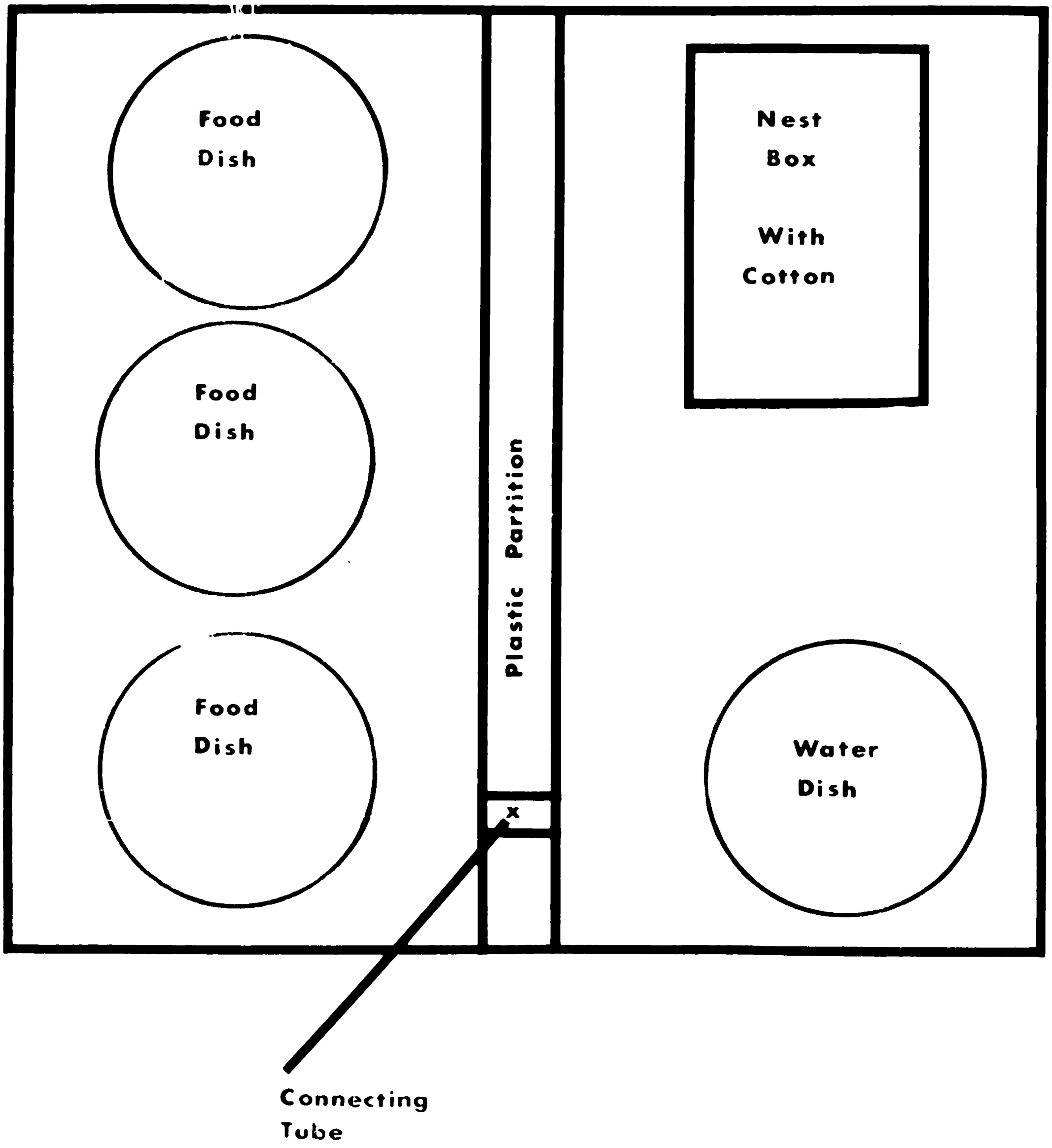
Each mouse was housed individually in a double plastic cage (Figure 1), comprised of two of the plastic cages previously described connected by a one inch section of one inch diameter Plexiglas tubing. One side of the cage contained a nest box, nesting cotton and water. The other side of the cage contained the three food dishes, each with a different food-odor combination.

Adult mice were weighed and placed in the cage with three weighed food dishes. At three day intervals for 27 days the mouse and food dishes were weighed. At each weighing the cotton with odor stimulus was changed, and the positions of the food dishes were rotated in a random sequence to prevent the development of a position habit. Food was added where necessary to maintain adequate food levels in all the dishes.

Young mice were tested identically except that the longitudinal groups were weighed every two days. These mice were tested from weaning at 21 days of age until they were 61 days of age. Cross-sectional groups began at 21, 25, 29, 33, 37, 41, 45, 49, 53 and 57 days of age and mice in each group were tested for a two day interval.

Figure 1. Schematic drawing of the double plastic cage (top view with wire lid removed). One inch in the diagram equals two inches of the actual cage and equipment.

FIGURE 1


Food
Dish
Food
Dish
Food
Dish
Plastic Partition
x
Nest
Box
With
Cotton
Water
Dish
Connecting
Tube

Food consumption was obtained by subtracting the weight of the food dish at the end of the time interval from the weight of the dish at the beginning of the interval. This value was corrected for humidity changes by a factor determined from control dishes placed in the experimental chamber. The corrected weight of food consumed from each dish was then used to compute the dependent variable; the percentage of the diet taken from each food-odor combination per day.

Analysis

For young mice tested longitudinally and all adult groups a mean percentage and standard error was calculated and graphed. The data were analyzed within each odor across the ten test groups using a one way analysis of variance followed by Duncan's New Multiple Range test (Li, 1964). This analysis tested for differences in species preferences, ages, and between wild caught and domestic stocks. Variance in preferences (called here fixity) was computed within each test group across the three odors. A mouse with complete fixity would take 100 percent of its food from one of the food-odor combinations and no food from the other two combinations, which would produce a variance across odors of 3333.33. For each test group the variance computed was divided by this figure of 3333.33 to produce a ratio. Any deviation from complete fixity would result in a ratio less than 1.00. The lower the ratio computed in this manner, the

less fixed are the odor preferences of that particular group.

Differences between cross-sectional and longitudinal test groups of young mice were tested within each odor using a parametric paired t-test. The basis for pairing was the age variable. Mice in the two groups were compared for the same ages. These tests were made within each odor to insure the independence of the measures on each food-odor combination.

Results

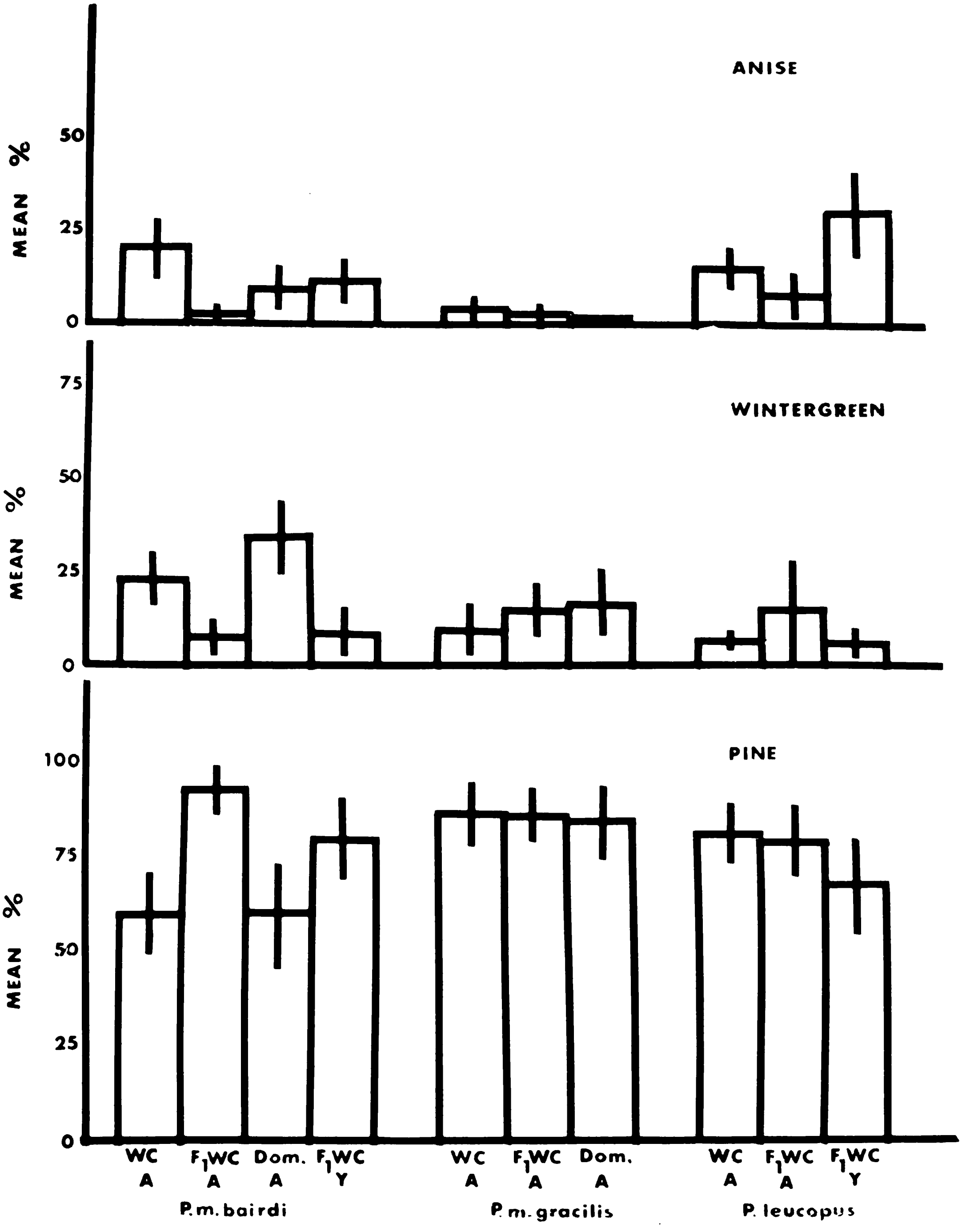
Results of the first experiment are shown in Table 1 and Figure 2. The one way analyses of variance within each odor showed no significant differences across the ten test groups for any of the three food-odor combinations (anise, $F=0.10$, $DF=9,86$, $p > .10$; wintergreen, $F=0.95$, $DF=9,86$, $p > .10$; pine, $F=0.41$, $DF=9,86$, $p > .10$). The Duncan's Multiple Range tests run on each analysis failed to show any significantly different groups. In all groups the pine odored food was preferred (Figure 2). Pine comprised from 58 percent of the diet for wild caught and domestic *P. m. bairdi*, to 92 percent of the diet for the F_1WC *P. m. bairdi* stock. Young F_1WC *P. m. bairdi* and *P. leucopus* also showed a strong preference for the pine odored food. Wintergreen was the second most preferred food-odor combination in eight of the ten test groups. Mice ate from this odor for between

Table 1. Mean percentage (± 1 S. E.) of the diet consumed from each of the three food odor combinations.

Species	Age	Strain	Odor			Variance Ratio
			Anise	Wintergreen	Pine	
P. m. bairdi	Young	F_1WC	10.7(4.5)	9.0(4.5)	80.3(8.9)	0.50
P. m. bairdi	Adult	WC	19.2(8.1)	22.2(8.4)	58.7(10.0)	0.15
P. m. bairdi	Adult	F_1WC	2.4(2.0	5.8(4.2)	91.9(6.2)	0.77
P. m. bairdi	Adult	Dom.	7.9(5.6)	34.6(10.6)	57.5(11.7)	0.18
P. m. gracilis	Adult	WC	3.5(2.1)	11.2(4.7)	85.4(4.7)	0.61
P. m. gracilis	Adult	F_1WC	1.8(1.4)	13.6(6.6)	84.6(6.6)	0.61
P. m. gracilis	Adult	Dom.	0.8(0.8)	16.1(8.5)	83.1(8.4)	0.57
P. leucopus	Young	F_1WC	29.0(10.9)	3.7(1.6)	67.3(11.8)	0.31
P. leucopus	Adult	WC	12.8(6.2)	6.0(1.9)	81.2(7.4)	0.52
P. leucopus	Adult	F_1WC	5.7(5.7)	15.0(14.1)	79.3(9.5)	0.48
	Total		9.4(0.2)	13.7(0.5)	76.9(0.7)	

Figure 2. Mean percentage (± 1 S. E.) of the diet eaten from each of the three food-odor combinations (A=adult; Y=young; WC=wild caught; F_1WC=first generation laboratory reared; Dom.=domestic).

FIGURE 2


ANISE
WINTERGREEN
PINE
MEAN %
0
25
50
75
100
WC A
F1WC A
Dom. A
F1WC Y
WC A
F1WC A
Dom. A
WC A
F1WC A
F1WC Y
P. m. bairdi
P. m. gracilis
P. leucopus

4 percent (young F_1WC *P. leucopus*), and 35 percent (domestic adult *P. m. bairdi*) of their diet. Anise odored food was not eaten for more than 15 percent of the diet except by adult wild caught *P. m. bairdi* (19 percent) and young F_1WC *P. leucopus* (29 percent). Across all groups the average diet composition was 9.4 (± 0.2) percent anise, 13.7 (± 0.5) percent wintergreen, and 76.9 (± 0.7) percent pine. These data showed a strong preference for pine odored food and no significant differences in the preferences across all the test groups within each odor. No differences were found between young and adult mice, among species, or between wild caught and domestic stocks of either *P. m. bairdi* and *P. m. gracilis*.

The mice in these groups were tested for periods of time ranging from 27 days (adult mice) to 40 days (young mice). No measurable deviations from the observed preference levels were recorded during these time periods.

Young mice with little or no prior feeding experience showed the preference for pine odored food immediately. Comparisons of the cross-sectional and longitudinal groups of young mice, using t-tests, revealed no significant differences for any of the three food-odor combinations (pine, $t=0.8$, $p > .10$; wintergreen, $t=0.9$, $p > .10$; pine, $t=1.1$, $p > .10$; all with DF=7). Groups of young mice tested once showed the same pine preference at each test age as mice of similar ages tested longitudinally.

Examination of the degree of fixity of these odor preferences (Table 1), as measured by the within group across odors variance, showed that F_1WC *P. m. bairdi* adults showed the most fixity (ratio=0.77), while the other two adult *P. m. bairdi* groups showed the lowest degrees of fixity (ratio=0.15 and ratio= 0.18). *P. m. gracilis* showed a consistently high preference fixity (ratio $>$ 0.57). The ratios for the two groups of young mice were relatively low among the test groups, being 0.50 for *P. m. bairdi* and only 0.31 for *P. leucopus*. Adult *P. leucopus* also showed a lower degree of fixity than most of the other test groups (ratio=0.48).

CONTROL EXPERIMENTS

A number of control experiments were necessary to establish: (1) that the mice were not using a position habit; (2) that the mice were not differentiating intensity changes within an odor; (3) that the pine shavings in the rearing and holding cages had no effect on the preferences; (4) that the mice could not differentiate soiled (used by other mice) food from unsoiled food; (5) that dish preferences were not present; and (6) that seasonal shifts in the species odor preferences did not occur. The first control, that for position habit, was accomplished by rotating the positions of the dishes in a random sequence, at least once every three days. This rotation method was used throughout all of the experiments. The other controls for dish preference, intensity, seasonal shifts, food condition and shavings experience were tested as described separately below.

Control for Odor Intensity

The cotton with the odor stimulus at the bottom of each food dish was changed every three days. While I was unable to control for possible differences in the vapor pressure and intensity present for each odor, this method of replacement kept the same intensity present for each odor. It was

necessary, however, to make certain that the mice could not differentiate fresh odor, just placed in the bottom of the food dish, from odor three days old. As an extra margin the determination was also made for odors six days old.

Young and adult F_1WC _P_. _m_. _bairdi_ and _P_. _leucopus_ were tested with 12 mice per group. Each mouse was presented with three food dishes in the double plastic cage apparatus described previously (Figure 1). The three food dishes contained the same odor stimulus which had been put on the cotton at different days. One dish was supplied with food and odor six days prior to the start of the test, one dish was set up three days prior to the beginning of the test, and one dish was prepared with odor stimulus and food on the day the test began. In effect this created a graded series of intensities representing 0, 3 and 6 day old odors. Each dish and the mouse were weighed for two three day intervals. A fresh dish was added in place of the 6 day old dish at the middle weighing interval to maintain the same graded series of intensities.

The dependent variable was the percentage of the diet taken from each of the three odor intensities. The data were the same for the two time intervals, so the results were computed over the entire six day test period. The results were analyzed separately within each odor. The twelve mice in each age and species group were randomly divided into three groups of four mice each. For each mouse in the first group

Table 2. Summary of the results of the intensity control test. Mean percentage (± 1 S. E.) of the diet eaten from each intensity.

Odor		Anise			Wintergreen			Pine		
Intensity		0	3	6	0	3	6	0	3	6
Group	N									
P. m. bairdi										
Young	12	41.6 (11.5)	23.7 (8.4)	34.7 (11.1)	29.0 (12.6)	32.9 (13.3)	34.5 (12.9)	40.3 (12.5)	30.6 (12.6)	29.1 (11.4)
Adult	12	29.6 (11.2)	40.0 (10.7)	30.5 (12.8)	21.6 (12.6)	29.4 (12.0)	49.0 (12.6)	28.0 (11.9)	37.9 (13.9)	34.1 (8.9)
P. leucopus										
Young	12	16.8 (10.8)	38.6 (15.6)	44.6 (14.4)	37.4 (13.3)	29.4 (10.1)	33.2 (11.5)	33.7 (12.8)	33.8 (8.2)	32.6 (12.8)
Adult	12	27.8 (12.4)	34.0 (11.7)	38.3 (11.4)	47.6 (15.1)	28.3 (13.0)	24.1 (19.0)	34.4 (13.7)	26.4 (9.0)	39.2 (11.9)

only the percentages for the 0 day intensity were used. Similarly the 3 and 6 day intensity data were used from the mice in the other two groups. In this way the results could be analyzed across the different intensities without violating the assumption of independence. Within each odor a three way analysis of variance was run using three levels of intensity, two species, and two ages. The three analyses showed no significant species, age, or intensity differences within any of the three odors. The F-ratios for intensity differences within each odor were; pine, $F=1.3$, $p > .10$; wintergreen, $F=1.4$, $p > .10$; anise, $F=1.4$, $p > .10$; each with $DF=2,37$. Thus, changing the cotton with the oil odor stimulus at three day intervals controlled for constant intensity within each odor and for constant relative intensities between different odors.

Control for Dish Preference

It might be argued that the preferences in the first experiment were due to the mouse marking a preferred dish in some manner, and then returning to the dish, regardless of the odor stimulus present or its position. To test dish preferences I presented each mouse with three food dishes, all with the same odor and intensity and rotated the positions of the dishes every other day. The amount of food consumed at each dish was measured for three intervals of three days each. Young and adult F_1WC *P. m. bairdi* and

P. leucopus were tested with sixteen mice in each group. From this total, groups of four mice each were tested with anise, wintergreen and pine odor and with plain laboratory chow, the control stimulus in later experiments.

The dependent variable was the percentage of the diet taken from each dish per day. The results were analyzed for dish preference within each mouse, using a Kruskal-Wallis one-way analysis of variance (Siegel, 1956). The results of these analyses are shown in Table 3 as the ratio of the number of animals exhibiting a dish preference out of the four mice tested within each group. The analyses indicated that only 3 mice out of 64 tested showed a significant dish preference. There were no trends within species, ages, or the different food-odor combinations tested. The result obtained, that is three significant cases of dish preference out of 64 tests, would be expected on the basis of chance. The mice in these studies were not using a dish preference.

Seasonal Control

Experiments on species odor preferences were initially conducted during January of 1969. The strategy experiments were not concluded until September of 1969, and the bulk of the experience study was conducted during the summer months. A control experiment tested the hypothesis that the odor preferences of the mice had not changed from January to July. The procedures were exactly as described for the initial

Table 3. Summary of the dish preference control test. The ratio presented is the number out of four animals tested in each group showing a significant dish preference.

Group	N	Odor Anise	Wintergreen	Pine	Control
P. m. bairdi					
Young	16	0/4	0/4	1/4	0/4
Adult	16	0/4	0/4	0/4	1/4
P. leucopus					
Young	16	0/4	0/4	0/4	0/4
Adult	16	0/4	1/4	0/4	0/4

determinations of species odor preferences, except that only two groups, adult F_1WC P. m. bairdi and P. leucopus were tested. As before, 10 mice were tested in each group for nine three-day intervals. The dependent variable was the percentage of the diet taken from each food-odor combination. To test the hypothesis concerning seasonal shifts in odor preferences, paired parametric t-tests were used. Each odor was analyzed separately within each species. The basis for pairing the January and July test animals was the time interval variable.

The results are shown in Table 4 where the means and standard errors are presented along with the t values and probabilities. Odor preferences in these groups did not shift significantly ($p > .10$) from January to July. This makes it unlikely that the odor preferences within any of the stocks changed during the total experimental period of 10 months.

Shavings Effect

All of the mice used in these studies were housed with pine shavings bedding until the start of the test procedure. Young mice were on shavings for up to 21 days and adult mice from birth until 90-150 days of age. The pine shavings smelled similar to the pine odor used in these studies. This control experiment tested the hypothesis that mice

Table 4. Results of the seasonal control test. Mean percentage (± 1 S. E.) of the diet eaten from each food-odor combination.

Group	N	Odor	January	July	t(DF=8)	P
P. m. bairdi	20					
		Anise	2.5(1.3)	5.4(1.5)	0.20	NS
		Wintergreen	12.8(0.7)	15.8(4.4)	1.28	NS
		Pine	84.7(2.0)	78.8(3.9)	1.08	NS
P. leucopus	20					
		Anise	10.8(5.2)	15.1(7.4)	0.79	NS
		Wintergreen	9.7(3.3)	14.7(3.1)	0.42	NS
		Pine	78.9(3.2)	71.2(8.8)	0.71	NS

reared either with or without the pine shavings in the cage would show the same odor preferences.

Pregnant mice were placed in bare plastic cages with only a small amount of nesting cotton and the necessary laboratory chow and water. The offspring were raised from birth in a room without any of the pine shavings. At 21 days of age the mice were given the standard species odor preference test with all three food-odor combinations. Both *P. m. bairdi* and *P. leucopus* young were tested. The test period lasted six days. Four mice were tested in each group, each mouse from a different litter. These mice were compared with another group raised under standard laboratory conditions with pine shavings and in the mouse colony room, where the shavings odor and other possible olfactory stimuli were always present.

Mann-Whitney U nonparametric t-tests were used to compare these groups (Siegel, 1956). Again the odors were tested separately because they lacked independence if tested in the same analysis. None of the U values was significant, indicating that the species preferences for pine odor were not the result of being raised on pine shavings (Table 5).

Soiled Versus Unsoiled Food

The food used in these studies was stored for up to three weeks in large bins, and re-used. Each bin contained food of only one odor. The food in each bin was mixed before

Table 5. Results of the test for the effect of pine shavings on food-odor preferences. Mean percentage (± 1 S. E.) of the diet eaten from each food-odor combination.

		Odor		
Group	N	Anise	Wintergreen	Pine
P. m. bairdi				
Shavings	4	8.3(1.1)	15.3(2.4)	76.4(3.2)
No shavings	4	7.0(1.2)	12.6(2.3)	80.4(1.6)
U(DF=3) =		5	7	5
Prob.		NS	NS	NS
P. leucopus				
Shavings	4	5.8(3.5)	11.9(2.5)	82.3(2.6)
No shavings	4	6.1(1.5)	13.7(1.7)	80.2(1.0)
U(DF=3) =		6	5	6
Prob.		NS	NS	NS

Table 6. Results of the test for soiled versus unsoiled food. Mean percentage (± 1 S. E.) of the diet eaten from each food type.

Group	N	Unsoiled Food	Soiled Food
P. m. bairdi	8	44.5(10.6)	55.5(11.7)
P. leucopus	8	53.6(8.9)	46.4(9.7)

re-use to insure a random mixture of the age of the food. This control experiment tested the hypothesis that the mice did not discriminate between re-used and unused food. Both foods were a mixture from bags of food opened one to three weeks previously. Thus, the age of the food was not a factor.

One group of eight adult mice was tested from each species: P. m. bairdi and P. leucopus. Two food dishes were presented to each mouse, one with previously used food and one with the unused food. The food dishes were weighed for two time periods of two days each. The data were lumped for the entire experimental period and the dependent variable was calculated as the percentage of the diet taken from each type of food.

Results of the analysis of variance performed on these data (Table 6) showed no significant species effects ($F=1.45$, $p > .10$, $DF=1,12$), food effects ($F=0.97$, $p > .10$, $DF=1,12$), or interaction effects ($F=1.68$, $p > .10$, $DF=1,12$). The results of this test indicated that the mice were not discriminating between previously used and unused food.

EXPERIENCE

Purposes

This experiment tested the effect of prior olfactory experience on the preference for a particular food-odor combination by examining: (1) whether young mice were more affected by the experience than adult mice; and (2) whether there were any species differences in modifiability of odor preferences.

Test Subjects

Four groups of mice were used in this study: (1) young *P. m. bairdi*; (2) adult *P. m. bairdi*; (3) young *P. leucopus*; and (4) adult *P. leucopus*. All mice were from F_1WC stocks. The young mice used were all 21 days of age at the beginning of the experiment and the adult mice were between 90 and 150 days of age (mean = 121 ± 2). A one-way analysis of variance was used to test the hypothesis that the average age of the adult mice was the same for each test group. The data and the analysis are presented in Table 9. There were no significant age differences across the test groups ($F=0.26$, $p > .10$, $DF=7,184$).

Procedure

The design of the entire experiment is summarized in Tables 7 and 8. The independent variables manipulated were: (1) age at time of experience; (2) age at time of testing; (3) quality of experience; (4) types of tests used; and (5) genetics (see King, 1958).

The apparatus used in the conditioning process was the double plastic cage previously described (Figure 1), with food dishes on one side and water and nesting cotton on the other side. Each mouse was conditioned individually for two weeks with all three food-odor dishes present, but only one food-odor combination available for eating. Control mice were presented with three dishes, all containing laboratory chow, but no oil odors. A mouse was trained to associate food with one of the odors by allowing it to eat from only the dish with the conditioning odor. The other two food-odor combination dishes were blocked by a wire mesh screen that prevented the mouse from obtaining food. The mouse was positively reinforced for going to one odor for food, and was negatively reinforced (no food) at the other two dishes. Control mice were allowed access to only one food dish, the other two being blocked by similar wire mesh screens.

Genetics was manipulated by testing both _P_. _m_. _bairdi_ and _P_. _leucopus_. Both young and adult mice were used to determine any aging effects. The quality of the experience was varied by using the three food-odor combinations and

Table 7. Design for the study of the effect of experience on odor preferences. The same design was repeated for each of the four test groups.

Conditioned Odor (2 weeks)	Immediate Test		Late Test	
	Consummatory Test	Appetitive Test	Consummatory Test	Appetitive Test
Anise	n=6	n=6	n=6	n=6
Wintergreen	n=6	n=6	n=6	n=6
Pine	n=6	n=6	n=6	n=6
Control	n=6	n=6	n=6	n=6

Test Groups:

Peromyscus maniculatus bairdi: Young(21 days of age) N=96
Adult(90-150 days of age) N=96

Peromyscus leucopus noveboracensis: Young(21 days of age) N=96
Adult(90-150 days of age) N=96

Table 8. Summary of the ages at which the mice were conditioned and tested. Age given is that at start of the conditioning or test period.

Age Class	Age at Conditioning	Immediate Test Age	Late Test Age
Young	21	35	65
Adult	90-150	104-164	119-194

Table 9. Mean ages (± 1 S. E.) of adult mice used in each test group in the experience study.

Species	*P. m. bairdi*				*P. leucopus*			
Test	Stick	Stick	Cage	Cage	Stick	Stick	Cage	Cage
Time of Test	Early	Late	Early	Late	Early	Late	Early	Late
Mean age	118.5	124.3	123.0	118.9	120.8	119.3	121.6	123.5
Std. error	3.6	3.8	5.1	3.6	5.2	3.4	4.5	5.2

F=0.26 DF=7,184 $p > .10$

laboratory chow. The age at the time of testing was manipulated by giving mice tests immediately after the experience and one month later.

The mice were conditioned for a two-week period. Every three days the positions of the food dishes were rotated in a random sequence to prevent the development of a position habit, and the odor stimulus was refreshed with new oil, to maintain the constant intensities of the different odors. At the end of the conditioning period mice were either tested immediately, or one month later. During the interim month the mice were housed in a single plastic cage with normal laboratory chow, water and nesting cotton (no shavings). These two groups were designed to test the immediate effects of the olfactory experience and its perseveration. The same mice were not used in both the early (immediate) and late (one month) test groups.

Two types of tests were used. Behavioral sequences usually involve both an appetitive searching phase and a consummatory phase (Craig, 1918; Lorenz, 1960). The appetitive phase is a variable sequence of searching behaviors, i.e., for a nest, for food, or for a mate; and the consummatory phase is a more stereotyped pattern of terminating the sequence, i.e., eating, sleeping, or copulating. The two tests employed in the present study measured these two phases for the feeding sequence. The consummatory phase was tested by measuring the amount of each food-odor combination

consumed when all three food-odor combinations were present. The appetitive phase was measured by the number of sticks a mouse chewed through to get to the food behind the sticks.

Appetitive and consummatory tests were given to separate groups of mice. Each test was given to separate groups of mice tested immediately and to mice tested one month after the conditioning experience. The consummatory test involved presenting each mouse with three food dishes containing the three food-odor combinations. The amount of food consumed at each dish was determined by weighing the dishes daily for four days. Control mice were tested with the three food-odor combinations and provided the baseline percentages used for comparing with the results of the experimental groups.

The appetitive test apparatus consisted of a ten gallon aquarium partitioned at one end by a two inch thick board, extending the height and width of the aquarium. The board had three openings at the bottom. In each opening 14 balsa wood sticks were arranged in three rows of 5, 4 and 5 sticks each (see King et al., 1968). Behind each opening was one of the three food-odor combinations. The test animal was placed in the chamber at the beginning of its activity period, approximately 14 hours since it had last eaten. The dependent variable was the food-odor combination behind the opening through which the mouse chewed to obtain food. If experience was effective in conditioning the mouse to associate food with a particular odor, then the mouse should chew

through the sticks in front of that particular food-odor combination. Control mice were tested in a similar manner. All tests lasted 24 hours, or until the mouse died. The positions of the three food-odor combinations behind the barrier were shifted at random between test subjects to control for any species specific position bias.

Results

During the olfactory experience a number of mice died before completing the two weeks prescribed. Each dead mouse was replaced by another of the same age and species. The frequencies of deaths in each group were compared against a probability of equal deaths in each conditioning treatment using a Chi-square test (Table 10). It was necessary to lump the young and adult mice within each species to obtain frequencies large enough to test. The results were not significant for P. leucopus ($X^2=7.46$, DF=3, $p > .05$), but were significant for P. m. bairdi ($X^2=54.02$, DF=3, $p < .001$). For P. m. bairdi more deaths occurred in the anise treatment groups than would be expected on the assumption of equal probability for all treatments. Fewer deaths occurred in the control group than would be expected. Gut analyses of the dead P. m. bairdi indicated that they were probably dying from starvation. All of the deaths that occurred during the conditioning phase occurred during the first three days of the procedure. Body weights of the dead mice showed that they

Table 10. Frequency of deaths during the olfactory conditioning process.

Odor Test Group	N	Anise	Wintergreen	Pine	Control	X^2 (DF=3)	Prob.
P. m. bairdi	192	35	8	7	1	54.02	.001
P. leucopus	192	13	14	4	8	7.46	.10

had lost up to 40 percent of their original pre-experiment body weight. Mice surviving the conditioning experience did not show a weight loss. The first experiment showed that F_1WC _P_. _m_. _bairdi_ had a strong preference for the pine odored food (91.9 percent of the diet), and virtually avoided the anise food-odor combination (2.4 percent of the diet). This aversion may account for the high death rate in _P_. _m_. _bairdi_.

Appetitive Test

In Table 11 the data from the appetitive test are presented as the number of mice out of six test subjects in each group that chewed through the sticks in front of the food-odor combination with which they had been given conditioning experience. These ratios were added within each species and age group. The totals and the probability determined by a sign test (Siegel, 1956) are shown in Table 10. A one-tailed test was used because the effects of the experience could only be in one direction (pilot study). The data were analyzed separately for each test period: immediate (early) and late (one month later).

Young _P_. _m_. _bairdi_ strongly preferred the food-odor combination to which they had been conditioned when tested immediately (17/18, $p < .001$), and they retained this preference over one month (14/18, $p < .02$). Conditioning was equally effective with each of the three food-odor combinations. Adult _P_. _m_. _bairdi_ were not significantly affected

Table 11. Results of the appetitive test. The ratio is the number of mice out of six in each test group chewing through the sticks in front of the conditioned food-odor combination. (A=anise; W=wintergreen; P=pine).

Odor	Early Test					Late Test				
	A	W	P	Total	Prob.	A	W	P	Total	Prob.
Group										
P. m. bairdi										
Young	6/6	6/6	5/6	17/18	.001	4/6	5/6	5/6	14/18	.02
Adult	2/6	2/6	5/6	9/18	NS	1/6	3/6	4/6	8/18	NS
P. leucopus										
Young	5/6	6/6	6/6	17/18	.001	4/6	4/6	6/6	14/18	.02
Adult	5/6	6/6	6/6	17/18	.001	4/6	4/6	6/6	14/18	.02

by the conditioning experience, either immediately, or one month later.

Both young and adult P. leucopus were significantly affected in their food-odor preferences by the prior conditioning to exactly the same extent: immediate test (17/18, $p < .001$), and late test (14/18, $p < .02$). During the early test all combinations were equally effective (5/6 or 6/6), but during the test one month later the pine (6/6) was more effective than either wintergreen (4/6), or anise (4/6).

The results from the appetitive tests given to control mice (Table 12) showed that these mice were not associating any of the test odors with food. Twenty-three of the 48 control mice died during the appetitive test without ever chewing through the sticks to obtain food. The deaths during the test were equally distributed among the four species and age groups. Of the 144 mice conditioned to associate one of the three test odors with food, only 17 died during the appetitive test, with the maximum number of deaths being two in one cell, which occurred only once. These deaths were probably also due to the failure of the mice to associate food with any of the test odors.

In summary, young P. m. bairdi and young and adult P. leucopus all showed strong effects of the conditioning experience in the appetitive test. Adult P. m. bairdi were not significantly affected by the odor conditioning. Test groups that showed significant experience effects when tested

Table 12. Results of control mice in the appetitive test. Early and late tests combined. The ratio is the number out of 12 mice in each group chewing through each food-odor combination, or dying. (A=anise; W=wintergreen; P=pine)

	Response			
Group	A	W	P	Dead
P. m. bairdi				
Young	1/12	2/12	1/12	8/12
Adult	1/12	1/12	5/12	5/12
P. leucopus				
Young	1/12	2/12	4/12	5/12
Adult	2/12	1/12	4/12	5/12
Totals	5/48	6/48	14/48	23/48

immediately after the olfactory experience also showed significant effects when tested one month later.

Consummatory Test

The results of the consummatory test were analyzed for the first day (one) and the last day (four) of the test in order to discern a shift in preference over the test period. The dependent variable used was the percentage of the diet eaten from each food-odor combination. The mean percentages and standard errors for all test groups on day one are shown as bar histograms in Figures 3, 4, 5, and 6.

Since all groups preferred the pine odored food, the experimental groups were compared with the control groups within each food-odor combination. For example, an individual mouse from each test group was compared with the control group baseline to determine whether the experimental mouse had eaten a greater percentage of the food-odor combination on which it had been conditioned than had the control. The number of mice out of six in each test group that ate a greater percentage of their diet from the conditioned food-odor combination than the control mean was computed as a ratio, exactly as in the analysis of the appetitive test.

The results of the analyses for day one (Table 13) and day four (Table 14) were identical, indicating that there was no waning of the experience effect during the four day test period. Young P. m. bairdi showed a significant experience effect at both the early and late test periods, while adult

Figure 3. Mean percentage ($\pm$ 1 S. E.) of the diet consumed from each food-odor combination for day one of the test, both immediately and one month after conditioning in young _P_. _m_. _bairdi_. Conditioning odors indicated as: A=anise; W=wintergreen; P=pine; C=control.

FIGURE 3

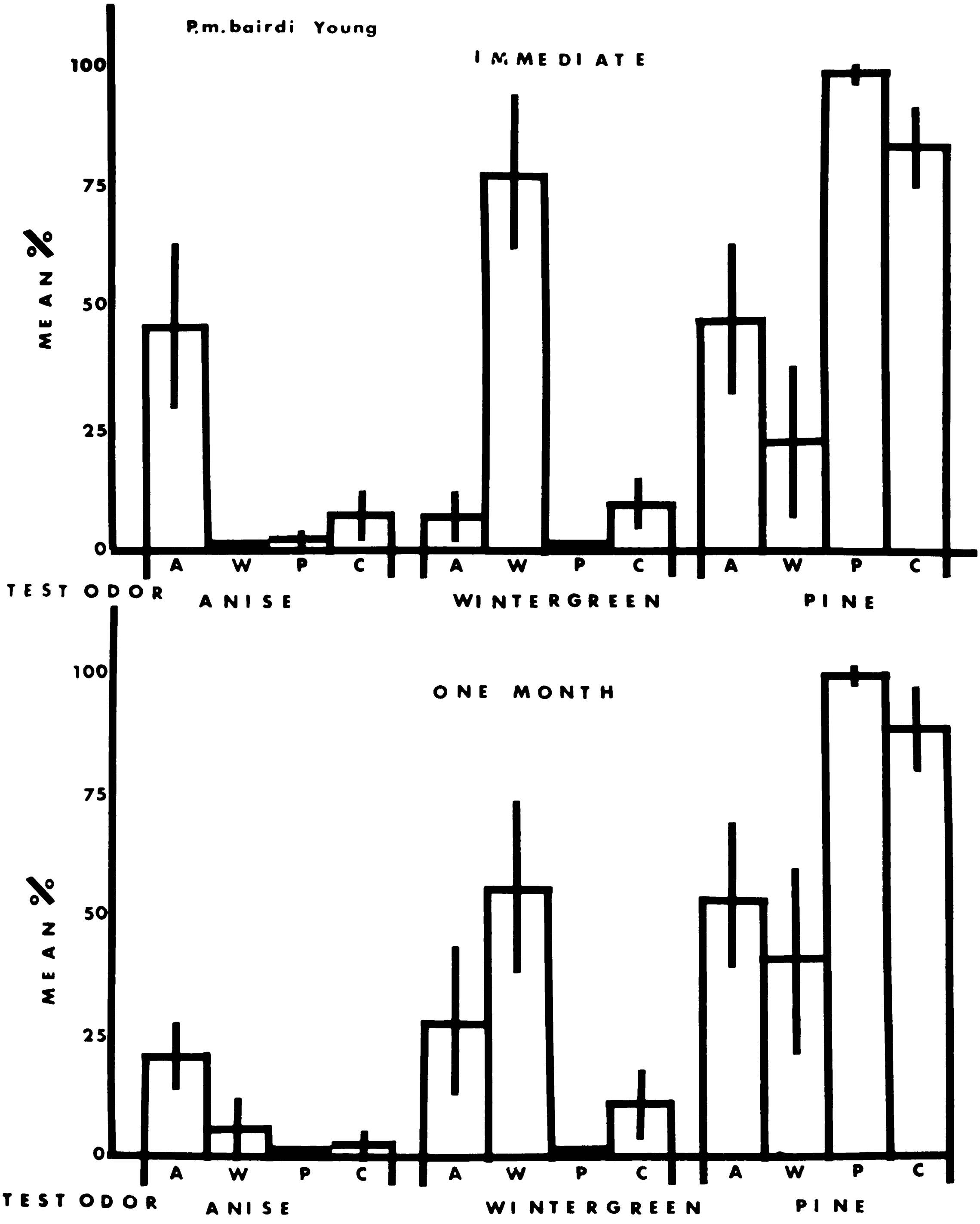

P.m.bairdi Young
IMMEDIATE
MEAN %
100
75
50
25
0
A W P C
A W P C
A W P C
TEST ODOR
ANISE
WINTERGREEN
PINE
ONE MONTH
MEAN %
100
75
50
25
0
A W P C
A W P C
A W P C
TEST ODOR
ANISE
WINTERGREEN
PINE

Figure 4. Mean percentage (± 1 S. E.) of the diet consumed from each food-odor combination for day one of the test, both immediately and one month after conditioning in adult P. m. bairdi. Conditioning odors indicated as: A=anise; W=wintergreen; P=pine; C=control.

FIGURE 4

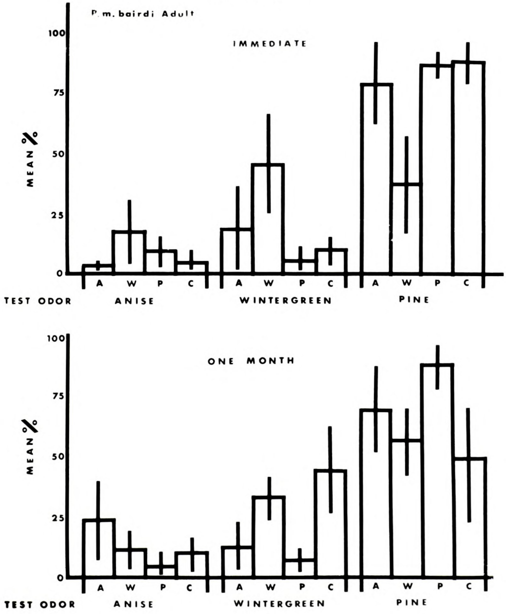

P. m. bairdi Adult
IMMEDIATE
MEAN %
100
75
50
25
0
A
W
P
C
TEST ODOR
ANISE
WINTERGREEN
PINE
ONE MONTH

Figure 5. Mean percentage (± 1 S. E.) of the diet consumed from each food-odor combination for day one of the test, both immediately and one month after conditioning in young _P_. _leucopus_. Conditioning odors indicated as: A=anise; W=wintergreen; P=pine, C=control.

FIGURE 5

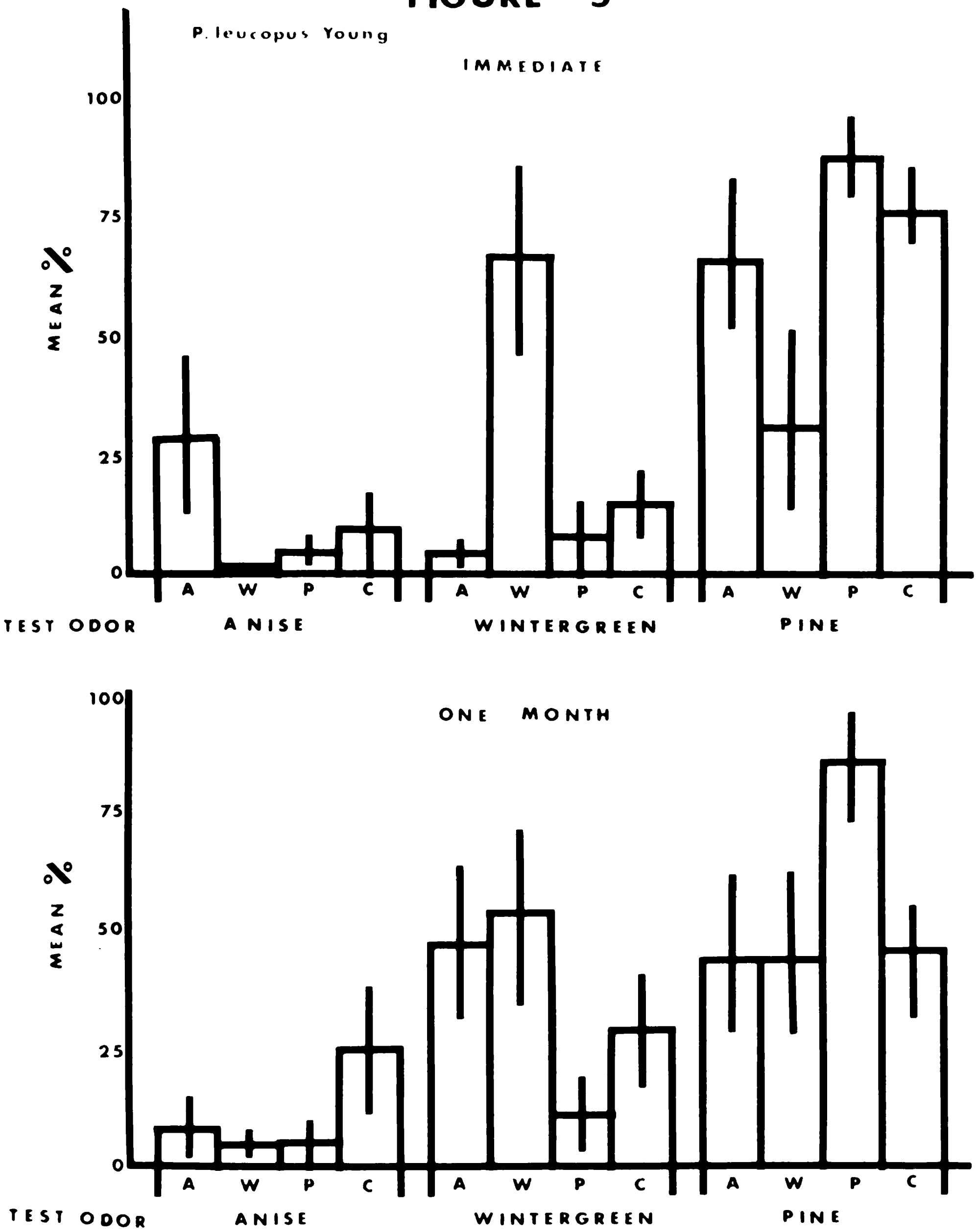

P. leucopus Young
IMMEDIATE
MEAN %
100
75
50
25
0
A
W
P
C
A
W
P
C
A
W
P
C
TEST ODOR
ANISE
WINTERGREEN
PINE
ONE MONTH
MEAN %
100
75
50
25
0
A
W
P
C
A
W
P
C
A
W
P
C
TEST ODOR
ANISE
WINTERGREEN
PINE

Figure 6. Mean percentage (± 1 S. E.) of the diet consumed from each food-odor combination for day one of the test, both immediately and one month after conditioning in adult *P. leucopus*. Conditioning odors indicated as: A=anise; W=wintergreen; P=pine; C=control.

FIGURE 6

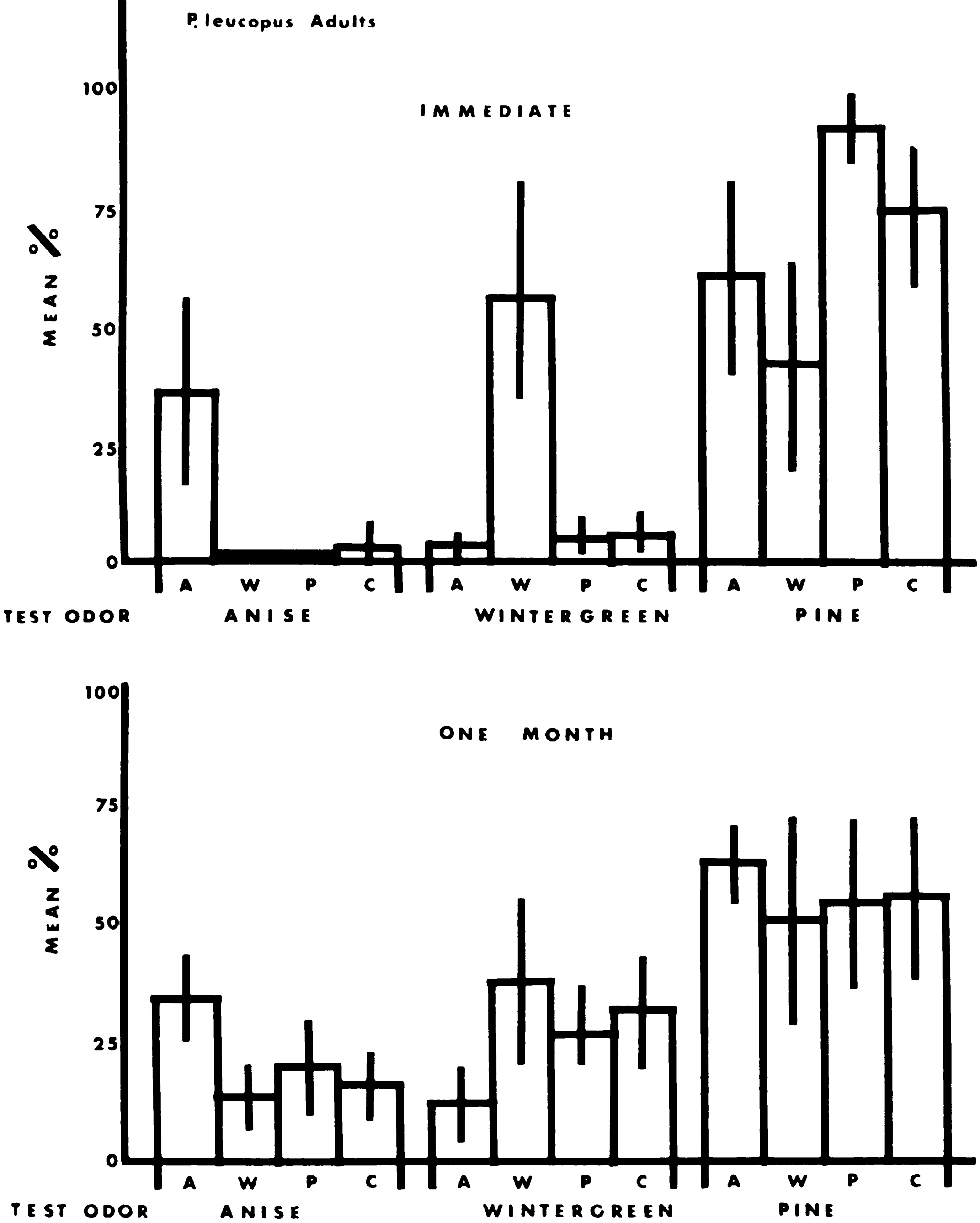

P. leucopus Adults
IMMEDIATE
MEAN %
100
75
50
25
0
A
W
P
C
A
W
P
C
A
W
P
C
TEST ODOR
ANISE
WINTERGREEN
PINE
ONE MONTH
MEAN %
100
75
50
25
0
A
W
P
C
A
W
P
C
A
W
P
C
TEST ODOR
ANISE
WINTERGREEN
PINE

Table 13. Number of animals out of six that ate a greater percentage of their diet from the conditioned odor than controls during day one of the consummatory test. (A=anise; W=wintergreen; P=pine.)

	Early Test					Late Test				
Odor	A	W	P	Total	Prob.	A	W	P	Total	Prob.
Group										
P. m. bairdi										
Young	5/6	5/6	6/6	16/18	.001	4/6	4/6	6/6	14/18	.02
Adult	2/6	3/6	5/6	10/18	NS	1/6	4/6	5/6	10/18	NS
P. leucopus										
Young	4/6	5/6	5/6	14/18	.02	1/6	4/6	5/6	10/18	NS
Adult	4/6	5/6	5/6	14/18	.02	4/6	3/6	5/6	12/18	NS

Table 14. Number of animals out of six that ate a greater percentage of their diet from the conditioned odor than controls during day four of the consummatory test. (A=anise; W=wintergreen; P=pine.)

	Early Test					Late Test				
Odor	A	W	P	Total	Prob.	A	W	P	Total	Prob.
Group										
_P_. _m_. _bairdi_										
Young	4/6	4/6	6/6	14/18	.02	4/6	5/6	5/6	14/18	.02
Adult	2/6	3/6	5/6	10/18	NS	1/6	3/6	5/6	9/18	NS
_P_. _leucopus_										
Young	4/6	5/6	5/6	14/18	.02	2/6	1/6	6/6	9/18	NS
Adult	4/6	4/6	6/6	14/18	.02	4/6	3/6	5/6	12/18	NS

P. m. bairdi were not significantly affected by the olfactory conditioning. Both young and adult *P. leucopus* showed a significant conditioning effect during the test period immediately after the experience, but one month later the effect was not significant (Tables 13 and 14).

Figures 3, 4, 5, and 6 illustrate that the significant experience effects during the immediate test, particularly with anise and wintergreen odored food, waned in magnitude in the groups of mice tested one month after conditioning. In some instances the effects of the experience were no longer significant one month later, particularly in both young and adult *P. leucopus*, as was previously noted using the sign tests.

The results of both the appetitive and consummatory tests are summarized in Table 15. Young *P. m. bairdi* were affected by the early conditioning during both test periods and as measured by both tests, whereas adult *P. m. bairdi* were not significantly affected by the experience. Both young and adult *P. leucopus* were significantly affected by the experience as measured by both tests during the immediate test period. During the test one month later, results showed that both young and adult *P. leucopus* were significantly affected as determined by the appetitive test, but not as determined by the consummatory test.

Table 15. Summary of the effects of early experience. Plus (+) indicates a significant overall effect and a minus (-) indicates no significant effect.

Group	Appetitive Test	Consummatory Test
P. m. bairdi		
Young-immediate test	+	+
Young-late test	+	+
Adult-immediate test	-	-
Adult-late test	-	-
P. leucopus		
Young-immediate test	+	+
Young-late test	+	-
Adult-immediate test	+	+
Adult-late test	+	-

STRATEGY

Purposes

The purposes of this experiment were: (1) to describe the strategies used by P. m. bairdi and P. leucopus in obtaining food from three different food-odor combinations; (2) to test the hypothesis that the mice would locate a preferred food-odor combination by odor rather than position habit; and (3) to test the reactions of the mice to a novel food-odor combination.

Test Subjects

The mice used in this experiment were young and adult F_1WC P. m. bairdi and P. leucopus. Four mice were tested in each species-age group.

Apparatus

The test apparatus was a wooden cage 1' by 1½' by 10" deep with a clear Plexiglas top. Each of three openings in one side of the cage was fitted with a short passageway containing a swinging door. Removable food hoppers were attached to the end of each passageway. Each hopper contained

a different food-odor combination. To obtain food, the mouse swung open the door, activating a microswitch, which remained activated throughout the period the mouse was eating at the hopper. The frequency and duration of feeding were recorded on a Rustrak Model 92 event recorder (Rustrak Instrument Company, Manchester, New Hampshire) with a chart speed of one inch per hour, and with Sodeco four digit counters (Landis and Gyr, Inc., New York, New York). Food consumption was measured to the nearest 0.1 gram with a Shadowgraph scale (Exact Weight Scale Company, Columbus, Ohio). The three dependent variables were: (1) the number of times each food-odor combination was visited; (2) the amount of food consumed from each food-odor combination; and (3) the pattern and duration of visits to the different food-odor combinations.

Procedure

The mice were tested individually for a period of 26 days. During the first 20 days of the test, three food hoppers with anise, wintergreen and pine food-odor combinations were affixed to the ends of the passageways. During the final six days of the test, the anise food-odor combination was replaced by food with the odor of sassafrass oil.

At the beginning of the test the mouse and foods were weighed. Each test day the foods were weighed, the number of counts at each door were recorded, the event recorder chart paper was marked with the time, and the positions of the food hoppers were changed. The odor stimuli were refreshed every three days with new oil. At the conclusion of the test the mouse was weighed again. Since weighing and handling the mouse daily would probably have made the patterns of feeding unrepresentative, the weights of the mouse at the beginning and at the end of the test were averaged to provide a mean body weight for the entire period.

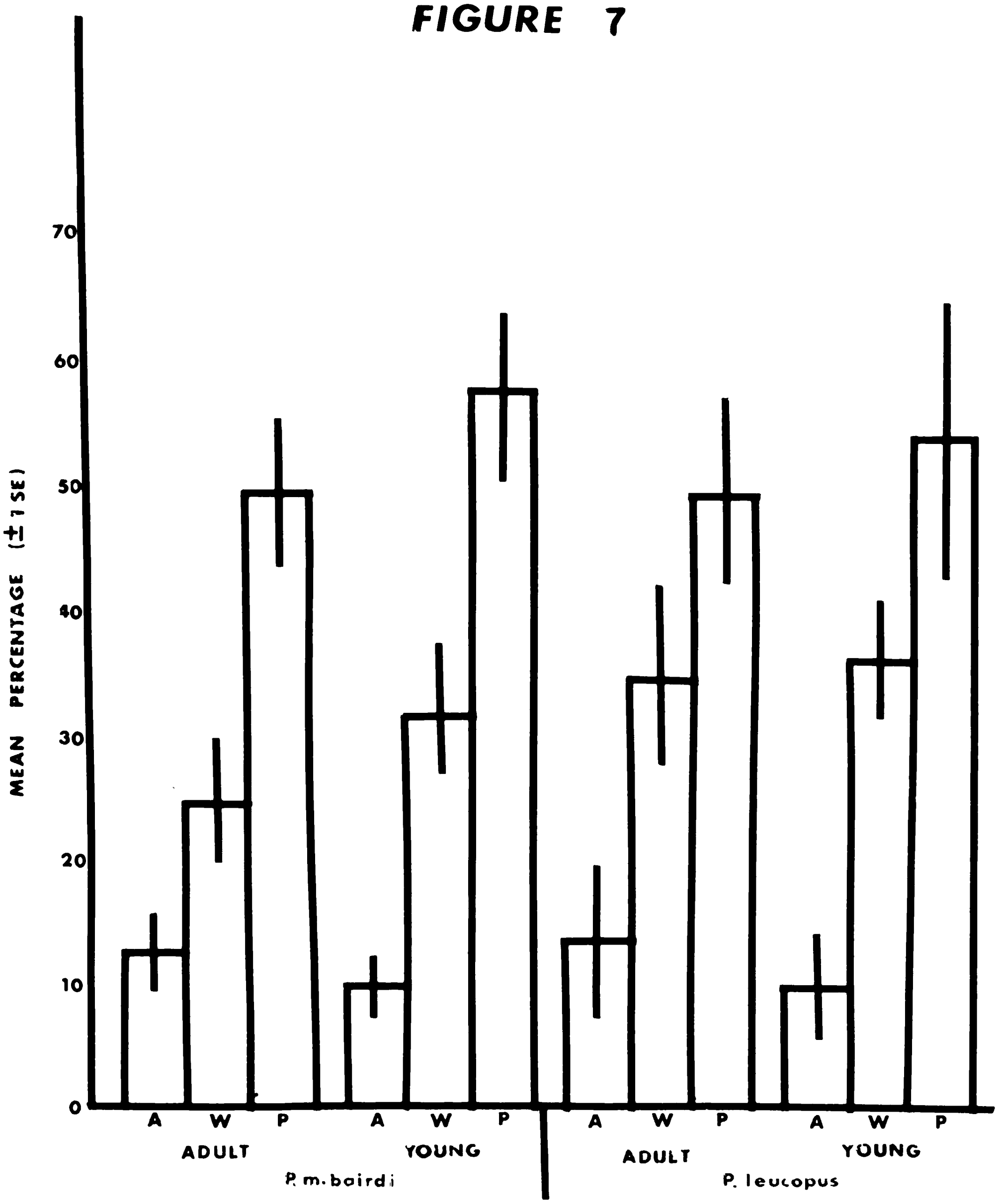
Results

The percentage of the diet consumed from each of the three food-odor combinations during days 1-20 was computed for each day and correlations were run between the consumption at each odor and the number of counts recorded from the counter at that food hopper. For each of the sixteen mice the correlation was highly significant ($.80 < r < .92$, DF=18). The count data were, therefore, eliminated from the analyses.

The mean percentage of the diet eaten from each of the food-odor combinations was computed for each group over the first 20 days of the test period. These data are graphed as bar histograms with standard errors in Figure 7. Mice in the various groups did not differ significantly in the percentage of the diet eaten from each of the food-odor combinations. The standard errors for the four values at any one food-odor combination were all overlapping. The histograms also show that the mice in all four groups preferred the pine odored food, as in the earlier preference tests.

Figure 7. Mean percentage (± 1 S. E.) of the diet consumed from each of the three food-odor combinations (A=anise; W=wintergreen; P=pine).

FIGURE 7


MEAN PERCENTAGE (± 1 SE)
0
10
20
30
40
50
60
70
A
W
P
A
W
P
A
W
P
A
W
P
ADULT
YOUNG
ADULT
YOUNG
P. m. bairdi
P. leucopus

Strategy was analyzed by collecting the data from the event recorder charts, and translating it onto sheets marked off in hours and days. The amount of time spent at each food-odor combination was transformed to an ordinal scale by rating the amount of activity each hour as follows: zero if the mouse was not at the combination at all, 1 if the mouse fed 0-5 minutes, 2 if the mouse fed for 5-15 minutes, 3 if the mouse fed for 15-30 minutes, and 4 if the mouse fed for more than 30 minutes of the hour at that combination. The ordinal scale was chosen on the basis of the distribution of the length of feeding activity periods. The data then provided a running record of the amount of time spent per hour at each of the three possible food-odor combinations for each mouse. These records were analyzed to determine four aspects of the feeding strategy.

(1) The number of days the mouse went to all three food-odor combinations during the initial activity bout (defined as the active period prior to the first inactive period of 60 minutes or longer) was analyzed. The 20 day experimental period was divided into the first ten days (1-10) and the second ten days (11-20). The mean number of days that all three food-odor combinations were visited during the initial activity period was computed for each of the four groups of four mice each. These means and standard errors are presented in Table 16. A ratio was computed for each mouse by dividing the mean number of days all three food-odors were visited

Table 16. Mean number (± 1 S. E.) of days, out of 10, that all three food-odor combinations were visited during the initial activity bout.

Species	*P. m. bairdi*		*P. leucopus*	
Age	Young	Adult	Young	Adult
Time				
Days 1-10	2.3(0.6)	5.0(1.2)	4.0(1.3)	5.5(0.6)
Days 11-20	6.5(0.9)	5.2(1.4)	7.0(1.3)	6.8(0.2)
Mean Ratio $\frac{1\text{-}10}{11\text{-}20}$	0.46(0.18)	0.97(0.03)	0.56(0.13)	0.83(0.13)

during the first ten days, by the mean for the second ten days. These ratios were then averaged for each group. The mean ratios and their standard errors are shown in Table 16. An analysis of variance was performed on these ratios. The main treatment factors were species and age, with four replicates per cell (Table 17). Only the age factor was significant ($F=8.88$, $DF=1,12$, $p < .001$). Young *P. m. bairdi* and *P. leucopus* did not visit all three food-odor combinations on as many days during the first ten days of the test as did adult mice. Table 16 shows that young mice did visit the same mean number of combinations as adult mice during days 11-20.

(2) A mouse's use of a position habit was tested by recording the initial feeding response, each test day, after the food hoppers had been weighed and replaced in different positions. The response was recorded as a position habit (P) if the mouse went to the door where its preferred food-odor combination had been the previous day. The behavior was recorded as a following response (F) if the mouse went to the preferred food-odor combination regardless of its position. This implies that the mouse used the odor cue to locate the combination it preferred. The response was recorded as neutral if the mouse went to the remaining position, neither the position habit nor the following response. Of 320 responses only 15 percent were neutral; this response was not considered in the analysis.

Table 17. Analysis of variance on the food-odor combinations visited during the initial activity bout.

Factor	DF	Mean Square	F	Prob.
Species	1	0.002	0.03	NS
Age	1	0.613	8.88	.001
Species x Age	1	0.062	0.90	NS
Error	12	0.069		
Total	15	(SS=1.505)		

The data were divided into two time blocks; the first ten days of the test (1-10) and the second ten days (11-20). The data were computed as a ratio for each mouse; the number of position responses divided by the number of following responses. The mean ratios and standard errors for each species and age group are shown in Table 18. These ratios were subjected to a three-way analysis of variance with one measure repeated, and with four replicates per cell. The main factors were age and species and the repeated measure was time blocks.

The analysis (Table 19) showed significant species differences ($F=5.01$, $DF= 1,12$, $p < .05$), and a significant trials effect ($F=12.49$, $DF=1,12$, $p < .005$). There were no significant age or interaction effects. During the first ten days _P_. _m_. _bairdi_ had a very high P/F ratio (> 3.4), indicating a strong use of position habit. During the second ten days the ratio for _P_. _m_. _bairdi_ was less ($P/F=0.4$ and $P/F=1.1$), indicating the mice were using both position habit and following responses. Young and adult _P_. _leucopus_ used the position habit only slightly more than the following response during the first ten days, and switched to the following response during the second ten days of the test.

(3) Differences in feeding strategy were analyzed from the number of food-odor combinations visited per hour of activity per mouse per day. These were averaged for the four mice in each test group for four-day blocks (1-4, 5-8, 9-12,

Table 18. Mean ratio (± 1 S. E.) of position responses to following responses.

Days		1-10	11-20
Group	N		
P. m. bairdi			
Young	4	3.4(1.2)	1.1(0.3)
Adult	4	4.6(2.0	0.4(0.1)
P. leucopus			
Young	4	1.3(0.1)	0.3(0.1)
Adult	4	1.9(0.7)	0.6(0.2)

Table 19. Three-way analysis of variance with one measure repeated for the ratio of position responses to following responses.

Factor	DF	SS	MS	F	P
Between Subjects	15	50.52			
Age	1	0.98	0.98	0.34	NS
Species	1	14.58	14.58	5.01	.05
Species x Age	1	0.08	0.08	0.03	NS
Error Between Subjects	12	34.88	2.91		
Within Subjects	16	89.85			
Trials	1	37.84	37.84	12.49	.005
Trials x Ages	1	2.65	2.65	0.87	NS
Trials x Species	1	8.82	8.82	2.91	NS
Trials x Species x Ages	1	1.12	1.12	0.37	NS
Error Within Subjects	12	39.39	3.03		
Total	31	140.37			

13-16 and 17-20). This meant that there were five (trials 1-5) four-day blocks during the 20-day test. The maximum number of food-odor combinations that a mouse could visit in one hour would be three. Thus, the maximum value that the mean could have would be 3.0. The higher the mean value, the greater the tendency for the mice to visit all three combinations during each hour of activity.

The means and standard errors for these data are presented in Table 20. These data were subjected to a three-way analysis of variance with one measure repeated and four replicates per cell. The two main factors were species and age and the repeated factor was trials. The analysis (Table 21) showed that the age factor approached significance (F=4.35, DF=1,12, $.10 > p > .05$). The trials effect was significant (F=4.24, DF=4,48, $p < .001$). Duncan's New Multiple Range test breakdown of these means (Table 22) showed that trials 1 and 2 were significantly different from trials 3, 4, and 5, and trials 2 and 3 were not different from each other. The breakdown of the trials by age interaction (F=4.31, DF=4,48, $p < .001$), showed that the most critical difference in the experiment was that young mice on trials 1 and 2 were visiting significantly fewer food-odor combinations than adult mice on all trials and young mice on trials 3, 4, and 5 (Table 22). This means that young mice visited fewer food-odor combinations per hour during the initial 8 days of the test than adults, but during the 12 days following, the mice of all ages and

Table 20. Mean number (± 1 S. E.) of food-odor combinations visited per hour of activity per mouse per day.

Species		*P. m. bairdi*		*P. leucopus*	
Age		Young	Adult	Young	Adult
Days	Trial				
1-4	1	1.34(0.11)	1.72(0.08)	1.31(0.09)	1.77(0.11)
5-6	2	1.41(0.09)	1.83(0.13)	1.43(0.12)	1.80(0.10)
9-12	3	1.77(0.17)	1.80(0.11)	1.77(0.13)	1.85(0.11)
13-16	4	1.75(0.10)	1.69(0.06)	1.79(0.13)	1.70(0.06)
17-20	5	1.69(0.13)	1.81(0.08)	1.83(0.12)	1.79(0.11)

Table 21. Three-way analysis of variance with one measure repeated on the food-odor combinations visited per hour.

Factor	DF	SS	MS	F	P
Between Subjects	15	1.981			
Species	1	0.023	0.023	0.19	**NS**
Age	1	0.522	0.522	4.35	$.05 < p < .10$
Age x Species	1	0.006	0.006	0.05[a]	**NS**
Error Between	12	1.435	0.120		
Within Subjects	64	3.468			
Trials	4	0.710	0.178	4.24	.001
Trials x Age	4	0.722	0.181	4.31	.001
Trials x Species	4	0.009	0.002	0.04[a]	**NS**
Trials x Species x Age	4	0.027	0.007	0.06[a]	**NS**
Error Within	48	2.000	0.042		
Total	80	5.449			

[a] These **F** values are unusually low. The analysis was re-checked several times and these values are correct.

Table 22. Duncan's New Multiple Range Test. Those means not subtended by the same line are significantly different ($p < .05$).

(1) Trials

Trial No.	1	2	3	4	5
Mean	1.56	1.62	1.73	1.78	1.80

(2) Trials x Age Y=young A=adult

Age	Y	Y	A	A	Y	Y	Y	A	A	A
Trial	1	2	4	1	5	3	4	5	2	3
Mean	1.37	1.42	1.70	1.75	1.76	1.77	1.77	1.80	1.81	1.83

species performed at the same level, visiting on the average 1.7 to 1.8 food-odor combinations per hour of activity.

(4) The data were also analyzed by counting the number of times each mouse switched food-odor combinations per day. A switch was recorded every time a mouse fed for five minutes at one hopper with no excursions to other food-odor combinations, followed by at least five minutes feeding at a different hopper. There was no prescribed time limit for the interval between feeding at the two different food-odor combinations. Intermittent feeding at the same food-odor combination would, therefore, not involve any switching behavior.

The data were divided into two ten-day blocks (1-10 and 11-20). The mean number of switches per day was calculated for each mouse for each ten-day block, and was used as the dependent variable. The species and age group means and standard errors are presented in Table 23. These data were subjected to a three-way analysis of variance with one measure repeated, and four replicates per cell. The main factors were age and species and the repeated factor was 10-day blocks. The analysis (Table 24) showed that only the trials effect was significant (F=8.01, DF=1,12, $.01 < p < .025$). This difference was due to the fact that all of the mice showed more switching behavior during the second ten days of the test. The species difference approached significance (F=4.53, DF=1,12, $.05 < p < .06$). A Duncan's New Multiple

Table 23. Mean number (± 1 S. E.) of switches between food-odor combinations per day.

Days		1-10	11-20
Group	N		
P. m. bairdi			
Young	4	1.5(0.2)	2.0(0.3)
Adult	4	1.3(0.1)	1.8(0.3)
P. leucopus			
Young	4	1.6(0.1)	2.4(0.3)
Adult	4	3.9(0.4)	4.0(0.4)

Table 24. Three-way analysis of variance of the number of switches between food-odor combinations per day.

Factor	DF	SS	MS	F	P
Between Subjects	15	41.81			
Age	1	2.94	2.94	1.46	NS
Species	1	9.14	9.14	4.53	$.05 < p < .10$
Age x Species	1	5.52	5.52	2.74	NS
Error Between	12	24.21	2.02		
Within Subjects	16	10.11			
Trials	1	3.85	3.85	8.01	$.01 < p < .025$
Age x Trials	1	0.05	0.05	0.10	NS
Species x Trials	1	0.38	0.38	0.79	NS
Age x Species x Trials	1	0.06	0.06	0.12	NS
Error Within	12	5.76	0.48		
Total	31	51.92			

Range test (Table 25) showed that the adult P. leucopus switched food sources significantly more often than any of the other three test groups, which did not differ from each other.

On day 21 of the test, a food hopper with sassafrass oil as the odor stimulus replaced the anise food-odor combination. The last six days of the test measured the responses of the mice to this novel food-odor combination. The data were computed as percentages of the diet eaten from the novel combination, for the first day of the test and for all six days of the test combined. The means and standard errors are shown in Table 26. The response did not change from the first day to all six days combined. A two-way analysis of variance was performed on the data for all six days (Table 27). The main factors were species and age with four replicates per cell. A significant species by age interaction (F=7.68, DF=1,12, $.01 < p < .025$) was broken down using Duncan's New Multiple Range test (Table 28). This showed that the difference was due to the avoidance reaction of the young P. m. bairdi. The young P. m. bairdi consumed only 18.8 percent of their diet from the sassafrass odored food, while young P. leucopus and adult mice of both species ate at least 50 percent of their diet from the novel combination during the six test days.

Table 25. Duncan's New Multiple Range test. Those means not subtended by the same line are significantly different ($p < .05$).

(1) Species x Age

Species	*P. m. bairdi*	*P. m. bairdi*	*P. leucopus*	*P. leucopus*
Age	adult	young	adult	young
Mean	1.55	1.75	2.00	3.95

Table 26. Mean percentage (± 1 S. E.) of the diet eaten from the novel food-odor combination.

Group	First Test Day	All Six Test Days
P. m. bairdi		
Young	21.0(7.9)	18.8(8.0)
Adult	76.3(14.0)	64.9(17.0)
P. leucopus		
Young	74.1(16.1)	61.8(5.6)
Adult	55.3(19.4)	50.1(7.1)

Table 27. Two-way analysis of variance of the percentage of the diet eaten from the novel food-odor combination during all six test days.

Factor	DF	MS	F	P
Species	1	791.02	1.81	NS
Age	1	1185.09	2.71	NS
Species x Age	1	3355.29	7.68	$.01 < p < .025$
Error	12	436.91		
Total	15	(SS=10574.32)		

Table 28. Duncan's New Multiple Range Test. Those means not subtended by the same line are significantly different ($p < .05$).

(1) Species x Age

<table>
<tr><td>Species</td><td>*P. m. bairdi*</td><td>*P. leucopus*</td><td>*P. leucopus*</td><td>*P. m. bairdi*</td></tr>
<tr><td>Age</td><td>young</td><td>adult</td><td>young</td><td>adult</td></tr>
<tr><td></td><td>18.8</td><td colspan="3">$\underline{50.1 \qquad\qquad 61.8 \qquad\qquad 64.9}$</td></tr>
</table>

DISCUSSION

At the outset it was proposed that variation in food habits among species of small mammals are attributable to differences in the inherited capacity of each species to be affected by ontogenetic experiences. Variation was investigated with regard to fixity of species food-odor preferences, modifiability of food-odor preferences, and the strategies used by the mice to locate food. The principle conclusion was that similar olfactory experiences differentially modified the food-odor preferences of the two species. Using a restricted set of food cues, *P. leucopus* showed more flexibility in its feeding behavior than *P. m. bairdi*. Since both species showed the same pine preference, and since the same cues were used to study modifiability and strategy in both species, there must be an inherited species difference in the capacity to respond to these stimuli.

Results from both the experience and strategy studies support the conclusion that *P. leucopus* are more flexible in their feeding behavior than *P. m. bairdi*. While both young and adult *P. leucopus* were significantly affected by olfactory conditioning, only young *P. m. bairdi* shifted

food-odor preferences in accord with the experience treatment. Analysis of the feeding strategies indicated that young P. m. bairdi avoided a novel food-odor combination, whereas the other three test groups all consumed more than 50 percent of their diet from the novel source. Thus, even early in development the P. m. bairdi are more restricted in their feeding behavior. At maturity P. leucopus switched feeding sites more frequently than P. m. bairdi, and the P. leucopus initially adopted a strategy using both position habit and following responses, whereas P. m. bairdi used a position habit almost exclusively.

I will now briefly discuss the conclusions and explanations for each separate experiment, to relate these findings to the overall theme of species variation in feeding behavior.

Genetics

The central question in the first experiment was whether the food-odor preferences of the mice were inherited. Three approaches were taken: (1) a description of odor preferences for the various species and age groups; (2) a comparison of longitudinal and cross-sectional developmental groups of young mice; and (3) a test for possible relaxed selection for the odor preferences in domesticated stocks of mice.

The results (Table 1 and Figure 2) showed a consistent pine preference in all experimental stocks. There are

several explanations for these results. (1) The pine preference may be a generalized trait inherited by all the mice. (2) The pine shavings on which the mice were housed prior to experimentation may have produced a consistent experiential effect resulting in the uniform pine preference. A test of this hypothesis (Table 5) showed that the pine shavings in the cage did not affect the odor preference pattern. (3) Lastly, the dam's milk may have contained some factor which affected the olfactory preferences of the mice. This hypothesis could be tested only if there were some means of artificially feeding the mice from birth.

Longitudinal and cross-sectional groups of young mice did not differ significantly in their odor preferences ($p > .10$). The preference for pine odored food was immediate and was not a function of maturation. The immediate (day 21) preference for pine odored food could be used as an argument for a genetic component in the olfactory preferences. Further study is necessary to determine the odor preferences of the mice from birth until 21 days of age.

The hypothesis that laboratory confinement relaxed selection pressures on the odor preferences of domestic stocks of _P_. _m_. _bairdi_ and _P_. _m_. _gracilis_ was not supported by the data (Figure 2). Differences between wild caught and domestic stocks would have provided evidence for the overall genetic hypothesis. Alternative explanations for these results include: (1) selection pressures were not relaxed

under laboratory conditions which maintained the selection for odor preferences, and (2) the odor preferences are entirely experiential.

Individual differences in olfactory preferences provided the best evidence for genetic components. Eleven of 96 mice tested showed clear preferences (c.a. 80 percent of the diet) for the anise or wintergreen odored food. Individual differences have been used previously as an argument for inheritance of behavior patterns (Free, 1958; Hamburg, 1967; Vale and Vale, 1969). Experimental tests of the genetic hypothesis would involve selectively breeding mice that showed a fixed preference for anise or wintergreen (or even pine) odored food. Selection for a particular food-odor combination would confirm a genetic hypothesis. In a similar type selection study Nachman (1959) showed that saccharin preference in rats was inherited.

The major conclusion from this first experiment was the consistent preference pattern for the pine odored food. This preference may be inherited, but only as a generalized character. Genetic variability among the three odors used was low and not systematically related to species, age, or domestication. The consistent baseline was important for interpretation of the experience manipulation and for describing age and species specific feeding strategies. It will be necessary to conduct further investigations, such as the selection experiment, to determine the extent of genetic

variation in these species and the degree of inheritance of these food-odor preferences.

Experience

Against this consistent background, experience was manipulated to determine the modifiability of the odor preferences. The results of this experiment are summarized in Table 15. The two species differed in the degree of preference modification. The key group in this species variation was the adult _P_. _m_. _bairdi_. The olfactory preferences of both young _P_. _leucopus_ and _P_. _m_. _bairdi_ were significantly ($p < .05$) modified by early experience. This is consistent with most previous findings on early experience effects (see Beach and Jaynes, 1954; Scott, 1962). Adult mice of these two species differed radically in the modifiability of their olfactory preferences. Adult _P_. _leucopus_ were significantly ($p < .05$) affected, but _P_. _m_. _bairdi_ adults showed no modification of odor preferences. The only odor to have a significant effect on the adult _P_. _m_. _bairdi_ was pine, but there was already a strong baseline predisposition to prefer pine odored food. There are two hypotheses which may explain this adult variation between the two species: (1) an age specific effect, with rigidity of food habits developing differentially in both species, and (2) species differences in the perception of the laboratory experiences which lead to rigidity or maintain flexibility of food-odor preferences.

(1) Two parameters were manipulated in testing the effects of olfactory experience; age at conditioning and age at testing. It is my hypothesis that the age of experience was the critical variable. In support of my position, mice 66 days of age (see Table 8) were morphologically and reproductively adult. Also, outside of the experience provided in the study, mice of all ages were exposed to the same uniform environment, the only variable being the length of exposure to that environment. It could also be argued, however, that the two weeks of olfactory conditioning constituted a larger proportion of the total experience of mice only 66 days of age than for mice 105 days of age or older, in which case, the age of testing might be important.

Assuming that the age of experience was the critical variable, the overall effect may be described as a probability curve with age as the abcissa and the probability of being reinforced for selecting any food-odor combination and learning the food-cue association as the ordinate. For many behaviors, in a variety of animals, this probability curve is higher in young animals (see Scott, 1962; King, 1968b), and declines with age. In this study _P. m. bairdi_ appeared to follow this pattern, with high modifiability in young mice, but virtually no shifts in adult preferences where the probability curve had declined to a low level. The probability of learning the food-cue association was uniquely identical for both young and adult _P. leucopus_.

This species variation is supported by two further points of evidence. One, already discussed was the relatively high fixity of the pine preference in adult P. m. bairdi contrasted with lower fixity in young P. m. bairdi and young and adult P. leucopus. The second point comes from the deaths recorded during the conditioning phase. Among the P. leucopus, the deaths (39) were equally divided between young (18) and adult (21) mice. However, for P. m. bairdi 42 (82 percent) of the 51 deaths occurred in the adult test group, indicating that young mice associated new odors with food more readily than adults.

(2) The laboratory environment may have provided a sufficiently broad set of experiential stimuli for adult P. leucopus to retain a high degree of flexibility in their food-odor preferences. The same laboratory environment may not be broad enough for P. m. bairdi, so at 90 days of age they are no longer very flexible in their food-odor preference. Testing this hypothesis would require manipulation of the perceptual worlds of the mice to provide young P. m. bairdi with a wider range of stimuli prior to the olfactory conditioning experience. The species differences in preference modifiability could also have resulted from an interaction of a species aging effect (1) and the differential interpretation of the breadth of the experiential world provided by the laboratory (2).

The fact that young and adult P. leucopus showed significant experience effects during the late test period in the appetitive test, but not in the consummatory test (Table 15) also deserves explanation. Two alternative explanations are: (1) the effect of the experience waned during the 30 day interval between conditioning and testing; and (2) there was some difference between the two tests. Since the interval was the same for both tests, the second alternative appears more reasonable. In the appetitive test mice were forced to select a position at which to chew through the sticks to obtain food. In this test there was no immediate food reinforcement. In the consummatory test the mice were free to sample all three food-odor combinations immediately. The interaction of the 30 day interval between conditioning and testing with the nature of the test may explain the nonsignificant results obtained for these two groups in the late consummatory test.

Strategy

The last experiment was designed to investigate species and age differences in feeding strategies of the mice. The major conclusions of this study support the species variation in feeding behavior observed in the study of experience.

(1) Young mice adopted a conservative strategy for the first half of the test, feeding each day at the first food-odor combination encountered which provided nutritional

reinforcement. During days 11-20 of the experiment the experiential worlds of the mice expanded, and so did their strategy. Measures of the number of food-odor combinations visited during the initial activity bout and during each hour of activity increased during days 11-20. As I have already discussed the adoption of a conservative strategy by young mice would strengthen their early feeding habits which were restricted to one or several foods.

(2) Young P. m. bairdi reverted again to a conservative strategy when a novel food-odor combination was introduced. This group consumed only 18 percent of its diet from the sassafrass odored food, whereas each of the other three test groups (adult P. m. bairdi; young and adult P. leucopus) consumed more than 50 percent of their diet from the novel combination (Table 26). Thus, the species variation in feeding behavior is already manifested early in the ontogeny of the mice.

(3) Species differences in feeding strategy were also shown with adult mice. Two of the measures tested, use of a position habit and switching behavior, support this statement. In contrast measures of food-odor combinations visited initially each day and per hour of activity showed no species variation. The adult P. m. bairdi strategy shifted dramatically from a position habit during days 1-10 to a following response during the second ten days of the test. Adult P. leucopus showed a similar shift, but the magnitude of the

change was much reduced. This species difference cannot be explained from these data unless *P. m. bairdi* had stronger position habits during days 1-10.

Adult *P. leucopus* switched feeding sites signigicantly more frequently than any other test group (Table 25). In keeping with a conservative strategy, young mice of both species would be expected to switch feeding sites less frequently than adults, which they did. But, adult *P. m. bairdi* exhibited the least amount of switching behavior of all the test groups. This finding supports the conclusion that the feeding behavior and strategy are more flexible in adult *P. leucopus*, in agreement with the results of the experience study.

Since my eventual goal is to relate food habit to distribution, I will briefly reiterate what is known of the distributions and food habits of *P. m. bairdi* and *P. leucopus*. *P. m. bairdi* is found exclusively in grassland, cultivated or open field habitats, while *P. leucopus* is found in a much wider variety of habitat types (Nicholson, 1941; Blair, 1940; Linduska, 1950; Whitaker, 1966; personal trapping records). *P. leucopus* is characteristically found in woodland habitats, but it is also readily trapped in fields of all types, in brushy areas, and in and around buildings. The only complete study of the food habits of these two species (Whitaker, 1966) showed that *P. leucopus* tended to select a more varid diet, particularly if all types of habitats were considered.

From the distributions of these two species I would hypothesize that P. leucopus would exhibit a wider variation in feeding behavior. For the parameters I investigated, this hypothesis is valid. Young mice of both species were affected by early experience, but even at an early age P. m. bairdi avoided new foods. In adult mice there were distinct species differences in the flexibility of feeding habits. Adult P. m. bairdi showed a higher fixity for pine odored food than any other group, they switched feeding sites less often than any other group, and olfactory conditioning experience did not signigicantly alter their food-odor preferences. In contrast P. leucopus retained their flexibility, switched feeding sites frequently and showed strong modifiability of food-odor preferences. This argument has proceeded from the distribution to the types of feeding behaviors shown by the mice. A more meaningful approach would be to argue from the behaviors of the mice to the distributions observed in nature.

It appears from the current study that the degree of variation exhibited by a species in its feeding behaviors, might be a predictor of its distribution. That is, a species which, as a population, exhibits a wider spectrum of variation in its feeding habits could occupy more types of habitats. The present study has determined that species do vary in the degree of variation exhibited in feeding habits and the present study has tested several factors which can affect

this variation. Now, a proposed direct test of the hypothesis that feeding behavior affects distribution may be presented.

Since I am concerned with food habits and distribution, I will assume, for the purposes of this experiment, that the mice live in food worlds. The critical question is whether the species variation in feeding behavior in one world can be used as a predictor of the number of different worlds in which the mice will live. A series of interconnected pens could be set up with, for example, four food types in each pen. As one set of independent variables the types (quality) and quanities of the foods available in each pen could be manipulated. Two species (P. leucopus and P. m. bairdi) and two rearing treatments in a cross-classified design will be used to produce the mice to be tested in the apparatus. The two rearing treatments would provide the mice with either a restricted or a varied early feeding environment. The two dependent variables would be the amount of time spent in each pen and the food consumption at the various food types in each pen.

From the current study on species variation in feeding behavior I would predict the following results for this suggested experiment: (a) P. leucopus will distribute their time evenly among the different pens, while P. m. bairdi will be restricted to fewer pens; and (2) within each pen P. leucopus will select a more varied diet than P. m. bairdi. The early experience treatment would be expected to significantly modify the food preference pattern.

SUMMARY

1. It was proposed that variation in food habits among species of small mammals are attributable to inherited capacities which are affected by ontogenetic experiences with food cues.

2. *Peromyscus maniculatus bairdi* and *P. leucopus noveboracensis* were used to examine preferences for three food-odor combinations made from three essential oils combined with laboratory chow. Food-odor preferences were determined for three taxa, two stocks of different breeding history (wild caught and domestic), and two age groups. All groups exhibited a strong preference for the pine food-odor combination and there were no significant differences among the groups.

3. The mice did not: (1) discriminate odor intensities; (2) use a dish preference; (3) discriminate soiled from unsoiled food; (4) prefer pine because of pine shavings present in their rearing cages; nor did their preference change over seasons.

4. Mice conditioned for two weeks to associate one of the three odors with food and control mice given laboratory chow without the experimental odors produced the following

results in an appetitive and consummatory test given immediately after and one month after the olfactory conditioning experience:

a. Young _P_. _m_. _bairdi_ and young and adult _P_. _leucopus_ were significantly affected by the olfactory experience, but adult _P_. _m_. _bairdi_ were not.

b. Groups of mice that showed a significant effect during the immediate test also showed a significant effect when tested one month later.

5. Feeding strategies were tested in young and adult mice of both species by recording the duration, frequency, sequences, and amount of food consumed at the three food-odor combinations.

a. Young mice initially adopted a conservative feeding strategy, but changed to that strategy used by all mice later.

b. Young _P_. _m_. _bairdi_ avoided a novel food-odor combination, whereas the other test groups consumed more than 50 percent of their diet from the new source.

c. Both species shifted from a position habit (days 1-10) to a following response (days 11-20), using an odor cue to locate food.

d. Adult _P_. _leucopus_ switched feeding sites more frequently than any other test group.

6. It was concluded that _P_. _leucopus_ showed more flexibility in feeding behavior than _P_. _m_. _bairdi_.

a. Food habits were discussed in relation to the distributions of the mice.
b. The variation in feeding behavior shown by different species may be a predictor of the number of habitats it will occupy.

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