Contributions to the development of effective food baits and pheromone lures for capturing mice and rats

by

Antonia Elizabeth Musso

B.Sc., Simon Fraser University, 2012

Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Pest Management

in the
Department of Biological Sciences
Faculty of Science

SIMON FRASER UNIVERSITY

Spring 2016

Approval

Name:	**Antonia Elizabeth Musso**
Degree:	**Master of Pest Management**
Title:	***Contributions to the development of effective food baits and pheromone lures for capturing mice and rats***
Examining Committee:	**Chair:** Dr. Elizabeth Elle Professor

Dr. Gerhard Gries
Senior Supervisor
Professor

Dr. Jenny Cory
Supervisor
Professor

Dr. Douglas Ransome
External Examiner
Program Head, Ecological Restoration Programs
British Columbia Institute of Technology

Date Defended/Approved: April 21, 2016

Ethics Statement

The author, whose name appears on the title page of this work, has obtained, for the research described in this work, either:

a. human research ethics approval from the Simon Fraser University Office of Research Ethics

or

b. advance approval of the animal care protocol from the University Animal Care Committee of Simon Fraser University

or has conducted the research

c. as a co-investigator, collaborator, or research assistant in a research project approved in advance.

A copy of the approval letter has been filed with the Theses Office of the University Library at the time of submission of this thesis or project.

The original application for approval and letter of approval are filed with the relevant offices. Inquiries may be directed to those authorities.

Simon Fraser University Library
Burnaby, British Columbia, Canada

Update Spring 2016

Abstract

My research aimed to improve trap captures of mice and rats by incorporating food cues and pheromone signals into a bait complex. I show that a food bait consisting of cereals, fructose, soy lecithin and a semiochemical blend in safflower oil, suspended in a gelatine/water solution, mediates feeding by mice and rats in the laboratory and capture of wild mice in the field. Traps baited with bedding soiled by caged male mice attracted juvenile and adult female mice, indicating the presence of a sex pheromone in soiled bedding. Analyses of male and female bedding odorants by gas chromatography-mass spectrometry show that the known primer pheromone components 3,4-dehydro-*exo*-brevicomin (DEB) and 2-*sec*-butyl-4,5-dihydrothiazole (DHT) were present in male bedding. In a field experiment, traps baited with DEB and DHT captured four times more female mice than corresponding control traps, indicating that DEB and DHT are sex attractant pheromone components of male house mice.

Keywords: *Mus musculus*, *Rattus norvegicus*, sex attractants, pheromone, 3,4-dehydro-*exo*-brevicomin, 2-*sec*-butyl-4,5-dihyrdrothiazole

To lab animals everywhere, without them, we could not build a safe and healthy world.

Acknowledgements

First, I would like to thank my senior supervisor Dr. Gerhard Gries for giving me a chance to show my stuff as a graduate student. Your consistent and continued optimism has kept me going when things weren't working and made me strive to do better when they were. Regine Gries, you are the heart of the lab, your many words of wisdom, comfort, and scepticism have truly made me a better scientist. Dr. Jenny Cory, your assistance as a committee member has been invaluable, thank you for keeping me on track.

Dr. John Borden and Dr. Steve Takács, thank you for all of your assistance in the field and advice during the long and sometimes redundant meetings. Thank you to Dr. Huimin (Robby) Zhai, without your phenomenal synthetic chemistry a lot of this thesis would not have been possible.

My fellow Gries lab members Bekka Brodie, Sean McCann, Catherine Scott, Eloise Rowland, Mike Hrabar, thank you for welcoming me into the lab and making me feel at home. Matt Holl, Nathan Derstine, Sebastian Ibarra, Joshua Pol, Stephanie May, Veronika Lambinet, Adam Blake, Dan Peach, and Lydia Stepanovic thank you for all of your varied support and feedback at lab meetings! I am so lucky to have had all of your brilliant minds around me for the last three years.

I could not have completed this work without the unwavering support of my friends and family. Mom, grandma, granddad, for all of your love and help, knowing you are happy that I do what I love makes living in relative poverty easier. My non-biological sister Andrea Carter, your boundless support has been felt even from 1200 km away. Pentafriend, Natalie, Jasmin, Sarah, and Kasia thank you for loving me for who and everything I am, helping me escape and for understanding when I am too busy to hang out. Very special thanks to my partner Andrew Cook for being my anchor, ladder, and comfort, you have been whatever I need to help me get to where I want to be.

My cats, Kezia (RIP) and Morris you have done as much for me as my human friends, I hope I communicate this well enough in the form of treats and toys.

Table of Contents

Approval ii
Ethics Statement iii
Abstract iv
Dedication v
Acknowledgements vi
Table of Contents vii
List of Tables ix
List of Figures x

Chapter 1. Introduction 1
1.1. Commensal rodents as pests 1
Adaptations by commensal rodents to human activities 1
Methods of rodent management and their limitations 2
1.2. Research goals 4
1.3. Literature Cited 4

Chapter 2. Development of feeding stimulants for house mice (*Mus musculus*) and brown rats (*Rattus norvegicus*) 9
2.1. Abstract 9
2.2. Introduction 10
2.3. Materials and Methods 11
2.3.1. Lab animals 11
2.3.2. Survey of food items as feeding stimulants 12
2.3.3. Survey of most preferred food items as feeding stimulants 12
2.3.4. Re-building baby food as a feeding stimulant for rodents 13
2.3.5. Field trial of composition #14 (2012) 15
2.4. Results 15
2.4.1. Survey of food types as feeding stimulants 15
2.4.2. Survey of most preferred food items as feeding stimulants 15
2.4.3. Re-building baby food as a feeding stimulant for rodents 16
2.4.4. Field test of Composition #14 (2012) 17
2.4.5. Agar and carrageenan 17
2.5. Discussion 18
2.6. Literature Cited 20
2.7. Tables and Figures 22

Chapter 3. Effect of house mouse pheromones on behavioural responses of mice in the laboratory and on captures of wild mice in the field 34
3.1. Abstract 34
3.2. Introduction 35
3.3. Materials and Methods 38
3.3.1. Lab animals 38

3.3.2. O1: Laboratory-test the effect of bedding soiled with urine and feces of female or male mice on the responses of female and male mice....38
3.3.3. O2: Field-test the effect of bedding soiled by females or males on captures of wild mice....40
3.3.4. O3: Analyse odorants emanating from male- or female-soiled bedding....40
3.3.5. O4: Laboratory-test the effect of candidate male pheromone components on responses of females and males....42
3.3.6. O5: Field-test the effect of candidate male pheromone components on captures of wild mice....43
3.4. Results....44
3.4.1. O1: Laboratory-test the effect of bedding soiled with urine and feces of female or male mice on the responses of female and male mice....44
3.4.2. O2: Field-test the effect of bedding soiled by females or males on captures of wild mice....44
3.4.3. O3: Analyse odorants emanating from male- or female-soiled bedding. 45
3.4.4. O4: Laboratory-test the effect of candidate male pheromone components on responses of females and males....45
3.4.5. O5: Field-test the effect of candidate male pheromone components on captures of wild mice....46
3.5. Discussion....46
3.6. Literature Cited....49
3.7. Tables and Figures....55

Chapter 4. Concluding summary....63

List of Tables

Table 2.1 List of food items tested in food choice bioassays, their brand names and suppliers. 22

Table 2.2 Ingredients and the percent (%) present in compositions (C) #1-24. 24

Table 2.3 Physical characteristics of compositions (C) #1, 3-7. 26

Table 2.4 Mean consumption (%) of food items [Heinz baby food, Provoke®, various compositions (see Table 2.2)] by house mice in 2- or 3-food choice experiments (Figure 2.1, B). Data of 3-food choice experiments were analysed by Oneway ANOVA followed by the Tukey HSD test, and data of 2-food choice experiments were analyzed by the Students t-test. Within 3-food choice experiments, mean consumption rates associated with different letters are statistically different from one another. 27

Table 2.5 Effect of specific food compositions (C) (see Table 2.2) and Provoke® on attraction (1^{st} choice) and on the first food sampled (1^{st} fed) by house mice in T-tube olfactometer bioassays (see Figure 2.1, C). 28

Table 3.1 List of research objectives (O) and stimuli (S) tested in laboratory or field experiments (Exps.) 1-10 for the behavioural responses of laboratory-strain or wild house mice 55

List of Figures

Figure 2.1 (A) Design of feeding bioassay for rats. Glass aquarium (three in total; L × W × H = 60 × 30 × 40 cm) housing 3 or 4 Petri dishes (5 cm diam) with a specific food item in each corner. A single rat was released into the centre of each aquarium. (B) Design of feeding bioassay for mice; rectangular clear Plexiglas arena (five in total; 60 × 30 × 40 cm) with a food item in a Petri dish (5 cm diam) in opposite corners. A single mouse was released into the centre of each arena. (C) T-tube olfactometer design used to test behavioural responses of mice to test stimuli in laboratory experiments; (1) clear Plexiglass arena (40 × 20 × 30 cm) for placement of a mouse at the onset of a bioassay; (2) glass T-tube (stem: 65 cm long, side arms: 45 cm long, all 10 cm in diam); (3a, 3b) Plexiglass arenas (each 60 × 30 × 40 cm) housing test stimuli; (4a, 4b) Petri dish (5 cm diam) with food bait. 29

Figure 2.2 Mean differential consumption of food items (see reference numbers in Table 2.1) by brown rats in each of food-choice experiments A-F. In each replicate (N=3) of experiments A-F, a single rat was offered a choice of four different food items (2 g each). Food consumption was determined by weighing the food at the onset and end of a replicate (see methods for details). 30

Figure 2.3 Mean differential consumption (with 95% confidence intervals) of those four food items most preferred by brown rats in food-choice experiments A-F (Figure 2.2). In each replicate (N=18), a single brown rat was offered a choice of these four food items (2 g each). Food consumption was determined by weighing each food item at the onset and end of a replicate (see methods for details). There was no detectable preference for any of the four food items (Oneway ANOVA $F_{3,68} = 2.61$, $p = 0.06$). 31

Figure 2.4 Differential captures of house mice in paired snap traps baited with Composition #14 and the leading commercial rodent bait Provoke® Mice (Bell Laboratories Inc.) in a field experiment (31 Aug. to 03 Sept. 2012) on a farm in Richmond, BC (Photo: G. Gries). 32

Figure 2.5 Flow chart of developmental steps leading to the production of an effective rodent bait. Work presented in this chapter pertains to the first two steps. Steps from formulation chemistry to commercial bait production have input from the industrial partner. 33

Figure 3.1 Graphical illustration of the experimental design used in laboratory and field experiments. (A, B) T-tube olfactometer with release arena (1; 40 cm × 20 cm × 30 cm), T-Tube (2; stem: 65 cm long, side arms: 45 cm long, all 10 cm in diam), and stimulus arenas (3a, 3b; 60 cm × 30 cm × 40 cm), each housing a Petri dish (5 cm diam) with a food bait (4) and (*i*) an Erlenmeyer flask containing soiled or clean bedding (5a, 5b) (A) or (*ii*) a 20-ml scintillation vial (6a, 6b) in a 150-ml beaker (7a, 7b) containing mineral oil laced, or not, with the two pheromone components 2-*sec*-butyl-4,5-dihydrothiazole (DHT) and 3,4-dehydro-*exo*-brevicomin (DEB) (B). (C, D, E) Paired trap boxes (8) each fitted with a baited Victor® snap trap (9) and containing (*i*) clean bedding or bedding soiled (s) by caged female mice (C) or caged male mice (D) or (*ii*) a scintillation vial containing 10 g of mineral oil laced, or not with DEB and DHT (E)..... 56

Figure 3.2 Effect of bedding soiled by caged female house mice on behavioural responses of adult female mice (Experiment 1) and adult male mice (Experiment 2). In all experimental replicates (N=35), the mouse was given a choice between stimuli presented in bioassay arenas 3a or 3b (see Figure 3.1, A). The following data were recorded and analyzed: (*i*) the treatment or control arena that the mouse entered first with all four paws (first choice data), (*ii*) the arena in which it first fed on the food bait ("first-feeding data"), and (*iii*) the proportion of time it spent in each arena ("time spent data"). In each experiment, the Asterisk (*) denotes a significant preference for a test stimulus (Students t-test; CI = confidence interval)..... 57

Figure 3.3 Effect of bedding soiled by caged male house mice on behavioural responses of adult female mice (Experiment 3) and adult male mice (Experiment 4). In all experimental replicates (N=27), the mouse was given a choice between stimuli presented in bioassay arenas 3a or 3b (see Figure 3.1, A). The following data were recorded and analyzed: (*i*) the treatment or control arena that the mouse entered first with all four paws (first choice data), (*ii*) the arena in which it first fed on the food bait ("first-feeding data"), and (*iii*) the proportion of time it spent in treatment or control arenas ("time spent data"). The Asterisk (*) denotes a significant preference for a test stimulus (Students t-test; CI = confidence interval)..... 58

Figure 3.4 Numbers of house mice captured in field experiments 5 and 6 during late summer and fall of 2014 in snap traps inside paired trap boxes (N=73) containing bedding soiled, or not, by either caged female mice (A) or caged male mice (B) (see methods for detail)..... 59

Figure 3.5 (A) Total ion chromatograms of volatile components emanating from urine- and feces-soiled bedding of adult male (top) and adult female (bottom) house mice. Note: 3,4-dehydro-*exo*-brevicomin (DEB) is about seven times more abundant in volatiles of males than in volatiles of females, and 2-*sec*-butyl-4,5-dihydrothiazole (DHT) is a male-specific component. (B) Gas chromatographic analyses on a chiral GC column of (*i*) synthetic racemic DEB (top), (*ii*) synthetic (1*R*,5*S*,7*R*)-DEB (middle), and (*iii*) DEB produced by male and female mice. Note: (a) male mice and female mice produce (1*R*,5*S*,7*R*)-DEB and (1*S*,5*R*,7*S*)-3,4-DEB at a 3:1 ratio; (b) only (*S*)-2-(*sec*-butyl)-4,5-dihydrothiazole is produced by male mice (Cavaggioni et al. 2003); (c) the identity of compounds in B was confirmed by GC-MS analyses. 60

Figure 3.6 Effect of the synthetic 3,4-dehydro-*exo*-brevicomin (DEB) and 2-*sec*-butyl-4,5-dihydrothiazole (DHT; 1:1 ratio; 2 mg total; formulated in 10 g of mineral oil) on behavioural responses of individually tested, group-housed house mouse females (Experiment 7), group-housed males (Experiment 8) and singly-housed males (Experiment 9). In all experimental replicates (N = 26), the mouse was given a choice between stimuli presented in bioassay arenas 3a or 3b (see Figure 3.1, B). The following data were recorded and analyzed: (*i*) the treatment or control arena that the mouse entered first with all four paws (first choice data), (*ii*) the arena in which it first fed on the food bait ("first-feeding data"), and (*iii*) the proportion of time it spent in treatment or control arenas ("time spent data"). The asterisk (*) denotes a significant preference for a test stimulus (Students t-test; CI = confidence interval). 61

Figure 3.7 Effect of synthetic male mouse sex pheromone components 3,4-dehydro-*exo*-brevicomin (DEB) and 2-*sec*-butyl-4,5-dihydrothiazole (DHT) (1:1 ratio; 2 mg total; formulated in 10 g of mineral oil) on trap captures of adult and juvenile male and female house mice in field experiment 10 (22 January to 25 September 2015). In all replicates (N=124), mice were given a choice between paired trap boxes, each box fitted with a food-baited snap trap, and the treatment box, but not the control box, also baited with DEB & DHT. Trap captures differed between adult females, adult males, juvenile females or juvenile males (Logistic regression analyses; residual deviance = 7.71, $P = 0.0004$). As indicated by different letter superscripts on bars, the probability of adult female mice to be captured in pheromone–baited traps relative to control traps (0.8) was significantly greater than the probabilities of adult males (0.33), juvenile females (0.37), or juvenile males (0.42) (Tukey multiple comparisons of trapping probabilities; $P < 0.05$). 62

Chapter 1. Introduction

1.1. Commensal rodents as pests

Adaptations by commensal rodents to human activities

Rodents in the family Murinae are some of the most prolific and damaging pests worldwide (Singleton *et al*. 1999). Best known species in Murinae are the commensal rodents: (*i*) house mouse, *Mus musculus* L.*,* (*ii*) black rat (alias ship rat, house rat, roof rat, fruit rat, Alexandrian rat), *Rattus rattus* L.*,* and (*iii*) brown rat (alias Norway rat, Norwegian rat, common rat, street rat, sewer rat, wharf rat, Hanover rat), *Rattus norvegicus* (Berkenhout 1769). These species are associated with humans and are referred to as commensal (eating at the same table) rodents. Contrary to the ecological concept of commensalism, where one member of a relationship benefits while the other is unaffected, "commensal" rodents at high population levels have numerous adverse effects on humans. When inadvertently introduced by humans into new habitats or ecosystems, these invasive rodents have detrimental impacts on native wildlife. Indeed, commensal rodents are more accurately referred to as synanthropic rodents as they are widely regarded as pests and invasive species (Battersby 2015).

Species in the genera *Mus* and *Rattus* have evolved alongside humans since the mid Pleistocene (1 – 2.5 million years ago), and have dispersed all over the globe with early humans in the late Pleistocene (~11,700 years ago; Macdonald *et al*. 2015). With their high reproductive rates, synathropic rodents adapt quickly to new selective pressures (Macdonald *et al.* 2015), including those put on them by humans. *Rattus and Mus* species adapted to human activities, adopted the human diet, and evolved trap avoidance behaviours. The ancestors of murine rodents are believed to have originated in Asia (Macdonald *et al.* 2015). They were granivorous but had dental specializations that enabled adoption of a generalist diet over evolutionary time (Tiphaine *et al.* 2013).

This generalist diet, in turn, allowed these rodents to live almost anywhere and even become predators; modern rats hunt other rodents and birds.

Mice and rats are present in diverse habitats including urban, agricultural and natural settings, where they cause problems for humans and wildlife (Singleton *et al*. 1999). As household pests, mice and rats not only feed on and contaminate food, they also cause significant structural damage by their nesting, chewing and gnawing activities (Smith and Meyer 2015). For example, they use insulation material in attics and walls to build nests and if they perish in these inaccessible areas, they cause considerable distress to human residents. Chewing on electrical wires is not only frustrating for homeowners but is also a considerable fire risk (Smith and Meyer 2015).

Rats and mice plague agricultural crops, food stores and livestock production facilities (Lund 2015). For example, in Indonesia rats cause 17% pre-harvest losses in rice, which could feed 25 million Indonesian people for one year (Singleton 1999). The 1993 mouse plague in southern Australia caused an estimated $64.5 million (AUS) in damages to standing crops, food stores, infrastructure, and property (Buckle and Pelz 2015). On poultry farms, they eat eggs and even kill and eat young birds. This predatory behaviour is also a problem in natural ecosystems, especially islands. Rats and mice that have adapted to being nest predators can greatly reduce populations of ground-nesting birds (Atkinson 1985, Howald *et al.* 2007, Wanless *et al.* 2007, Jones *et al.* 2008, Angel *et al.* 2009, Simberloff 2009, Towns *et al.* 2009).

Methods of rodent management and their limitations

For as long as commensal rodents have been pests for humans, humans have been trying to trap them. Baked clay traps for small rodents have been found as early as 5,000 years ago in Northern Africa, Pakistan and Northern India (Drummond 2005). It is likely that humans were trapping small rodents even earlier than that but pertinent records are not as well preserved as baked clay traps. Selective pressure of trapping resulted in many counter-adaptations by mice and rats, such as neophobia (the fear of new objects), which helped them avoid getting trapped and killed. Cautious rodents do not readily enter objects like bait boxes in their habitat. Neophobic behaviour has been

well documented in wild mice and rats as a trap avoidance strategy (Inglis *et al*. 1996, Brigham and Sibly 1999).

Toxic baits for mice and rats are reported as early as 2,200 years ago in classical Greece and Rome (Smith and Secoy 1975). Rodenticides can have an acute or chronic lethal effect with reference to the speed at which they kill a rodent. Acute rodenticides that typically kill a rodent after a single feeding bout were exclusively used until the advent of chronic rodenticides, namely anticoagulants, in 1950. Anticoagulants inhibit the synthesis of blood-clotting factors and subject rodents to internal bleeding and massive haemorrhaging. Unlike acute rodenticides, anticoagulants must be ingested repeatedly and may take up to 10 days to cause death.

First-generation anticoagulant rodenticides included warfarin and related compounds. Between 1950 and 1970, they were commercialized and sold widely all over the world. In 1958, warfarin-resistant populations of *R. norvegicus* were found in Scotland (Boyle 1960) and later across all continents. Warfarin-resistant populations of *R. rattus* (Saunders 1978) and *M. musculus* (Dodsworth 1961, Rowe and Redfern 1965) were also detected. In response to the ever increasing resistance of mice and rats to first-generation rodenticides, second-generation rodenticides (SGRs) were developed which are much more acute than first-generation rodenticides. Although there is some concern that rodents may develop resistance to SGRs, SGRs are currently the most widely used method of rodent control.

Although SGRs revolutionized rodent management and are considered most effective, there is rising concern about their secondary toxicity in non-target animals. Most countries require rodenticides to be placed in exclusionary devices to prevent non-target animals and humans from consuming or tampering with the poisonous bait. However, rodents that have ingested SGRs exhibit impaired behaviour and become easy targets for predators (Cox and Smith 1992; Thomas *et al.* 2011). Over time, predators accumulate SGRs from fed-on prey in their own bodies, causing both sub-lethal and lethal effects. This is evident in (*i*) birds of prey such as American kestrels, *Falco sparverius* (Rattner *et al*. 2011) and red tailed hawks, *Buteo jamaicensis* (Murray and Tseng 2008, Thomas *et al.* 2011), (*ii*) small mammalian predators such as foxes, *Vulpes vulpes*, and polecats, *Mustela putorius* (Berny *et al.* 1997, Shore *et al.* 1999), and

(*iii*) even large mammalian predators such as bobcats, *Lynx rufus*, and mountain lions, *Puma concolor* (Riley *et al*. 2007).

1.2. Research goals

The research presented in my thesis aims to improve the attraction and capture of mice and rats, and thus to reduce our reliance on SGRs for rodent control. To achieve these goals, I wanted to take a two-pronged approach that addresses the need of these animals to both feed and communicate. By incorporating both food cues and communication signals into a bait complex, I was hoping to overcome the neophobic trap avoidance behaviour of mice (and rats) and to expedite their captures in snap traps.

In Chapter 2, I focus on food cues. I present the methods I applied to develop effective feeding stimulants that are to be used in conjunction with food-based, long-range semiochemical attractants. The data presented in this chapter are an integral part of a larger study that was undertaken to develop commercially viable and competitive rodent baits that can be deployed to increase the efficacy of snap traps.

In Chapter 3, I focus on communication signals of house mice. There are abundant anecdotal and peer-reviewed reports (e.g., Rowe 1970, Daly *et al.* 1980, Drickamer 1995, 1997) that traps which have previously captured a mouse are more likely to capture another mouse than clean traps, possibly because traps with prior captures carry some kind of a mouse scent. Therefore, I investigated whether mouse urine, and specifically male mouse urine, contains pheromones that can be deployed to attract (female) mice to trap boxes, overcome their neophobia, and increase their captures in snap traps.

1.3. Literature Cited

Angel, A., R. M. Wanless, and J. Cooper. 2009. Review of impacts of the introduced house mouse on islands in the Southern Ocean: are mice equivalent to rats? Biological Invasions 11:1743–1754.

Atkinson, I. A. E. 1985. The spread of commensal species of *Rattus* to oceanic islands and their effects on island avifaunas. Conservation of Island Birds 3:35–81.

Battersby, S. A. 2015. Rodents as carriers of disease. Pages 81-100 *in* A. P. Buckle and R. H. Smith, editors. Rodent pests and their control. 2nd edition. CAB International, Boston, MA, USA.

Berkenhout, J. 1769. Pages XIII and 233 *in* Outlines of the natural history of Great Britain and Ireland, containing a systematic arrangement and concise description of all the animals, vegetables, and fossiles which have hitherto been discovered in these kingdoms. Vol. I. Comprehending the animal kingdom. Elmsly London, UK.

Berny, P. J., T. Buronfosse, F. Buronfosse, F. Lamarque, and G. Lorgue. 1997. Field evidence of secondary poisoning of foxes (*Vulpes vulpes*) and buzzards (*Buteo buteo*) by bromadiolone, a 4-year survey. Chemosphere 35:1817-1829.

Boyle, C. M. 1960. Case of apparent resistance of *Rattus norvegicus* Berkenhout to anticoagulant poisons. Nature 188:517.

Brigham, A. J., and R. M. Sibly. 1999. A review of the phenomenon of neophobia. Pages 67–84 *in* Advances in vertebrate pest management. Filander Verlag, Furth, Germany.

Buckle, A. P. and H. J. Pelz. 2015. Pages 247-258 *in* A. P. Buckle and R. H. Smith, editors. Rodent pests and their control. 2nd edition. CAB International, Boston, MA, USA.

Cox, P. and R. H. Smith. 1992. Rodenticide ecotoxicology: pre-lethal effects of anticoagulants on rat behaviour. Pages 165-170 *in* J. E. Borreco, R. E. Marsh, editors. Proceedings of the 15th Vertebrate Pest Conference. University of California, Davis, California, USA.

Daly, M., M. I. Wilson, and P. Behrends. 1980. Factors affecting rodents' responses to odours of strangers encountered in the field : experiments with odour-baited traps. Behavioural Ecology and Sociobiology 6:323–329.

Dodsworth, E. 1961. Mice are spreading despite such poisons as warfarin. Municipal Engineering London 3746:1668.

Drickamer, L. C. 1995. Odors in traps: does most recent occupant influence capture rates for house mice? Journal of Chemical Ecology 21:541–555.

Drickamer, L. C. 1997. Responses to odors of dominant and subordinate house mice (*Mus domesticus*) in live traps and responses to odors in live traps by dominant and subordinate males. Journal of Chemical Ecology 23:2493–2506.

Drummond, D. 2005. Mouse traps: a quick scamper through their long history. North American Trap Collectors Association, INC, Galloway, OH.

Howald, G., C. J. Donlan, J. P. Galván, J. C. Russell, J. Parkes, A. Samaniego, Y. Wang, D. Veitch, P. Genovesi, M. Pascal, A. Saunders, and B. Tershy. 2007. Invasive rodent eradication on islands. Conservation Biology 21:1258–1268.

Inglis, I. R., D. S. Shepherd, P. Smith, P. J. Haynes, D. S. Bull, D. P. Cowan, and D. Whitehead. 1996. Foraging behaviour of wild rats (*Rattus norvegicus*) towards new foods and bait containers. Applied Animal Behaviour Science 47:175–190.

Jones, H. P., B. R. Tershy, E. S. Zavaleta, D. A. Croll, B. S. Keitt, M. E. Finkelstein, and G. R. Howald. 2008. Severity of the effects of invasive rats on seabirds: A global review. Conservation Biology 22:16–26.

Lund, M. Commensal rodents. 2015. Pages 19-32. *in* A. P. Buckle and R. H. Smith, editors. Rodent pests and their control. 2nd edition. CAB International, Boston, MA, USA.

Macdonald, D. W., M. G. P. Fenn, and M. Gelling. Natural History of Rodents: Preadaptations to Pestilence. 2015 Pages 1–18 *in* A. P. Buckle and R. H. Smith, editors. Rodent pests and their control. 2nd edition. CAB International, Boston, MA, USA.

Murray, M., and F. Tseng. 2008. Diagnosis and treatment of secondary anticoagulant

rodenticide toxicosis in a red-tailed hawk (*Buteo jamaicensis*). Journal of Avian Medicine and Surgery 22:41–46.

Rattner, B. A., K. E. Horak, S. E. Warner, D. D. Day, C. U. Meteyer, S. F. Volker, J. D. Eisemann, and J. J. Johnston. 2011. Acute toxicity, histopathology, and coagulopathy in American kestrels (*Falco sparverius*) following administration of the rodenticide diphacinone. Environmental Toxicology and Chemistry 30:1213–1222.

Riley, S. P. D., C. Bromley, R. H. Poppenga, , F. A. Uzal, L. Whited, and R. M. Sauvajot. 2007. Anticoagulant exposure and notoedric mange in bobcats and mountain lions in urban Southern California. Journal of Wildlife Management 71:1874–1884.

Rowe, F. P. 1970. The response of wild house mice (*Mus musculus*) to live-traps marked by their own and by a foreign mouse odour. Journal of Zoology 162:517–520.

Rowe, F. P., and R. Redfern. 1965. Toxicity tests on suspected warfarin resistant house mice (*Mus musculus* L.). Journal of Hygiene 63:417–425.

Saunders, G. R. 1978. Resistance to warfarin in roof rat in Sydney, NSW. Search 9: 39-40.

Shore, R. F., J. D. S. Birks, and P. Freestone. 1999. Exposure of non-target vertebrates to second-generation rodenticides in Britain, with particular reference to the polecat *Mustela putorius*. New Zealand Journal of Ecology 23:199–206.

Simberloff, D. 2009. Rats are not the only introduced rodents producing ecosystem impacts on islands. Biological Invasions 11:1735–1742.

Singleton, G. R., H. Leirs, L. A. Hinds, and Z. B. Zhang. 1999. Ecologically-based management of rodent pests: re-evaluating our approach to an old problem. Pages 17-29 *in* G. R. Singleton, L. Hinds, H. Leirs, Z. Zhang, editors. Ecologically-Based Management of Rodent Pests. ACIAR, Canberra, Australia.

Smith, R. H. and A. N. Meyer. 2015. Rodent control methods: non-chemical and non-lethal chemical, with special reference to food stores. Pages 101-122 *n* A. P. Buckle

and R. H. Smith, editors. Rodent pests and their control. 2nd edition. CAB International, Boston, MA, USA.

Smith, A. E., and D. M. Secoy. 1975. Forerunners of pesticides in classical Greece and Rome. Journal of Agricultural Food Chemistry 23:1050–1055.

Thomas, P. J., P. Mineau, R. F. Shore, L. Champoux, P. A. Martin, L. K. Wilson, G. Fitzgerald, and J. E. Elliott. 2011. Second generation anticoagulant rodenticides in predatory birds: Probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada. Environment International 37:914–920.

Tiphaine, C., C. Yaowalak, C. Cyril, G. R. Helder, M. Jacques, T. Paul, V. L. Monique, V. Laurent, and L. Vincent. 2013. Correlated changes in occlusal pattern and diet in stem murinae during the onset of the radiation of old world rats and mice. Evolution 67:3323–3338.

Towns, D. R., D. A. Wardle, C. P. H. Mulder, G. W. Yeates, B. M. Fitzgerald, G. Richard Parrish, P. J. Bellingham, and K. I. Bonner. 2009. Predation of seabirds by invasive rats: Multiple indirect consequences for invertebrate communities. Oikos 118:420–430.

Wanless, R. M., A. Angel, R. J. Cuthbert, G. M. Hilton, and P. G. Ryan. 2007. Can predation by invasive mice drive seabird extinctions? Biology Letters 3:241–244.

Chapter 2. Development of feeding stimulants for house mice (*Mus musculus*) and brown rats (*Rattus norvegicus*)

Some aspects of this chapter are part of a PCT patent application PCT/CA2014/050435 with the following authors: Antonia E. Musso, Stephen J. Takács, Regine M. Gries, and Gerhard J. Gries

2.1. Abstract

The efficacy of baits for mice and rats hinges upon both potent long-range semiochemicals that attract rodents and feeding stimulants that prompt a feeding response, thereby triggering the capture mechanism of snap traps or mediating ingestion of lethal agents. My objectives were to determine effective feeding stimulants and to investigate an optimal dose of a proven-effective semiochemical blend ("Entice": 2-hydroxy-3-methylcyclopent-2-en-1-one, 2,3-butandione, butyric acid, 6-methyl-(*E*)-2-hepten-4-one, γ-octalactone, 3-methylbutanal). In laboratory four-choice experiments, I tested a large variety of food types for the feeding preference of laboratory-strain brown rats, *Rattus norvegicus.* Heinz Mixed Cereal Baby Food (HMCBF) was one of the rats' top food choices. A simplified version of HMCBF (Composition #14), comprised of oat flour, rice flour, wheat bran, safflower oil, soy lecithin, fructose, a gelatine/water solution and 0.001% of "Entice" in safflower oil, also elicited good feeding responses by house mice, *Mus musculus,* and attracted house mice in T-tube olfactometer experiments. In a proof-of-concept field experiment, paired snap traps baited with Composition #14 captured seven house mice, whereas corresponding traps baited with Provoke® Mice (a leading commercial mouse bait) captured only a single mouse, indicating the superior effectiveness of Composition #14. Further experiments then focused on improving physical characteristics of Composition #14, and on testing the effect of select preservatives to suppress microbial growth and thus stabilize the composition.

2.2. Introduction

The brown rat (*Rattus norvegicus*) and house mouse (*Mus musculus*) are some of the most damaging pests worldwide (Singleton *et al.* 1999). Having co-existed and dispersed with humans for over 10,000 years, mice and rats are now present and persistent on every continent except Antarctica (Macdonald 2015). Inhabiting both natural and man-made environments, and thriving on an opportunistic, omnivorous diet, these rodents have become some of the most successful mammals on the planet.

While there is some literature on food preferences of rats and mice (Barnett and Spencer 1953, Pennycuik and Cowan 1990), most of the pertinent information as to what food type constitutes a suitable or perfect trap bait for rodents is garnered from anecdotal evidence. From peanut butter to bacon fat, there are many different kinds of home "remedies", with peanut butter being the most popular and widely reported by pest management companies and operators. As effective as peanut butter is as a rodent bait, it contains allergens and by law must no longer be used by pest management professionals. Thus, hypoallergenic alternatives to peanut butter baits are much needed.

Effective rodent baits placed in lethal traps must contain (*i*) long-range semiochemicals (message bearing chemicals) that attract rodents to bait; and (*ii*) feeding stimulants that induce feeding on bait, which is typically placed on the trip mechanism of the trap, thus triggering a spring-loaded bar or jaw that kills the rodent. Alternatively, feeding on the bait mediates ingestion of lethal substances. In snap traps, many rodents can simply lick the bait out of the trip mechanism without triggering the trap to snap. An ideal feeding stimulant is not only tasty to rodents, it is also formulated in such a way that it is difficult to remove during feeding.

The work presented in this chapter builds on research by Takács *et al.* (2013) who developed a six-component blend of semiochemicals characteristic of the smell of certain candy (2-hydroxy-3-methylcyclopent-2-en-1-one), cheese (2,3-butandione, butyric acid), hazelnuts and coconuts (6-methyl-(*E*)-2-hepten-4-one, γ-octalactone), and chocolate (3-methylbutanal). When formulated in wet-rendered lard (Tenderflake®, Maple Leaf Foods, Mississauga, Ontario), this blend of semiochemicals, termed "Entice", attracted significantly more house mice and brown rats in laboratory bioassays relative to

a control. However, field tests of Entice formulated in lard did not yield significant trap captures of rodents compared to the leading commercial bait brand Provoke® (Bell Laboratories Inc., Madison, WI 53704, USA) which it had out competed in the lab in attractiveness. It was hypothesized that wild-type rodents did not readily consume the lard, and thus did not trigger the snap mechanism that would have captured them.

Subsequent laboratory bioassays revealed that dry-rendered lard procured from a local deli increased the feeding response by mice. Adding cut-up “cracklings” or “pork rind” (fried pig skin), to Entice compositions with dry- and wet-rendered lard further increased the feeding responses of mice. However, the same formulations did not induce appreciable feeding by brown rats (Musso, unpublished data). My objective was to design a bait composition of feeding stimulants that induced feeding by both mice and rats.

This objective proved challenging because house mice and brown rats prefer different types of food as described above, and in the literature (Barnett and Spencer 1953, Rowe *et al*. 1974, Pennycuik and Cowan 1990, Morris *et al.* 2012). Drawing on the food preference survey for mice in Takács *et al.* (2013), I ran an equivalent survey for brown rats, and then screened for those food types (feeding stimulants) that induced feeding by both house mice and brown rats. These food types, together with the Entice semiochemicals, were then formulated into a commercial rodent bait prototype.

2.3. Materials and Methods

2.3.1. Lab animals

CD-1® strain house mice and BN/Crl strain brown rats at 4 and 8 weeks of age, respectively, were obtained from Charles River Laboratories International Inc. (Saint-Constant, QC J5A 2E7, Canada). On arrival, 20 rats or 20 mice were assigned to four groups of five males each and four groups of five females for each species, accommodated in cages (50 × 40 × 20 cm) lined with commercial corncob bedding (Anderson’s Bed o’cobs, The Andersons Inc. Maumee, OH 43537, USA), provided with Nalgene toys and running wheels (Jaimesons Pet Food Distributers, Richmond, BC V4G

1C9, Canada), and provisioned with commercial rodent food (LabDiet® Certified Rodent Diet, LabDiet, St. Louis, MO 64144, USA) and water *ad libitum*. The Animal Care Services of Simon Fraser University monitored and cared for all animals. Animal rooms were kept at a consistent relative humidity and temperature, and a reversed photoperiod (12L:12D), with the scotophase running from 13:00 h to 01:00 h to facilitate behavioural bioassays. When male animals reached puberty, those individuals that became aggressive were removed from their groups and housed singly to prevent injury to their cage mates. Only healthy animals were used in behavioural bioassays. All bioassays were run during scotophase between 13:00 h and 21:00 h, observations were facilitated by dim red light.

2.3.2. Survey of food items as feeding stimulants

Twenty-three food items (Table 2.1) were tested for the feeding responses of rats. For each bioassay, a single male rat was placed in a rectangular glass aquarium (60 × 30 × 40 cm) without a lid, given a choice between 3-4 types of food (Figure 2.1, A) and observed for 2 h under dim red light. Rats were deprived of food, but not water, for 12-16 h before a bioassay. Each food item was put in a Petri dish (5 cm diam), which was randomly assigned to one of the four corners of the aquarium. Dry foods (1 g) were ground (if necessary) and mixed with water (1 mL) to form a paste, whereas moist foods (2 g) were mashed (if necessary). Petri dishes were weighed before and after a bioassay to determine, for each food item, the amount that was consumed. Additional Petri dishes were filled with 2 g of each food item tested and kept outside the glass aquaria to estimate the weight loss due to water evaporation during the 2-h bioassay period. Each set of food choices was tested in three separate replicates with a single male rat, allowing me to calculate the mean consumption of each food type. Over all feeding response bioassays, each individual rat was tested only once.

2.3.3. Survey of most preferred food items as feeding stimulants

After the four most preferred food items (Heinz baby food cereal, honey oat cereal, oats, rat chow) had been determined (see Results 2.4.1; Figure 2.2), they were tested in 4-choice bioassays (see above; Figure 2.1, A) with 15 single male rats and

three single female rats. Consumption of each food type was determined as described above. Data were analysed by Oneway ANOVA, using JMP® 11.2.0 (Copyright © 2013 SAS Institute Inc.).

2.3.4. Re-building baby food as a feeding stimulant for rodents

Heinz Mixed Cereal Baby Food (HMCBF) turned out to be one of the most preferred foods of brown rats (see results), contrasting with dry-rendered lard as the top food choice of mice (Takács *et al*. 2013). Considering that HMCBF was the secondary food choice for mice and that lard was not an effective feeding stimulant for rats, HMCBF was selected as a template to design a feeding stimulant composition effective for both mice and rats.

HMCBF is comprised of oat flour, wheat flour, rice flour, oligofructose, dicalcium phosphate, safflower oil, soy lecithin, vitamins (niacinamide, riboflavin, thiamine mononitrate), and iron. While several ingredients such as the vitamins and minerals are important nutrition for babies, they are not needed for a rodent feeding stimulant, and thus were omitted from the composition. Included in the composition were (*i*) cereals (a blend of oats, and long-grained white rice), (*ii*) all-purpose white wheat flour, (*iii*) safflower oil (high heat, refined; Spectrum®, Lake Success, NY 11042, USA), (*iv*) fructose (Sigma Aldrich) and (*v*) granulated soy lecithin (Xenex Labs Coquitlam, BC V3K 6C2, Canada). A gelatine solution, prepared - as per package instructions - by boiling water with Knox® Unflavoured Gelatine (The Kraft Heinz Company, Northfield, IL 60093, USA), was added to the composition to provide moisture and allow formation of a paste.

Once the base composition of cereals, fructose, soy lecithin and gelatine/water was in place (Compositions 1-10, Table 2.2), the Entice semiochemicals were added to the safflower oil (0.33% of total composition) at concentrations of 1, 0.1, 0.01 or 0.001% w/v (Compositions 11-14, Table 2.2). As preservatives, sorbic acid and calcium propionate were admixed with the composition (Compositions 15-18, Table 2.2). Subsequently, the liquid portion of the composition was switched from gelatine to agar, and later to agar and carrageenan (Compositions 19-24, Table 2.2), which were dissolved in water during boiling and then added to the dry ingredients of the composition.

Various permutations of the composition were tested in either food choice bioassays or T-tube olfactometer bioassays with house mice. Food choice bioassays were run in five rectangular clean Plexiglass arenas (each 60 × 30 × 40 cm; L × W × H) with two food choices presented in Petri dishes (5 cm diam) placed in opposite corners of the arena (Figure 2.1, B). A single mouse deprived of food, but not water, for 4-6 h was released in the centre of each arena and allowed to sample and feed for 2 h under dim red light. Recorded were (*i*) the food it sampled first, (*ii*) any obvious feeding preferences, and (*iii*) the amount of each composition consumed (determined by weighing each composition before and after a bioassay).

The T-Tube olfactometer (Figure 2.1, C) consisted of three clear Plexiglass arenas with vented lids (**1**: 40 cm × 20 cm × 30 cm; **3a** and **3b**: each 60 cm × 30 cm × 40 cm) interconnected by a Pyrex glass T-tube (**2**; stem: 65 cm long, side arms: 45 cm long, all 10 cm in diam). For each bioassay, a 1-g aliquot of one of two test compositions was placed in a Petri dish (5 cm diam; **4a** or **4b**) and randomly assigned to one of two opposite corners of the arena. Subsequently, arenas were covered with lids and composition-derived odorants including the Entice semiochemicals were allowed to diffuse through the arenas and T-tube for 5 min. Then, a single mouse (food- but not water-deprived for 4-6 h) was placed in the release arena (**1**) and allowed to enter and explore the T-tube (**2**) on its own accord in response to test stimuli. For each bioassay, a single mouse was tested, recording the following data: (*i*) the stimulus arena (**3a** or **3b**) the mouse entered first with all four paws ("first choice" data), (*ii*) the feeding stimulus (**4a** or **4b**) which it fed on first ("first-feeding" data), and (*iii*) its position every 15 seconds. The bioassay was considered complete when the mouse fed on one of the food stimuli or 30 min had passed. Mice that did not make a first choice were excluded from statistical analyses.

Following each replicate (N=143), food choice arenas and the entire T-tube olfactometer set-up were cleaned with Percept™ disinfectant detergent (Virox Technologies Inc. Oakville, ON L6H 6R1), and were wiped with Nature's Miracle Pet Stain and Odour Remover (Spectrum Brands, Blacksburg, VA 24060, USA) to remove any remnant urine odours that may have been deposited. All equipment was left to dry for 30 min before being used in subsequent bioassays.

2.3.5. Field trial of composition #14 (2012)

Composition #14 proved effective in laboratory bioassays (see results), and thus was tested in a field experiment (31 August to 03 September 2012) to gauge its effect on responses and captures of wild house mice. At the recommendation of a local pest management company, the experiment was set up in the dry storage shed of a farm in Delta (British Columbia) that had persistent mouse presence. Experimental replicates (N=22) were placed along the interior walls of the shed. Each of the 22 replicates consisted of paired snap traps (Victor® M325 M7 Pro mouse trap, Woodstream Co. Lititz, PA 175543, USA), with 50-cm spacing between traps in each pair, and at least 2 m between pairs. By random assignment, one trap in each pair was baited with Composition #14 and the other with Provoke® (Bell Laboratories Inc., Madison, WI 53704, USA), a leading commercial bait. Traps were checked and re-baited on 01 and 03 September, terminating the experiment on 04 September 2012.

2.4. Results

2.4.1. Survey of food types as feeding stimulants

Of the many food types (Table 2.1) tested for food preference choices by rats, rats consumed particularly large amounts of Rat Chow (93.3%), Honey Oat Cereal (91.7%), Oats (65%) and Heinz Mixed Cereal Baby Food (HMCBF) (69.1%) (Figure 2.2). Rats also consumed large amounts of vanilla pudding (75%) or oatmeal cookies (61%), but these two food items were discounted in anticipation of difficulties to formulate them as a commercial bait.

2.4.2. Survey of most preferred food items as feeding stimulants

In 4-food-choice feeding bioassays, rats consumed on average 43.4% of HMCBF ; 38.5% of Rat Chow; 36.3% of Honey Oat Cereal; and 13.7% of Oats (Figure 2.3). These differences in food consumption were not statistically significant (Oneway ANOVA; $F_{3,68} = 2.61$, $p = 0.06$). It is noteworthy that these four food items are all cereal-based and that the HMCBF contains the fewest ingredients.

2.4.3. Re-building baby food as a feeding stimulant for rodents

Ingredients selected from the HMCBF (Table 2.2) were prepared in 24 separate compositions, of which most were tested for the feeding response of mice. Compositions #1 and #3-7 (Table 2.3) were not tested in bioassays but were kept in the laboratory for assessment of their physical properties. Compositions #2 and #8-10 (Table 2.2) differed in the ratio of dry to wet ingredients and the ratio of cereal flours, and as a result, affected the feeding response of mice in relation to HMCBF and Provoke® (Exp. #1-4; Table 2.4).

All-purpose white flour was a common constituent of compositions #1-9 but seemed to adversely affect both the taste and consistency of these compositions. This prompted me to replace the white flour with whole-wheat flour, which contains both wheat germ and bran. Adding more wheat bran provided the desired consistency in the composition (Compositions #9 and 10; Table 2.4). Guided by the positive feeding responses of mice to compositions containing wheat bran, follow-up compositions (#10 onward; Table 2.2) substituted wheat bran entirely for wheat flour (all-purpose and whole wheat).

The effect of Entice semiochemicals formulated in safflower oil (Compositions #11-16; Table 2.2) on responses of mice was tested in T-tube olfactometer bioassays. When mice were given a choice between Composition #11 (containing 1% Entice in safflower oil) and composition #10 (lacking Entice in safflower oil), mice first chose Composition #10 5 times and Composition #11 only 2 times (Table 2.5). Interestingly, mice also preferentially fed on composition #10. The mice that did feed on composition #11 were observed to spend a lot of time grooming their faces after coming in contact with the composition, implying that the concentration of Entice might have been too high, thus causing aversive responses. Consequently, follow-up compositions were altered to contain Entice semiochemicals in safflower oil at concentrations of only 0.1%, 0.01% and 0.001%, and were then tested for the response of mice in the T-tube olfactometer. Composition #14 (Table 2.2) with a 0.001% concentration of Entice was only slightly more attractive than Provoke® (first-choice responses: 11 to Entice; 8 to Provoke®; Table 2.5) but was fed on preferentially by 18 of the 19 mice tested (Table 2.5). These

results provide evidence that the dose of food-derived semiochemicals is critically important for the behavioural responses of mice.

The effect of food preservatives (sorbic acid, calcium propionate) on feeding responses by mice was tested in Compositions #15-18. When sorbic acid accounted for 0.2% of composition #15, two out of five mice fed on Provoke® rather than on composition #15 (Table 2.5). Those mice that first fed on Composition #15 did so reluctantly and consumed only small amounts. Based on the feeding responses of two other mice, there was emerging evidence that even 0.1% of sorbic acid in Composition #16 reduced feeding responses to levels well below those prompted by compositions void of preservatives. Calcium propionate at 1% in Composition #17, as an alternative to sorbic acid, still deterred feeding but at 0.1% in Composition #18 did not (Table 2.5). Unfortunately, composition #18 developed microbial growth within three days. At this stage, a formulation chemist was consulted for advice on appropriate food preservatives.

2.4.4. Field test of Composition #14 (2012)

In this proof-of-concept field test, seven mice were captured in traps baited with Composition #14, and one mouse was captured in a trap baited with Provoke® (Figure 2.4). In one additional replicate, the trap baited with Composition #14 was sprung but no mouse was captured. Observations on the field performance of Composition #14 revealed two deficiencies: (1) the liquid component of the composition separated out over time, and (2) the composition - once placed in the bait receptacle of a snap trap - dried out over the course of one night. Armed with this information, follow-up laboratory work focused on modifications of the composition that would help prevent the separation of water and interfere with the drying process.

2.4.5. Agar and carrageenan

While Composition #14 proved to be an effective trap bait for capturing wild mice in a field experiment, the physical properties of the composition were suboptimal and prompted alterations. These alterations included the addition of agar, and subsequently agar and carrageenan, to the wet ingredients of Compositions #19-24 (Table 2.4).

Composition #24 had the most ideal consistency and performed much better than Provoke® in a two choice feeding arena bioassay ($F_{1,18} = 1113.24$, $p < 0.0001$).

2.5. Discussion

The research presented in this chapter illustrates the start of a long development process (Figure 2.5) leading to an effective trap bait for capturing rodents. As one early part of this process, I investigated the feeding preferences of laboratory-strain house mice and brown rats to assemble a composition of feeding stimulants that could then be "coupled" with long-range attractants such as the Entice semiochemicals. In combination, the long-range attractants and feeding stimulants, not only lure rodents but also triggers feeding, which mediates their trapping or poisoning. The effectiveness of a bait under development is exemplified by Composition #14, with outperformed the effectiveness of Provoke®, the leading commercial bait for mice.

Selecting suitable preservatives, or preservatives in optimal amounts, for supressing microbial growth in a composition without adversely affecting the feeding propensity of mice or rats proved challenging. For example, when calcium propionate as one possible preservative was added to a composition at 1%, it deterred feeding by mice but when it was added at only 0.1%, it failed to supress microbial growth over time.

The retention of moisture in a composition proved to be another challenge. Rodents can easily remove compositions that dry out quickly from the bait receptacle of a snap trap without triggering the snapping mechanism. This phenomenon was evident in the field experiment (data not shown), where many traps baited with Composition #14 were found with the bait missing but the snapping mechanism not triggered. Therefore, the tackiness of a composition is an essential characteristic that contributes to its efficacy as a bait. My attempts to address this challenge by adding agar and carrageenan as food thickeners to select compositions were met with limited success.

Other important characteristics of an effective and commercially viable composition such as viscosity, stability, pH, shelf- and field-life as well as a method of delivery will have to be investigated in future studies. During the course of my study,

Composition #14 was delivered with a spatula from a sealable jar to the bait receptacle of snap traps. While this mode of bait delivery was acceptable for a proof-of-concept research experiment, it would not gain acceptance by pest management professionals that often need to bait many traps in a short period of time and that are accustomed to a squeeze tube or bottle as facile bait dispensers.

Realizing the intricacies of bait formulation chemistry, and accepting the fact that formulation chemistry is as much art as science and hinges on years of professional experience, an expert formulation chemist (Dr. Emma Rozenberg) was hired to assist with the task of bait formulation. Drawing on her vast professional experience, Dr. Rozenberg recommended specific preservatives, water-retaining agents, and other select bait stabilizing ingredients that ultimately led to compositions that met the specific requirements of a commercial bait. Throughout this process, the basic framework of Composition #24 remained the same.

Dr. Rozenberg's compositions were tested in >200 laboratory bioassays with mice and rats between February and July 2013 (data not shown). Once the overall best composition had been determined, it was termed, affectionately, "SFU Best Bait". The composition of SFU Best Bait is described in the patent application "New compositions and methods for attracting and stimulating feeding by mice and rats" (WO 2014/186885; PCT/CA2014/050435). SFU Best Bait contains Parabens (Liquipar PN: Phenoxyethanol, Methylparaben, Ethylparaben, Propylparaben, Butylparaben) as preservatives, which bind water molecules, thus making them unavailable for microbial growth. SFU Best Bait also contains "binding syrup" composed of glycerine and Gum Arabic FT powder to afford bait tackiness (see above). Indeed, this binding syrup, in the correct proportion, kept the bait tacky even after one week in the field (Musso, personal observation).

Realizing further that brown rats are more omnivorous than house mice and do seek meat and fish as part of their diet, SFU Best Bait for brown rats was modified to include salmon oil (instead of safflower oil) and dimethyl trisulfide as an indicator of fresh meat (a recently deceased animal). SFU Best Bait "Rats" and SFU Best Bait "Mice" were then tested in eight two-choice field experiments against each of the prominent commercial rodent baits (Provoke® Mouse and Provoke® Rat, Bell Laboratories Inc., Madison, WI, USA; Pro-Pest® Professional Lure for rats and mice, JF Oakes LLC,

Yazoo City, MS, USA; LiphaTech® Rat & Mouse Attractant™, Milwaukee, WI, USA) for the response of wild brown rats and wild house mice. Both SFU Best Baits were also field tested against peanut butter (Skippy® Peanut Butter, Hormel Foods Co, Austin, MN, USA), the golden standard bait for mice and rats. Even though peanut butter, due to its peanut-derived allergens, is now prohibited as a rodent bait, the latter experiment was run to further gauge the effectiveness of the SFU Best Baits. In all of these experiments, snap traps baited with the SFU Best Bait captured significantly more mice, or significantly more rats, than paired snap traps baited with one of the commercial baits, or baited with peanut butter (data not shown), attesting to the superior performance of the SFU baits.

In conclusion, the data presented in this chapter along with additional data gathered by the Gries-laboratory, reveal that rodent baits can be "designed" that are not only highly effective but also tailored for a target species. The design entails the same basic composition of feeding stimulants but attains some degree of specificity by incorporating odorants that represent food types favoured by a target species. The SFU Best Baits that we developed through the course of my research, and through follow-up research by lab mates, outperform all other commercial rodent baits. They have the potential to make snap trapping more effective and to replace lethal bait stations for rodents which are currently considered more effective than any other trapping technology but wreak havoc on predators that consume poisonous rats and mice.

2.6. Literature Cited

Barnett, S. A., and M. M. Spencer. 1953. Experiments on the food preferences of wild rats (*Rattus norvegicus* Berkenhout). The Journal of Hygiene 51:16–34.

Macdonald, D. W., M. G. P. Fenn, and M. Gelling. 2015. Natural History of Rodents: Preadaptations to Pestilence. Pages 1–18 *in* A. P. Buckle and R. H. Smith, editors. Rodent pests and their control. 2nd edition. CAB International, Boston, MA, USA.

Morris, C. F., D. McLean, J. A. Engleson, E. Patrick Fuerst, F. Burgos, and E. Coburn. 2012. Some observations on the granivorous feeding behavior preferences of the

house mouse (*Mus musculus* L.). Mammalia 76:209–218.

Pennycuik, P. R., and R. Cowan. 1990. Odor and food preferences of house mice, *Mus musculus*. Australian Journal of Zoology 38:241–247.

Rowe, F. P., A. Bradfield, and R. Redfern. 1974. Food preferences of wild house-mice (*Mus musclus* L). Journal of Hygiene 73:473–478.

Singleton, G. R., H. Leirs, L. A Hinds, and Z. B. Zhang. 1999. Ecologically-based management of rodent pests: re-evaluating our approach to an old problem. Pages 17-29 *in* G. R. Singleton, L. Hinds, H. Leirs, Z. Zhang, editors. Ecologically-Based Management of Rodent Pests. ACIAR, Canberra, Australia.

Takács, S. J., R. M. Gries, and G. J. Gries. 2013. Compositions and methods for attracting and stimulating feeding by mice and rats. International Publication Number WO 2013/003946 A1. Wold Intellectual Property Organization.

2.7. Tables and Figures

570 **Table 2.1 List of food items tested in food choice bioassays, their brand names and suppliers.**

#	Food item	Brand name	Supplier
1	Oats	Rogers Porridge Oats	Rogers Food Ltd., Armstrong, BC V0E 1B0 Canada
2	Wheat flour	Robin Hood Original All Purpose Flour	Smucker Foods of Canada Corp., Markham, ON L3R 0P3 Canada
3	Barley	Golden Chef Pot Barley	Golden Boy Foods Ltd., Burnaby, BC V5A 4V8 Canada
4	Rice	No Name Long Grain White Rice	Loblaw Foods Inc., Brampton, Ontario L6Y 5S5 Canada
5	Pea	Green Split Peas (dry)	Golden Boy Foods Ltd., Burnaby, BC V5A 4V8 Canada
6	Corn starch	Canada Corn Starch	ACH Food Companies Inc., Memphis, TN 38016 USA
7	Lentil	Red Split Lentils (dry)	Golden Boy Foods Ltd., Burnaby, BC V5A 4V8 Canada
8	Beans	Small White Beans (dry)	Golden Boy Foods Ltd., Burnaby, BC V5A 4V8 Canada
9	Baby food	Heinz mixed cereal	Heinz Canada, North York, ON M2N 7K5 Canada
10	Honey oat cereal	Honey Bunches of Oats	Post Foods Canada Corp., Niagara Falls, ON L2E 6T8 Canada
11	Rat chow	Lab Diet 50001	PMI Nutrition International LLC., Brentwood, MO 63144 USA
12	Apple seeds	n/a	Purchased from local grocery store
13	Vanilla pudding	Snack Pack Vanilla	ConAgra Foods Canada, Mississauga, ON L4V 1W5 Canada
14	Banana	n/a	Purchased from local grocery store
15	Custard	Ambrosia Devon Custard	Premier Food Group. Spalding, Lincolnshire PER 9EQ UK
16	Applesauce	Western Family Applesauce	Overwaitea Food Group LP, Vancouver, BC V6B 4E4 Canada
17	Sweet potato	n/a	Purchased from local grocery store
18	Dried soup	Knorr Vegetable Soup mix	Unilever Canada, Toronto, ON M4W 3R2 Canada
19	Gravy	Knorr classic brown roast gravy	Unilever Canada, Toronto, ON M4W 3R2 Canada
20	Oatmeal cookies	Dad's Oatmeal Cookies	Kraft Canada, Scarborough, Ontario M1P 2Z4 Canada

Table 2.1 continued

#	Food item	Brand name	Supplier
21	Fish food	Nutrafin basix staple food	Rolf C. Hagen Inc., Montreal, QC H4R 1E8 Canada
22	Artichoke	n/a	Purchased fresh from local grocery store
23	Macaroni & Cheese	No Name Macaroni and Cheese Dinner	Loblaw Foods Inc., Brampton, Ontario L6Y 5S5 Canada

Table 2.2 Ingredients and the percent (%) present in compositions (C) #1-24.

C	Ingredients	%	C	Ingredients	%	C	Ingredients	%
1	Oat flour	20.8	2	Oat flour	11.60	3	Oat flour	12.00
	Rice flour	6.70		Rice flour	11.60		Rice flour	12.00
	Fructose	1.70		Safflower oil	0.75		Safflower oil	0.25
	Soy lecithin	0.29		Soy lecithin	0.50		Soy lecithin	0.25
	Safflower oil	0.29		Fructose	0.50		Fructose	0.50
	Gelatine/Water solution	69.0		Gelatine/Water solution	75.0		Gelatine/Water solution	75.0
4	Oat flour	11.5	5	Oat flour	12.8	6	Oat flour	14.9
	Rice flour	12.5		Rice flour	12.8		Rice flour	3.20
	Safflower oil	0.50		Wheat flour	9.00		Wheat flour	9.14
	Soil lecithin	0.25		Safflower oil	0.29		Safflower oil	0.29
	Fructose	0.50		Soy lecithin	0.14		Soy lecithin	0.14
	Gelatine/Water solution	75.0		Fructose	0.86		Fructose	0.85
				Gelatine/Water solution	71.4		Gelatine/Water solution	71.4
7	Oat flour	14.9	8	Oat flour	5.70	9	Oat flour	14.3
	Rice flour	9.40		Rice flour	11.4		Rice flour	5.14
	Wheat flour	3.20		Wheat flour	10.0		Whole wheat flour	5.00
	Safflower oil	0.29		Safflower oil	0.29		Wheat bran	0.85
	Soy lecithin	0.14		Soy lecithin	0.29		Safflower oil	0.29
	Fructose	0.86		Fructose	0.86		Soy lecithin	0.14
	Gelatine/Water solution	71.4		Gelatine/Water solution	71.4		Fructose	2.86
							Gelatine/Water solution	71.4

Table 2.2 continued

C	Ingredients	%	C	Ingredients	%	C	Ingredients	%
10	Oat flour Rice flour Wheat bran Safflower oil Soy lecithin Fructose Gelatine/Water solution	16.7 9.33 5.00 0.33 0.33 1.60 66.7	11	Composition #10 with 1.0% "Entice" in safflower oil		12	Composition #10 with 0.1% "Entice" sin safflower oil	
13	Composition #10 with 0.01% "Entice" in safflower oil		14	Composition #10 with 0.001% "Entice" in safflower oil		15	Composition #14 with 0.2% sorbic acid	
16	Composition #14 with 0.1% sorbic acid		17	Composition #14 with 1% Calcium propionate		18	Composition # 14 with 0.1% Calcium propionate	
19	Oat flour Rice flour Wheat bran Fructose Soy lecithin Safflower oil Water, 0.35% agar, 0.3% Calcium propionate	14.3 8.00 4.29 1.43 0.29 0.29 71.4	20	Oat flour Rice flour Wheat bran Fructose Soy lecithin Safflower oil Water, 0.35% agar, 0.3% Calcium propionate	20.0 11.2 6.00 2.00 0.4 0.4 60.0	21	Oat flour Rice flour Wheat bran Fructose Soy lecithin Safflower oil Water, 0.35% agar, 0.3% Calcium propionate	16.7 9.33 5.00 1.67 0.33 0.33 66.7
22	Composition #21 with 2% carrageenan powder added to agar solution during boiling		23	Composition #21 with 0.5% carrageenan powder		24	Oat flour Rice flour Wheat bran Fructose Soy lecithin Safflower oil Water, 0.35% agar, 0.7% carrageenan, 0.3% ca propionate	14.2 7.94 4.26 1.42 0.28 1.00 70.9

Table 2.3 Physical characteristics of compositions (C) #1, 3-7.

C	Physical characteristics
1	Consistency not smooth (ratio of wet to dry ingredients too narrow?)
3	Consistency too runny
4	Consistency too runny, grainy
5	Too much wheat, too little oat, sweetness is optimal
6	Grainy, still too much wheat flour
7	Grainy, too much oat flour, or wheat flour is not ideal

Table 2.4 **Mean consumption (%) of food items [Heinz baby food, Provoke®, various compositions (see Table 2.2)] by house mice in 2- or 3-food choice experiments (Figure 2.1, B). Data of 3-food choice experiments were analysed by Oneway ANOVA followed by the Tukey HSD test, and data of 2-food choice experiments were analyzed by the Students t-test. Within 3-food choice experiments, mean consumption rates associated with different letters are statistically different from one another.**

Exp. #	Treatments tested	*N*	Mean % fed	*P*	Observations on composition
1	Heinz baby food Composition #2	10	96.4 45.9	$p = 0.01$	Consistency problems
2	Heinz baby food Composition #8 Provoke®	10	a 81.4 b 49.9 b 21.7	$p < 0.0001$	Less oat flour but graininess is not improved; wheat flour is not working well in consistency
3	Heinz baby food Composition #9 Provoke®	5	a 75.6 a 49.5 b 27.5	$p = 0.05$	Includes WW flour and WB instead of AP flour. WB seems to provide better taste and texture than wheat flour (WW or AP flour)
4	Heinz baby food Composition #10 Provoke®	10	a 95.3 b 51.8 c 32.1	$p < 0.0001$	When WB replaced WW or AP flour; notice increase in feeding
5	Composition #19 Composition #18	10	45.8 90.7	$p < 0.003$	Agar solution dries out slower than gelatin solution
6	Composition #20 Provoke®	5	91.0 12.2	$p < 0.0001$	Formulation too stiff
7	Composition #21 Provoke®	10	91.3 18.1	$p < 0.0001$	Altered ratio of dry to wet ingredients
8	Composition #21 Composition #22	10	80.6 59.6	$p = 0.19$	2% Carrageenan made bait solid and decreased feeding response
9	Composition #21 Composition #23	10	78.9 55.6	$p = 0.075$	Too little carrageenan; water separated from bait within 24 h
10	Composition #24 Provoke®	10	95.3 13.3	$p < 0.0001$	0.7% Carrageenan provided best consistency; consumed more readily than Provoke®

WW = Whole Wheat
AP = All Purpose
WB = Wheat Bran

Table 2.5 Effect of specific food compositions (C) (see Table 2.2) and Provoke® on attraction (1st choice) and on the first food sampled (1st fed) by house mice in T-tube olfactometer bioassays (see Figure 2.1, C).

590

Exp. #	C # tested	*N*	1st choice	1st fed	Observations on composition
1	Composition #10 Composition #11	7	5 2	4 2	Presence of "Entice[a]" semiochemicals (1% in safflower oil) in Composition #11 reduces feeding response by mice
2	Composition #12 Provoke®	4	1 3	4 0	Lower dose of Entice (0.1% in safflower oil) makes Composition #12 still less attractive than Provoke® but there is preferential feeding on Composition #12
3	Composition #13 Provoke®	5	1 4	4 1	Aversion to Composition #13 with strong scent of Entice (0.01% of safflower oil)
4	Composition #14 Provoke®	19	11 8	18 1	Composition #14 with Entice (0.001% of safflower oil) is not more attractive than Provoke® but is preferentially fed on
5	Composition #15 Provoke®	5	3 2	3 2	Mice do not readily feed on Composition #15 containing sorbic acid (0.2%) as a food preservative. Composition has bitter taste.
6	Composition #16 Provoke®	2	0 2	2 0	Composition #16 containing sorbic acid at only 0.1% is still not readily fed on by mice
7	Composition #17 Provoke®	2	0 2	1 0	Composition #17 containing calcium propionate (1%) in lieu of sorbic acid is also not readily fed on by mice
8	Composition #18 Provoke®	9	6 3	8 1	Composition #18 containing 0.1% calcium propionate does not deter feeding but allowed microbial growth within 3 days.

[a] 2-hydroxy-3-methylcyclopent-2-en-1-one, butyric acid, 2,3-butadione, 3-methylbutanal, 6-methyl-(*E*)-2-hepten-4-one, γ-octalactone

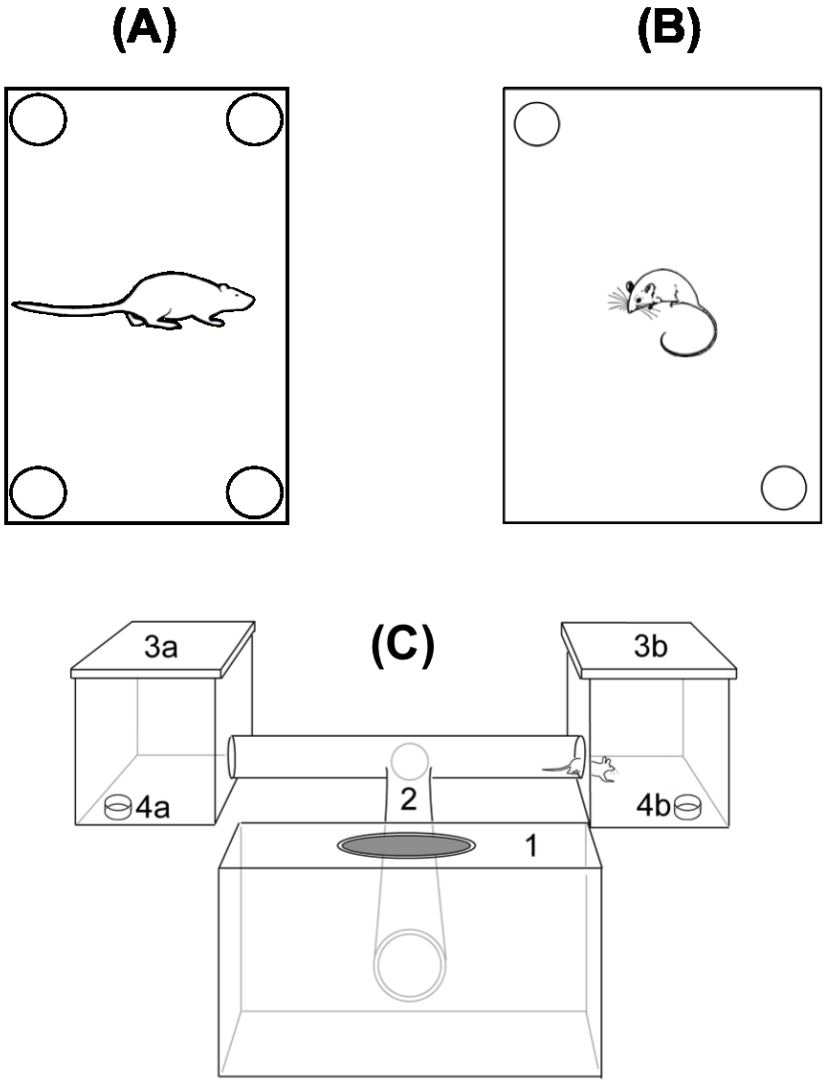


Figure 2.1 **(A) Design of feeding bioassay for rats. Glass aquarium (three in total; L × W × H = 60 × 30 × 40 cm) housing 3 or 4 Petri dishes (5 cm diam) with a specific food item in each corner. A single rat was released into the centre of each aquarium. (B) Design of feeding bioassay for mice; rectangular clear Plexiglas arena (five in total; 60 × 30 × 40 cm) with a food item in a Petri dish (5 cm diam) in opposite corners. A single mouse was released into the centre of each arena. (C) T-tube olfactometer design used to test behavioural responses of mice to test stimuli in laboratory experiments; (1) clear Plexiglass arena (40 × 20 × 30 cm) for placement of a mouse at the onset of a bioassay; (2) glass T-tube (stem: 65 cm long, side arms: 45 cm long, all 10 cm in diam); (3a, 3b) Plexiglass arenas (each 60 × 30 × 40 cm) housing test stimuli; (4a, 4b) Petri dish (5 cm diam) with food bait.**

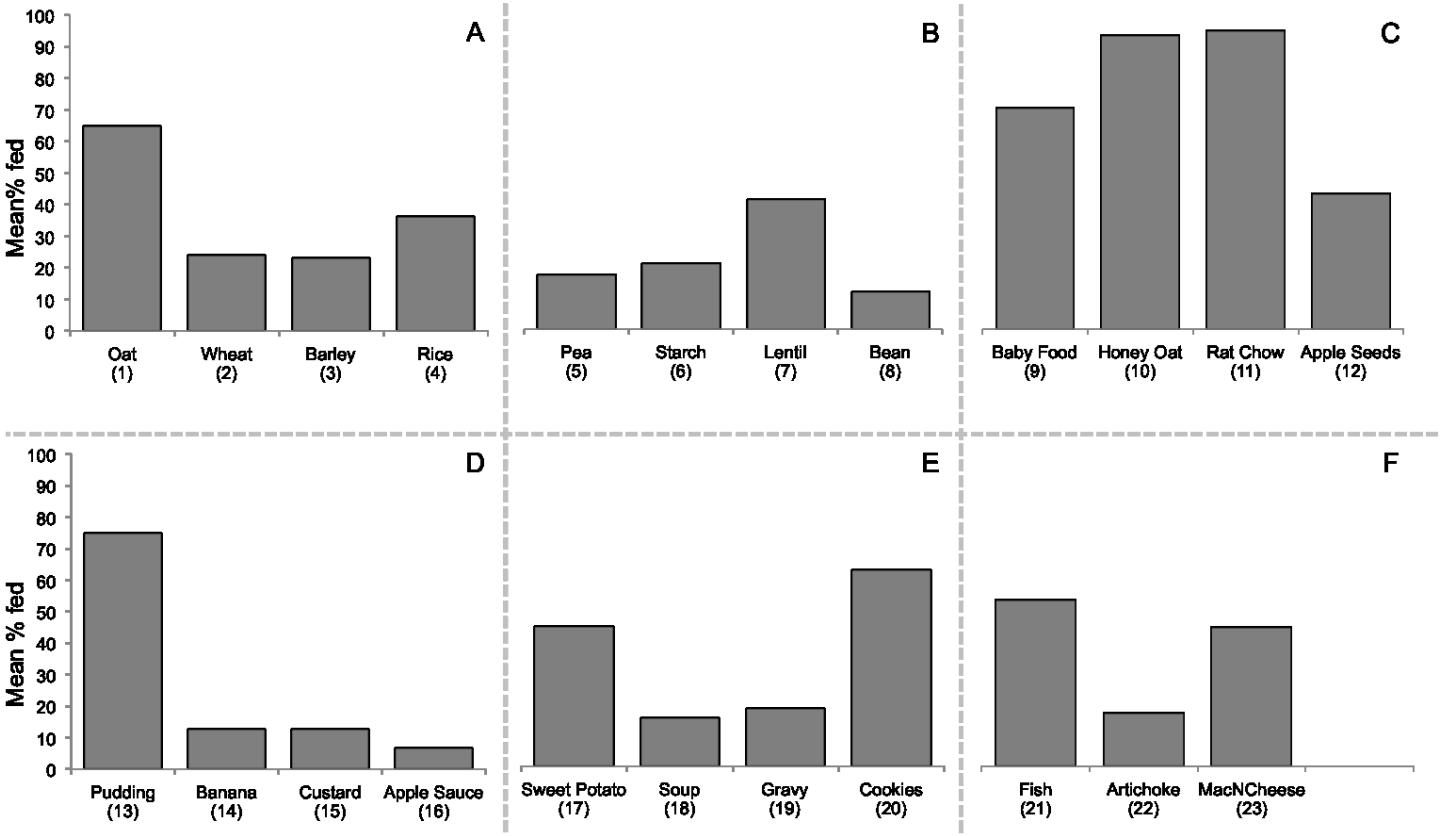


Figure 2.2 **Mean differential consumption of food items (see reference numbers in Table 2.1) by brown rats in each of food-choice experiments A-F. In each replicate (N=3) of experiments A-F, a single rat was offered a choice of four different food items (2 g each). Food consumption was determined by weighing the food at the onset and end of a replicate (see methods for details).**

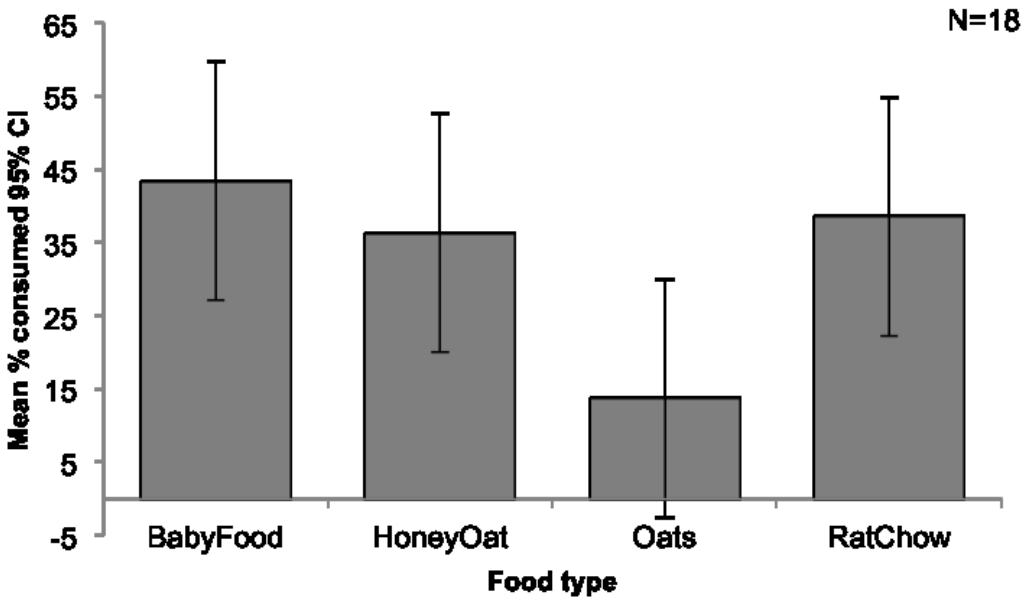


Figure 2.3 **Mean differential consumption (with 95% confidence intervals) of those four food items most preferred by brown rats in food-choice experiments A-F (Figure 2.2). In each replicate (N=18), a single brown rat was offered a choice of these four food items (2 g each). Food consumption was determined by weighing each food item at the onset and end of a replicate (see methods for details). There was no detectable preference for any of the four food items (Oneway ANOVA $F_{3,68} = 2.61$, $p = 0.06$).**

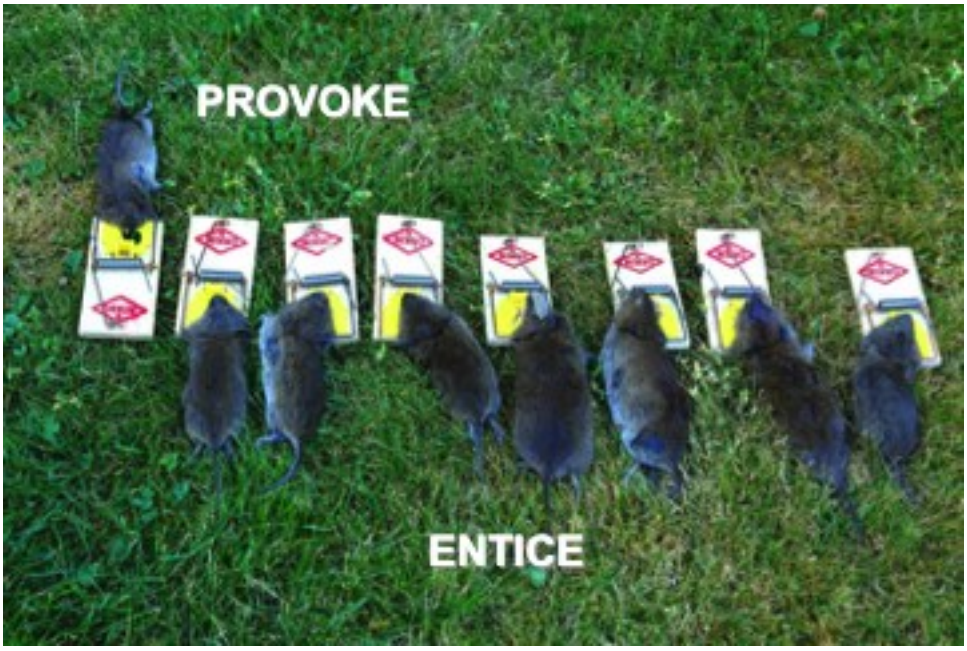


Figure 2.4 **Differential captures of house mice in paired snap traps baited with Composition #14 and the leading commercial rodent bait Provoke® Mice (Bell Laboratories Inc.) in a field experiment (31 Aug. to 03 Sept. 2012) on a farm in Richmond, BC (Photo: G. Gries).**

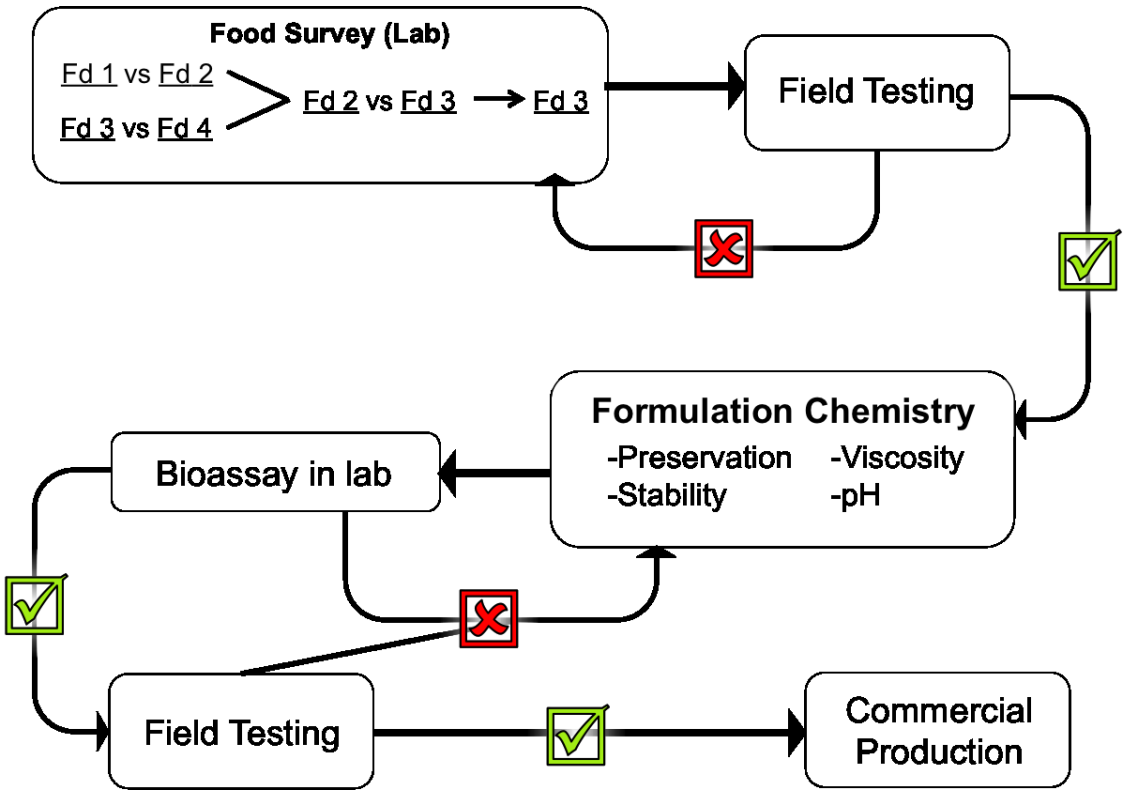


Figure 2.5 **Flow chart of developmental steps leading to the production of an effective rodent bait. Work presented in this chapter pertains to the first two steps. Steps from formulation chemistry to commercial bait production have input from the industrial partner.**

Chapter 3. Effect of house mouse pheromones on behavioural responses of mice in the laboratory and on captures of wild mice in the field

Some parts of this chapter have been submitted as a provisional US patent application with the following authors: Antonia E, Musso, Regine Gries, Huimin Zhai, and Gerhard Gries

3.1. Abstract

Urine of male house mice (*Mus musculus*) is well known to have primer pheromone effects on the reproductive physiology of female mice. Urine-mediated releaser pheromone effects that trigger certain behavioural responses are much less understood, and no field studies have investigated whether urine deposits by male or female mice, or synthetic mouse pheromones could increase trap captures of mice. In field experiments, I baited traps with bedding soiled by urine and feces of caged female or male mice, and recorded captures of mice in such baited traps or in corresponding control traps containing clean bedding. Traps baited with female bedding preferentially captured adult males, whereas traps baited with male bedding preferentially attracted juvenile and adult females, indicating the presence of male- and female-specific sex pheromones in soiled bedding. Analyses of headspace volatiles emanating from soiled female or male bedding by gas chromatography-mass spectrometry revealed that 3,4-dehydro-*exo*-brevicomin (DEB) was seven times more prevalent in males and that 2-*sec*-butyl-4,5-dihydrothiazole (DHT) was male-specific. In a follow-up field experiment, traps baited with DEB and DHT captured significantly more female mice than corresponding control traps, thus clearly indicating that DEB and DHT are sex attractant pheromone components of house mouse males. My study provides impetus to identify the sex attractant pheromone of female mice, and to develop synthetic mouse pheromones as lures to enhance the efficacy of trapping programs for mouse control.

3.2. Introduction

House mice (*Mus musculus*) are one of the most notorious pests worldwide. They inhabit urban, agricultural, industrial, and natural environments (Singleton *et al.* 1999). They cause damage by feeding on and soiling stored food products, inflicting structural damage to buildings, and spreading diseases such as Leptospirosis (Smith and Meyer 2015). As an invasive species, mice threaten ground nesting bird populations, especially on islands (Howald *et al.* 2007, Wanless *et al.* 2007, Angel *et al.* 2009, Simberloff 2009).

The most common methods of mouse control used today are trapping, and baiting with anticoagulant rodenticides. Rodenticides have become the preferred method because they are less work intensive than trapping, and are generally considered more effective. To prevent poisoning of non-target wildlife, pets or humans, rodenticide-laced baits are kept in locked plastic or metal boxes with small entry holes. While bait boxes prevent many non-target vertebrates from consuming bait directly, there is rising concern that poisoned prey causes secondary toxicity in predators of rodents. This has been documented for (*i*) birds of prey such as American kestrels, *Falco sparverius* (Rattner *et al*. 2011), and red tailed hawks, *Buteo jamaicensis* (Murray and Tseng 2008, Thomas *et al*. 2011), (*ii*) small mammalian predators such as foxes, *Vulpes vulpes,* and polecats, *Mustela putorius* (Berny *et al*. 1997, Shore *et al*. 1999), and (*iii*) even large predators such as bobcats, *Lynx rufus*, and mountain lions, *Puma concolor* (Riley *et al.* 2007).

Snap traps are a viable alternative to anticoagulant rodenticides as they are lethal and require no toxic substances. Snap traps can also be kept in boxes to prevent non-target vertebrates from getting trapped or killed. Once a snap trap has caught a mouse, it must be reset to capture another mouse. This makes baited snap traps more work intensive to service than poison bait stations. However, if one could design baits for snap traps that are very efficient and even target specific members of a mouse population, then snap traps could be a better alternative to rodenticides.

Mouse urine is a complex composition of chemical signals for communication. The signals differ between male and female mice and are dependent on hormone levels (Schwende *et al.* 1986). Mice constantly mark their habitat with urine deposits, thereby

conveying information about their sex, age (Osada *et al.* 2008), experience and health (Ehman and Scott 2001), or their group membership (Nakamura *et al.* 2007). Urine-marking is especially important for males that engage in competitive scent marking and stake out territory (Hurst 1990). Indeed, male mice have a paintbrush-like penis that facilitates efficient deposition of urine marks (Maruniak *et al.* 1975). Urine marks remain fresh for about a week due to large protein complexes, termed major urinary proteins (MUPs), that bind volatile ligands and serve as "slow-releasers" (Hurst *et al.* 1998). MUPs, however, are not simply scent dispensers; they are polymorphic and are expressed in different patterns that provide information about the identity of the marking animal (Hurst *et al.* 2001). A mouse senses MUPs with its Vomeronasal Organ (VNO) when it makes direct contact with a urine mark (Maruniak *et al.* 1985). Thus, a male urine mark comprises two groups of semiochemicals: (*i*) volatile components that convey information about health and dominance of the marker and that are sensed from a distance with the main olfactory system and (*ii*) non-volatile components (MUPs) that convey information about the marker's identity and that are detected by the VNO upon direct contact with a urine mark.

When a male mouse enters another male's territory, the intruder assesses urine marks of the resident male to gauge his competitiveness, and to decide whether or not to "counter-mark" around them. When the resident male, in turn, finds his marks counter-marked, he may mark over the counter-marks. Male-seeking females assess these competitive olfactory displays and choose the male who deposited the most recent mark even if does not belong to the resident male (Rich and Hurst 1999). Females glean a lot of information by checking marks from potential mates and seek older, more experienced males as mates (Osada *et al*. 2008).

Mouse urine has been shown to have several primer pheromone effects, as follows: the oestrous cycles of group-housed females are supressed ("Lee-Boot effect"; Vander Lee and Boot 1955) but can be re-activated and accelerated in the presence of male urine ("Whitten effect"; Whitten 1956); (*ii*) the presence of an unfamiliar male blocks pregnancy in a female that is paired with a stud male ("Bruce effect"; Bruce 1959); and (*iii*) male urine causes early onset of puberty in juvenile female mice ("Vandenbergh effect"; Vandenbergh 1969).

The primer pheromone components mediating some of these effects are known. For example, 2-*sec*-butyl-4,5-dihydrothiazole (DHT) and 3,4-dehydro-*exo*-brevicomin (DEB) in combination, but not singly, cause both the Whitten effect (Jemiolo *et al.* 1986) and the Vandenbergh effect (Novotny *et al.* 1999). DHT and DEB, together with α- and β-farnesene, also have releaser pheromone effects such as (*i*) inducing sniffing by females (Jemiolo *et al.* 1985), prompting investigatory behaviour by males, and triggering male aggression (Novotny and Harvey 1990). Unlike DHT and DEB, the two farnesenes are not present in bladder urine but are secreted by the preputial gland and added to the urine during elimination. While DHT- and DEB-titres are similar in dominant and subordinate males, dominant males have larger preputial glands and thus add larger amounts of the two farnesenes to their urine (Harvey *et al*. 1989). As a blend, the four pheromone components may provide a complex message with two kinds of information. DHT and DEB may signal the presence of a residential male occupying a territory, and α- and β-farnesene may then signal his social dominance.

While the (pheromonal) constituents of male mouse urine have been well characterized, the feasibility of exploiting them for control of mice is relatively under-studied. In a laboratory experiment, Volfová *et al.* (2011) demonstrated that house mice (subspecies *M. musculus musculus*) spent more time in bait stations containing wood shavings soiled with mouse urine than in bait stations containing clean wood shavings. However, Volfová *et al*. (2011) did not investigate whether the presence of urine affects the propensity of mice to feed on a bait or to enter trap boxes in field experiments.

My overall objective was to investigate whether house mouse pheromones can be exploited for mouse control. My specific objectives (O) were to: (O1) laboratory-test the effect of bedding soiled with urine and feces of female or male mice on the responses of female and male mice; (O2) field-test the effect of bedding soiled by females or males on captures of wild mice; (O3) analyse odorants emanating from male- or female-soiled bedding; and (O4, O5) test the effect of candidate male pheromone components on responses of females and males in the laboratory (O4) and on captures of wild mice in the field (O5).

3.3. Materials and Methods

3.3.1. Lab animals

CD-1® strain house mice, 4 weeks of age, were obtained from Charles River Laboratories International, Inc. (Saint-Constant, QC J5A 2E7). On arrival, mice were assigned to four groups of five males each and four groups of five females each, accommodated in cages (50 × 40 × 20 cm) lined with commercial corncob bedding (Anderson's Bed o'cobs, The Andersons Inc. Maumee, OH 43537, USA), provided with Nalgene toys and running wheels (Jaimesons Pet Food Distributers, Richmond, BC V4G 1C9, Canada), and provisioned with commercial rodent food (LabDiet® Certified Rodent Diet, LabDiet, St. Louis, MO 64144, USA) and water *ad libitum*. All animals were housed and cared for by Animal Care Services of Simon Fraser University. Animal rooms were kept at a consistent relative humidity (50%) and temperature (21 °C), and a reversed photoperiod (12L:12D), with the scotophase running from 13:00 h to 01:00 h to facilitate behavioural bioassays. When male mice reached puberty, those individuals that became aggressive were removed from their groups and housed singly to prevent injury to their cage mates. Only healthy animals were used in behavioural bioassays. All bioassays were run between 13:00 h and 21:00 h.

3.3.2. O1: Laboratory-test the effect of bedding soiled with urine and feces of female or male mice on the responses of female and male mice

The response of male and female mice to soiled bedding collected from male and female cages was tested in the lab with a T-tube olfactometer (Exp. 1-4; Table 3.1), which was illuminated by dim red light to facilitate observations. The T-Tube olfactometer (Figure 3.1, A) consisted of three clear Plexiglass arenas with vented lids (**1**: 40 cm × 20 cm × 30 cm; **3a** and **3b**: each 60 cm × 30 cm × 40 cm) interconnected by a Pyrex glass T-tube (**2**; stem: 65 cm long, side arms: 45 cm long, all 10 cm in diameter). For each bioassay, a 1-g aliquot of a cereal-based food bait (Musso *et al*. 2014) in a Petri dish (5 cm diam; **4a** or **4b**) was placed in one corner of arena **3a** and **3b**.

An Erlenmeyer flask (150 mL; **5a** or **5b**) containing 50 g of either clean (control) or soiled (treatment) bedding was randomly assigned to arenas **3a** and **3b.** Immediately before a bioassay, bedding was collected from the corners of mouse cages, each containing 3-5 mice that had soiled new bedding over the course of 2-5 days. Erlenmeyer flasks were sufficiently tall so that mice could not come in contact with the test bedding. When a female mouse was bioassayed for her response to female-soiled bedding, the bedding material was removed from a cage other than the test female's "home cage". The same concept was applied for male mice, thus ensuring that any animal did not just respond to its familiar home cage scent. When all stimuli were in place, arenas were covered with lids and odorants from the bedding were allowed to diffuse through the arenas and T-tube for 5 min.

For each bioassay, a single mouse (food- but not water-deprived for 4-6 h) was placed in the release arena (**1**) and allowed to enter and explore the T-tube (**2**) on its own accord in response to test stimuli. For each bioassay, the following data were recorded: (*i*) the response arena (**3a** or **3b**) the mouse entered first with all four paws ("first choice" data), (*ii*) the arena (**4a** or **4b**) where it fed first on the food bait ("first-feeding" data), and (*iii*) the position of the mouse every 15 seconds. The bioassay was considered complete when the mouse fed on one of the food baits or 30 min had passed. Mice that did not make a first choice were excluded from statistical analyses.

Following each replicate (N=62), the arenas and T-tube were cleaned with a Percept® disinfectant detergent (Virox Technologies Inc. Mississauga ON L5N 5M4, Canada) and wiped with 70% ethanol.

For each experiment, first-choice data and first-feeding data were analyzed using a Chi-square test ($\alpha = 0.05$). Time-spent data were analyzed by comparing the mean proportions of time points recorded in control and treatment arenas, using Students t-test ($\alpha = 0.05$). All data were analyzed using JMP® 11.2.0 (Copyright © 2013 SAS Institute Inc.).

3.3.3. O2: Field-test the effect of bedding soiled by females or males on captures of wild mice

Field data were collected in experiments 5 and 6 (Table 3.1) at two poultry farms in the Fraser Valley of British Columbia during late summer and fall of 2014. Paired trap boxes (N=73) (PROTECTA® Mouse, Bell Laboratories Inc. Madison, WI 53704, USA) were set up indoors, in the attic above a barn, or outdoors along walls of a barn exterior, with 0.5 m between the two boxes in each pair, and 2 m between pairs. Each trap box contained a clean snap trap (Victor® M325 M7 Pro mouse trap, Woodstream Co. Lititz, PA 175543, USA) that was baited with a food lure (Musso *et al.* 2014). The two experiments were run to test for the effect of female bedding (Exp. 5) or male bedding (Exp. 6) on captures of mice (Figure 3.1, C, D). Treatment boxes contained bedding that had been soiled by urine and feces of male or female mice caged in SFU's Animal Care Facility, whereas control boxes contained clean, unsoiled bedding. Treatments of male or female bedding were alternated throughout the field sites, each paired with a control.

Traps were checked every 24-48 h and treatment bedding was replaced with soiled bedding collected that day to ensure consistent odour. The sex and relative age (juvenile or adult, based on mouse size and development of genitalia) of trapped mice were recorded.

3.3.4. O3: Analyse odorants emanating from male- or female-soiled bedding

Urine- and feces-stained bedding was removed and replaced with fresh bedding at weekly intervals. Soiled bedding (100 g) from males or females was placed into separate cylindrical Pyrex glass chambers, each connected to a Pyrex glass tube (15 cm × 5 mm OD) filled with 200 mg of the absorbent Porapak Q. Charcoal-filtered air was drawn through each chamber and the Porapak Q volatile trap at 1 l per min for 24 h. Processing clean bedding from a control cage without mice in the same way helped differentiate between bedding and rodent volatiles in subsequent analyses. Porapak Q-captured volatiles were desorbed with two consecutive rinses of pentane (2 ml) and ether (2 ml). Pentane and ether extracts were combined and after adding dodecyl

acetate as an internal standard for compound quantification were concentrated under a stream of nitrogen to 250 µl per sample.

Aliquots (2 µl) of Porapak Q extracts of soiled bedding odorants from male and female house mice were analyzed on a Varian Ion Trap GC-MS fitted with a DB-5 MS GC column (30 m × 0.25 mm ID), setting the injector port and ion trap to 250 °C and using helium as the carrier gas (35 cm per sec), with the following temperature program: 50 °C for 5 min, 10 °C per min until 280 °C (10 min). Odorants were identified by comparing their retention indices (relative to straight chain alkanes) and mass spectra with those reported in the literature and with those of authentic standards that were available from previous work, purchased from suppliers (Sigma Aldrich Comp., St. Louis, MO 63103, USA; Aldrich Chemical company Inc., Milwaukee, WI 53201, USA; Alfa Aesar, Heysham, LA3 2XY England), or synthesized (2-*sec*-butyl-4,5-dihydrothiazole and 3,4-dehydro-*exo*-brevicomin).

To isolate the candidate pheromone component 3,4-dehydro-*exo*-brevicomin (DEB) (see 3.4.3 in Results) from male and female house mice for chiral GC analysis, Porapak Q extracts of soiled-bedding odorants were subjected to high-performance liquid chromatography (HPLC), using a Waters high-performance liquid chromatograph (600 Controller, 2487 Dual Absorbance Detector, Delta 600 pump; Waters Corporation, Milford, MA, USA) fitted with a Synergy Hydro reverse phase C18 column (250 mm × 4.6 mm, 4 µm; Phenomenex, Torrance, CA, USA) which was eluted with an isocratic mixture of 70% acetonitrile and 30% water. DEB eluted between 5 and 6 minutes.

To determine the absolute configuration of DEB produced by male and female mice, the 5- to 6-minute HPLC fraction was extracted with pentane and concentrated 10-fold for chiral GC analyses. Aliquots were analyzed isothermally (90 ˚C) in split mode using a Hewlett Packard 5890 gas chromatograph (GC) and an Agilent 5973 Mass Selective Detector coupled to a 6890 GC, both GCs fitted with a Chirasil-Dex-CB column [25 m × 0.25 mm; Varian Inc. (now Agilent Technologies Inc., Santa Clara, CA, USA) Lake Forest, CA, USA)]. Both the GC injector and flame ionization detector were to 250 ˚C.

The second candidate pheromone component, 2-*sec*-butyl-4,5-dihydrothiazole (DHT) (see 3.4.3 in Results), was not isolated for chiral GC analyses because a previous report indicated that mice produce the *S*-configuration of this compound (Cavaggioni *et al.* 2003).

3.3.5. O4: Laboratory-test the effect of candidate male pheromone components on responses of females and males

With evidence that 2-*sec*-butyl-4,5-dihydrothiazole (DHT) and 3,4-dehydro-*exo*-brevicomin (DEB) were present in male bedding that tested in laboratory and field experiments (see results), the responses of group-housed adult females (Table 3.1; Experiment 7), group-housed adult males (Table 3.1; Experiment 8), and singly-housed adult males (Table 3.1; Experiment 9) to DHT and DEB were tested in the T-tube olfactometer (Figure 3.1, C; apparatus dimensions as in B). For each replicate, a single mouse was deprived of food, but not water, for 4-6 h before it was placed into arena **1** which was illuminated by dim red light to facilitate observations of the mouse's position. The mouse was allowed to enter the stem of the T-tube (**2**) on its own accord in response to test stimuli, which were randomly assigned to arena **3a** or **3b**.

Both arenas were baited with 1 g of a cereal-based food bait (Musso *et al*. 2014) in a Petri dish (**4**; 5 cm diameter). The treatment stimulus also comprised 2 mg of a 1:1 blend of DHT and DEB (each >98% chemically pure), which was formulated in 10 g of mineral oil and pipetted into a 20-ml scintillation vial (**5a**; Wheaton Industries Inc., Millville, NJ 08332,USA). A control scintillation vial (**5b**) contained the same amount of mineral oil but lacked the two volatile compounds. Each scintillation vial was placed in a 150-ml beaker (**6a**, **6b**), which was covered with wire mesh (Phifer Inc., Tuscaloosa, Alabama, 35403-1700, USA) to prevent mice from contacting the vial or its contents.

For each bioassay, a single mouse was tested and the following data were recorded: (*i*) the treatment or control arena the mouse entered first with all four paws ("first choice data"), (*ii*) the arena in which it first fed on the food bait ("first-feeding data"), and (*iii*) the position of the mouse at each of 40 15-sec intervals. Position data were then used to calculate the proportion of time a mouse spent in the treatment or control arena ("time spent data").

Following each replicate (N=26), the arenas and T-tube were cleaned with a Percept® disinfectant detergent (Virox Technologies Inc. Mississauga ON L5N 5M4, Canada) and wiped with 70% ethanol.

For each experiment, first-choice data and first-feeding data were analyzed using a Chi-square test ($\alpha = 0.05$). Time-spent data were analyzed by comparing the mean proportions of time points recorded in control and treatment arenas, using Students t-test ($\alpha = 0.05$). All data were analyzed using JMP® 11.2.0 (Copyright © 2013 SAS Institute Inc.).

3.3.6. O5: Field-test the effect of candidate male pheromone components on captures of wild mice

Field experiment 10 was run in the Greater Vancouver Area and the Fraser Valley of British Columbia between 22 January and 25 September 2015, testing the effect of synthetic DHT and DEB on trap captures of wild mice. Experimental replicates were set up along the interior walls of a retirement home, a floral conservatory, and a horse barn, and along the exterior walls of a poultry barn. Each replicate (N=124) consisted of paired trap boxes (PROTECTA® Mouse, Bell Laboratories Inc., Madison, WI 53704, USA) and snap traps (Victor® M325 M7 Pro mouse trap, Woodstream Co. Lititz, PA 175543, USA) (Figure 3.1, D), with 50-cm spacing between the boxes in each pair, and at least 2 m between pairs.

The snap trap in each box was baited with a food lure (Musso *et al*. 2014) and one randomly assigned box in each pair was also baited with the synthetic blend of DHT and DEB. The compounds were formulated in 10 g of mineral oil and dispensed from a custom-made scintillation vial, whereas the corresponding scintillation vial in the control box contained only mineral oil. All boxes and snap traps were checked every 48-72 h, and test stimuli (food lure, DHT and DEB in mineral oil, mineral oil) were replaced at each visit. Whenever mice had been captured, their age (juvenile or adult) and sex were recorded, and new trap boxes and snap traps were deployed, thus ensuring that the odour of captured mice did not affect future captures.

Capture data of mice in treatment and control traps were analysed using a logistic regression model performed in R (v.3,4,3, Urbanek & Bibiko; R Development Core Team 2015) with age-sex category and location as factors. Age-sex categories were adult female, adult male, juvenile female, and juvenile male mice. The probabilities of trapping mice in these four groups were analyzed by multiple comparisons using a Tukey test ($\alpha = 0.05$) comparing a family of four estimates.

3.4. Results

3.4.1. O1: Laboratory-test the effect of bedding soiled with urine and feces of female or male mice on the responses of female and male mice

In T-tube olfactometer experiments 1 and 2, adult females (Exp. 1) and adult males (Exp. 2) spend significantly more time in arenas containing bedding soiled by females than in arenas with clean bedding (Exp. 1: $t = 4.30$, $p = 0.0002$; Exp. 2: $t = 4.30$, $p = 0.0002$; Figure 3.2). However, the presence of female-soiled bedding did not affect the choice of females or males as to which arena to enter first (Females: $X^2 = 1.00$, $p = 0.32$; Males: $X^2 = 2.58$, $p = 0.11$; Figure 3.2) or to feed in first (Females: $X^2 = 2.25$, $p = 0.13$; Males: $X^2 = 0.22$, $p = 0.64$; Figure 3.2).

In T-tube olfactometer experiments 3 and 4 which tested bedding soiled by males *versus* clean bedding as stimuli, females did not spend more time in arenas containing soiled bedding ($t = 0.22$ $p = 0.83$; Figure 3.3), but males did ($t = 5.23$ $p < 0.0001$; Figure 3.3). The presence of male-soiled bedding did not affect the choice of females or of males as to which arena to enter first (Females: $X^2 = 0.08$, $p = 0.78$; Males: $X^2 = 1.14$, $p = 0.29$; Figure 3.3) or to feed in first (Females: $X^2 = 1.92$, $p = 0.17$; Males: $X^2 = 1.14$, $p = 0.29$; Figure 3.3).

3.4.2. O2: Field-test the effect of bedding soiled by females or males on captures of wild mice

As many as 30 and 43 mice were captured in (field) experiments 5 and 6, respectively, but specific trap catch data of juvenile and adult male and female mice

(Figure 3.4) were still too low to warrant logistic regression analyses. Nonetheless, these data allow some tentative interpretations, as follows: traps containing bedding soiled by females seem to (*i*) be avoided by adult females and by juvenile males, (*ii*) sought by adult males, and (*iii*) ignored by juvenile females (Figure 3.4, A). Traps containing bedding soiled by males seem to be (*i*) sought by juvenile and adult females, (*ii*) avoided by juvenile males, and (*iii*) ignored by adult males (Figure 3.4, B).

3.4.3. O3: Analyse odorants emanating from male- or female-soiled bedding.

Many odorants were common in Porapak Q headspace volatile extracts of bedding soiled by male or female mice, but 2-*sec*-butyl-4,5-dihydrothiazole (DHT) was male-specific and 3,4-dehydro-*exo*-7-ethyl-5-methyl-6.8-dioxabicyclo[3.2.1]octane (= 3,4-dehydro-*exo*-brevicomin) (DEB) was 7 times more prevalent in headspace volatiles of male bedding (Figure 3.5, A). Therefore, these two compounds were deemed candidate male pheromone components.

Chiral GC analyses of racemic DEB (containing both optical isomers), enantioselectively synthesized (1*R*,5*S*,7*R*)-DEB, and mouse-produced HPLC-isolated DEB revealed that both male and female mice produce (1*R*,5*S*,7*R*)-DEB and (1*S*,5*R*,7*S*)-DEB at a 3:1 ratio (Figure 3.5, B).

3.4.4. O4: Laboratory-test the effect of candidate male pheromone components on responses of females and males

Females and singly housed males spent significantly more time in arenas containing the blend of DHT and DEB than in control arenas [Exp. 7 (females): $t = 2.97$, $P = 0.007$; Exp. 9 (singly-housed males): $t = 3.44$, $P = 0.006$; Figure 3.6]. In contrast, group-housed males spent significantly less time in arenas containing the blend of DHT and DEB than in control arenas (Exp. 8, $t = -2.24$, $P = 0.042$; Figure 3.6).

3.4.5. O5: Field-test the effect of candidate male pheromone components on captures of wild mice

Snap traps in treatment boxes baited with DEB and DHT in mineral oil captured 20 adult female house mice, whereas snap traps in control boxes captured only five adult females (Exp. 10; Figure 3.7). Conversely, snap traps in treatment boxes baited with DHT and DEB captured only 14 adult male house mice, whereas snap traps in control boxes captured 29 adult males. Captures of juvenile mice in treatment and control boxes were similar.

Logistic regression analyses of trapping data revealed significant differences (residual deviance = 7.71 p = 0.0004) based on the age/sex group of mice [adult females, adult males, juvenile females or juvenile males]. The model showed no effect of location because trends of age-sex category captures in treatment and control traps were similar across all locations. Multiple comparisons of trapping probabilities further indicated that the blend of DEB and DHT had a significant effect (Tukey; $p < 0.05$). The probability of an adult female mouse being captured in a treated trap relative to the control was 0.8, whereas the probabilities for adult males, juvenile females, or juvenile males were 0.33, 0.37, and 0.42, respectively.

3.5. Discussion

My data provide proof of concept that house mouse pheromones could be developed to improve the efficacy of trapping programs for mouse control. This conclusion is based on definitive field data revealing a strong pheromone effect on attraction and capture of wild mice. Specifically, (*i*) bedding soiled by caged female mice preferentially attracted adult male mice (Figure 3.4, A), (*ii*) bedding soiled by caged male mice preferentially attracted juvenile and adult female mice (Figure 3.4, B), and (*iii*) the synthetic 2-component sex pheromone of male mice significantly enhanced the probability of attracting adult female mice (Figure 3.7). The study also underlines the importance of testing wild animals in mammalian pheromone research. My laboratory data obtained with inbred, laboratory-strain house mice, did not completely mirror field data, and apparently have less predictive value than field data to conclusively determine odorants with attractive or repellent pheromonal function.

The search for the pheromones that mediated the attraction of wild mice in field experiments started with Porapak Q captures of headspace volatiles emanating from soiled bedding. Analyzing aliquots of these Porapak Q extracts by GC-MS revealed complex volatile blends associated with soiled bedding. While many odorants were common in headspace volatiles of female and male bedding (Figure 3.6), two compounds "stood out" in males. 3,4-Dehydro-*exo*-brevicomin (DEB) was seven times more abundant in volatiles of males than in volatiles of females, and 2-*sec*-butyl-4,5-dihydrothiazole (DHT) was a male-specific component, consistent with previous literature reports (Liebich *et al*. 1977; Schwende *et al.* 1986). Prevalence or specificity of these two components in males strongly implied a sex pheromone function and warranted their syntheses for field-testing. Because males produce both enantiomers of DEB (Figure 3.6, B), field-testing the effect of each enantiomer was deemed not necessary. With previously reported evidence that male mice produce only the *S*-enantiomer of DHT (Cavaggioni et al. 2003), we did consider the synthesis and field-testing only of the *S*-enantiomer. However, taking into account that racemic DEB and racemic DHT are functional as a primer pheromone (Jemiolo *et al.* 1986; Novotny *et al.* 1995), and that optically-active DHT would be prohibitively expensive as a component of the pheromone lure, racemic instead of optically-active DHT was synthesized and field-tested.

α- and β-Farnesene – two other previously reported pheromone components of male house mice (Harvey *et al* 1989, Novotny and Harvey 1990, Jemiolo *et al.* 1991, Novotny *et al.* 1999, Röck *et al.* 2006, Zhang *et al.* 2007, 2008, Osada *et al* 2008) – were not detectable in our analyses. Because our methods to gather and identify the farnesenes were proficient and on a par with methods previously used (Novotny *et al.* 1976, Schwende *et al.* 1986*,* Harvey *et al.* 1989; Osada *et al.* 2008), it seems that our particular strain of mice may have produced these farnesenes at very low levels. Alternatively, the absence of α- and β-farnesene in our analytes may stem from group-housing males and removing dominant (aggressive) males from groups, thereby diminishing potential farnesene content in soiled-bedding analyte. As the presence of α- and β-farnesene has the effect of shortening the time subordinate male mice spend investigating urine deposits (Novotny *et al*. 1990), both compounds were considered not

essential, or even counterproductive, for the development of a pheromone lure and were thus excluded from the pheromone blend prepared for field experiments.

The synthetic pheromone blend of DEB and DHT had a significant effect on captures of female mice (Figure 3.7). Trap boxes baited with DEB and DHT captured four times more adult female mice than corresponding control boxes. Moreover, there is emerging evidence that this blend also affects the behaviour of male mice. Trap boxes baited with DEB and DHT captured 14 adult males, whereas corresponding control boxes captured 29 male, revealing a potential adverse effect of the male pheromone on the response of males. Near-identical data were recently obtained in a field study with brown rats, *Rattus norvegicus* (Takács *et al.* 2016). Trap boxes baited with synthetic male sex pheromone captured 10 times more female rats, but 5 times fewer male rats, than corresponding control boxes, again revealing sex-specific attraction and avoidance responses, respectively.

While bedding soiled by females had a positive effect on the response of adult males in the lab (Figure 3.2, Exp 2), and on captures of wild adult males in the field (Figure 3.4, A), the pheromone components mediating the response remain unknown. Differential analyses of headspace volatiles from female and male bedding (Figure 3.5) revealed no female-specific components that would have indicated a potential pheromonal function. It is conceivable, however, that the female-produced sex pheromone is linked to the oestrus cycle of females and that the pheromone is detectable only in a specific phase of the cycle. Changes in the relative abundance of odorants in relation to the oestrus cycle have already been reported in mice (Schwende *et al.* 1986) but a pheromone function has not yet been assigned to any of these odorants. To determine the sex pheromone of female mice, it may be necessary to track the presence and relative abundance of odorants in each of the proestrus, estrus, metestrus and diestrus phases, and then to bioassay those compounds for attraction of males that are present only in a distinct phase, or that undergo the most significant changes in relative abundance through the cycle.

That juvenile males avoided trap boxes baited with female or male bedding (Figure 3.4), likely sensing the sex pheromone of adult females or males, is consistent with life history traits of house mice. Weaned by their mother, wandering young males

likely attempt to avoid encounters with territorial adult males to reduce the risk of a fight and injury (Hurst 1990). Young males may avoid adult females because they may possibly be in the company of an unfamiliar dominant male.

In conclusion, I report the first evidence that the synthetic male mouse sex pheromone enhances trap captures of wild female mice. I provide further evidence that the female sex pheromone – once identified – could be deployed to enhance captures of wild male mice. Baiting some traps in any trapping program with only a food bait will address the phenomenon that young males avoid traps baited with male or female sex pheromone. Operational implementation of mouse pheromones could improve the efficacy of control tactics for mice.

3.6. Literature Cited

Angel, A., R. M. Wanless, and J. Cooper. 2009. Review of impacts of the introduced house mouse on islands in the Southern Ocean: are mice equivalent to rats? Biological Invasions 11:1743–1754.

Berny, P. J., T. Buronfosse, F. Buronfosse, F. Lamarque, and G. Lorgue. 1997. Field evidence of secondary poisoning of foxes (*Vulpes vulpes*) and buzzards (*Buteo buteo*) by bromadiolone, a 4-year survey. Chemosphere 35:1817–1829.

Bruce, H. M. 1959. An exteroceptive block to pregnancy in the mouse. Nature 184:105.

Cavaggioni, A., C. Mucignat-Caretta, and Giuseppe Zagotto. 2003. Absolute configuration of 2-*sec*-butyl-4,5-dihydrothiazole in male mouse urine. Chemical Senses 28:791–797.

Ehman, K. D., and M. E. Scott. 2001. Urinary odour preferences of MHC congenic female mice, *Mus domesticus*: implications for kin recognition and detection of parasitized males. Animal Behaviour 62:781–789.

Harvey, S., B. Jemiolo, and M. Novotny. 1989. Pattern of volatile compounds in dominant and subordinate male mouse urine. Journal of Chemical Ecology 15:2061–2072.

Howald, G., C. J. Donlan, J. P. Galván, J. C. Russell, J. Parkes, A. Samaniego, Y. Wang, D. Veitch, P. Genovesi, M. Pascal, A. Saunders, and B. Tershy. 2007. Invasive rodent eradication on islands. Conservation Biology 21:1258–1268.

Hurst, J. L. 1990. Urine marking in populations of wild house mice *Mus domesticus* Rutty. I. Communication between males. Animal Behaviour 40:209–222.

Hurst, J., D. Robertson, U. Tolladay, and R. Beynon. 1998. Proteins in urine scent marks of male house mice extend the longevity of olfactory signals. Animal Behaviour 55:1289–97.

Hurst, J. L., C. E. Payne, C. M. Nevison, A. D. Marle, R. E. Humphries, D. H. L. Robertson, A. Cavaggioni, and R. J. Beynon. 2001. Individual recognition in mice mediated by major urinary proteins. Nature 414:631–634.

Jemiolo, B., J. Alberts, S. Sochinski-Wiggins, S. Harvey, and M. Novotny. 1985. Behavioural and endocrine responses of female mice to synthetic analogues of volatile compounds in male urine. Animal Bahviour 33:1114–1118.

Jemiolo, B., S. Harvey, and M. Novotny. 1986. Promotion of the Whitten effect in female mice by synthetic analogs of male urinary constituents. Proceedings of the National Academy of Sciences of the United States of America 83:4576–4579.

Jemiolo, B., T. M. Xie, and M. Novotny. 1991. Socio-sexual olfactory preference in female mice: attractiveness of synthetic chemosignals. Physiology and Behavior 50:1119–1122.

Liebich H. M., A. Zlatkis, W. Bertsch, R. Van Dahm, and W. K. Whitten. 1977. Identification of dihydrothiazoles in urine of male mice. Biological Mass Spectrometry 4:69-72

Maruniak, J. A., C. Desjardins, and F. H. Bronson. 1975. Adaptations for urinary marking in rodents: prepuce length and morphology. Journal of Reproduction and Fertility 44:567-570.

Maruniak, J. A., C. J. Wysocki, and J. A. Taylor. 1985. Mediation of male mouse urine marking and aggression by the vomeronasal organ. Physiology and Behaviour: 37:655-657.

Murray, M., and F. Tseng. 2008. Diagnosis and treatment of secondary anticoagulant rodenticide toxicosis in a red-tailed hawk (*Buteo jamaicensis*). Journal of Avian Medicine and Surgery 22:41–46.

Musso, A. E., S. J. Takács, R. M. Gries, and G. G. Gries. 2014. New compositions and methods for attracting and stimulating feeding by mice and rats. International Publication Number WO 2014/186885 A1. Wold Intellectual Property Organization.

Nakamura, K., T. Kikusui, Y. Takeuchi, and Y. Mori. 2007. The critical role of familiar urine odor in diminishing territorial aggression toward a castrated intruder in mice. Physiology and Behavior 90:512–517.

Novotny, M., M. L. Lee, and K. D. Bartle. 1974. Some analytical aspects of the chromatographic headspace concentration method using a porous polymer. Chromatographia 7:333-338.

Novotny, M., and S. Harvey. 1990. Chemistry of male dominance in the house mouse, *Mus domesticus*. Cellular and Molecular Life Sciences 46:109–113.

Novotny, M. V., T. -M. Xie, S. Harvey, D. Wiesler, B. Jemiolo, and M. Carmack. 1995. Stereoselectivity in mammalian chemical communication: male mouse pheromones. Experientia 51:738-743.

Novotny, M. V, W. Ma, D. Wiesler, and L. Zídek. 1999. Positive identification of the puberty-accelerating pheromone of the house mouse: the volatile ligands associating with the major urinary protein. Proceedings Royal Society B: Biological Sciences 266:2017–2022.

Osada, K., T. Tashiro, K. Mori, and H. Izumi. 2008. The identification of attractive volatiles in aged male mouse urine. Chemical Senses 33:815–823.

Rattner, B. A., K. E. Horak, S. E. Warner, D. D. Day, C. U. Meteyer, S. F. Volker, J. D. Eisemann, and J. J. Johnston. 2011. Acute toxicity, histopathology, and coagulopathy in American kestrels (*Falco sparverius*) following administration of the rodenticide diphacinone. Environmental Toxicology and Chemistry 30:1213–1222.

Rich, T., and J. Hurst. 1999. The competing countermarks hypothesis: reliable assessment of competitive ability by potential mates. Animal Behaviour 58:1027–1037.

Riley, S. P. D., C. Bromley, R. H. Poppenga, , F. A. Uzal, L. Whited, and R. M. Sauvajot. 2007. Anticoagulant exposure and notoedric mange in bobcats and mountain lions in urban Southern California. Journal of Wildlife Management 71:1874–1884.

Röck, F., S. Mueller, U. Weimar, H. Rammensee, and P. Overath. 2006. Comparative analysis of volatile constituents from mice and their urine. Journal of Chemical Ecology 32:1333–1346.

Schwende, F. J., D. Wiesler, J. W. Jorgenson, M. Carmack, and M. Novotny. 1986. Urinary volatile constituents of the house mouse, *Mus musculus*, and their endocrine dependency. Journal of Chemical Ecology 12:277–296.

Shore, R. F., J. D. S. Birks, and P. Freestone. 1999. Exposure of non-target vertebrates to second-generation rodenticides in Britain, with particular reference to the polecat *Mustela putorius*. New Zealand Journal of Ecology 23:199–206.

Simberloff, D. 2009. Rats are not the only introduced rodents producing ecosystem impacts on islands. Biological Invasions 11:1735–1742.

Singleton, G. R., H. Leirs, L. A Hinds, and Z. B. Zhang. 1999. Ecologically-based management of rodent pests: re-evaluating our approach to an old problem. Pages 17-29 *in* G. R. Singleton, L. Hinds, H. Leirs, Z. Zhang, editors. Ecologically-Based Management of Rodent Pests. ACIAR, Canberra, Australia.

Smith, R. H., and A. N. Meyer. 2015. Rodent control methods: non-chemical and non-lethal chemical, with special reference to food stores. Pages 101-122 *in* A. P. Buckle and R. H. Smith, editors. Rodent pests and their control. 2nd edition. CAB International, Boston, MA, USA.

Takács, S., R. Gries, H. Zhai, and G. Gries. 2016. The sex attractant pheromone of male brown rats: identification and field experiment. Angewandte Chemie International Edition 55:1-6.

Thomas, P. J., P. Mineau, R. F. Shore, L. Champoux, P. A. Martin, L. K. Wilson, G. Fitzgerald, and J. E. Elliott. 2011. Second generation anticoagulant rodenticides in predatory birds: Probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada. Environment International 37:914–920.

Vandenbergh, J. G. 1969. Male odor accelerates female sexual maturation in mice. Endocrinology 84:658–660.

Van der Lee, S., and L. M. Boot. 1955. Spontaneous pseudopregnancy in mice. Acta Phsiologica et Pharmacologica Neerlandica 4:422.

Volfová, R., V. Stejskal, R. Aulický, and D. Frynta. 2011. Presence of conspecific odours enhances responses of commensal house mice (*Mus musculus*) to bait stations. International Journal of Pest Management 51:35-40.

Wanless, R. M., A. Angel, R. J. Cuthbert, G. M. Hilton, and P. G. Ryan. 2007. Can predation by invasive mice drive seabird extinctions? Biology Letters 3:241–244.

Whitten, W. K. 1956. Modification of the oestrous cycle of the mouse by external stimuli associated with the male. Journal of Endocrinology 13:399–404.

Zhang, J. X., X. P. Rao, L. Sun, C. H. Zhao, and X. W. Qin. 2007. Putative chemical signals about sex, individuality, and genetic background in the preputial gland and urine of the house mouse (*Mus musculus*). Chemical Senses 32: 293–303.

Zhang, J. X., Y. J. Liu, J. H. Zhang, and L. Sun. 2008. Dual role of preputial gland secretion and its major components in sex recognition in mice. Physiology and Behaviour 95:388–394.

3.7. Tables and Figures

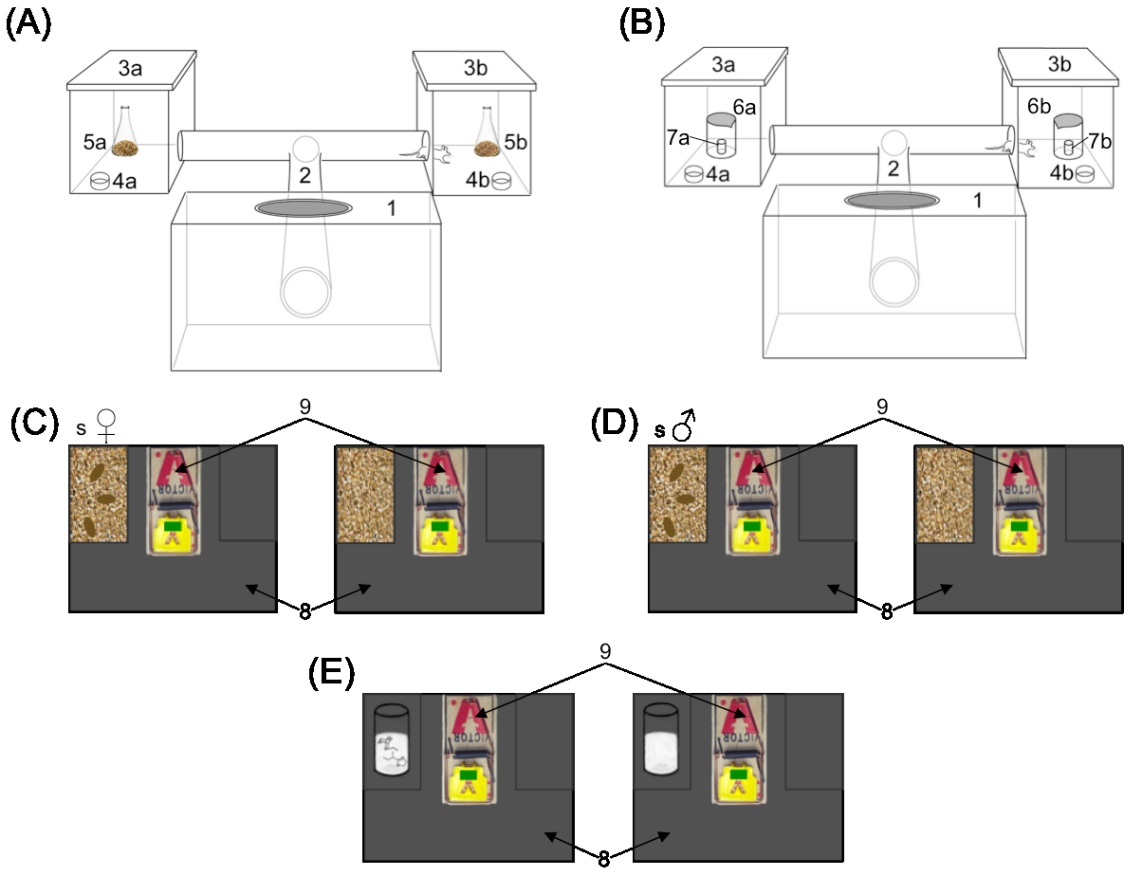
Table 3.1 List of research objectives (O) and stimuli (S) tested in laboratory or field experiments (Exps.) 1-10 for the behavioural responses of laboratory-strain or wild house mice

Objectives (O)	Exps.	Location	S1	S2	Mice tested
O1: Test: the effect of bedding soiled with urine and feces of ♀ or ♂ mice on responses of lab-strain ♀ and ♂ mice	1, 2	Lab	♀ bedding[a]	Clean bedding[b]	♀ or ♂
	3, 4	Lab	♂ bedding[a]	Clean bedding[b]	♀ or ♂
O2: Field-test the effect of bedding soiled by females or males on captures of wild mice	5	Field	♀ bedding[a]	Clean bedding[b]	Wild mice
	6	Field	♂ bedding[a]	Clean bedding[b]	Wild mice
O3: Analyze odorants emanating from bedding soiled by ♂ or ♀ mice					
O4: Test the effect of candidate male pheromone components on responses of lab-strain ♂ or ♀ mice	7	Lab	DHT/DEB[c]	Mineral oil	Group-housed ♀
	8	Lab	DHT/DEB[c]	Mineral oil	Group-housed ♂
	9	Lab	DHT/DEB[c]	Mineral oil	Singly-housed ♂
O5: Test the effect of candidate male pheromone components on captures of wild mice	10	Field	DHT/DEB[c]	Mineral oil	Wild mice

[a]50 g of bedding soiled by urine or feces of 3-5 laboratory-kept male or female mice over the course of 2-5 days

[b]50 g of clean bedding

[c]2 mg of a 1:1 blend of 2-*sec*-butyl-4,5-dihydrothiazole and 3,4-dehydro-*exo*-brevicomin (each >98% chemically pure), formulated in 10 g of mineral oil.



1210 **Figure 3.1** **Graphical illustration of the experimental design used in laboratory and field experiments. (A, B) T-tube olfactometer with release arena (1; 40 cm × 20 cm × 30 cm), T-Tube (2; stem: 65 cm long, side arms: 45 cm long, all 10 cm in diam), and stimulus arenas (3a, 3b; 60 cm × 30 cm × 40 cm), each housing a Petri dish (5 cm diam) with a food**
1215 **bait (4) and (*i*) an Erlenmeyer flask containing soiled or clean bedding (5a, 5b) (A) or (*ii*) a 20-ml scintillation vial (6a, 6b) in a 150-ml beaker (7a, 7b) containing mineral oil laced, or not, with the two pheromone components 2-*sec*-butyl-4,5-dihydrothiazole (DHT) and 3,4-dehydro-*exo*-brevicomin (DEB) (B). (C, D, E) Paired trap boxes**
1220 **(8) each fitted with a baited Victor® snap trap (9) and containing (*i*) clean bedding or bedding soiled (s) by caged female mice (C) or caged male mice (D) or (*ii*) a scintillation vial containing 10 g of mineral oil laced, or not with DEB and DHT (E)**

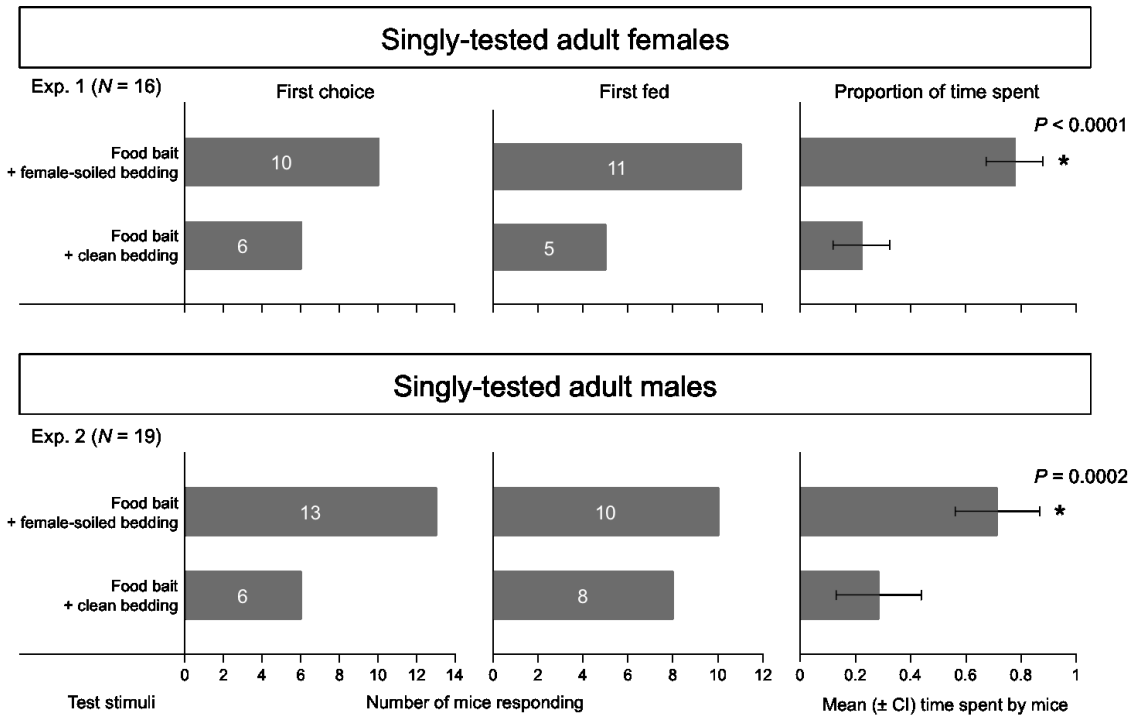


Figure 3.2 **Effect of bedding soiled by caged female house mice on behavioural responses of adult female mice (Experiment 1) and adult male mice (Experiment 2). In all experimental replicates (N=35), the mouse was given a choice between stimuli presented in bioassay arenas 3a or 3b (see Figure 3.1, A). The following data were recorded and analyzed: (*i*) the treatment or control arena that the mouse entered first with all four paws (first choice data), (*ii*) the arena in which it first fed on the food bait ("first-feeding data"), and (*iii*) the proportion of time it spent in each arena ("time spent data"). In each experiment, the Asterisk (*) denotes a significant preference for a test stimulus (Students t-test; CI = confidence interval).**

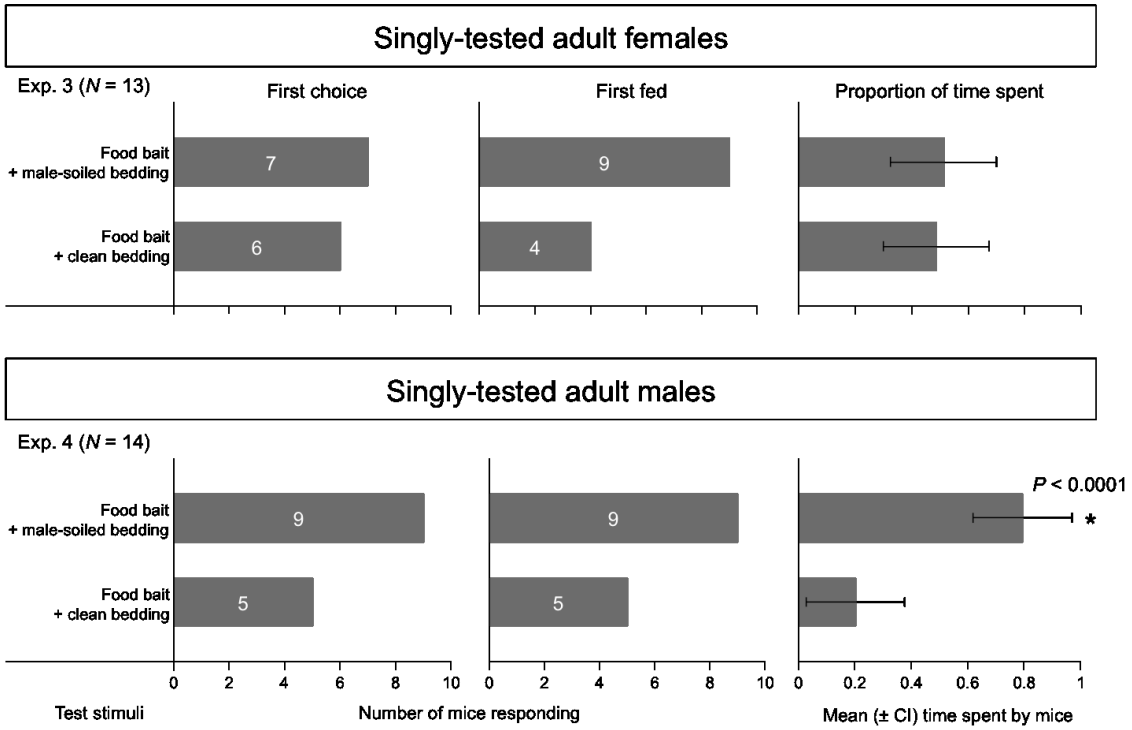


Figure 3.3 **Effect of bedding soiled by caged male house mice on behavioural responses of adult female mice (Experiment 3) and adult male mice (Experiment 4). In all experimental replicates (N=27), the mouse was given a choice between stimuli presented in bioassay arenas 3a or 3b (see Figure 3.1, A). The following data were recorded and analyzed: (*i*) the treatment or control arena that the mouse entered first with all four paws (first choice data), (*ii*) the arena in which it first fed on the food bait ("first-feeding data"), and (*iii*) the proportion of time it spent in treatment or control arenas ("time spent data"). The Asterisk (*) denotes a significant preference for a test stimulus (Students t-test; CI = confidence interval)**

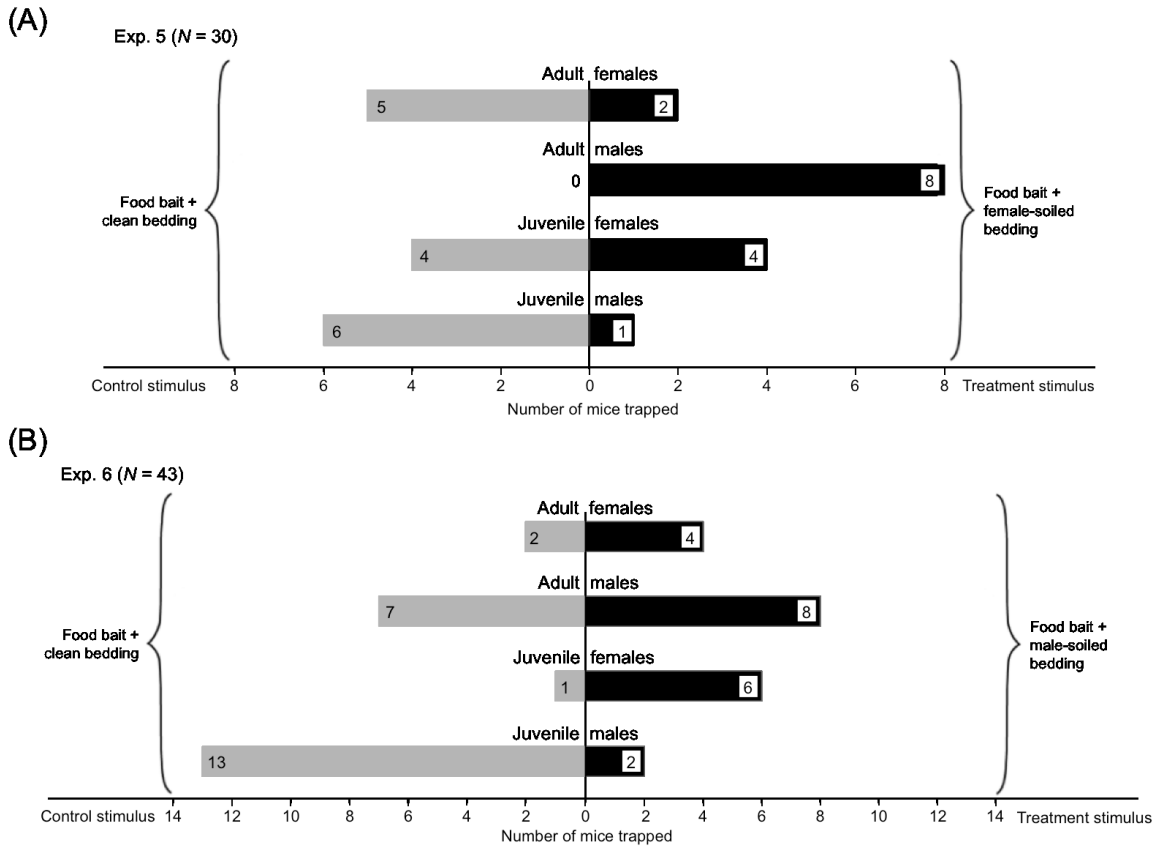


Figure 3.4 **Numbers of house mice captured in field experiments 5 and 6 during late summer and fall of 2014 in snap traps inside paired trap boxes (N=73) containing bedding soiled, or not, by either caged female mice (A) or caged male mice (B) (see methods for detail).**

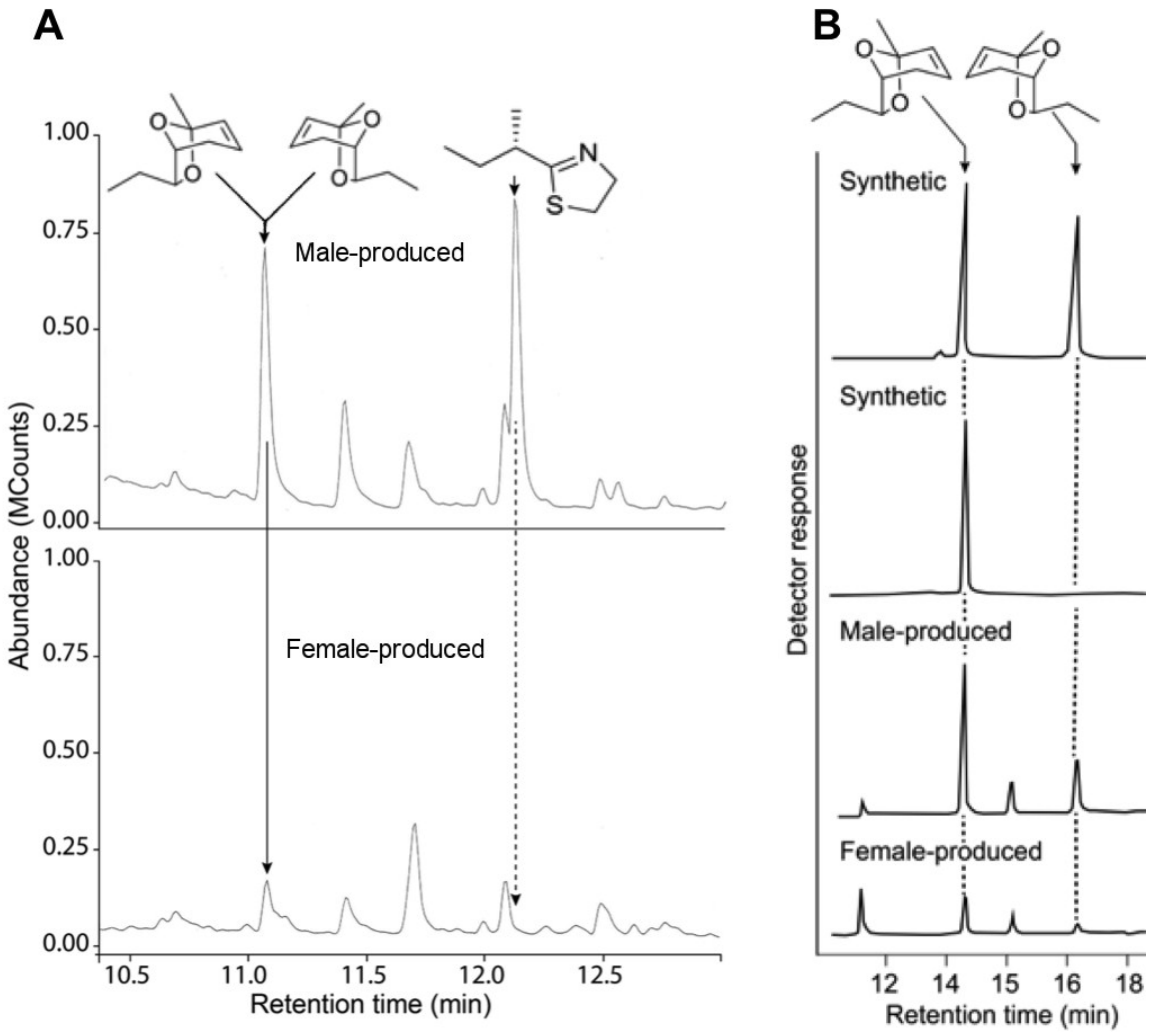


Figure 3.5 (A) Total ion chromatograms of volatile components emanating from urine- and feces-soiled bedding of adult male (top) and adult female (bottom) house mice. Note: 3,4-dehydro-*exo*-brevicomin (DEB) is about seven times more abundant in volatiles of males than in volatiles of females, and 2-*sec*-butyl-4,5-dihydrothiazole (DHT) is a male-specific component. (B) Gas chromatographic analyses on a chiral GC column of (*i*) synthetic racemic DEB (top), (*ii*) synthetic (1*R*,5*S*,7*R*)-DEB (middle), and (*iii*) DEB produced by male and female mice. Note: (a) male mice and female mice produce (1*R*,5*S*,7*R*)-DEB and (1*S*,5*R*,7*S*)-3,4-DEB at a 3:1 ratio; (b) only (*S*)-2-(*sec*-butyl)-4,5-dihydrothiazole is produced by male mice (Cavaggioni et al. 2003); (c) the identity of compounds in B was confirmed by GC-MS analyses.

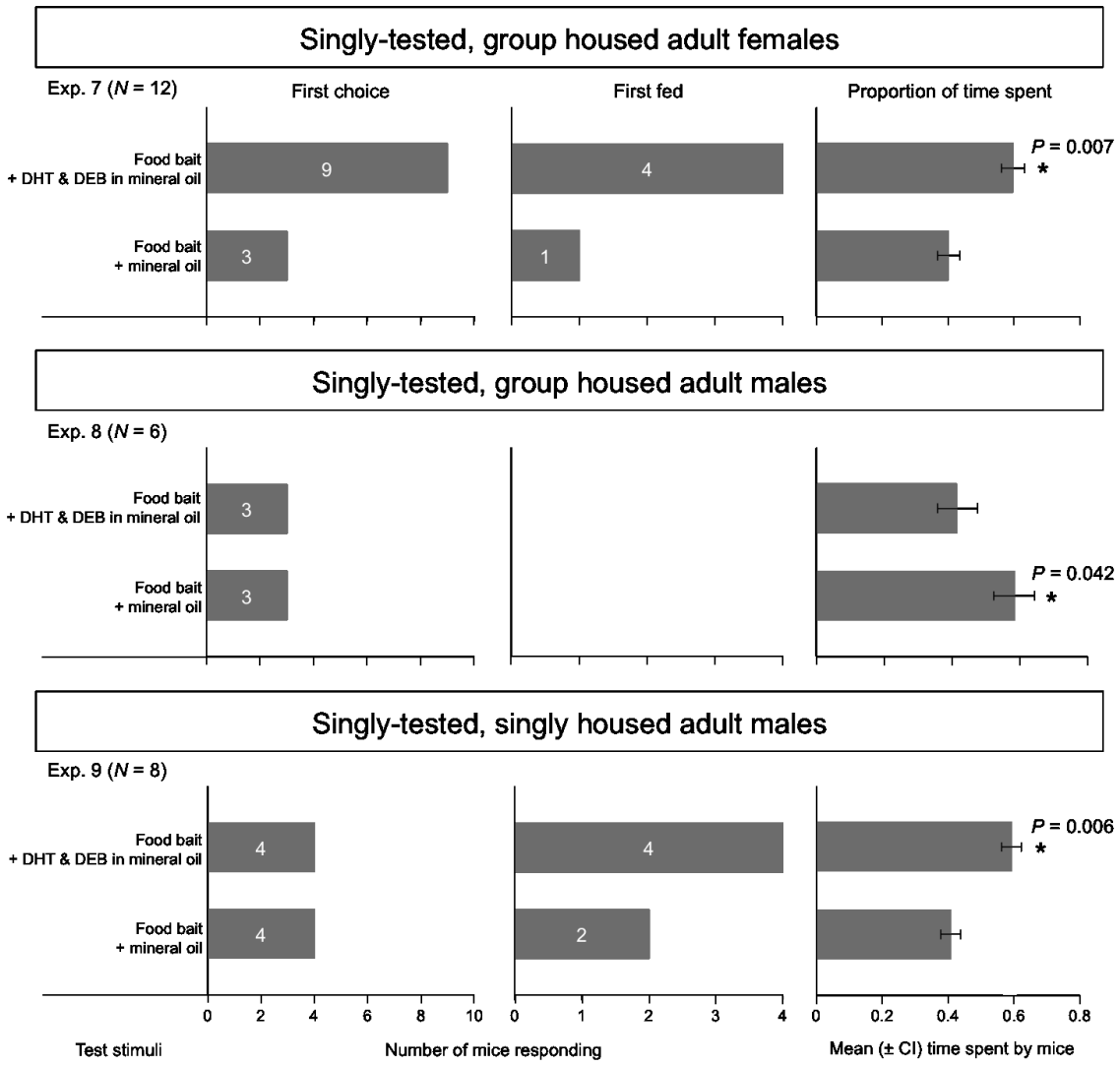


Figure 3.6 **Effect of the synthetic 3,4-dehydro-*exo*-brevicomin (DEB) and 2-*sec*-butyl-4,5-dihydrothiazole (DHT; 1:1 ratio; 2 mg total; formulated in 10 g of mineral oil) on behavioural responses of individually tested, group-housed house mouse females (Experiment 7), group-housed males (Experiment 8) and singly-housed males (Experiment 9). In all experimental replicates (N = 26), the mouse was given a choice between stimuli presented in bioassay arenas 3a or 3b (see Figure 3.1, B). The following data were recorded and analyzed: (*i*) the treatment or control arena that the mouse entered first with all four paws (first choice data), (*ii*) the arena in which it first fed on the food bait ("first-feeding data"), and (*iii*) the proportion of time it spent in treatment or control arenas ("time spent data"). The asterisk (*) denotes a significant preference for a test stimulus (Students t-test; CI = confidence interval).**

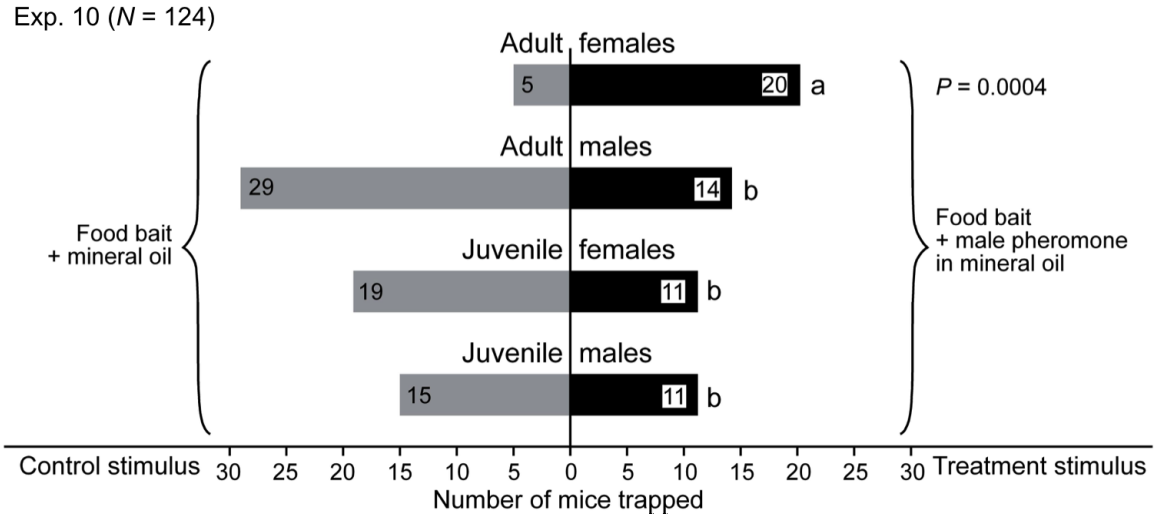


Figure 3.7 **Effect of synthetic male mouse sex pheromone components 3,4-dehydro-*exo*-brevicomin (DEB) and 2-*sec*-butyl-4,5-dihydrothiazole (DHT) (1:1 ratio; 2 mg total; formulated in 10 g of mineral oil) on trap captures of adult and juvenile male and female house mice in field experiment 10 (22 January to 25 September 2015). In all replicates (N=124), mice were given a choice between paired trap boxes, each box fitted with a food-baited snap trap, and the treatment box, but not the control box, also baited with DEB & DHT. Trap captures differed between adult females, adult males, juvenile females or juvenile males (Logistic regression analyses; residual deviance = 7.71, *P* = 0.0004). As indicated by different letter superscripts on bars, the probability of adult female mice to be captured in pheromone–baited traps relative to control traps (0.8) was significantly greater than the probabilities of adult males (0.33), juvenile females (0.37), or juvenile males (0.42) (Tukey multiple comparisons of trapping probabilities; *P* < 0.05).**

Chapter 4. Concluding summary

My major findings and their implications can be summarized as follows:

- Both house mice and brown rats prefer feeding on cereal-based food.
- The particular composition of feeding stimulants that was readily consumed by both house mice and brown rats comprises cereals (oat flour, rice flour, wheat bran), safflower oil, soy lecithin and fructose, suspended in a gelatine water solution.
- Adding a six-component blend ("Entice") of food-based semiochemicals (2-hydroxy-3-methylcyclopent-2-en-1-one, 2,3-butandione, butyric acid, 6-methyl-(*E*)-2-hepten-4-one, γ-octalactone, 3-methylbutanal) to this composition of feeding stimulants made it as attractive as the leading commercial brand Provoke® in laboratory bioassays.
- In a proof-of-concept field experiment with paired traps, traps baited with composition #14 which contained the above feeding stimulants and 0.001% "Entice" in safflower oil, captured seven mice whereas traps baited with Provoke® captured one single mouse.
- The development of the above feeding stimulant composition coupled with Entice that was readily consumed by both mice and rats constitutes an important and foundational step forward the advancement of effective rodent baits.
- In a field experiment, trap boxes baited with bedding soiled by urine and feces of caged female mice attracted wild adult male mice, indicating the presence of a sex attractant pheromone in bedding produced by female mice.
- In another field experiment, trap boxes baited with bedding soiled by urine and feces of caged male mice attracted wild juvenile and adult female mice, indicating the presence of a sex attractant pheromone in bedding produced by male mice.
- Analysing headspace volatiles emanating from male and female bedding by coupled gas chromatography-mass spectrometry revealed that 3,4-dehydro-*exo*-brevicomin

(DEB) was seven times more abundant in analytes of males and that 2-*sec*-butyl-4,5-dihydrothiazole (DHT) was a male-specific component.

- Isolating DEB from male and female bedding by high-performance liquid chromatography and analysing the isolate by chiral gas chromatography revealed that both male and female mice produce (1*R*,5*S*,7*R*)-DEB and (1*S*,5*R*,7*S*)-DEB at a 3:1 ratio.
- In laboratory olfactometer experiments, adult female and singly housed adult male mice spent more time investigating arenas baited with a synthetic blend of DEB and DHT formulated in mineral oil than in control arenas containing only mineral oil.
- In another laboratory olfactometer experiment, group-housed male mice spent more time in arenas containing the mineral oil control than in arenas baited with the blend of DEB and DHT in mineral oil, suggesting an adverse effect of the pheromone blend on the behaviour of group-housed males.
- In a field experiment with paired trap boxes, boxes baited with a food bait and the synthetic blend of DEB and DHT in mineral oil captured significantly more female mice than corresponding trap boxes baited with a food bait and mineral oil only, indicating that the two-component blend has a releaser pheromone function.
- My data show that DEB and DHT are sex attractant pheromone components of male mice. Synthetic pheromone can be used to enhance trap captures of wild female mice. Operational implementation of this technology could help control populations of mice in urban and agricultural settings.
- Future research should focus on the identification of the sex attractant pheromone produced by female mice.