Heritability and Characteristics of Catnip Response in Two Domestic Cat Populations

By

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Table of Contents

Table of Figures

Table of Tables

Abstract

The domestic cat response to catnip is unique in nature as it represents a repeatable, recognizable behavioral response to an olfactory stimulus that appears to have little evolutionary significance. There is clear variation in response between cats and this has been attributed to genetic factors in the past. These factors are explored in this study using behavioral observation after presenting of catnip to cats in two different research colonies with different environmental and genetic backgrounds. The response trait is defined and Gibbs sampling methods are used to explore a mixed model for the trait to determine genetic effects. Heritabilities obtained in the two colonies for the most significant response behaviors, the head over roll and cheek rub, were 0.511 and 0.794 using catnip spray and dried catnip respectively. No clear Mendelian mode of inheritance was ascertained in either colony. The variation in response behaviors and intensity seen in the two colonies reflects the complex nature of expression of the catnip response, but there is a clear genetic influence on the feline predisposition to responding.

1 INTRODUCTION

Cat owners have noted the response of cats to catnip throughout the ages as an interesting and unique phenomenon. Catnip can be used as behavioral enrichment for domestic and wild cats in captivity [1-3] and is common in toys and treats marketed for cats. The response has been characterized in a variety of manners in the scientific literature, with a Mendelian mode of inheritance hypothesized [4]. The exact mechanisms through which catnip affects feline behavior, however, remain to be elucidated to date. This research aims to determine more precisely the way in which this naturally occurring model of chemical alteration of normal behavior and mood patterns is inherited in cats.

1.1 The catnip plant

Nepeta cataria, otherwise known as the catnip plant, is a hardy perennial herb of the family Lamiaceae. The chemical constituent of the catnip plant's essential oil thought to lead to the response seen in cats is a well characterized isomer of the methylcyclopentane monoterpenoid nepetalactone [5].

1.1.1 Lamiaceae

The plant family Lamiaceae contains aromatic herbs such as the culinary spices oregano, thyme, basil and mint. Various human populations over time

have used other plants from Lamiaceae for medicinal or spiritual-ritual purposes. For example, the herb *Clinopodium douglasii*, commonly known as yerba buena, was documented by a number of western Native American tribes as a remedy for fevers, colds and insomnia, among other uses [6]. The Mazatec Indians of Oaxaca, Mexico have traditionally employed an infusion from the leaves of a Lamiaceate, *Salvia divinorum* (Epling and Jativa-M) to induce "visions" in their divinatory rites. The psychoactive effects induced in humans by inhalation or ingestion of *S. divinorum* have been described as vivid and potent, but shorter in duration than those of synthetic narcotics such as lysergic acid diethylamide (LSD) [7].

1.1.2 Terpenoids

Chemists classify nepetalactone as a terpenoid, a class of structurally and functionally diverse lipids composed of isoprene units. Terpenoids are found throughout all domains of life as steroid precursors, plant and insect hormones, cell membrane ligands and intracellular signaling molecules. Many plant terpenoids exert physiological effects on species outside of their own domain. Tetrahydrocannabinol (THC), a major cannibinoid constituent of *Cannabis* spp., and salvinorin A*,* found in *S. divinorum,* are terpenoids known for their psychoactive properties in humans. Classical hallucinogenic/dissociative chemicals such as LSD are alkaloids and act primarily on the 5-HT2A serotonin receptor [8]. However, salvinorin A, a non-alkaloid diterpenoid, acts as a potent kappa-opoid receptor agonist [9] and dopamine receptor D2 (D2R) partial agonist [10]. To date, salvinorin A is

the only naturally occurring psychotropic chemical known to have this psychopharmaceutical profile.

1.1.3 Human uses of catnip

N. cataria itself has a long and fabled history of utility to human populations. Teas and infusions made from catnip are thought to have a soothing, calming effect, and have been used by practitioners of herbal medicine to treat various anxiety-related maladies [11]. Catnip poultices have been applied to alleviate minor inflammation, particularly toothaches [11]. Catnip has been used to ameliorate gastrointestinal discomfort and to regulate body temperature [12]. Catnip has also been added as filler to preparations of marijuana or tobacco or smoked on its own to relieve respiratory illness. One series of case reports suggested psychotropic effects induced by smoking catnip [13], but this study has long been disputed due to mislabeling of the plant figures in the article and lack of repeatability.

The catnip constituent nepetalactone is an extremely effective insect repellent [14, 15] and shows antimicrobial activity against various strains of *Staphylococcus aureus* [16] and other bacterial pathogens [17].

1.2 The feline response to catnip

The most familiar property of the catnip plant continues to be its allure to domestic cats. Catnip attracts cats in virtually any form, from the natural plant cultivated in gardens and wild on the roadside to commercially produced dried and ground catnip leaves and liquid sprays containing the plant's essential oil. Many non-domesticated members of the Felidae harbor a

similar affinity for catnip. In one study, lions and jaguars in particular tended to respond to catnip, whereas bobcats and cougars did not [18]. Most catnip research done to date, however, primarily focuses on the behavioral effects seen in the response of the domestic cat, *Felis silvestris catus*.

1.2.1 Behaviors of catnip response

Pet owners and laypersons describe the feline response to being presented with catnip using such anthropomorphic terms as "high" and "euphoric" by pet owners and laypersons. From a behavioral science perspective, the observed responses documented in the literature show slight variation between studies. Response behaviors can generally be described as natural feline behaviors normally expressed in other contexts. Repeatedly documented response behaviors include sniffing [4], chewing and licking with head shaking [4], cheek and body rubbing [4], head-over rolling [4], digging [19], batting at [19] and grabbing the catnip containing object with front paws [20], biting [21], and kicking with rear claws [21]. Some behaviors exhibited by undomesticated felids share elements with those shown by domestic cats, but others are unique and catnip's behavioral effects appear to vary between feline species [18].

1.2.2 Catnip response and sexual behavior

The head-over roll is a prominent catnip response component primarily expressed outside of the response condition by estrus females. Thus, some theories concerning the mechanism of catnip response focus on activation of sex-related behaviors [19]. Catnip is sometimes hypothesized to be similar to a pheromone found in male cat urine. In Todd's study, a small group of cats,

both male and female, exhibited rubbing and rolling in response to male cat urine [4].

In 1966, Palen and Goddard performed a series of experiments to examine the similarities between the catnip response and sexual behavior more closely. Palen and Goddard attributed the sniffing, licking and chewing behaviors observed in earlier studies to the presence of the dried leaves and not to behavioral effects induced by catnip itself. Seeking to isolate effects induced by catnip odor, they used a synthetic catnip oil preparation to induce response behaviors in six intact male and female cats first alone, then with a rat and finally with a cat-sized object [19]. Overall, exposure to catnip spray increased the frequency of rolling and head shaking, and of a rapid front paw digging motion not previously characterized in the response [19]. The spray decreased the attention given to prey and increased attention to the "cat sized" object [19]. In addition, tests on another group of cats indicated no influence of sex, age or neuter status, and that previously exposed cats responded similarly as they had to past exposures [19]. Palen and Goddard concluded that the head-over roll exhibited in the catnip response was the same as that performed during estrus, and that the other behaviors demonstrated during the response were either statistically not different from cats not exposed to catnip, attributed to of the use of dry catnip leaves, or were actually other female estrus behaviors directed towards the "cat-sized" object [19].

With many researchers building the case for pheromone-like activity of catnip, in 1985 Hart and Leedy performed behavioral experiments to test vomeronasal organ (VNO) involvement. The VNO is a specialized auxiliary olfactory organ found in many mammals that serves primarily to recognize pheromones. In these experiments, vomerectomized cats' reactions to catnip were consistent with response behaviors prior to surgery, including sniffing, licking, chewing, head-shaking, rolling, object-focused rubbing and play behavior [21]. However, removal of the olfactory bulb in cats with or without the VNO intact virtually eliminated all response behaviors on exposure to catnip [21]. These experiments supported the repeatability of the non-sexual behaviors displayed in response to catnip with an olfactory route to response. Hart and Leedy proposed involvement of multiple neural systems governing species-specific pleasure-related behaviors initiated primarily through olfactory input following the cat sniffing the herb [21].

1.2.3 Physiological control of catnip response

Other studies hypothesized catnip as a hallucinogenic to cats with response behaviors mediated by reward-seeking regions of the brain. In 1972, R.C. Hatch found that administration of a variety of drugs manipulated the characteristics and duration of response [20]. Sedatives had a tendency to dramatically decrease or abolish the response in previously responding cats, as did diphenylhydantoin, which may indicate the necessity of an altered neural state for the reaction [20]. Diphenylhydantoin actually blocked response to catnip for up to four weeks post-administration [20]. Coadministration of anticholinergic and antiserotonergic agents drastically

shortened or abolished the response, though either class of drug administered alone caused statistically insignificant changes in overall response with qualitative alteration of certain phases reported [20]. This suggests a degree of reciprocity and redundancy in these systems as they relate to response. Amphetamine administration blocked response, and morphine and chlorpromazine attenuated the effects [20]. Hatch discussed the alteration of the voluntary control of the response by many of these agents and by additional environmental, temperament, or mood conditions. Hatch concluded that the response is complex and multifactorial with regards to expression and discussed neurochemical pathways affected by the drugs used in the study and pleasure seeking behavior.

1.2.4 Onset and duration of response

A precise age of onset of attraction and response to catnip in susceptible cats has not been characterized to date. Todd indicated that kittens younger than six to eight weeks do not respond, and that response does not reliably develop until three months of age, but did not include experimental results supporting the claim [4]. Todd also described an avoidance response in young kittens exhibited regardless of whether they would ultimately become responders or non-responders [4]. Palen and Goddard documented responders at two months of age [19], though, and Todd mentioned a case of a seven week old kitten exhibiting a full response [4]. Nevertheless, Todd's estimate of three months has been cited in both mainstream and scientific literature as the age at which a cat’s response status can be determined. The duration of the response is variable, with a mean of ten to

fifteen minutes, followed by an hour long refractory period during which the cat will not respond to further exposure to the plant or its oils [4].

1.2.5 Genetics of response

The reported variation in response behaviors and the tendency of some cats to be disinterested in or not respond to catnip prompted research into genetic mechanisms potentially affecting expression of the traditional response. Todd observed a family of 30 Siamese cats and classified them as responders or non-responders, then examined the resulting pedigree [4]. This pedigree was consistent with autosomal dominant inheritance of the response trait [4]. Todd acknowledged a class of "partial responders" that did not correspond with individuals assumed heterozygous by pedigree, and attributed this variation to other mitigating factors inhibiting normal expression of the response [4]. In a separate population of 84 cats, the results showed gene frequency of 0.45 for the response condition and 0.55 for non-response, yielding a minimum of 69% responders in that population under Hardy-Weinberg equilibrium [4]. Todd's results, published in 1962, comprise the only scientific source concerning the inheritance and frequency of the catnip response to date.

Hatch disputed Todd's theory of genetic control of the response, given that in his experiments, cats who did not respond to catnip under normal conditions would respond if injected with compounds that attenuated the response in responders [20]. Hatch attributed the variation in response primarily to environmental factors and individual cat temperament [20], essentially claiming "nurture" over "nature".

1.3 Behavior and sensitivity genetics

The aforementioned nature vs. nurture debate has in recent years become less of a dichotomy. Development of the vast majority of behaviors and behavioral traits can be attributed to interplay between environmental and genetic factors. Strategies for studying the influence of genetics on behavior in humans, dogs and mice have utilized methods from many different genetics subfields. Studies concerning genetic influences on behavior in cats are scant, however.

1.3.1 Mendelian influences on perception and behavior

Todd's assertion of catnip response as an autosomal dominant trait is interesting as there are few examples of Mendelian inherited behavioral patterns in the literature. Mendelian inherited differences in sensory perception could certainly exert effects on behavior, however, though the effects may not be clear-cut. In humans, the ability to taste phenylthlocarbamide (PTC) is an autosomal dominant variation in taste perception. Links between cigarette smoking and PTC tasting have been hypothesized and studied for many years [22]. Some groups have postulated that people who can taste PTC are less susceptible to becoming dependent on smoking cigarettes than those who are non-tasters [23]. In cohorts of smokers studied, the frequency of PTC tasters is indeed lower than that found in the general population [24]. Additionally, in one study, smokers who were PTC tasters scored significantly lower than non-tasters on surveys that assessed nicotine dependence and positive reinforcement from smoking [23]. Interestingly, a higher proportion of non-tasters preferred brands of

cigarettes with higher nicotine yield than tasters [23]. Whether higher nicotine content is an important factor in dependence or could potentially cause genetic tasters to become non-tasters still remains unknown. This circular relationship between perception, behavior and genetics indicates that each aspect could influence response or reaction to a substance, even if a major gene effect exists.

1.3.2 Genetics of susceptibility

It stands to reason, then, that many behavioral phenotypes can be attributed to inheritance of alleles that contribute to development of these patterns given the presence of other environmental and genetic conditions. For example, variation in behavior within dog breeds is generally smaller than that between breeds, but the presence of this variation despite human persistence in breeding practices to preserve uniformity and maximize utility reflects the complexity of the expression of behaviors. Complicating factors often muddle assessment of inheritance of specific behavioral patterns.

Some researchers have historically approached this problem through breeding or transgenically modifying animal models to exhibit certain behavioral tendencies, studying differences in genetics and neurobiology and testing the frequency of other observed behaviors in controlled conditions. For example, Roman high-avoidance (RHA) and low-avoidance (RLA) lines of rats were selectively bred long ago based specifically on speed of avoidant behavior acquisition in a shuttlebox [25]. As a result of breeding for this temperament trait, the two lines have exhibited significant divergence in dopamine system function [26] and in behavioral and neurochemical

responses to various drugs [27] and environmental stressors [28]. The strains also differ in their propensity to seek drugs [29] and to show signs of addiction or dependence [30]. These differences illustrate quite clearly that underlying genetic factors can affect all steps of a response, from the acquisition and integration of sensory information to outward behavior in response to the information. However, it is also clear that expression of the phenotypes attributed to these genes is subject to modification by external and intrinsic factors [31]. Thus, finding the extent of the contribution of such 'susceptibility' genes will require examination of the phenotype in a large population and the use of statistical methods that can accurately estimate the extent that various factors contribute to the variation.

1.4 Analysis of complex traits

From obsessive-compulsive disorder in humans to offspring size and fertility in beef cattle, complex inheritance historically has been studied using a variety of statistical techniques. Interpretation of the genetic association studies performed currently using hundreds of thousands of single nucleotide polymorphism (SNP) markers still rests on the assertion that the traits under scrutiny have a heritable component. Elucidating the influence of the heritable factors on the differences between individuals using a pedigree and more clearly defining complex phenotypes prior to undertaking an association analysis will inform the initial design of such a study.

1.4.1 Quantifying complex phenotypes

It is difficult to assess the heritability of a particular phenotype without first defining a repeatable method to uniformly describe it. Even those that can be measured in defined units such as body mass, leg length or wing span often must be converted to other units or otherwise represented in order to facilitate genetic analysis. When the phenotype in question is variable and behavioral, with the possibility for subjective interpretation, the situation gets even more complicated. Even in human studies, where one can reasonably assess a person's perception and motivations through personal interview, various schemes and rating scales have been needed to properly study the genetics of relatively common psychiatric disorders.

One example of this type of analysis was published in 1999, to further study the genetics behind obsessive-compulsive disorder. In this case, four factor-analytic symptom dimensions were used to define the shared components of this heterogenous phenotype [32]. A second study around the same time examined attention deficit hyperactivity disorder by simply using DSM-IIIR symptom count as a semicontinuous, quantitative variable [33]. Over a decade later, and even with high-throughput methods of genotyping individuals, current studies of these disorders still lack uniformity in their definitions of the heritable phenotypes. There has been a recent trend in the field, however, back towards refining the phenotypes to get to the genes. Some emerging research studies endophenotypes amongst unaffected relatives of individuals affected with psychiatric disorders and conditions to

determine what factors segregate in families with a presdisposition towards these disorders [34-36].

In animal studies using non-traditional model organisms, the additional problem of human interpretation of animal behavior is introduced into phenotyping practices. Recording of the occurrence of specific behavioral patterns within a given observation removes some of that variability but also limits possibilities for quantitative analysis.

1.4.2 Statistical methods for heritability and segregation

Modeling complex phenotypes in humans is often fairly straightforward due to the relative simplicity of most human family structures. In one recent schizophrenia study [37], endophenotypes were subjected to computation of parent-offspring and sib-sib correlations, and then selected for further heritability and segregation analysis based on these correlations. Narrow-sense heritability for suspected heritable traits was assessed using variance component analysis, with covariates age and sex. They determined that five of the thirteen originally selected endophenotypes showed viable heritability estimates across 25 pedigrees [37]. There are many assumptions that must be made through this method, though, such as the unrelatedness of the founders and of individuals integrated through marriage. In many animal pedigrees, these assumptions are erroneous and could lead to false interpretation of results. Therefore, alternate means must be used for analysis.

Various methods of determining genetic parameters have been used in these more complex pedigrees in a number of species. Animal geneticists have long studied inheritance of traits important to production in agriculturally relevant species through Bayesian inference and maximum likelihood estimation of variance components attributable to genetic and other factors. These methods have recently been applied in studies of complex traits and relationships between traits in dogs, as well [38]. To date, no cat populations or traits have been subjected to such an analysis.

1.5 Objectives of this research

In order to use modern genomic methods such as genome wide association to search for loci associated with the catnip response, the heritable response components must be more clearly defined and Mendelian segregation must be tested in another pedigree. If there is insufficient evidence to support Mendelian segregation, the heritability of response behaviors will be examined to determine the phenotypic observations that will maximize the utility and yield from a genomic study. Since the expression of the response can be affected by non-genetic factors as well, and heritability estimates describe specific populations, two distinct populations will be observed in this study.

2 MATERIALS AND METHODS

2.1 Populations

Two breeding populations of domestic cats were tested to examine the response to catnip. Both are research colonies with well-maintained pedigrees and consistent environmental exposures, including human interactions.

2.1.1 Feline Nutrition and Pet Care Center

The Feline Nutrition and Pet Care Center (FNPCC) colony is housed on the campus of the University of California at Davis. It has been maintained as a specific pathogen free (SPF) facility since 1977, with cats bred for the purpose of conducting nutritional studies and AAFCO feeding trials. Cats are removed from the facility through sales to research and teaching laboratories and through adoption. Due to the SPF nature of the facility, new cats are very rarely introduced to the breeding population, in effect forming a closed population. Health and breeding logs are maintained for queens in the colony, and kittens are logged in and identified through unique integers assigned sequentially by year of birth and tattooed on the pinnae. Written records detailing parental, date of birth and color information are currently available for kittens born at the colony since 1984.

2.1.2 FNPCC housing

Mature queens are typically housed in group cages holding 10-12 cats. Immature kittens and young toms are similarly housed unless territorial aggression is noted. Mature toms used for breeding remain in individual

cages when not being used in an active protocol. Breeding is on an as-needed basis and is harem style, with one tom placed in a group cage with multiple queens.

2.1.3 Feline Genetics Research colony

The Feline Genetics Research (FGR) colony, also located on the UC Davis campus, is composed of cats bred on-site or acquired through donation or purchase. The purpose of the colony is to establish lines of cats that segregate for naturally occurring traits and diseases within or across breeds, facilitating further study of these attributes. Health and breeding records are kept on individual cats and a pedigree is maintained. Founder cats representing various breeds are frequently introduced to the FGR population. As such, this population as a whole represents a higher level of genetic diversity than a typical breed or the FNPCC population.

2.1.4 FGR colony housing

Cats at the FGR colony are generally housed in smaller groups than at the FNPCC. Breeding practices are largely one tom to one or two queens at a time. The majority of cats are group housed, but the number of adult cats housed together rarely exceeds four. Immature and non-breeding cats are housed in an open room of 10 or less cats or a walk-in cage of 6 or less cats. Mature breeding toms are individually housed when not breeding, or in small groups if tolerated.

2.2 Catnip response observations

Cats over 6 months of age were tested for catnip response in both colonies. At both facilities, the observations were performed in the cats' normal enclosures in order to minimize the behavioral effects of environmental disturbance. For similar reasons, observers had minimal contact with the cats while the observations were taking place.

2.2.1 Testing at the FNPCC

The FNPCC catnip exposure trials used commercially produced dried catnip from a single batch obtained from Cosmic Cat Toys (Hagerstown, MD) (Figure 3; appendix). A single subject AB design (observations with control followed by exposure and comparison of states within an individual) was employed. Cats were first offered a length of Coflex (Andover Healthcare; Salisbury, MA) that had not come into contact with catnip, rolled into a ball shape (Figure 3; appendix). Any interactions with this control object were observed and noted. After 15 minutes, the Coflex-only ball was removed and replaced with a similar length of Coflex that had been stored in the catnip barrel and was filled with catnip. Similarly, cats were observed for 15 minutes and interactions with the catnip ball were observed and noted. Due to caging changes and cats being removed from the population, cats were observed from one to three times each by one of three observers.

2.2.2 Testing at the FGR colony

For the FGR catnip trials, a commercially available catnip-infused spray produced by Worldwise pet products (San Rafael, CA) was used (Figure 3; appendix). Cats were first observed for 15 minutes in their enclosures, and

general demeanor and any specific behaviors were noted. The catnip spray was then sprayed onto the floor or shelving of enclosures and the cats were observed for 15 minutes. Specific response-related coded behaviors were observed and noted during and after exposure to the catnip-infused spray. These cats were observed three times each, with at least a week separating each observation. The same individual performed all observations.

2.3 Data recording

2.3.1 Pedigree format

Available breeding records for the two colonies were compiled including animal ID, sire, dam, and sex. If only one parent was known for a given sibship, as was the case for some cats in the FNPCC population, the other was coded as a uniquely numbered unknown in order to preserve the sibling relationship. Animals with no parental records included in the pedigree were considered to be unrelated founders, with '0' in both the sire and dam categories indicating founder status.

2.3.2 Observational data format

The observational data were entered into a spreadsheet program including animal ID, age in years at the time of observation, date of observation, observer and all response behaviors observed. For the FNPCC data, only behaviors noted in response to the catnip ball that were not noted in response to the control ball were included. All behaviors noted within the first fifteen minutes of exposure to catnip spray were included for the FGR observations.

2.3.3 Behavioral observations

Documented catnip behavioral responses include sniffing, chewing and licking with head shaking, cheek and body rubbing, head-over rolling [4], digging, batting at [19] and grabbing the catnip containing object with front paws [20], biting and kicking with rear claws [21]. After review of catnip response behaviors with the other observers, the lead observer (observer 1), recorded catnip trial observations with observers 2 and 3 on two separate occasions each. Observers recorded catnip responses independently and compared observations after trials. Observer 1 was available for consultation during all trials.

2.4 Data analysis

2.4.1 Selection of behaviors for analysis

To reduce inter-observer variation and subjectivity of interpretation, repeatability and reliability of response behaviors in the study conditions were assessed qualitatively throughout the observational time period to determine which behaviors could be further analyzed as attributed to the catnip response.

2.4.2 Defining the traits

The cheek rub and the head over roll, as well as expression of both in a single trial, were considered as categorical threshold traits for estimation of heritability. The phenotypes for each were coded as 1 for affected and 0 for unaffected for each observation, with animal ID, sex (male = 1, female = 2), age range, observation date, observer and observation number. This method assumes an underlying continuous normally distributed variable (θ) related

to the observable phenotype by a set of fixed thresholds. In the case of a binary trait, the three fixed thresholds are x0 = −∞, x1 = 0 and x2 = ∞. The unobservable θ, which is controlled by continuous genetic and environmental terms, can be translated into the observable phenotypes through comparison to these thresholds (for an unaffected animal, θ must be within the boundaries of x0 and x1, whereas for an affected animal it must be between x1 and x2).

2.4.3 Estimating the heritability

Since there is an underlying continuous distribution assumed, the algebraic form of the models for θ is similar to those used for continuous phenotypes:

FNPCC: $\theta_{ijkl} = \mu + sex_i + age_j + a_k + u_l + e_{ijkl}$

FGR: $\theta_{ijklm} = \mu + sex_i + age_j + observation_k + a_l + u_m + e_{ijklm}$

Additive genetic contribution (a_l), permanent environmental effects (u_m) and the residual (e_{ijklm}) are assumed as random effects with zero means and variances of σ_a^2, σ_u^2 and σ_e^2 respectively. The covariance in phenotypes of relatives is accounted for in the additive genetic effect and assumed as normally distributed, using additive relationships among all animals in the pedigree for the covariance structure. Permanent environmental effects represent non-genetic, animal specific random effects seen as a result of multiple testing. The residual variance is fixed at 1.0, with no loss of generality, and $\sigma_p^2 = \sigma_a^2 + \sigma_u^2 + \sigma_e^2$ is the total variance. Heritability can be estimated as $h^2 = \sigma_a^2/(\sigma_a^2 + \sigma_u^2 + \sigma_e^2)$.

A mixed model Bayesian strategy was used to estimate unknown fixed effects and σ_a^2. Prior densities of the fixed effects were assumed to be uninformed, or "flat." Random residuals were assumed as normally distributed, with variance fixed at 1.0 and a null mean. The prior densities for additive genetic variance and permanent effect variance were assumed to be an inverted Wishart distribution with the expected prior mean started at 1.0 and 0.2 respectively, with 3 degrees of certainty, the lowest possible confidence in this mean.

A Gibbs sampling algorithm was used to estimate the distributions of unknown parameters. In this method, a sequence of random variables, the Gibbs sample, is iteratively generated from the known conditional distributions of parameters given the data's likelihood function. The Gibbs sample thus comprises the basis for estimation of the desired parameters. In this case, the Gibbs sampling and analysis were performed with the software MTGSAM, and the manual for this program provides theoretical justification and a more complete description of the process.

For each trait, 350,000 Gibbs sample chains were generated. The first 50,000 samples were discarded. A thinning rate of 40 was applied to the samples. Thus, the final Gibbs sample generated was 7,500 for each trait. The R package coda[1] (version 0.13-5) was used for analysis of these generated Gibbs samples, to examine convergence, autocorrelation and

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[1] Martyn Plummer, Nicky Best, Kate Cowles and Karen Vines (2006). CODA: Convergence Diagnosis and Output Analysis for MCMC, R News, vol 6, 7-11

posterior distribution parameters. Variance components and the estimated heritabilities of the traits were determined from the posterior distributions.

3 RESULTS

3.1 Pedigree characteristics

3.1.1 FNPCC

The FNPCC pedigree used in the analysis spans 25 years, from 1984 to 2009, and includes 3,638 unique individuals. The average inbreeding coefficient in the 2,670 inbred animals in the pedigree is 0.145, as determined by the MTGSNRM routine of the MTGSAM package. The average inbreeding coefficient for the 180 tested cats is 0.103 with a range from 0.0 – 0.397. This is a conservative estimate due to the number of individuals with missing or incomplete parentage information. The complete records, including animal ID, sex, sire and dam for each cat are available and location is referenced in the appendix.

3.1.2 FGR

The pedigree for the FGR colony includes 178 unique individuals over a period of approximately 8 years from 2002 to 2010. In this pedigree, 49 individuals are inbred, with an average inbreeding coefficient of 0.123. The complete records, including ID number, sex, sire and dam for each cat are available and location is referenced in the appendix.

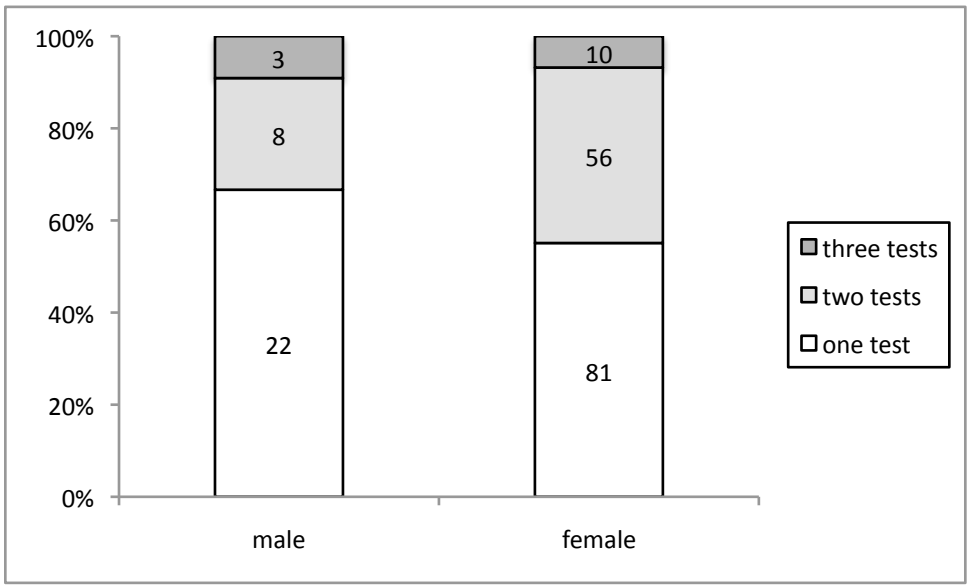
3.2 Catnip test results

3.2.1 FNPCC

One hundred and eighty cats were observed for catnip response from 1-3 times each. Seventy-seven (43%) were tested multiple times and one hundred and three (57%) were tested once for a total of 270 observations.

Figure 1 shows the male vs. female observation occurrences. In this population, males are generally removed from the population more frequently and at a younger age than females. Thus, the population sampled reflects the overall demographics of the colony and fewer males could be observed multiple times. Cats were observed on 17 different occasions, by 1 of 3 observers each time. More detailed information for each of the 180 cats tested is included in Table 7 (appendix).

Figure 1: Illustration of total males and females tested multiple times



3.2.2 FGR

These 30 cats were observed 3 times each, on the same days by the same observer, giving 90 total observations. Observational data for these cats is shown in Table 1.

Table 1: FGR observational data

Cat ID	*Sex*	*Age*	*Trial 1*	*Trial 2*	*Trial 3*
12010	F	2	0	0	0
12173	F	2	0	0	0
12359	F	2	0	0	0
13229	M	1	0	0	0
13230	F	1	0	0	0
13233	M	1	0	0	0
13380	M	1	0	0	1
13237	F	1	0	1	0
13065	F	1	0	2	2
11894	M	2	1	0	1
9890	F	3	1	1	0
11662	F	3	1	1	0
12170	F	2	1	1	2
10699	F	3	1	1	2
13228	M	1	1	2	0
8639	F	3	1	2	2
9882	M	3	2	0	0
13892	M	1	2	0	0
5692	M	3	2	0	1
11893	F	2	2	0	2
8637	M	3	2	1	0
5338	F	3	2	1	1
9969	M	3	2	1	1
13232	F	1	2	2	0
12357	F	2	2	2	1
12168	M	2	2	2	2
12355	M	2	2	2	2
12680	F	1	2	2	2
13225	F	1	2	2	2
13227	M	1	2	2	2

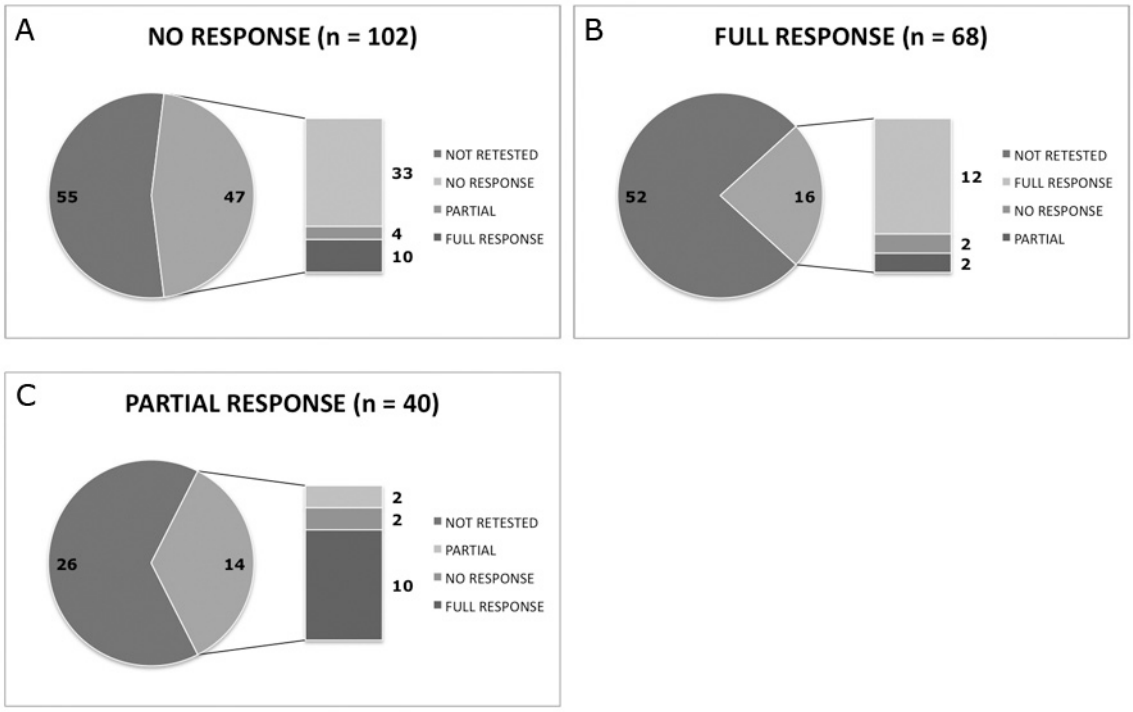
0 = no response behaviors, 1 = partial response (CR or RL), 2 = full response

3.2.3 Variation over multiple tests

In both colonies, some cats exhibited variation in responses over multiple observations. In the FNPCC, of the 77 cats tested multiple times, 29 (38%) varied in response between the tests. Cats that exhibited only one of the response behaviors on the first trial were most likely to vary on subsequent

observation, though 30% of non-responders and 25% of full responders had a variable subsequent response, as seen in Figure 2. In the FGR, 19 out of 30 cats tested (63%) had variable responses over the three observations, shown in Table 2.

Figure 2: FNPCC response variation



Variation in responses in the FNPCC population by response on first test. Inset is the set of cats tested multiple times from each initial response group.

Table 2: FGR response variation

TRIAL 1	*TRIAL 2*	*NR*	*PR*	*R*
NR	NR	6	1	0
NR	PR	1	0	0
NR	R	0	0	1
PR	NR	0	1	0
PR	PR	2	0	2
PR	R	1	0	1
R	NR	2	1	1
R	PR	1	2	0
R	R	1	1	5

Variation in responses in the FGR population by responses on first and second trial.

3.3 Analysis of response

In both populations, cats were grouped by age for analysis as shown in Table 3.

Table 3: Observation summaries

	Males		*Females*		*Total*
FNPCC	R	NR	R	NR	
1-2 years	8	4	14	24	50
2-4 years	5	4	31	22	62
4-6 years	2	5	15	15	37
6-11 years	3	2	14	15	34
FGR					
6 months-1 year	2	4	4	2	12
1-3 years	3	0	3	3	9
3-6 years	3	1	5	0	9

7 females changed age groups between observations. R = full or partial responder. NR = nonresponder

3.3.1 Simple segregation analysis

Table 4 shows observations from FNPCC nuclear families with at least one offspring and both sire and dam tested for response. For this analysis, partial responders are included as responders.

Table 4: FNPCC simple segregation analysis

Sire	*Dam*	*Offspring*	*R*	*NR*
Responder	Responder	10	7	3
Responder	Non-responder	17	8	9
Non-responder	Responder	17	10	7
Non-responder	Non-responder	9	3	6

3.3.2 Gibbs sample analysis

Analysis of the generated Gibbs samples for the genetic variance components and male-female contrasts is presented in Table 5. The Gibbs sampler did not achieve acceptable convergence for analysis of the "either" trait in the FNPCC population and as such the trait was not subjected to further analysis (data not included). Convergence was attained for the cheek rub and head over roll in the FNPCC population, but autocorrelation was high in these samples as well. The "both" trait in the FNPCC population converged well and had the least autocorrelation among the generated Gibbs samples.

Table 5: Gibbs sample analysis

	Mean	Median	SD	Convergence (P value)	95% HDR
FNPCC CR					
Genetic Variance	12.72	10.17	9.68	-1.35 (0.18)	1.35, 31.7
Female-Male	0.72	0.69	0.69	-0.45 (0.65)	-0.67, 2.13
FNPCC RL					
Genetic Variance	20.25	15.03	15.98	-0.09 (0.93)	1.65, 51.36
Female-Male	2.08	1.88	1.18	-0.2 (0.84)	-0.04, 4.52
FNPCC BOTH					
Genetic Variance	5.83	4.90	3.79	0.99 (0.32)	0.56, 13.39
Female-Male	0.54	0.52	0.53	2.14 (0.03)*	-0.51, 1.57
FGR CR					
Genetic Variance	2.72	2.06	2.43	-0.16 (0.87)	0.36, 6.90
Female-Male	-0.33	-0.30	0.59	0.36 (0.72)	-1.51, 0.84
FGR RL					
Genetic Variance	2.86	2.22	2.29	0.67 (0.50)	0.28, 7.04
Female-Male	0.38	0.37	0.57	1.21 (0.23)	-0.79, 1.49
FGR BOTH					
Genetic Variance	3.53	2.48	3.48	0.54 (0.59)	0.12, 10.09
Female-Male	-0.08	-0.07	0.58	0.38 (0.70)	-1.25, 1.07
FGR EITHER					
Genetic Variance	3.84	2.66	4.15	-0.19 (0.85)	0.15, 0.60
Female-Male	-0.81	-0.74	0.71	0.01 (0.99)	-2.25, 0.46

Genetic variance and female vs male contrasts for the traits and trait combinations. 95% HDR including 0 indicates no significant difference between sexes. Convergence measured using Geweke's statistic. *$p < 0.05$ indicates a possible problem with convergence.

Heritability estimates drawn from these Gibbs samples by MTGSAM were sensitive to the input parameters for the closely related FNPCC colony. For the FGR colony, estimates were similar for all traits. These heritabilites are given in Table 6.

Table 6: Heritability estimates

	cheek rub	head over roll	cheek rub and head over roll
FNPCC	0.891	0.924	0.794
FGR	0.523	0.547	0.511

Determined from a Gibbs sample of 7,500 values

4 DISCUSSION

4.1 Repeatability and reliability

In this study, two different colonies of cats were observed for catnip response using different methods of catnip delivery and observation. As such, comparison of the response between colonies is a difficult endeavor. However, it is interesting to note that the response behaviors exhibited and frequencies of response were similar in the two colonies despite these differences. One area in which the colonies were quite divergent was in the consistency of the response in individual cats from trial to trial, though in both colonies there were clear responders and non-responders that did not vary through multiple trials with a clear lack of consistency seen in those cats with a partial response on any observation.

4.1.1 FNPCC variability

Nearly 30% of the cats that were observed multiple times had some variation in response from test to test. As Hatch showed in his demonstration of the effects of neuropharmaceuticals on the response, the neurochemical and general arousal state of a cat at the time of testing can serve to change the duration, intensity or even presence of response [20]. Thus in some cases the response could be suppressed or enhanced by factors outside of experimental control. For example, social factors in a group-housing situation where cats have been housed together for a lengthy period of time could limit responses of lower-ranking or more timid individuals in the presence of

their more confident peers. Additionally, stress related hormonal and biochemical changes could exert an effect on response. Therefore, a low-stress, individually housed situation is likely to provide the least interference with phenotyping for catnip response.

4.1.2 FGR trials

In the FGR, the between test variability in response was higher than that seen in the FNPCC. It is difficult to assess whether this can be attributed to factors within the population or differences in efficacy between the two types of catnip. The population is, as a whole, more genetically diverse and more well socialized than the FNPCC population due to its more frequent influx of new members and non-SPF status. While it is possible that this variability influences the lack of consistency across trials, another possible explanation is that the spray catnip does not promote as robust and consistent of a response as that of dried catnip. Follow-up studies to partition this variability could examine responses of a group of cats after exposure to both types of catnip delivery.

4.1.3 Effect of multiple testing on modeling

The Gibbs sampling method used data from individual observations of each cat as its basis for constructing the samples, so it was possible to estimate the components of variance attributed to temporary vs. permanent environmental effects. Repeatability of a measurement is defined by the intraclass correlation, which is the sum of genetic variance and general environmental variance divided by the phenotypic variance. This is the proportion of single measurement variance due to permanent genetic and

environmental differences between individuals and necessarily provides an upper limit for heritability. Since the genetic variance is high for these traits, the temporary environmental effects (1 – intraclass correlation) uncovered by the multiple testing are a small proportion of variance and represent the low yield from multiple measurements in the FNPCC population. Conversely, the effects of the higher degree of variability in the FGR population could falsely decrease the heritability estimated from this set of observations.

4.2 Defining the heritable response

Todd's genetic study noted 4 components to the response: prolonged sniffing, head shake, cheek rubbing and head over roll [4]. In both of the observed populations, a prolonged sniffing period and the head shake were unreliable predictors as to whether a cat would proceed to the more recognizable response components on that test or subsequent tests. A number of cats that demonstrated both the object-directed cheek rubbing and head over roll behavior did not sniff the catnip for prolonged periods or shake their heads either prior to or following the demonstration. These less prominent behaviors are intuitively more difficult to reliably record and more privy to inter-observer variation, which inevitably leads to a decrease in repeatability of observational results. For this reason, only the cheek rub and head over roll behaviors are examined in further detail with respect to heritability.

4.2.1 Head over roll

The head over roll exhibited in the catnip response bears similarity to that observed in female cats in estrous. No significant differences were noted in

this trait between males and females in either colony, however. As both colonies are breeding colonies, insufficient numbers of neutered animals were available for testing to contrast with the intact cats. Caution should be exercised when observing intact cats for the response as rolling by estrous females could lead to false classification as a responder if the head over roll is considered itself to be a defining response behavior.

4.2.2 Cheek rub

The cheek rubbing behavior directed towards the catnip-containing object or surface is similar to behaviors seen in sexual and scent marking contexts. Some cats in this study did exhibit cheek rub behaviors towards the non-catnip-containing control Coflex ball or towards other cats or surfaces in their enclosures. For this reason, only cats that exhibited the behavior towards the catnip ball either alone or prior to directing it towards other surfaces or cats were included as responders. Due to this restriction, the frequency of the behavior in response to catnip could perhaps be higher than that reported here.

4.2.3 Defining the response threshold

Though the majority of the partial responders re-tested were responders on subsequent trials, the potential for classification error is high when only displaying one of the two aforementioned behaviors is considered as the threshold for response classification. Ongoing work with this data will consider the partial response condition as an intermediate and examine the feasibility of a trichotomous trait model for catnip response, which was an idea proposed by Todd in his original segregation analysis [4]. From the

standpoint of defining the response for a future genome wide association study, these partial responders should be excluded as either responders or non-responders if the trait is to be considered as a dichotomous trait.

4.3 Genetic analysis and heritability

Heritability of a trait describes the proportion of phenotypic variance that can be attributed to genetic variance within a population. Heritability estimates for catnip response within the two populations in this study varied based on the definition of the trait. The high Gibbs sample autocorrelation led to a small sample size for the partial response traits in the FNPCC population. This could falsely inflate the heritability estimate. A more reasonable sample size was estimated for the full response trait in this population, leading to higher confidence in its heritability of 0.794. In the FGR population, the trend in the heritability was similar.

4.3.1 Mode of inheritance

In contrast to Todd's earlier study wherein an autosomal dominant mode of inheritance for catnip response was reported [4], neither of the populations examined in this study exhibited a clear Mendelian mode of inheritance for response. However, the heritability and simple segregation analysis support clear genetic effects that could be examined in further detail using genome wide association to search for loci involved.

Conclusions

Overall, these populations yielded a variable but high degree of heritability for the catnip response. The most heritable and repeatable metric for classification of cats as responders for the purpose of further genomic study of the trait appears to be exhibition of both the cheek rub and head over roll in the same observation with dried catnip. To find genes that play a role in response behaviors, it is important to reduce environmental variability as much as possible and to preferentially select cats exhibiting a qualitatively strong response with both of these components.

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APPENDIX

Figure 3: Catnip used in both trials

Table 7: FNPCC observation results

Cat ID	*Sex*	*Trials*	*Trial 1*	*Trial 2*	*Trial 3*
101335	F	1	NR		
101338	F	1	NR		
102058	F	1	NR		
102101	F	1	NR		
102194	F	1	NR		
102213	F	1	NR		
102231	F	1	NR		
102254	F	1	NR		
103022	F	1	NR		
103036	F	1	NR		
103061	F	1	NR		
103077	F	1	NR		
104331	F	1	NR		
105073	F	1	NR		
105184	F	1	NR		
105187	F	1	NR		
105209	F	1	NR		
105226	F	1	NR		
105342	F	1	NR		
107014	F	1	NR		
107158	F	1	NR		
107213	F	1	NR		
107235	F	1	NR		
107296	F	1	NR		
107300	F	1	NR		
108047	F	1	NR		
108048	F	1	NR		
108072	F	1	NR		
108076	F	1	NR		
108083	F	1	NR		
108084	F	1	NR		
108097	F	1	NR		
108098	F	1	NR		
108099	F	1	NR		
108101	F	1	NR		
108111	F	1	NR		
108137	F	1	NR		
108145	F	1	NR		
108155	F	1	NR		
108159	F	1	NR		
108161	F	1	NR		
108164	F	1	NR		
108169	F	1	NR		
109041	F	1	NR		

Cat ID	*Sex*	*Trials*	*Trial 1*	*Trial 2*	*Trial 3*
103064	M	1	NR		
103121	M	1	NR		
104228	M	1	NR		
106113	M	1	NR		
106263	M	1	NR		
106382	M	1	NR		
107100	M	1	NR		
108010	M	1	NR		
108022	M	1	NR		
108030	M	1	NR		
108080	M	1	NR		
106188	F	1	CR		
107212	F	1	CR		
108136	F	1	CR		
105377	M	1	CR		
106963	M	1	CR		
108018	M	1	CR		
104067	F	1	RL		
105197	F	1	RL		
106253	F	1	RL		
108074	F	1	RL		
108158	F	1	RL		
108031	M	1	RL		
100399	F	1	BOTH		
102060	F	1	BOTH		
102169	F	1	BOTH		
103080	F	1	BOTH		
103224	F	1	BOTH		
104131	F	1	BOTH		
104168	F	1	BOTH		
104227	F	1	BOTH		
104391	F	1	BOTH		
105173	F	1	BOTH		
105236	F	1	BOTH		
105299	F	1	BOTH		
105304	F	1	BOTH		
105340	F	1	BOTH		
106197	F	1	BOTH		
106321	F	1	BOTH		
106455	F	1	BOTH		
106555	F	1	BOTH		
107017	F	1	BOTH		
107030	F	1	BOTH		
107140	F	1	BOTH		
108041	F	1	BOTH		
108057	F	1	BOTH		
108102	F	1	BOTH		
108103	F	1	BOTH		

Cat ID	*Sex*	*Trials*	*Trial 1*	*Trial 2*	*Trial 3*
108157	F	1	BOTH		
108160	F	1	BOTH		
108163	F	1	BOTH		
108166	F	1	BOTH		
102048	M	1	BOTH		
106467	M	1	BOTH		
107275	M	1	BOTH		
108001	M	1	BOTH		
108015	M	1	BOTH		
108077	M	1	BOTH		
108147	M	1	BOTH		
100413	F	2	NR	NR	
101047	F	2	NR	NR	
101307	M	2	NR	NR	
102229	F	2	NR	NR	
102230	F	2	NR	NR	
102557	F	2	NR	NR	
103060	M	2	NR	NR	
103062	F	2	NR	NR	
103063	F	2	NR	NR	
103122	M	2	NR	NR	
104045	F	2	NR	NR	
104373	F	2	NR	NR	
104374	F	2	NR	NR	
104380	F	2	NR	NR	
105199	F	2	NR	NR	
105296	F	2	NR	NR	
106306	F	2	NR	NR	
106313	F	2	NR	NR	
106323	F	2	NR	NR	
106427	F	2	NR	NR	
107002	F	2	NR	NR	
107018	F	2	NR	NR	
107031	F	2	NR	NR	
107157	F	2	NR	NR	
107164	F	2	NR	NR	
107306	F	2	NR	NR	
107350	F	2	NR	NR	
107353	F	2	NR	NR	
103469	F	2	NR	CR	
104100	F	2	NR	CR	
104109	F	2	NR	CR	
99294	M	2	NR	RL	
102050	F	2	NR	BOTH	
104160	F	2	NR	BOTH	
105211	F	2	NR	BOTH	
105245	M	2	NR	BOTH	
105271	F	2	NR	BOTH	

Cat ID	*Sex*	*Trials*	*Trial 1*	*Trial 2*	*Trial 3*
105338	F	2	NR	BOTH	
105339	F	2	NR	BOTH	
106863	F	2	NR	BOTH	
107199	F	2	NR	BOTH	
107299	F	2	CR	NR	
104156	F	2	CR	CR	
104226	F	2	CR	CR	
104400	F	2	CR	CR	
101266	F	2	CR	BOTH	
102049	F	2	CR	BOTH	
102133	F	2	CR	BOTH	
104037	F	2	CR	BOTH	
104332	F	2	CR	BOTH	
107120	F	2	CR	BOTH	
108034	F	2	CR	BOTH	
105188	F	2	RL	BOTH	
105294	F	2	BOTH	NR	
107348	M	2	BOTH	NR	
106557	F	2	BOTH	RL	
101551	M	2	BOTH	BOTH	
104172	F	2	BOTH	BOTH	
104389	F	2	BOTH	BOTH	
106567	F	2	BOTH	BOTH	
107023	F	2	BOTH	BOTH	
107027	F	2	BOTH	BOTH	
107132	F	2	BOTH	BOTH	
108007	M	2	BOTH	BOTH	
364	M	3	NR	NR	NR
102244	F	3	NR	NR	NR
106582	F	3	NR	NR	NR
107265	F	3	NR	NR	NR
107290	F	3	NR	NR	NR
106238	F	3	NR	BOTH	BOTH
105350	F	3	CR	NR	NR
104324	F	3	RL	BOTH	BOTH
108029	M	3	BOTH	CR	BOTH
104035	F	3	BOTH	BOTH	BOTH
105056	M	3	BOTH	BOTH	BOTH
105202	F	3	BOTH	BOTH	BOTH
108011	F	3	BOTH	BOTH	BOTH

NR = no response; CR = cheek rub; RL = head over roll; BOTH = both in a single observation

Files on central laboratory server:

FNPCCpedigree.xlsx: Pedigree information for the FNPCC containing 3876 cats dating from 1984-2010.

FNPCCobservations.xlsx: Full data recorded from observations taken at the FNPCC.

FGRpedigree.xlsx: Pedigree information for the FGR containing 178 cats dating from 2000-2010.

FGRobservations.xlsx: Full data recorded from observations taken at the FGR.

MTGS82_xx: Modeling information from MTGSAM.

MTGS83_xx: Log information from Gibbs sample analysis obtained from MTGSAM.