

UNIVERSIDADE DE ÉVORA

ESCOLA DE CIÊNCIA E TECNOLOGIA

DEPARTAMENTO DE MEDICINA VETERINÁRIA

Evaluation of the relation between tameness and coat color in cats

Avaliação da relação entre a cor e o temperamento em gatos

Maria Teresa Leça Pereira Umbelino

Orientação:

Doutor Alfredo Manuel Franco Pereira

Doutor Gonçalo da Graça Pereira

Mestrado Integrado em Medicina Veterinária

Dissertação

Évora, 2014



UNIVERSIDADE DE ÉVORA

ESCOLA DE CIÊNCIA E TECNOLOGIA

DEPARTAMENTO DE MEDICINA VETERINÁRIA

Evaluation of the relation between tameness and coat color in cats

Avaliação da relação entre a cor e o temperamento em gatos

Maria Teresa Leça Pereira Umbelino

Orientação:

Doutor Alfredo Manuel Franco Pereira

Doutor Gonçalo da Graça Pereira

Mestrado Integrado em Medicina Veterinária

Dissertação

Évora, 2014

Dedication

To my sister-in-law Inês, simply because her strength and positive energy make this world a better place.

Acknowledgments

There are a few people that were essential to me over the last few years and that helped making this dream come true. No words can truly express how much they mean to me, but I could not write this thesis without acknowledging them.

My mother, who has always known me better than myself, and always encourages me to become the best possible version of myself.

My father, siblings and family, who always supported and helped me fight for my dreams, in more ways than anyone can possibly imagine. If I am here today it is thanks to them.

Doutor Gonçalo da Graça Pereira, who took me under his wing and went above and beyond to make my dream of learning more about animal behavior come true. His help and support were so much more than what I could hope for.

Theresa DePorter, DVM, MRCVS, dip. ECAWBM, dipl. ACVB Veterinary Behaviorist and Melissa Spooner, LVT, VTS (Behavior), BS, KPA-CTP, Behavior Technician who taught me so much during my stay in Michigan. Their effort to teach and support me was very clear, and I am forever thankful for the change I had to learn from them.

Ann Mattise, DVM, and her family who became my own family while I was in Michigan. Their warmth and support were my home away from home.

Dr. Abel Almeida and the entire Centro Veterinário de Sintra team, who made me feel a part of the team as well and made sure I was fully prepared for my USA adventure regarding general small animal practice. It was an honor to be a part of the team for a few months.

Doutor Alfredo Pereira, who kindly accepted to support and help me in this project, making sure everything was in order every step of the way, regardless of his very busy schedule.

Doutor Paulo Infante, for his precious help in analyzing and interpreting the statistical data.

Kevin Lossner, who has not only been a great personal friend but also took the task of revising my thesis as a native speaker, making sure the final version had the best possible English.

My dear friends Ana Bota, Francisco Brandão, Francisco Silva, João Camacho, João Fragoso and Liliana Silva who have always supported me in every single moment and with whom I can always count on, regardless of how far away they may be whenever I need them.

Doutora Elisa Bettencourt and Dr. Nuno Alexandre, for all their help and support in the arrangements for my externships.

And finally, to Figo, Ginja and Marujo, my dear pets who are no longer with me but who helped me find that Veterinary was my true passion. To Romã and Salsa, for brightening up my days every day. And to Pitanga, who helped me realize how wonderful the world of animal behavior is. I simply can't imagine a life where I would not have met any of them.

Abstract

Evaluation of the relation between tameness and coat color in cats

This study evaluated the relation between coat color and tameness in domestic cats.

To evaluate tameness, we used an existing tool, the Cat Stress Score (CSS) and created a new tool, the Oakland Approachability Scale for Cats (OASC) that gathered information regarding the motivation to interact with the observer. Coat color and patterns were also observed and

registered.

We could not find statistical evidences to support that there is a relation between tameness and

coat color.

However, we found that the initial position in cage, response to the observer's hand approach and attempting to stroke accurately determine how tame a cat is. Future studies may use only these variables in order to evaluate a larger number of cats in the same amount of time. This new assessment tool created may also allow shelters to further their evaluation of each cat and

better determine the type of care they need.

Key words: cat, tameness, coat, piebald markings

IV

Resumo

Avaliação da relação entre a cor e o temperamento em gatos

Este estudo avaliou a relação entre a cor da pelagem e a mansidão em gatos domésticos.

Para avaliar a mansidão, utilizou-se uma ferramenta já existente, a *Cat Stress Score* (CSS), e criou-se uma nova ferramenta, *a Oakland Approachability Scale for Cats* (OASC). As cores e padrões da pelagem fora igualmente avaliados e registados.

Não se encontraram resultados estatisticamente significativos que demonstrassem a relação entre a cor da pelagem e a mansidão.

Contudo, determinou-se que a posição inicial na jaula, a resposta dada pelo gato à aproximação da mão e a sua resposta à tentativa de carícia determinam o nível de mansidão. Trabalhos futuros poderão utilizar apenas estas variáveis, permitindo a avaliação de um maior número de gatos no mesmo tempo. A nova ferramenta de avaliação criada poderá também permitir a gatis uma avaliação mais profunda de cada gato e assim determinar melhor o tipo de cuidados que necessitam diariamente.

Palavras chave: gato, mansidão, pelagem, malhas brancas

V

Index

Dedication	I
Acknowledgments	II
Abstract	IV
Resumo	V
Index	
Figures index	VII
Abbreviations index	
Objective	
Literature review	
The role of tameness in domestication	2 4 7
Considerations prior to the experimental work	20 23
Material and Methods Time spent evaluating each cat Selection of cats to include in the study Cat evaluations Statistical analysis	25 25 26
Results	30
Discussion	35
Conclusions	37
Bibliography	
Appendix 1: Assessment tools used to evaluate the cats	i vi vi vii

Figures index

Figure 1 – Hand reared lamb in a home environment (2013)	3
Figure 2 – Piebald markings in a horse, dog, cat and cow (2014)	6
Figure 3 – Cat learning to wear a harness (Torres, 2013)	7
Figure 4 – Five week old puppy and kitten socializing (2009)	9
Figure 5 – Feline agonistic behavior (adapted from Overall, 2013)	12
Figure 6 – Social play postures in kittens (adapted from Beaver, 2003)	14
Figure 7 – Solid colors in cats (adapted from Medlej, 2012)	17
Figure 8 – Tabby patterns in cats (adapted from Medlej, 2012)	18
Figure 9 – Piebald patterns in cats (adapted from Medlej, 2012)	19
Figure 10 – Cat wearing a collar at KCAS (2014)	28
Figure 11 – Example of a shelter chart (2014)	28
Figure 12 – Observer performing CSS (DePorter, 2014)	28
Figure 13 – Cat that scored six on the CSS with dilated pupils (2014)	28
Figure 14 - Gender distribution of evaluated cats	30
Figure 15 – CSS distribution	31
Figure 16 – Base color distribution	32
Figure 17 – Distribution of white markings	33

Abbreviations index

CSS - Cat-Stress-Score

CS - Color Scale

GABA - Gamma-AminoButyric Acid

HAT - Human-Approach-Test

HPA - Hypothalamic-Pituitary-Adrenal

KCAS - Kent County Animal Shelter

MMYM - Modified Meet Your Match

OASC - Oakland Approachability Scale for Cats

OCAS - Oakland County Animal Shelter

OS - Observer Score

Objective

Darwin (1875, cited in Trut, 1999) noticed that there are common features shared by distinct domestic species, that are less common in the wild. Later, Belyaev (1969, cited in Trut, 1999) also noticed this changes while he was studying the domestication process in foxes.

It is now known that the process of domestication implies adaptation to human handling and the environment we provide. Even slight changes in the genes that control hormones and neurotransmitters can lead to major differences in behavior as well as the phenotypic changes first observed by Darwin (Kukekova et al., 2012).

Even though these changes have been studied in several species, cats have not been contemplated in these studies. Considering that piebald markings have been associated with domestication on other species (Gulevich et al., 2010) and therefore associated with tameness, our hypothesis was that tamer cats would be more likely to show white markings.

Therefore, the main purpose of this study was to determine whether white markings on any part of the body have an influence or not in tameness in cats. We were particularly interested in assessing if white markings on the cat's face would influence their tame behavior. Relation between coat color and tame behavior was also investigated.

Since we could not find any assessment tool that would evaluate tameness in cats, we also determined that a new tool had to be created for the purpose of our study.

Our study also permitted to assess other relations, particularly between age, gender and number of days at the shelter with tame behavior.

Literature review

The role of tameness in domestication

Domestication can be defined as the process by which captive animals adapt to Man and the environment he provides. This means that domestication implies change; therefore the phenotype of the domesticated animal will differ from the phenotype of its wild counterparts. This adaptation to Man provided environments is achieved both by genetic changes over many generations and experiences during an animal's lifetime. Therefore, domestication can be viewed as both an evolutionary process and a developmental phenomenon (Price, 2002). This means that domestication consists of a permanent genetic modification that leads to a heritable predisposition toward human associations and thus we can define a domestic animal as one whose mate choice is influenced by humans and whose tolerance of humans and tameness is genetically determined (Driscoll et al., 2009).

The interaction of captive animals with humans is a major element in the process of domestication. The degree of tameness of individual animals to a great extent determines the nature of that interaction (Price, 2002).

In fact, domestication started with the selection of this key factor (Trut, 1999). It was crucial to select animals with reduced aggressiveness and fewer escape attempts, as well as reduced fear of humans (Albert et al., 2008; Cieslak et al., 2011).

Taming can be defined as animals losing their fear of humans and following human cues (Cieslak et al., 2011). The taming process is a learning experience that occurs during the lifetime of an individual, in which an animal's avoidance of people is reduced and willingness to approach people is increased. Therefore, tameness is a measure of the extent to which an individual is reluctant to avoid or motivated to approach (Price, 2002).

Studies in foxes and rats have shown how it is possible to select tameness in a few generations (Cieslak et al., 2011), using only genetic selection (Trut, 1999). When comparing tame and aggressive lines in this experiments, it was noted that the two lines had different reactions to humans, even when a litter was raised by a mother belonging to the other group, showing that tame behaviors have at least some level of genetic influence (Albert et al., 2008). The fox studies showed that about 35% of the variations in the foxes' defense response to the observer are genetically determined (Trut, 1999). The degree of tameness could be first noted before the pups were less than a month old (Price, 2002).

In a series of experiments designed to determine how tameness and aggression are genetically influenced in rats, a cross-fostering experiment was done. Two-day old pups were separated from their mother and exchanged by placing them into the cage of a mother from the other strain. Patterns of behavior in cross-fostered animals were almost identical to those of the lines from which derived, being radically different from the behavior of their foster mothers. It was concluded that cross-fostering had no influence on tame behavior, further showing the genetic influence in behavior (Albert et al., 2008).

Another study that used dairy goat twin kids further demonstrated the role of genetics in tameness. One individual of each set of twins was dam-reared, while the co-twin was hand-reared. Among their other findings, it was shown that the tamest hand-reared kids had co-twins that ranked most tame within the dam-reared group. It also became clear that despite this genetic tendency toward tameness, habituation also plays an important role in tameness, as over time the dam-reared group behavior got closer and closer to the one shown by the hand-reared group (Lyons et al., 1988).

Behavioral changes associated with domestication of mammals show how genes influence behavior, as domesticated species behavior differs dramatically from the behavior of their wild counterparts. Domestication clearly represents an evolutionary process involving the genotypic adaptation of mammals to the captive environment (Kukekova et al., 2012).

Although tameness clearly has genetic components, in some species it also derives heavily from behavioral experience. Young animals may learn tameness by modeling from parents or other tamed conspecifics to a certain degree, but in many species, a period of neonatal handling can exert a stronger influence on the development of tameness than modeling from conspecifics (Aengus and Millam, 1999). Appropriate neonatal handling may produce tameness, alter the stress response and improve immune competence in rats and chickens. It also decreased resistance to later handling in young parrots (Spier et al., 2004). In lambs, early handling and artificial feeding (Fig. 1) have a persistent impact of the stockperson-lamb interactions following the initial training period for artificial feeding on the subsequent lamb responses to their familiar stockperson (Boivin et al., 2000). In young foals, short sessions of neonatal handling reduced the prey response (Spier et al., 2004).



Fig. 1 - Hand reared lamb in a home environment

Genetics and domestication

For many years, scientists have studied animal domestication, and how domestication causes changes in every species behavior, morphology and even physiology (Trut, 1999).

Belyaev (1969, cited in Trut et al., 2009) believed that the genes that control behavioral variation play a key role in regulating development, leading to the variations that occur during domestication. One of the most interesting aspects of tameness is its close association with brain biochemistry (Price, 2002).

Although natural selection plays a considerable role in the evolution of many traits during the animal domestication process, human desire for particular secondary sexual characters will have a greatest influence in the development of a domestic species. Artificial selection is a conscious process generally considered to be performed only by humans (Driscoll et al., 2009). Clearly domestication involves the complex interplay of both random and intentional genetic mechanisms. It's extremely difficult to separate the effect each component may have, even in a laboratory (Price, 2002).

Darwin (1875, cited in Trut et al., 2009) realized certain features are shared by many domestic species as a result of their domestication. These conclusions remain true in our day. When subjected to domestication, different species evolved in the same direction (Trut et al., 2009). Domestication shows us how genomic variation contributes to complex differences in phenotypes, both in morphology and behavior. The differences between behavior in domestic species and their wild counterparts are among the strongest evidence of how genes influence behavior (Kukekova et al., 2012).

Common physical and physiological recurrences among domesticated mammals include: dwarfs and giants, piebald markings, wavy or curly hair, fewer vertebrae, shorter tails, rolled tails, and floppy ears as well as other manifestations of neoteny (Driscoll et al., 2009).

Neoteny is the retention of juvenile characteristics into adulthood. It can affect the whole developmental process or be restricted to certain developmental events. Neoteny is believed to have a link with successful domestication as traditional animal management practices allow for the retention of juvenile social behaviors. Environment may also play a role in behavioral neoteny, as juvenile behaviors are usually reinforced and adult-like agonistic behaviors are discouraged (Price, 2002).

Behaviorally, domestication did not alter a single trait but a variety of traits, including elements affecting mood, emotion, agnostic and affiliative behavior and social communication (Driscoll et al., 2009).

All domestic animals lost the wild type response to humans and their reproductive activity was enhanced. In many domesticates, we also have a similarity in morphological changes, such as body size and proportion or coat characteristics. Some of these attributes, like white markings, floppy ears and curly tails have been called morphological markers of domestication. These common factors suggest that there may be some key genes with many regulatory functions that may be targeted by selection under certain recurring conditions (Trut et al., 2009).

The appreciable metabolic and morphological changes that often accompany behavioral adaptation to the human environment usually lead to a significant dependence on humans for food and shelter (Driscoll et al., 2009).

One of the important aspects in the process of taming animals is the reduction of the stress response when it concerns human handling, which improves the tamed animal's welfare (Aengus and Millam, 1999). Chronic or very high elevations of stress responses have both short and long term effects on brain functions, leading to poor learning abilities, selective or disrupted memory retrieval and consequent inappropriate behavior with adverse welfare consequences (Mendl et al., 2001).

A fine balance between neurotransmitters and hormones regulates behavioral responses. Genes controlling that balance occupy a high level in the hierarchical system of the genome. This means that slight changes in those regulatory genes can give rise to a wide network of changes in the developmental processes they govern and therefore selecting for behavior may lead to other alterations in the animals' development (Trut, 1999).

Ku and Sachser (1999) comparison of guinea pigs and wild cavies showed that the organisms stress axes had increased reactivity towards their physical environment in the wild cavies. There was also reduced activity of the Sympathetic-adrenomedullary system in the guinea pigs, which could be explained by a physiological response to the reduced alertness, nervousness and sensitivity of the domestic animals. Epinephrine and norepinephrine serum levels were also increased in the wild cavies after a blood draw, indicating that human handling is more stressful to them. These differences show that the wild cavies have a higher reactivity in response to changes in their environment as well as to manipulation.

In rats, it was found that the activity of the Hypothalamic-Pituitary-Adrenal (HPA) axis decreased in tame rats, who also had lower levels of Gamma-AminoButyric Acid (GABA) and Serotonin and their adrenal glands were smaller than the aggressive lines, who in their turn had higher adrenal glands and lower levels of Taurine (Albert et al., 2008). It was also found that timid goats responded with higher levels of serum corticosteroids when approached by humans (Lyons et al., 1988).

After 12 generations of selective breeding, the basal levels of corticosteroids released by the adrenal cortex in the domesticated foxes had dropped to more than half the level of the control group. This level continued to drop as the experiment continued. The adrenal cortex in the tame foxes also showed a lower response when the foxes were subjected to emotional stress. These foxes even suffered changes in their serotonin system, thought to be the leading mediator inhibiting aggressive behavior. Compared with a control group, the brains of the domesticated foxes contained higher levels of serotonin and of tryptophan hydroxylase, the key enzyme of serotonin synthesis (Trut, 1999).

Genetics, coat changes and tameness

Darwin (1875, cited in Trut et al., 2009) noticed that there were similarities between the changes observed in different domestic animals. The author suggested that certain features are shared by many domesticated species as a result of their domestication.

This means that while selecting tame animals, other characteristics are also selected, leading to morphological and physiological changes, suggesting that tameness is linked to morphological characteristics, and that those relationships can be studied and identified (Trut, 1999).

One of the changes observed was the color pattern in animals' coats. Unlike their wild ancestors, domesticated species are commonly characterized by a great diversity of coat-color-associated genes. This selection for coat-color phenotypes started at the beginning of domestication (Cieslak et al., 2011).

One other curious aspect is that although white markings exist in non-domesticated animal

populations, the frequency of this trait is significantly higher in domestic animals (Fig. 2) (Gulevich et al., 2010).

The appearance of these new colors may be attributable to









Fig. 2 - Piebald markings in a horse, dog, cat and cow

changes in the timing of embryonic development, such as migration rate of the melanoblasts. Melanoblasts are the embryonic precursors of the pigment cells, the melanocytes, which give color to the animal's coat (Price, 2002). Melanocytes produce two types of pigments, black eumelanin and red phaeomelanin, which differ in amino acid content, solubility and structure. These pigments are produced in specific organelles called melanossomes. Once the melanossomes mature they are transferred to surrounding keratinocyte cells that produce the hair and skin (Kaelin and Barsh, 2013).

The process of pigment cell development is also crucial for the determination of mammalian coat coloration. Melanoblasts migrate over long distances from the neural crest to their final destinations. Melanoblast specification migration and melanocyte differentiation depend on a cascade of molecular signal pathways and transcription factors. The migrating melanoblasts have to reach the skin during certain developmental time windows. If they fail to do so, there will be areas lacking pigment cells, resulting in white patches. If the melanogenesis is impaired, there will be a complete lack of pigment, a phenotype called albinism (Cieslak et al., 2011).

In the farm fox experiment, the changes in standard coat color pattern appeared earlier than other changes, namely in the eight to tenth generations selected. The piebald star and brown mottling on the background of standard silver-black color are the most typical patterns (Trut et al., 2009). The piebald markings are among the most striking mutations in domestic animals. This pattern is found in dogs, horses, pigs, cows and foxes (Trut, 1999).

Gulevich et al. (2010) studied Norway rats and also showed that the white spotting of the offspring depended on the parents' behavior – tamer parents had descendants with white markings more often than more aggressive parents.

Domestication process in cats

The domestication process in cats occurred later than other animals and trough a different process (Driscoll et al., 2009). Cats were first brought into the home for religious reasons instead of utilitarian ones, like the rest of the domesticated species (Beaver, 2003).

In fact, cats are territorial and generally only well adapted to life in large social groups when food, shelter and opportunity for isolation are available (Price, 2002). Historically, it was also

common to argue that cats do not perform tasks and that even their utility as mousers is debatable (Driscoll et al., 2009). However, we now know that cats are capable of several types of learning. The major forms that we use to teach cats new behaviors are classical conditioning, operant conditioning, and social learning. If cats can learn new behaviors, they can be taught to perform tasks on cue (Case, 2010). It has also been considered that training cats in shelters may reduce their stress and increase their chances of being adopted (Hoff, 2009).



Fig. 3 – Cat learning to wear a harness, Torres, 2013

Cats are still relatively aloof in the company of people. The breeding of domestic cats has not been subjected to the same kind of control that other domestic species have and their freedom of movement has not been restricted (Price, 2002). Some even argue that cats are not fully domesticated, as they can fully revert to self-sufficiency (Beaver, 2003). The changes seen in cat breeds and their development in the past 50 years as well as changes observed in coats, ear shapes, social preferences and behaviors further support that the cat domestication is still actively ongoing in the present (Overall, 2013).

Unlike other animals whose domestication was influenced by artificial selection, cats were a product of mere natural selection. It seems likely that habitat choice of wild cats better fit for urban life was the mean of selection of behavioral genes affecting domestication (Driscoll et al., 2009). Therefore, cats have developed a rather unique commensal relationship with humans (Price, 2002). This symbiotic relationship did not require humans to modify or expand innate feline behavior, and that is clearly visible in the behavior patterns of cats in our day (Overall, 2013).

At its most basic, domestication is a dependence on humans for food, shelter, and control of breeding. Of the domestic cats living today, 97% or more are random-bred house cats, or are feral and intact. This means that the great majority of domestic cats choose their own mates. Most feral cats are also capable of finding their own food without human assistance.

On the other hand, domestic cats are polyestrous and have a wider variety of coat colors and patterns than those of the wildcat. They have also become social under domestication. And most importantly, cats have an overwhelming tolerance of people.

Considering all these factors, it can be argued that cat domestication is under 200 years old and may be incomplete (Driscoll et al., 2009).

Feline behavior

Development and socialization

There are several factors that can influence the timetable for individual development, such as genetics, maternal and environmental factors or sexual differences (Landsberg et al., 2013).

Kittens are usually born after a 63-day gestation. The condition of the queen during pregnancy is crucial to the kittens' development (Crowell-Davis, 2006). Restricted diets during gestation and lactation may lead to brain development deficits, developmental delays and reduced social attachment on kittens. Kittens may show decreased learning abilities and fearful or aggressive

behavior towards other cats. These effects may, in some cases be permanent (Vilanova, 2002). Maternal malnutrition may also lead to abnormalities in behavior and motor development (Landsberg et al., 2013). It has also been demonstrated that stress during gestation increases death of newborns. Additionally, when adults, the offspring of stressed mothers exhibited learning and memory impairments in a delayed alternation task as well as in passive avoidance conditioning (Lordi et al., 1997).

During the neonatal period, kittens are mainly guided by tactile, thermal and olfactory stimuli. Olfaction is present at birth and is fully mature by three weeks. By the fifth day the kittens can hear, and start orienting by sound by the seventh day (Landsberg et al., 2013). Kittens' eyes open at around nine days of age, but visual orientation and obstacle avoidance are not developed until four or five weeks and visual acuity is not usually achieved before three or four months of age. Hearing develops similarly to vision, with the hearing canal opening at around nine days of age. By their second week kittens are able to locate sound stimuli and by the third of fourth week they are able to recognize people and cats by sound (Vilanova, 2002). Walking does not begin until three weeks of age, at the same time kittens begin to regulate their body temperatures (Landsberg et al., 2013). At six or seven weeks kittens are able to move around in a manner similar to adults, but more complex aspects of motor function are not fully developed until their tenth or eleventh week (Vilanova, 2002).

Early handling by humans improves the social relationship between kittens and humans, but also leads to faster physical and central nervous system development. If kittens are calmly and softly handled from birth to their 45th day for five minutes they will be less fearful than non-handled kittens (Landsberg et al., 2013). Studies indicate that the most receptive time for human socialization is up to 7 weeks of age. The more opportunities a kitten has to have pleasant human handling, the friendlier it is likely to be toward people in the future. Kittens may also be influenced by the queen's behavior: if the queen is shy, reserved or fearful while socialization takes place, her offspring may learn these behaviors. Ideally, the mother should at least not be overly fearful of humans (Landsberg et al., 2013).

Like all social species, cats are born with the ability to learn social behavior if the proper social environment is provided. Social attachments are more easily formed during the socialization period (Crowell-Davis, 2007). Socializing kittens to other species may begin as early as two weeks of age and may only be extended up to seven weeks of age (Fig. 4) (Vilanova, 2002).

Fig. 4 – Five week old puppy and kitten socializing

Despite their socialization, adult cats will still show a great variability in their friendliness towards other animals, according to their personality types.

Factors that may influence personality types include genetics, early socialization and social or observational effects of mother and littermates (Landsberg et al., 2013).

At about two weeks, self-play starts to show as attempts to bat moving objects. This play progresses as the kittens' muscle coordination develops (Beaver, 2003). Playful social interactions usually begin when the kittens are four weeks old and are well developed by the time they reach seven weeks. Play, exploration of inanimate objects and locomotor play escalate at around seven or eight weeks and peak at 18 weeks, before declining (Landsberg et al., 2013). Despite this decrease, play will change but will never fully disappear as the cat gets older, if it has a play companion (Overall, 2013).

Predatory behavior may be affected by social or observational learning, weaning age, early socialization and maternal behavior, observing other cats, genetics and perhaps by competing with littermates (Landsberg et al., 2013). Kittens that are separated from their mother much earlier than normal will develop behavioral, emotional and physical abnormalities. Usually they become more fearful and aggressive, show great amounts of random locomotor activities and are less capable of learning. They are also slower at learning social skills, have more accidents during free play and show more aggressive social play (Turner and Bateson, 2014). However, even in the absence of maternal experience and learning, many cats still develop into competent hunters. The juvenile phase ends when the cat reaches sexual maturity, at which point the cat becomes increasingly independent (Landsberg et al., 2013).

Influence of genetics in temperament

Ideally, the sire should be outgoing and confident (Landsberg et al., 2013). Studies have shown that paternity has an adding effect to socialization: paternity will influence the tendency a kitten shows to approach and explore new stimuli, while socialization will have a more specific effect on how that cat interacts with people (Vilanova, 2002).

Crowell-Davis (2007) evaluated the response of a cat to a novel box showed that cats with friendly-fathers were quicker to approach, investigate and enter the box. This father-based response suggested boldness in approaching people or objects might be inherited (Crowell-Davis, 2007).

Social behavior

Historically, cats were considered to be solitary species. However, cats are in fact social animals. The core of the social group is a queen and her kittens. Food resources will determine if the family group will disperse or if they will develop a social organization. The formation of groups of related and familiar individuals around food resources is the first step in the development and organization of social behavior in the domestic cat. The smallest colonies consist of a queen and her kittens, while larger colonies are composed of several queens, often related, who cooperate in ways to facilitate the survival of their young (Crowell-Davis, 2007).

Individual members of a colony recognize each other and recognize strangers to their colony. Acceptance and integration of strangers is gradual and likely to be resisted. However, integration of kittens abandoned near a colony seems to be easier than the integration of an adult cat (Crowell-Davis, 2006). There may be a great disruption of the social order in the colony when a new cat joins the group (Crowell-Davis, 2007).

Studies show that cat are excellent observational learners, being able to master a task that does not involve skills their ancestor needed for survival simply by observing another cat performing that task. Instinctive imitation is important to mental development and self-preservation. It allows kittens to learn how to hunt rapidly by watching their mothers (Beaver, 2003). At first the queen brings dead prey to her kittens. After releasing live prey near her offspring, the queen will often demonstrate hunting techniques to them. Kittens then gradually practice under their mother's supervision. The relevance of the mother in social learning is further demonstrated by the fact that a calm and present mother will induce a faster socialization of the kittens with humans (Crowell-Davis, 2007).

Cats also show a high degree of trial-and-error learning, since search techniques in strange areas tend to be random, but each is only investigated once. Cats are also able to use transfer learning, where the animal uses information one problem to solve another. Motivational factors are an important part of learning and behavioral choice, with avoidance learning being widely used by this species (Beaver, 2003).

Communicative behavior

Vocal communications are used to transmit general messages and allow an individual to determine if there are any other cats nearby, allowing them to prevent direct confrontations

(Beaver, 2003). Vocalizations convey general information on four main contexts: social conflicts, sexual behavior, parental behavior and interactions with people (Landsberg et al., 2013).

There are at least 23 vocalization patterns in cats that can be divided into pure calls, which are homogeneous, and complex calls with major changes in frequency range, harmonic structure or pulse modulation (Beaver, 2003). From these 23 vocalizations, there are four that deserve a closer attention: meowing, purring, growling and hissing. Meowing appears in friendly interactions or in an attempt to gain attention. Meow can become a conditioned response very easily, if the cat realizes vocalizing is an effective way to obtain food, attention or access to a particular place (Landsberg et al., 2013). Purring is first observed in nursing kittens at around two days of age. As the kitten matures the purr will develop other meanings. A cat may purr in almost any situation, in experiences that are either pleasurable or distressing (Beaver, 2003). While purring in kittens may contribute to reinforce the mother-infant bond, adult purring is understood as an appeasing or attention-seeking signal (Landsberg et al., 2013). Growling is first produced by kittens when they are matured enough to escape with a piece of food. The queen uses it whenever she intends to warn her kittens to seek immediate shelter (Beaver, 2003). This is a high intensity, long duration and low frequency vocalization typically observed in aggressive interactions (Landsberg et al., 2013). Hissing is an involuntary reaction to surprise. The sound is produced as air is forced through a small oral opening while de cat is changing positions to view the approacher. Hissing is controlled by the amygdala and hypothalamus (Beaver, 2003). This autonomic defensive response is produced with the mouth opened and the teeth exposed (Landsberg et al., 2013).

Postural communication is also of major importance, as the cat uses various body postures as its primary methods of communication (Beaver, 2003). The size and shape of the body, position of the ears, size of pupils, size and position of the tail and visibility of weapons convey important messages to others (Fig. 5) (Landsberg et al., 2013).

Ears can be positioned in three different ways: an interested cat will have its ears forward, an unsure or bluffing cat will have them halfway and a frightened cat will have its ears flat and facing backward (Landsberg et al., 2013).

Eyes can also convey important messages: interested cats will look at the object of their interest, while cats that are avoiding altercations will avoid eye contact, by looking away or engaging in intensive washing. Friendly eye contact is

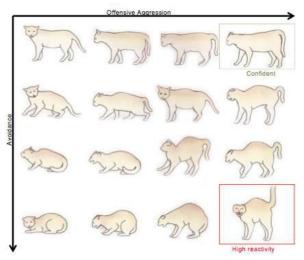


Fig. 5 - Feline agonistic behavior, adapted from Overall, 2013

soft and the cat may blink in an excessive way, while an aggressive stare is very intense (Landsberg et al., 2013).

Cat tails are very expressive and very rarely still. Vertically raised tails indicate amicable approach and familiar recognition (Landsberg et al., 2013). Tail wrapping around an individual is also an affiliative behavior, and is often transferred to other species. When the tail base is arched with the rest directed towards the floor it signals a confident cat, whereas a lowered tail will convey a message of uncertainty (Crowell-Davis, 2006). Flagging of the tail tip indicates a high level of arousal (Overall, 2013).

Body postures are also a good way to transmit important messages that can be read at a distance. An aggressive cat will have a straightforward posture, while a defensive cat will assume a lateral position, showing an arched back and piloerection. The appeasing posture consists of approaching another cat and rolling into the side or back (Landsberg et al., 2013).

Affiliative behaviors

Inside each colony, it is easy to identify preferred associates, which can be found together throughout the day in several locations and contexts. As cats become more familiar, they are more likely to display affiliative behaviors. Relatedness is also very important, as cat that are related to each other are even more likely to show affiliative behaviors than non-related cats of equal familiarity (Crowell-Davis, 2007). Preferred associates relations tend to persist in time, including when there are disruptions to the colony organization (Crowell-Davis, 2006).

There are several behaviors that cats display to demonstrate their affiliative relationships.

Nose touching is a greeting behavior, observed more often between preferred associates (Crowell-Davis, 2007). It likely serves as a way to exchange specific information, including specific odors from that individual and the colony (Crowell-Davis, 2006). Allogrooming refers to licking behavior directed at another cat. It typically occurs on the head or neck, when one cat licks another. The cat being groomed is usually very cooperative, and may even solicit the allogrooming by approaching another cat and lowering its head (Crowell-Davis, 2007). Allogrooming has been suggested as a behavior aimed to reinforce of social bonds (Landsberg et al., 2013), with a higher frequency between preferred associates (Crowell-Davis, 2006), but has also been seen in social conflicts, where the cat showing allogrooming may attack the other cat afterwards (Landsberg et al., 2013). Allorubbing occurs when two cats rub their heads, bodies and tails against each other, normally quite vigorously, often purring as they do so (Crowell-Davis, 2007). Allorubbing reinforces social bonds through the release of different neurotransmitters and neurohormones including dopamine, endorphins and oxytocin (Landsberg et al., 2013). It may also play a role in odor exchanging, facilitating the development

of a colony odor, shared by all the members, facilitating their identification as a part of their colony (Crowell-Davis, 2006). This is a key behavior that acts as a social cement (Overall, 2013). Resting together in close physical contact, even when there is enough space to spread out shows a close social bond between those cats (Crowell-Davis, 2007). It is understood as a sign of tolerance and indicates a good social relationship between the individuals that have expressed it (Landsberg et al., 2013).

Play is a well-known behavior of cats and is even displayed by adult feral cats living under poor nutritional conditions. There is a wide variety in how individual cats will display this behavior. This variation is probably determined by a combination of genetics, life experience and the timing of particular experiences with play (Crowell-Davis, 2007). Social play involves two or more cats. Initially, the various postures of social play are highly correlated with each other, but this interrelation is lost by the 12th week. "Belly up" is the first social play posture seen in kittens, followed by the "stand-up". The third type of social play being developed is the "side-step", followed by the "pounce" and "vertical stance". Between 38 and 41 days of age kittens begin to "chase" and about five days later, the "horizontal leap" appears. The final social play to develop

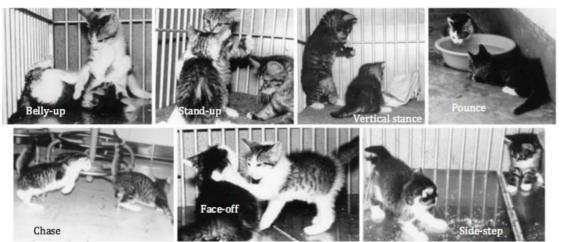


Fig. 6 - Social play postures in kittens, adapted from Beaver, 2003

is the "face-off", at around 48 days (Beaver, 2003). Fig. 6 illustrates the different postures of social play in kittens.

Several studies have been made on cat personalities, identifying tree major types: active/aggressive, timid/nervous and confident/easy-going (Overall, 2013; Bernstein, 2007). While some studies showed that proper handling by humans during early development help kittens to be friendlier towards humans, other studies have shown that some kittens are resistant to changing their original personality types despite the kind of human handling they may or may not have had (Bernstein, 2007). Cats socialized to people and those from friendly sires are not only friendlier to unfamiliar people, but they also show fewer signs of distress when approached and handled by strangers (Beaver, 2003).

Some of the affiliative behaviors are echoed in the social behavior towards humans. Allorubbing is transferred to humans by rubbing their legs, engaging in a species typical friendly greeting. A cat that has been well socialized to humans will have a greater tendency to rest in a human's lap, transferring to a human the act of resting in close physical contact. Greeting with the tail up and purring are other behaviors cats typically engage with humans (Bernstein, 2007). Tail wrapping around a human's leg is also very commonly seen as the cat is rubbing the leg, partially involving the leg with its tail (Crowell-Davis, 2006).

Aggression or fear will be displayed by the same body language, whether it is directed towards another cat or a human. Therefore, it is crucial to understand and recognize fear and aggression postures and avoid contact with cats displaying such behaviors, as that could result in an episode of aggression and contribute to perpetuating avoidance behaviors towards humans (Landsberg et al., 2013).

Colors in cats

If we consider all the possible variations of cat pelages, we have over 4000 possible colors. Describing each one would not be practical or useful and therefore this section is a summarized description of the solid colors and patterns, which in combination create the huge variety of pelages we see today.

Solid colors

Black occurs when the B gene forces the pigment producing cells at the root of the hairs to produce eumelanin. The rate of eumelanin production depends on temperature, with more intense black at lower temperatures. Chocolate results in the combination of the B gene with its recessive allele b, inducing a deformation of the pigment particles, which become longer and oval. The combination of the B gene with another allele, b^1 , results in an even paler color: cinnamon (Picardello, 1997). The allelic hierarchy $B > b > b^1$ corresponds to the dilution intensity, with darker alleles dominant to lighter alleles. The nature of the mutations and allelic relationships suggests that chocolate and cinnamon are partial and complete loss of function alleles, respectively (Kaelin and Barsh, 2013), causing a change in the shape of the eumelanin pigment granules to either an oval shape in chocolate phenotypes or a rod shape in cinnamon (Vella et al., 1999).

Blue is the result of action of the recessive maltese dilution gene *d*, which gives rise to a different distribution in the space of the pigment particles, causing lower color intensity. Lilac is the result of the action of the same gene on the chocolate coat color and fawn is the dilution of the cinnamon phenotype (Picardello, 1997). The maltese dilution locus produces a factor essential for even distribution of pigments throughout the hair. The recessive form of the gene causes pigment granules to enlarge and deposit unevenly in the hair shaft, causing the coat to appear diluted. The lighter shade visible to the human eye is caused by the increased amount of light that passes trough the hair (Vella et al., 1999).

The orange gene O transforms the black pigment, eumelanin, into different pigment particles, phaeomelanin, much more elongated (Picardello, 1997). This is accomplished by a biochemical diversion of those substances destined to become dark eumelanin into the alternate compound phaeomelanin, resulting in a lighter pigment granule with different optical properties, causing the red phenotype (Vella et al., 1999). The recessive gene o does not modify the eumelanistic phenotypes. The action of the maltese dilution gene will result in a cream phenotype (Picardello, 1997). The orange gene is sex-linked, and that means a male can either be O or o. Females however can also have the genotype Oo, which leads to some portions of the pelage being eumelanistic and some being phaeomelanistic, expressing a phenotype known as tortoiseshell or tortie (Kaelin and Barsh, 2013). As each of a female's cell must inactivate one X chromosome to avoid overproduction of factors, some cells will inactivate the O gene and other the o gene, resulting in the mosaic coat of orange and black (Vella et al., 1999).

There is a gene that modifies the maltese diluted colors, the *Dm* gene (Picardello, 1997). It is considered a modifier because it has no effect on dense colored animals. When it acts upon eumelanistic diluted color blue, it produces caramel; when it acts upon lilac, the phenotype is referred to as taupe (Vella et al., 1999). If the *Dm* gene acts upon phaeomelanistic colors red or cream it produces apricot. It is believed that the gene acts on both the shape and packaging of the pigment colors (Picardello, 1997).

White is transmitted due to the W gene, producing a complete depigmentation of the body. This results in an entirely white coat, pink nose and pink paw pads (Picardello, 1997). As the coat color is pure white, it is impossible to discover trough inspection which other genes are present in the genotype. In theory the white phenotype could also be due to an extreme piebald spotting, but this is highly unlikely to occur (Vella et al., 1999). The gene W may be epistatic over all genes. Determining whether dominant white is allelic or epistatic may be possible with additional genetic analysis (Kaelin and Barsh, 2013).

Another essential enzyme for pigment production is tyrosinase, produced at the albinism locus. The aromatic ring of the amino acid tyrosine gives eumelanin and phaeomelanin their color-producing properties and the enzyme is responsible for incorporating it in the pigments (Vella et

al., 1999). Allelic variation of the gene responsible for tyrosinase production gives rise to several phenotypes, such as acromegalism or albinism. The acromelanic phenotype is easily recognizable because pigment is usually restricted to the points here heat loss is greater: muzzle, ears, feet and tail – leading to the name colorpoint. Albinism leads to a complete depigmentation of coat and eyes, causing the coat to be white and eyes to be read. However true albinism is rare in cats, and the white phenotype is usually due to the *W* gene (Kaelin and Barsh, 2013).

The inhibitor of melanin gene *I* is responsible for the tipped colors in cats, where the coat is depigmented at the base and pigmented at the tips of each hair (Picardello, 1997). The extent of the dilution may depend on hair length and pigment type. The interaction of this gene with other color related genes or alleles gives rise to many of the phenotype-based nomenclature among cat fanciers and breeders (Kaelin and Barsh, 2013). Fig. 7 summarizes the solid colors described above.

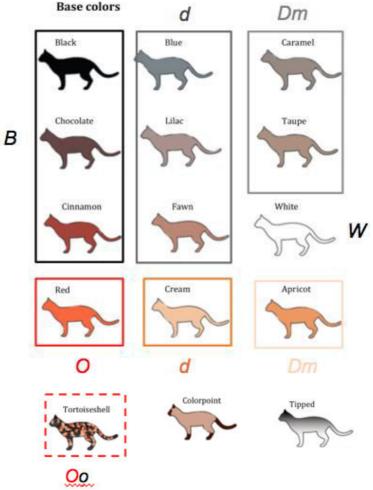


Fig. 7 - Solid colors in cats, adapted from Medlej, 2012

Tabby patterns

The agouti protein is responsible for a pattern that consists of black pigment against a yellowish ground color. As the hair grows eumelanin is deposited, but as the amount of agouti protein increases in the melanocyte, eumelanin production is inhibited, resulting in a shift to production of phaeomelanin that is then deposited into the hair, resulting in a hair that is black at the tip but yellow at the base. Cats have a second system of pigmentation that causes a marked reduction in the amount of the agouti protein receptors, or the agouti protein itself in certain areas of the skin, thus eliminating the agouti coloration on such areas and leading to the tabby patterns (Vella et al., 1999). There are four common and inheritable tabby patterns: mackerel, classic, spotted and ticked (Fig. 8) (Kaelin and Barsh, 2013).

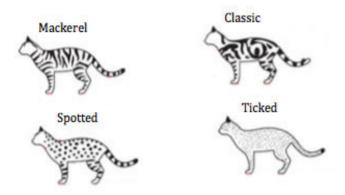


Fig. 8 - Tabby patterns in cats, adapted from Medlej, 2012

Mackerel cats have vertical, gently curving stripes on the side of the body. This phenotype is very common in mongrel populations (Vella et al., 1999).

In the classic pattern, the dark component is organized into whorls and spiral arrangements (Kaelin and Barsh, 2013). The blotched pattern is variable, but where the coalescence is extensive, a very dark tabby is produced (Vella et al., 1999).

Spotted cats have their dark components shaped into cheetah-like spots (Kaelin and Barsh, 2013).

The ticked phenotype has minimal tabby striping, leaving only the underlying agouti coloration-Little or no evidence of striping is observed in the body, but some may be observed in the face, legs and tail (Vella et al., 1999).

Piebald patterns

These patterns refer to white spotting in cats. White spotting is dominant over the absence of spotting and the degree of these markings is highly variable (Kaelin and Barsh, 2013).

Piebald markings may occur in conjunction with any color as an independent entity. Spotting may be limited to small marks on the chest or belly or can be at the other extreme, with the cat only showing small pigmented areas (Vella et al., 1999).

There are many piebald patterns, according to the amount of white that the cat shows. A detailed classification of white markings can be found on Fig. 9.

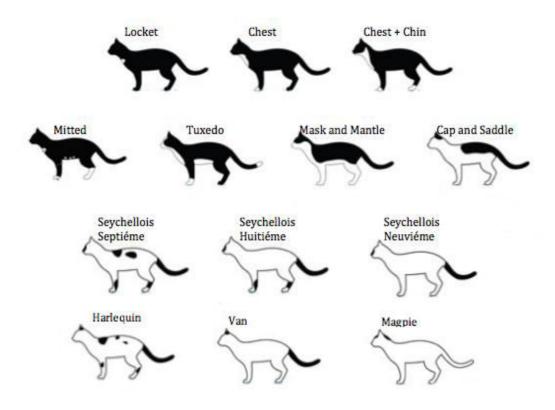


Fig. 9 - Piebald patterns in cats, adapted from Medlej, 2012

Considerations prior to the experimental work

Introduction to the evaluation methods

In order to evaluate tameness in cats, we decided to implement a two-part scale: Each cat should be evaluated by the Cat-Stress-Score (CSS) and the Oakland Approachability Scale for Cats (OASC). Finally, the coat phenotype of each cat would be registered in a Color Scale (CS) in order to assess any relations between coat phenotype and tameness.

The Cat-Stress-Score

Tamability determines how an animal can adapt to life among humans (Trut, 1999) and therefore, if an animal is tamer, it will have a lower stress level when being approached or manipulated by humans.

The CSS (appendix 3) was designed by Kessler & Turner (1997) to evaluate stress and adaptation of cats in boarding catteries. It provides a low cost, non invasive (Broadley et al., 2013) way to evaluate the stress level in cats.

Around 300 cats were observed in a pilot study, recording their behavior, to design the CSS. Then the researchers used the Cat-Assessment-Score (CAS) created by McCune (1994, cited in Kessler and Turner, 1997) adding active behavioral elements they observed and a "tense sleeping posture" (Kessler and Turner, 1999).

Eleven elements are observed using the CSS: activity, vocalization, head, eyes, pupils, ears, whiskers, body, belly, legs and tail. This provides us with a tool to assign a level of behavioral stress corresponding to a description of that attribute (Dybdall et al., 2007).

This assessment tool was found to have a 0.9 inter-rater reliability when used by trained observers; dropping to 0.75 when used by less formally trained observers (Kessler & Turner, 1997). Researchers commonly use it whenever they wish to evaluate stress levels in cats.

However, when using the CSS, all cats that appear to be sleeping or inactive may be scored low and be in fact stressed (Mccobb et al., 2005).

To avoid confusing a sleeping or feigned sleeping cat with a low stressed cat, we designed a second evaluation tool that would also provide better insight about how willing a cat is to approach and interact with humans, giving another insight on how tame that animal is.

In every animal, we observed each attribute and scored it, without engaging the cat and keeping a distance of approximately six feet. The CSS was performed along with the OASC.

Oakland Approachability Scale for Cats

This second scale was designed to assess how comfortable a cat was when the option of human interaction was available and if/how quickly they would engage.

If a cat was already showing high stress signals, engaging and manipulating it would only contribute to increase its discomfort and arousal.

Additionally, if a cat is already stressed and manipulation is forced, the risk for an aggressive event towards the observer is increased. If a cat is confined and can't escape from their threat, it will react aggressively when the critical distance between them and the threat is reached. The only way to alleviate this kind of stress in a shelter is by eliminating the source of fear, in this case, the observer (Beaver, 2003).

Since one of the principles of welfare is expressing appropriate behavior (Barnard et al., 2014) and stress does not contribute to this expression, we decided that if at any point a cat would prove to be highly stressed, we would not to go any further, remove the source of stress and respect their welfare. Therefore, while performing the OASC there were several key points where the test would be discontinued if the cat showed high levels of stress.

Kessler & Turner (1999) designed a Human-Approach-Test (HAT) to access socialization towards people in cats. This test consisted of greeting the cat, standing in front of the cage while touching the grating with one hand for one minute and finally opening the cage door for a few seconds before closing it again. Cats where scored on a six-point scale, from extremely friendly towards people to extremely unfriendly.

Moore & Bain (2013) combined the CSS with a Modified Meet Your Match (MMYM) while evaluating how quickly cats acclimated to a shelter. The MMYM was not performed if cats were visibly ill or if they showed aggression. While performing their tests, they used the observer's extended hand with a closed fist to interact with cats, as well as stroking and playing with toys. Taking these two tools into strong consideration and using them as our basis, we then elaborated a new and different scale we felt better suited our needs – the OASC (appendix 1).

This scale consisted of a two-part evaluation. The first portion was done with the cage door closed. After this closed-door evaluation, we would interrupt the OASC and perform the CSS. The CSS result would then determine if we should proceed to the second portion of the OASC evaluation that required an open cage door.

We decided to name the scale after the place where it was created, the purpose it serves and the species it should be used on.

The OASC also took into consideration the observers' opinion, and therefore we included in our scale a point that asked the observer to rate the cats' tameness according to his own personal opinion of the cat. This was named the Observers Score (OS).

Color scale

When we look at all possible coat phenotypes existent in cats, it becomes clear that it is hard to find a simple system that registers all options. Although we were specifically looking for presence or absence of piebald markings, we were concerned that scoring cats simply as piebald/not piebald would influence the opinion of observers and greatly limit our conclusions. For this reason, we designed a simplified model (appendix 4) of cat colors that included tabby patterns, piebald markings, base colors and other characteristics, such as being tortoiseshell, colorpoint or tipped.

Since describing all the different piebald and tabby patterns would be hard and susceptible to each observer's interpretations, we opted by clearly showing each pattern on a picture and then score each cat by comparison. Tabby patterns included mackerel, classic, spotted and ticked. Piebald markings included locket, chest, chest and chin, mitted, tuxedo, mask and mantle, cap and a saddle, seychellois septiéme, seychellois huitiéme, seychellois neuviéme, harlequin, van and magpie.

Base colors were also shown on picture to avoid differences between observers.

As we were also looking for information specifically on piebald faces, disregarding of what the pattern would be, we also included an item that simply asked if the cat had white markings on its face.

It is known that a complete white coat in cats can be caused by different genes, but resulting in the same phenotype (Kaelin and Barsh, 2013). However, white coats resulting from extreme white markings are less likely to occur (Picardello, 1997). Since there is no accurate way to distinguish the two genotypes based solely on phenotype, we previously decided that if a cat was completely white, it would not be considered as piebald.

Assessment tool used in the current study

Since we were looking for more than friendliness or acclimation to the shelter, the OASC was designed to assess friendliness, comfort and willingness to interact with the observer. The addition of the CSS, that was performed along with the OASC, allowed the observers to assess the stress level induced on each cat by the approach of a human to their cages. Registering colors allowed reliable correlation phenotypes with the scores obtained using the OASC.

To ensure that there was inter-observer validation, before the actual study was iniciated, several cats were evaluated twice by different observers and the scores obtained were compared. The results obtained between observers were similar, showing this tool is reliable even if used by different observers. Even though we verified the scale's reliability, the results for the actual study were collected by the same observer for all cats.

Inclusion/Exclusion factors

Age

Cats are born lacking sensor and motor responses. These responses are progressively developed on the first two months of their lives. As juveniles they also go through a socialization period and show a different range of behaviors than that of adult cats. The development of these responses, socialization and lack of sexual maturity will influence the cats' reaction when approached by humans. By the time they reach four months, kitten-like behavior will have disappeared, the socialization period is concluded and all adult responses, including sexual behaviors, are fully developed (Beaver, 2003).

To avoid the influence of juvenile behavior, a cat had to be older than 4 months in order to be included in our study.

Health considerations

When animals are in pain, they go through an unpleasant sensory and emotional experience. These unpleasant sensations will affect the animal's behavior: it may appear depressed and unresponsive to its surroundings (Barnard et al., 2014).

These changes in behavior would affect the cat's response to our tests, and therefore, in order to be included in our study, a cat could not be visibly sick or in pain. There were also concerns related to disease spreading across the shelter, and therefore cats that were visibly sick were excluded from our study.

Time spent by a cat in the shelter before evaluation

Several studies previously made, which scored cat stress levels in shelters, found that the first three days spent at a shelter are the ones cats feel more stress, which decreases after this first three days (Broadley et al., 2013; Dybdall et al., 2007). Therefore any cat in the shelter for less than three days was excluded from our study.

Housing

Cats' social behavior is characterized by avoidance of interactions (Beaver, 2003), meaning that the presence of another cat in the same housing would affect how each individual would interact with the observer. Therefore we decided that in order to be included in the study, cats had to be housed individually.

Disease spreading concerns

Some precautions had to be taken to avoid spreading diseases while performing our evaluations. The spread of disease is avoided by several measures, which include separate accommodations, strict movement control and hygiene procedures when in close contact with the cats. It is necessary to ensure contact between infectious agents and susceptible animals is reduced to a minimum (Möstl et al., 2013). The measures we took were avoiding contact with sick animals, avoiding moving cats from their cages and sanitizing the observer's hands after making physical contact with a cat during an observation or hourly if no contact was made with any cat during that time. By excluding sick cats, we hoped to limit our on exposure to pathogens that we could then spread to healthy cats. Keeping cats in their own cages avoided placing cats in an evaluation room that would have a high affluence of animals, increasing the risk for contamination. Sanitizing the observers' hands further reduced the risk of contamination and eliminated the odor from the previous cat and treats, which could influence the cat's behavior.

However, Neilson (2009) studied cats' reactions towards scented litter and showed that certain smells present in some detergents are aversive to cats, and that could lead to avoidance of the observers' hand. This study used a scent palette that included cedar, citrus, bleach, fish and floral scents, proved that cats had a preference for fish and bleach scents. Since cats show a preference for bleach rather than having an aversive reaction to it, this was the product we chose to use as sanitizer between observations.

Radford et al. (2009) studied Feline Calicivirus (FVC) infections stated that sodium hypochlorite (5% bleach) diluted at 1:32 is an effective disinfectant against FVC, so this was the concentration chosen for our study. We made 110.7 mL of a solution with the above-mentioned concentration by adding 2.42 mL of 8.25% bleach and 107.28 mL of water.

To make sure that the bleach smell did not cause any repulsion or strong attraction, we approached some cats and watched for their reactions to the smell of our sanitizer, in order to ensure we were not causing any bias. We concluded that the smell was not aversive but instead slightly appealing to most cats.

Material and Methods

Time spent evaluating each cat

While evaluating stress and socialization in cats, Kessler & Turner (1999) realized that some cats would act unfriendly at first, but as they got habituated to the evaluator became progressively friendlier. This suggests that the time spent by the observer close to the cat will influence its actions. Therefore, the duration that an observer spent evaluating each cat was predetermined. Time used in observations for the CSS varied from 30s (Moore and Bain, 2013), 2-3 minutes (Dybdall et al., 2007) to 5 minutes (Mccobb et al., 2005). After performing some trial evaluations using the CSS, it was determined that the time needed by our observers to conduct the CSS was one minute.

After trial evaluations we also determined that one minute and 30 seconds were necessary to perform the close up evaluation and 30 seconds to evaluate coat phenotype. Considering the time needed to perform the three evaluations, it was defined that each cat should be observed for a total of three minutes, that the observer would use to perform the CSS, OASC and finally the CS.

The fact that time spent observing a cat will affect its behavior was also considered when choosing the order in which our tests should be performed. The CSS and OASC were performed first to avoid increased stress from an observer standing too long observing color phenotype. As behavior is not a factor when evaluating a cat's phenotype, it was determined that this should be the last evaluation performed.

Selection of cats to include in the study

To avoid any bias, it was fundamental to randomly select which cats would be included in our study. At first we considered using a coin toss application to select our cats. The observer would stand in front of each cage and flip the coin. If the result was "heads" the cat would be evaluated, if the result was "tails" the cat was excluded from the evaluation.

However, after learning how many cats were in the shelters we decided that this method would not give us an appropriate number of evaluations. Therefore, we ultimately decided to evaluate each and every single cat in the shelters that met our inclusion criteria.

Cat evaluations

Cats from two shelters were evaluated in order to gather information for our study. These were the shelters that kindly showed availability to accept our visit and evaluations at the time of the study. The first shelter visited was Kent County Animal Shelter (KCAS), where a total of 14 cats were evaluated. These cats were distributed in several wings where treatment, movement and noise were similar. The second shelter visited was Oakland County Animal Shelter (OCAS), where we observed the remaining 41 cats included in this study. This shelter was composed of 3 types of wings. Two main wings where adoptable cats were kept, which allowed visitors, a stray wing, were newly arrived cats were placed was quieter and to which visitors had no access, and finally, there were a few adoptable cats in the lobby area.

Despite the differences in layout and size, in both shelters cats were kept on appropriate and clean cages and were provided with a settled environment. We found no relevant differences in the way cats were kept in these two shelters, and therefore that should not influence our results. In each shelter cats were assigned to each wing according to their health status and workers impression of where each cat may adjust better.

In both shelters cats had an information chart in their kennel door with important information regarding each individual. KCAS also had a collar on each cat with their identification number and general information (Fig. 10).

All the information necessary to decide if each cat met the inclusion criteria was collected from these individual charts attached to each individual's cage (Fig. 11). Name, shelter identification number, date of arrival and whether they were spayed or neutered were also included on these charts. Additional information such as friendliness to other cats or other animals or if the cat was found as stray or owner-relinquished was sometimes present, but not available for all cats.

After determining that they met all the inclusion criteria and none of the exclusion criteria, each cat was evaluated according to the following procedure, that each observer had access to (appendix 2):

- 1. Standing six feet from the cage, without interacting or engaging with the cat, we evaluated the cat's position inside.
- 2. At this point, the observers would begin the interactive evaluation, approaching the cage, placing their hand to the outside of the cage and keeping it still for ten seconds. The cat's reaction was recorded.
- The cat would then be called verbally for 3 seconds and coaxed to approach. The observer was careful to not make any sudden movements that could cause fear in the cat.
- 4. The observers would score the cat's initial response to the person approaching the cage.
- 5. Any changes in tail position in response to the human approaching the cage were also recorded.
- 6. At this point the CSS was performed (Fig. 12), evaluating all 11 parameters. The cat was then given a score from one to seven. The observers should also record if the cat seemed relaxed or to be feigning sleep if the cat slept through the CSS. In case of disruption, the evaluators should describe it and determine if this was enough to influence the CSS score or not. If the evaluators determined that the disruption disturbed the CSS, they would step away, wait to begin again.
- 7. Any body adjustments occurring during the CSS were also recorded.
- 8. While performing the CSS, the cat's eyes would also be observed, looking for affiliative or vigilant behaviors.
- 9. Vocalization responses during the CSS were also recorded.
- 10. If the cat scored higher than five on the CSS (Fig. 13) or one or two on the interactive evaluation, this next step would not be attempted and the cat would be scored one on the remaining items. From this point on, if the cat showed any form of clear aggressive behavior towards the observer, the test would also be discontinued. If the cat qualified to proceed with the test, the cage would be opened and the observer waited for 10 seconds before placing his hand inside the cage. The hand was placed on the first third and low on the cage. The hand was kept still for ten seconds while observing the cat's reaction.
- 11. Then, while keeping the hand already inside the cage opened and still, the observer would place a treat in that hand with his other hand. The cat's reaction was observed for ten seconds. The treat presented to each cat was a single bit of Wellness[®] Pure Delights Chicken and Lamb Jerky.
- 12. If the evaluation continued, the observers would keep their hand on the same place inside the cage, but closing their palm, rotating their hand and extending a single finger.

- 13. Finally, the observers would approach the cat without making any sudden movements towards it, stroking the cat and rubbing around its head and neck. This was only performed if the cat scored higher than two. If stroking the cat was the only thing that was not attempted, the cat would get the same score as in the previous item.
- 14. After performing the OASC and CSS according to the steps above, the observer would rate the cat as "very friendly and eager to approach", "friendly and interested to approach", "aloof and disinterested, neutral" or "very aloof, disinterested and/or aggressive to approach" according to their own personal impression of each cat after the interaction.
- 15. Then, each cat's coat was evaluated using the color scale.
- 16. Finally, a picture was taken of each cat's kennel chart and of the cat so that the information contained on the chart and the cat's coat markings were reliably registered for future use.

The entire evaluation process respected the welfare guidelines, without the use of invasive or painful procedures. At no point did we compromise the welfare of the cats included in our evaluations.



Fig. 10 - Cat wearing a collar at KCAS



Fig. 11 – Example of a kennel chart



Fig. 12 – Observer performing CSS, DePorter, 2014



Fig. 13 – Cat that scored 6 on the CSS, with dilated pupils

Statistical analysis

The data we collected was analyzed according different methods. Pearson's Chi-squared test was applied to find out the relation between the OS with the animal's sex. Since OS is an ordinal variable, Spearman correlation was applied to find out the relations between OS with age, days at the shelter, OASC and CSS. Spearman correlation was also used to analyze the relation between OASC and CSS since these variables do not follow a normal distribution.

Differences between piebald body for OASC and between piebald body for base colors were analyzed using an ANOVA fixed effects model. Homoscedasticity was verified by Levene test and normality was verified with qq-plot observation and with Kolmogorov-Smirnov test.

A t-test with Welch correction was used to compare OASC with presence or absence of piebald markings in the face.

To adjust a logistic regression model (the observer score was transformed in a dichotomous variable, considering that the event of interest occurs when the observer score is equal to 4), it was followed the methodology recommended by Hosmer et al. (2013): (1) it was computed an initial model accounting all the significant variables selected by the univariate model (P < 0.20); (2) a backward stepwise method was used to find a model with just significant variables (P < 0.05); it was verified if the variables that were not included in the initial model could be incorporated in the final model; interactions among variables were tested (P < 0.05). The assumption of linearity with the logit was tested. It was verified the existence of the outliers and data influence observations. Goodness of fit of the model was assessed by the Cessie van Houwelingen test. Using AUC, it was also verified how the model discriminates.

All statistical analyses were conducted using R project 3.0.1 software version, following packages: car, epi, Hmisc, nortest, rms.

Results

We observed a total of 55 cats for our study. 74.07% (N=41) of our observations were made at the Oakland County Animal Shelter (OCAS), while the remaining 25.93% (N=14) were conducted at the Kent County Animal Shelter (KCAS). The minimum number of days at the shelter for an evaluated cat was three days, while the longest a cat had been at the shelter 192 days when evaluated. The average number of days a cat was at the shelter prior to evaluation at the KCAS was 4.45 days, while at the OCAS the average was 33.24 days. Combining both shelters, cats were evaluated after spending an average of 26.74 days at the shelter.

The age of the cats evaluated ranged between six months and nine years, with an average of 2.82 years.

Most cats observed were spayed females (N=22, 41.51%), followed by intact females (N=17, 32.08%), intact males (N=8, 15.09%) and finally neutered males (N=6, 11.32%), as shown in fig.14.

On the evaluation of the initial position inside the cage, 21.82% (N=12) of

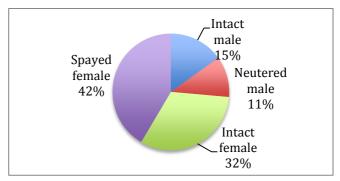


Fig. 14 - Gender distribution of evaluated cats

cats positioned in front of the cage and soliciting, 20% (N=11) of cats positioned in the front third of the cage, 25.45% (N=14) positioned in the middle third and 32.73% (N=18) of cats positioned in the back third of their cages.

During the closed cage evaluation, none of the cats showed a response that included growling, biting, swatting, charging or fleeing away. When a hand was approached outside of the cage, 45.45% (N=25) of cats responded by "watching, averting gaze, hissing or moving away", 40% (N=22) "immediately approached, friendly" and 14.55% (N=8) "approached within ten seconds".

When verbally called and coaxed to approach, 47.27% (N=26) chose to "watch, avert gaze, hiss or move away", 41.82% (N=23) "immediately approached friendly" and 10.91% (N=6) opted to "approach within ten seconds".

The initial response to a person approaching the cage of 45,45% (N=25) of cats was "watching, averting gaze, hissing or moving away", 34.55% (N=19) responded by "immediately approaching, friendly" and 20% (N=11) chose to "approach within ten seconds".

Regarding tail position, 70.91% (N=39) of cats responded with "tail level and even", 25.45% (N=14) with "tail up" and only 3.64% (N=2) showed "tail low, twitching".

Regarding the CSS score, 12.73% (N=7) of the cats scored one, 27.27% (N=15) scored two, 36.36% (N=20) scored three, 14.55% (N=8) scored four, 5,45% (N=3) scored five and 3.64% (N=2) scored six (fig. 15). No cats scored seven on the CSS. Cats from the KCAS had an average CSS score of three, while the average in OCAS was slightly lower, at 2.76. The combined average of all cats was 2.84.

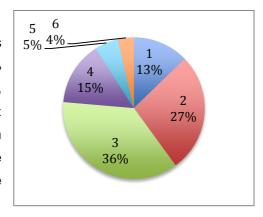


Fig. 15 - CSS distribution

While performing the CSS, 47.27% (N=26) of cats chose to "change position: approaching in a friendly manner", 40% (N=22) "remained in the same position" and 12.73% (N=7) "crouched, lowered or moved away".

The eye response to being observed during the CSS was "averting gaze" for 69.09% (N=38) of cats, "staring, watchful" for 20% (N=11) and 10.91% (N=6) chose to "blink".

The vocal response to being observed was "silent, quiet meow or open mouth meow, no sound" for 76.36% (N=42) of cats, while the remaining 23.64% (N=13) opted by "numerous meows and affiliative vocalizations".

The interactive evaluation with the open cage was not attempted at all on 3.64% (N=2) of the cats, as these cats scored six on the CSS and it was considered that this portion of the test would be too stressful and possibly result in injury to the observer and the cat. If the cats that scored lower on the CSS showed clear discomfort or distress at any time, the open cage evaluation would be discontinued immediately.

On the interactive evaluation with the open cage, 49.09% (N=27) of cats "approached and reached to touch the hand" placed inside the cage, 25.45% (N=14) "approached but didn't touch" and 21.82% (N=12) "watched, averted gaze, hissed or moved away".

When presented with a treat, 70.91% (N=39) of cats "refused food", 21.82% (N=12) "hesitated but took the food within ten seconds" and 3.64% (N=2) "approached and took food immediately from hand, eating it".

When extended a single finger, 41.82% (N=23) of cats "approached and reached to touch nose to finger", 34.55% (N=19) of cats "approached and sniffed the finger but didn't touch", 18.18%

(N= 10) "watched, averted gaze hissed or moved away", 1.82% (N=1) "growled, bit, swatted, charged or fled away".

When being stroked and rubbed around the neck and head, 54.55% (N=30) of cats responded by "leaning towards touch", 34.55% (N=14) "held position or leaned away from touch" and 1.82% (N=1) "averted gaze, hissed or moved away". This evaluation was not performed on 18.18% (N=10) of cats. 10.91% (N=4) were not evaluated and scored as a two and 7.27% (N=4) were not evaluated and scored as a one.

According to the observer's opinion, 34.55% (N=19) of cats were "very friendly and eager to approach", 30.91% (N=17) were "friendly and interested to approach", 23.64% (N=13) were "aloof and disinterested, neutral" and 10.91% (N=6) were "very aloof, disinterested and/or aggressive to approach".

After scoring the OASC and placing each score into the proper category, we found that 52.73% (N=29) of the evaluated cats were "very friendly and approachable", 41.82% (N=23) were "friendly and approachable" and only 5.45% (N=3) were considered "not friendly or approachable".

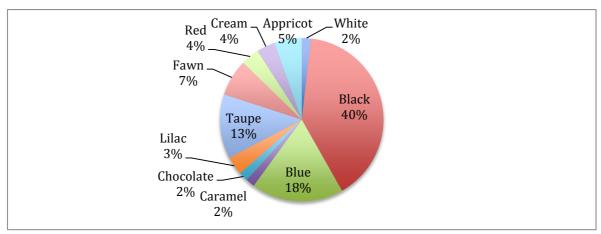


Fig 16 - Base color distribution

Concerning the base colors, we verified that 40% (N=22) of the observed cats were black, 18.18% (N=10) were blue, 12.73% (N=7) were taupe, 7.27% (N=4) were fawn and 5.45% (N=3) were apricot. The colors lilac, red and cream had 3.64% (N=2) each. White, caramel and chocolate had 1.82% (N=1) each (Fig. 16). There were no cinnamon cats. Despite being classified within a certain base color, 16.36% (N=9) of the observed females were tortoiseshell. The base color that was attributed to them was the one that seemed more visible in their coat.

Regarding tabby patterns, 58.18% (N=32) of cats did not show any tabby patterns, 40% (N=22) were mackerel and 1.82% (N=1) had a classic pattern.

The distribution of white markings in the body (Fig. 17) showed that 49.09% (N=7) had no white markings, 21.82% (N=12) had a mask and mantle and 12.73% (N=7) had a locket. Cap and saddle and tuxedo got 7.27% (N=4) of the cats each and 1.82% (N=1) was a seychellois septiéme. We did not observe any cats with white markings on their chest, chest and chin, mitted, seychellois heptiéme, seychellois neuviéme, harlequin, van or magpie.

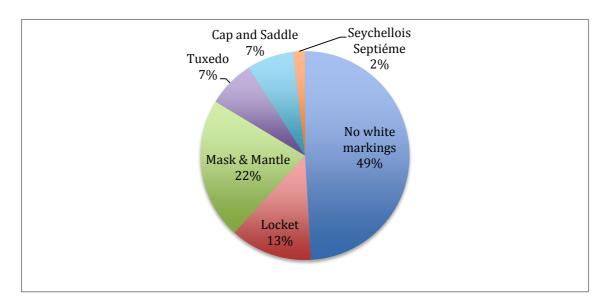


Fig. 17 - Distribution of white markings

When it came to white markings on the face, 61.82% (N=34) of cats did not have any white markings on their faces while the remaining 38.18% (N=21) had some sort of white marking on their faces.

In order to perform the statistical analysis of our small sample, the piebald patterns were grouped into 4 categories, according to the amount of white they presented: no white, traces of white, less than 50% of white coat and more than 50% of white coat.

The relation between the OASC and piebald body was studied and the statistic values obtained were $F_{2.51}$ =1.39 and p=0.258, demonstrating that there no significant differences in the OASC score averages for each piebald category.

When analyzing the relation between the OASC and piebald face the statistic values obtained were t_{52} =-1.29 and p=0,201, showing no significant difference on the OASC score for the two categories.

When analyzing the base colors, colors were once again grouped according to their genetic basis. The four categories were colors with the base gene W, colors with the base gene B, colors with the base gene O and colors under the effect of a dilution gene, d or d with Dm. The relation between base colors and the OASC was studied as in the relation between OASC and piebald body. The values obtained were $F_{2.51}$ =2.30 and p=0.110 showing that there was no statistical evidence to prove a difference in tameness for each color.

When analyzing a possible relation between the OASC score and the CSS, we used a Spearman correlation coefficient, with estimated values of rs=-0.30 and p=0.026, demonstrating a weak positive correlation between the two scores.

The relation between the OASC and the OS was also studied trough a Spearman correlation coefficient and showed a strong correlation between the two variables.

To further study the data, a logistic regression model was created, with OS as a dichotomous variable. Based on this model, we concluded that the scores for "position in cage", "approaching hand held up to the outside of the cage" and "stroke cat, rub around head and neck" were highly correlated with the OS. This model had a Nagelkerke R^2 =0.79, and a high discriminative capacity with AUC=0.96. Cessie van Houwelingen test had a p=0.34. The higher the score of the "position in cage", the less likely it is for a cat to be tame. A high score in "approaching hand held up to the outside of the cage" and "stroke cat, rub around head and neck" indicate a tamer cat.

It was not possible to show any relation between gender, age or number of days at the shelter and the tameness of the cats analyzed in our study.

Discussion

During this study, we collected a lot of data that could be inserted in a great amount of categories. However, we did not observe enough individuals to fill each category with an amount of cats that would allow a powerful statistical analysis. This meant that the creation of a statistical model to analyze our data was more difficult. Therefore, we chose to group some of these data into bigger categories in order to look for any tendencies and valuable information. Despite the fact that our sample did not allow us to verify our hypothesis, we were still able to get some valuable information that may allow a larger and more focused study in the future.

At this point we do not have enough information to verify if gender, age and number of days at the shelter have any influence in a cat's tameness. A larger study could find a relation that was not visible in our sample or show the same lack of tendency we had.

Regarding the lack of evidence that there is a relation between piebald markings or coat color and tameness in cats, there are two possible explanations. The first one is that there is no relation between these aspects. However, the fact that our study did not show statistical evidence of a relation is not reason enough to state that there really is no relation. The second explanation is that there may be a tendency that is not shown in our study due to our small sample. Once again, only a much larger study could help determine if there really is a tendency that was unnoticed due to our sample or not.

Our analysis found that there was a weak positive correlation between the OS and the CSS. This means that a tamer cat also showed a higher stress score. This may be due to the cat's anticipation of contact with the observer, leading to a higher display of stress signals at the time the observer was performing the CSS, but not in close contact to the cat. Waiting and anticipating the approach of the observer was in fact more stressful to the cats that wanted contact then it was to the cats that preferred to be left alone. This correlation may be weak due to the fact that a cat that does not wish to contact with the observer will also show a higher stress score when anticipating contact, and a small sample like ours may dilute this correlation.

We also reached the conclusion that the three variables "position in cage", "approaching hand held up to the outside of the cage" and "stroke cat, rub around head and neck" are highly correlated with tameness. In fact the correlation is so strong that these three variables alone can accurately predict the degree of tameness of a cat.

This means that in a future evaluation, it would be possible to use only these 3 variables to accurately predict the tameness of each cat, allowing for shorter evaluation times for each cat, which in turn means a larger number of cats evaluated in the same amount of time. This would

facilitate obtaining a larger sample of cats that could lead to more conclusions than the ones obtained by this study.

The relation between the initial position inside the cage and tameness states that the closer the cat is to the door, the less likely it is that the cat will be tame. This may be related either with escaping attempts, where the cats choose to be closer to the door as it makes it easier to escape from this position of the cage, or it could be related the vigilant behavior, as the cat can keep watch of the environment and the observer a lot better closer to the door, and then retreating when the observer approaches the cage door.

The reaction of the cat to a hand approaching the outside of the cage and to the attempt of stroking it is also highly related to tameness. In this case, the more eager the cat is to establish contact, the more likely it is that the cat is tame. This is related to how comfortable the cat is around humans and how much it really wishes to interact and establish physical contact with the observer.

We also found that the great majority of the cats refused a treat that was offered to them. While we expected tamer cats to take the treat without hesitation, this was not the case. Most cats chose to ignore the treat and interact with the hand that was offering it instead. This may indicate that physical contact was more important to these cats than the treat offered.

The fact that in both shelters cats had food available at the time we evaluated them, and therefore it was not likely they were hungry at the time, allowing them to place the need for social contact in front of the need for feeding.

Conclusions

When we look at previous research on the relations between coat color and temperament, there is no doubt that there are some linkages, but the extent of the interaction between coat characteristics and behavior is not yet understood. (Overall, 2013)

Despite our efforts to gain a better understanding of this relation in domestic cats, our study showed no significant relations between tameness and coat color. However, we do believe a new study, with a larger sample and particular adjustments to the measuring scales would possibly find a strong relation between these two variables.

Even with no statistical evidences that prove our hypothesis, this study gave us a chance to test the OASC, the tool we created to measure tameness. The results we got and the statistical analysis have shown us how we can improve this scale and make it more effective. The fact that we now know that the variables "position in cage", "approaching hand held up to the outside of the cage" and "stroke cat, rub around head and neck" can accurately measure tameness will allow a larger and more time effective study, as this will shorten greatly the time spent observing each cat, without loosing details.

We believe that these variables can determine how vigilant the cat is when the observer approaches, how whiling it is to investigate the observer and how interested it is in getting physical contact with the observer.

A larger study would also help clearing if the relation between gender, age or number of days at the shelter, and tameness really is inexistent or if our small sample simply did not show this tendency.

Regarding the behaviors towards the observer, we found interesting information that could be further studied, such as the refusal of food on tame cats, which preferred physical contact over food, or the fact that less tame cats were standing further in front on their cages, instead of standing in the back as expected.

Further studies could help us understand how high the value of physical contact is to tamer cats, specially compared with the value of a treat. It would be interesting to determine if this is only the case for tame cats with free access to food, or if a treat presented to a cat with a feeding schedule would be more valuable than the interaction with the observer.

It would also be important to investigate if the frequent restriction of human contact may be a stress factor for tamer cats. If that is the case, evaluating the degree of tameness and providing more frequent interactions with humans to tamer cats may help decrease their stress levels in shelters.

Concerning the position in the cage, it would be interesting to distinguish if the cats positioned in front of the cage are in fact more vigilant or if they are closer to the door in order to be better positioned to escape if necessary.

Bibliography

Aengus, W. L., & Millam, J. R. (1999). Taming parent- reared orange- winged Amazon parrots by neonatal handling. *Zoo Biology*, *18*(3), 177–187.

Albert, F. W., Shchepina, O., Winter, C., Römpler, H., Teupser, D., Palme, R., ... Pääbo, S. (2008). Phenotypic differences in behavior, physiology and neurochemistry between rats selected for tameness and for defensive aggression towards humans. *Hormones and Behavior*, *53*(3), 413–21.

Barnard, S., Pedernera, C., Velarde, A., & Villa, P. D. (2014). Welfare Assessment Protocol for Shelter Dogs.

Bateson, P. (2014). *The Domestic Cat*, 3rd Edition, ed. Turner, D. C., & Bateson, P, Cambridge University Press. ISBN 978-1-107-02502-8, pp. 17-19.

Beaver, B. V. (2003). *Feline Behavior: A guide for Veterinarians*, 2nd Edition, Saunders Elsevier Science. ISBN 0-7216-9498-5

Bernstein, P. (2007). Human Cat Relationship. In: *The Welfare of Cats*, ed. Rochlitz, I., Springer. ISBN 1-4020-3277-7, pp. 61-63.

Boivin, X., Tournadre, H., & Neindre, P. Le. (2000). Hand-feeding and gentling influence early-weaned lambs ' attachment responses to their stockperson. *Journal of Animal Science*, **78(4)**, 879–884.

Broadley, H. M., McCobb, E. C., & Slater, M. R. (2013). Effect of single-cat versus multi-cat home history on perceived behavioral stress in domestic cats (Felis silvestrus catus) in an animal shelter. *Journal of Feline Medicine and Surgery*, *16*(2), 137–43.

Case, L. P. (2010). *Canine and Feline Behavior and Training*. Delmar Cengage Learning[®]. ISBN 1-4283-1053-7, pp. 82-101.

Cieslak, M., Reissmann, M., Hofreiter, M., & Ludwig, A. (2011). Colours of domestication. *Biological Reviews of the Cambridge Philosophical Society*, **86(4)**, 885–99. Crowell-Davis, S. L. (2006). Comportamiento Social, Comunicación y Desarrollo del Comportamiento en Gatos. In: *Manual del comportamiento de pequeños animales*, ed. Horwitz, D., Mills, D., & Heath, S., Ediciones S. ISBN 978-84-87736-61-2, pp. 33-48.

Crowell-Davis, S. L. (2007). Cat Behaviour: Social Organization, Communication and Development. In: *The Welfare of Cats*, ed. Rochlitz, I., Springer. ISBN 1-4020-3277-7, pp. 1-22.

Driscoll, C. a, Macdonald, D. W., & O'Brien, S. J. (2009). From wild animals to domestic pets, an evolutionary view of domestication. doi:10.1073/pnas.0901586106

Dybdall, K., Strasser, R., & Katz, T. (2007). Behavioral differences between owner surrender and stray domestic cats after entering an animal shelter. *Applied Animal Behaviour Science*, **104(1-2)**, 85-94.

Gulevich, R. G., Plyusnina, I. Z., Prasolova, L. a., Oskina, I. N., & Trut, L. N. (2010). White spotting in Norway rats selected for tame behavior. *Journal of Zoology*, *280*(3), 264–270.

Hoff, A. (2009). Clicker Training of shelter cats.

Hosmer, D. W., Lemeshow, S., & Sturdivant, R. (2013). *Applied Logistic Regression*, 3rd Edition, John Wiley & Sons. ISBN 978-0-470-58247-3, pp. 10-70.

Kaelin, C. B., & Barsh, G. S. (2013). Genetics of Pigmentation in Dogs and Cats. *Annual Review of Animal Biosciences*, **1(1)**, 125–156.

Kessler, M. R., & Turner, D. C. (1997). Stress and Adaptaion of Cats (Felis silvestrus catus) Housed Sinlgy, in Pairs and in Groups in Boarding Catteries. *Animal Welfare*, *6(3)*, 243–254.

Kessler, M. R., & Turner, D. C. (1999). Socialization and Stress in Cats (Felis silvestris catus) Housed Singly and in Groups in Animal Shelters. *Animal Welfare*, *8(1)*, 15–26.

Ku, C., & Sachser, N. (1999). The Behavioral Endocrinology of Domestication: A Comparison between the Domestic Guinea Pig (Cavia aperea f. porcellus) and Its Wild Ancestor, the Cavy (Cavia aperea). *Hormones and Behavior*, *35(1)*, 28–37.

Kukekova, A. V, Temnykh, S. V, Johnson, J. L., Trut, L. N., & Acland, G. M. (2012). Genetics of behavior in the silver fox. *Mammalian Genome: Official Journal of the International Mammalian Genome Society*, *23*(1-2), 164–77.

Landsberg, G., Hunthausen, W., & Ackerman, L. (2013). *Behavior Problems of the Dog and Cat*, Saunders Elsevier. ISBN 978-0-7020-4335-2, pp. 20-26.

Lordi, B., Protais, P., Mellier, D., & Caston, J. (1997). Acute Stress in Pregnant RatsEffects on Growth Rate, Learning, and Memory Capabilities of the Offspring. *Physiology & Behavior*, *62*(5), 1087–1092.

Lyons, D. M., Price, E. O., & Moberg, G. P. (1988). Individual differences in temperament of domestic dairy goats: constancy and change. doi:10.1016/S0003-3472(88)80201-X

Mccobb, E. C., Patronek, G. J., Marder, A., Dinnage, J. D., & Stone, M. S. (2005). Assessment of stress levels among cats in four animal shelters. *JAVMA*, *226(4)*, pp. 548-555.

Mendl, M., Burman, O., Laughlin, K., & Paul, E. (2001). Animal Memory and Animal Welfare. *Animal Welfare*, *10(1)*, pp. 141-159.

Moore, A. M., & Bain, M. J. (2013). Evaluation of the addition of in-cage hiding structures and toys and timing of administration of behavioral assessments with newly relinquished shelter cats. doi:10.1016/j.jveb.2011.10.003

Möstl, K., Egberink, H., Addie, D., Frymus, T., Boucraut-Baralon, C., Truyen, U., ... Horzinek, M. C. (2013). Prevention of infectious diseases in cat shelters: ABCD guidelines. *Journal of Feline Medicine and Surgery*, **15**, 546–54.

Neilson, J. C. (2009). The latest scoop on litter.

Overall, K. L. (2013). *Manual of Clinical Behavioral Medicine for Dogs and Cats*, Elsevier Mosby. ISBN 978-0-323-00890-7, pp. 312-330.

Picardello, M. (1997). Feline Genetics: a Combinatorial Approach.

Price, E. O. (2002). *Animal Domestication and Behavior*, CABI Publishing. ISBN 0-85199-597-7, pp. 43-115.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Spier, S. J., Berger Pusterla, J., Villarroel, A., & Pusterla, N. (2004). Outcome of tactile conditioning of neonates, or "imprint training" on selected handling measures in foals. *Veterinary Journal (London, England: 1997)*, *168*(3), 252–8.

Trut, L. N. (1999). Early Canid Domestication: The Farm-Fox Experiment. *American Scientist*, **87(2)**, 160.

Trut, L., Oskina, I., & Kharlamova, A. (2009). Animal evolution during domestication: the domesticated fox as a model. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, *31*(3), 349–60.

Vella, C., Shelton, L., McGonagle, J., & Stonglein, T. (1999). Robinson's Genetics for Cat Breeders and Veterinarians, 4th Edition, Butterworth & Heinemann. ISBN 0-7506-40693, pp. 135-150.

Vilanova, X. M. (2002). *Etologia Clínica del Perro y del Gato*, 2nd Edition, MultiMédica. ISBN 84-932811-0-7, pp. 91-117.

Appendices

Appendix 1: Assessment tools used to evaluate the cats

Oakland Approachability Scale for Cats (OASC)
Name: ID: Age: Sex: Date: Location:	
This scale is intended to evaluate a cat's willingness to approach non-threatening person.	an unfamiliar,
Interpretation may be adversely affected by confounding factors or pain, learning due to experiences with people and fear. Kittens willing to approach. Cats who have recently been introduced to a may score poorly.	s may be more
Does this cat: Display medical or health conditions which may influence	behavior?
rr	es No
Date of arrival at the shelter? <3days Yes_, then wait to	evaluate.
Meets eligibility If yes to any above, does not meet eli	gibility 🗌
Distance evaluation Stand at 6 feet from the cage 1. Position in cage	
Positioned in front 1/3 cage and soliciting	4
Positioned in front 1/3 cage	3
Positioned in middle 1/3 cage	2
Positioned in back 1/3 cage	1
Cage front evaluation Step to the front of the cage wait 10 seconds to score	

nnroac	hing hand	hold un	to the	outcido	,
_			_		

2. Approaching hand held up to the outside	of
the cage	
Immediately approaches, friendly	4
Approaches within 10 seconds	3
Watches, averts gaze, hisses or moves away	2
Growls, bites, or swats. Charges or flees away	1

3. Verbally calling and coaxing to approach (3 second calling)	1	
Immediately approaches, friendly	4	
Approaches within 10 seconds		
Watches, averts gaze, hisses or moves away		
Growls, bites, or swats. Charges or flees away	1	

4. Initial response to person approaching cage		
4		
3		
2		
1		

5. Tail position change in response to person approaching cage		
Tail up	4	
Tail level and even	3	
Tail low, twitching	2	
Tail low, twitching/swishing side to side	1	

6. Perform CSS now.

Cat Stress Score evaluation CSS evaluation for 60 seconds. Evaluator to stand in natural posture close to the cage for causal viewing.
CSS 1 2 3 4 5 6 7
If the cat is sleeping throughout entire CSS: Cat appears to be relaxed and deeply sleeping Cat appears to be feigning sleep. Avoidant sleeping.
Disruptions to CSS Note if the CSS observations disrupted with by outside influences or factors. Describe Observer deemed not sufficient to disruption to influence CSS. Observer deemed sufficient to disruption to influence CSS. Necessary to move away and wait to begin again.
No disruptions occurred
7. Do do Do stores a director costa domina the CCC

7. Body Posture adjustments during the CSS	
Changes position: approaches in friendly manner	4
Remains in same position	3
Crouches, lowers or moves away	2
Crouches, flattens body. Lowers head. Charges or flees away.	1

8. Eyes in response to being observed during the CSS	
Blinking	4
Averted gaze	3
Staring, watchful	2
Staring, fixated and vigilant	1

9. Vocalization in response to being observed during	
the CSS	
Numerous meows and affiliative vocalizations	4
Silent. Quiet meow. Open mouth meow, no sound.	
Hiss	2
Growl	1

Interactive evaluation, open cage

Do not attempt if CSS >5 Do not attempt if cat scored 1 or 2 on Interactive Evaluation #7 and #8

Open cage, wait 10 seconds before and between each assessment Discontinue if any aggression or defensive actions shown (score =1 or 2)

If at any point in the interactive, open cage evaluation a cat shows clear aggressive behavior (growl, bite or charge) towards the observer, that cat should be scored 1.0 from that point on and the test would be discontinued.

10. Place hand in cage		Did not attempt. Score = 1.0
Approaches. Reaches to touch hand	4	
Approaches. Doesn't touch	3	
Watches, averts gaze, hisses or moves away	2	
Growls, bites, or swats. Charges or flees away	1	

11. Present treat/food		Did not attempt. Score = 1.0
Approaches. Takes food immediately from hand.	4	
Eats food		
Hesitates but takes food from hand within 10	3	
seconds		
Refuses food	2	
Refuses food. Growls, bites, or swats. Charges or	1	
flees away		

12. Extending single finger to cat		Did not attempt. Score = 1.0
Approaches. Reaches to touch nose to finger	4	
Approaches. Sniffs finger but doesn't touch	3	
Watches, averts gaze, hisses or moves away	2	
Growls, bites, or swats. Charges or flees away	1	

13. Stroke cat, rub around head and neck		Did not attempt. Score = 1.0 ☐ or 2.0 ☐
Leans toward touch	4	
Holds position or leans away from touch	3	
Averts gaze, hisses or moves away	2	
Growls, bites, or swats. Charges or flees away	1	

According to observer opinion, t	this cat is:			
 □ Very friendly and eager to approach⁴ □ Friendly and interested to approach³ □ Aloof and disinterested. Neutral.² □ Very aloof, disinterested and/or aggressive to approach¹ 				
OASC scoring: total score	48 – 37 = Very Friendly and Approachable 36 – 25 = Friendly and Approachable 24 – 12 = Not Friendly or Approachable			

Color Scale

Use Color Guide as reference and mark any option on this scale that applies to the cat being evaluated.

Base Colors	Other color characteristics	
White	Tortoiseshell	
Black	Colorpoint	
Blue	Tipped	
Caramel		
Chocolate		
Lilac		
Taupe		
Cinnamon		
Fawn		
Red		
Cream		
Apricot		

Tabby	White Markings
Mackerell	No
Classic	Locket
Spotted	Chest
Ticked	Chest + Chin
	Mitted
	Tuxedo
	Mask and Mantle
	Cap and Saddle
	Seychellois Septiéme
	Seychellois Heptiéme
	Seychellois Neuviéme
	Harlequin
	Van
	Magpie
	White markings on face

Appendix 2: Observer's instructions to the assessment tool

Every observer must read and understand this document before using the OASC or color scale

Guide Lines for performing OASC

Distance evaluation (item 1)

Standing at six from the cage, without interacting or engaging with the cat, evaluate the cat's position inside.

Cage front evaluation (items 2, 3, 4 & 5)

Step to the front of the cage and approach your hand to the outside of the cage and keeping it still for 10 seconds. When calling and coaxing the cat be careful to not make any sudden movements that can cause fear to the cat.

Score the cat's initial response your approach.

CSS evaluation (items 6, 7, 8 & 9)

Evaluate all 11 parameters individually. The cat will then be attributed an average score from one to seven.

Interactive evaluation, open cage: (items 10, 11, 12 & 13)

If the cat scored higher than 5 on the CSS or 1 or 2 on the interactive evaluation do not proceed to this part of the test and score cat as 1 for the remaining items. From this point on, if the cat shows any form of clear aggressive behavior towards you, discontinue the test.

If the cat is qualified to proceed on the test, open the cage and wait for 10 seconds before placing your hand inside the cage. The hand should be placed on the first third and low on the cage. Keep your hand still for 10 seconds while observing the cat's reaction.

To present the treat, keep the hand you already have inside the cage still and place a treat in it with your other hand.

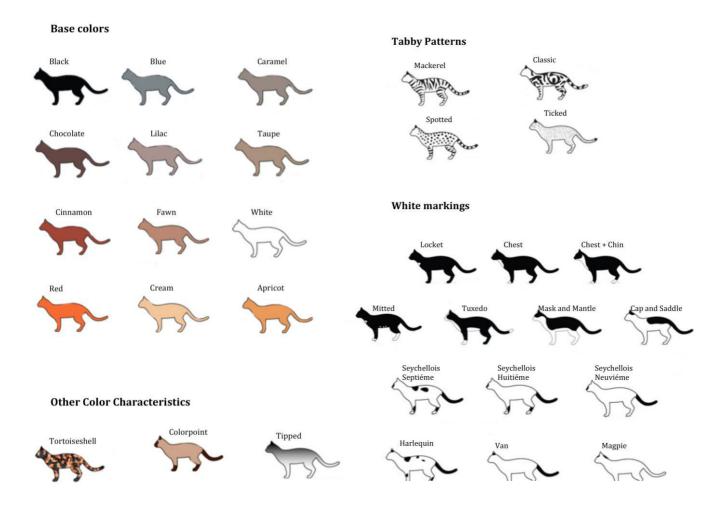
Keep your hand on the same place but close your palm, rotate your hand and extend a single finger.

Finally, approach the cat without making any sudden movements towards it, stroking the cat and rubbing around its head and neck. This will only be performed if the cat scored higher than two on the previous item. If stroking the cat was the only thing that was not attempted, the cat should get the same score as in the previous item.

Appendix 3: Cat Stress Score reference chart

			+		υ			
i: (or unspecified) = cat is inactive, a: = cat is active]	Activity	Sleeping or resting	Sleeping, resting, alert or active, may be playing	Resting awake or actively exploring	Cramped sleeping, resting or alert may be actively exploring, trying to escape	Alert, may be actively trying to escape	Motionless, alert or actively prowling	Motionless
	Vocal	None	None	Meow or quiet	Meow, plaintive meow or quiet	Plaintive meow, yowling, growling or quiet	Plaintive meow, yowling, growling or quiet	Plaintive meow, yowling, growling or quiet
	Whiskers	Lateral (normal)	Lateral or forward	Lateral or forward	Lateral or forward	Lateral or forward or back	Back	Back
	Ears	Half-back (normal)	Half-back or erected to front or back and forward on head	Half-back or erected to front or back and forward on head	Erected to front or back, or back and forward on head	Partially flattened	Fully flattened	Fully flattened back on head
	Pupils	Normal	Normal	Normal	Normal or partially dilated	Dilated	Fully dilated	Fully dilated
	Eyes	Closed or half opened, may be blinking slowly	Closed, half opened or normal opened	Normal	Widely open or pressed together	Widely opened	Fully opened	Fully opened
	Head	Laid on surface with chin up or on surface	Laid on surface or over the body, some movement	Over the body, some movement	Over the body or pressed to body, little or no movement	On the plane of the body, less or no movement	Near to surface, motionless	Lower than the body, motionless
	Tail	Extended or loosely wrapped	i: extended or loosely wrapped a: up or loosely downwards	May be twitching i: on the body or curved backwards a: up or tense downwards	i: close to the body a: tense downwards or curled forward, may be twitching	i: close to the body a: curled forward close to the body	i: close to the body a: curled forward close to the body	Close to the body
	Legs	Fully extended	i: bent, hind legs may be laid out A: when standing, extended	i: bent a: when standing, extended	i: bent a: when standing, hind legs bent in front extended	i: bent a: bent near to surface	i: bent a: bent near to surface	Bent
	Stomach	Exposed, slow ventilation	Exposed or not, slow or normal ventilation	Not exposed, normal ventilation	Not exposed, normal ventilation	Not exposed, normal or fast ventilation	Not exposed, fast ventilation	Not exposed, fast ventilation
	Body	Laid out on side or on back	i: laid ventrally or half on side or sitting a: standing or moving, back horizontal	i: laid ventrally or sitting a: standing or moving, body behind lower than in front	i: laid ventral, rolled or sitting a: standing or moving, body behind lower than in front	i: laid ventrally or sitting a: standing or moving, body behind lower than in front	i: laid ventrally or crouched directly on rop of all paws, may be shaking a: whole body near to ground, crawling, may be shaking	Crouched directly on top of all fours, shaking
(From Kassle	Score	1. Fully Relaxed	2. Weakly Relaxed	3. Weakly Tense	4. Very Tense	5. Fearful, Stiff	6. Very Fearful	7. Terrified

Appendix 4: Reference chart for the Color Scale



Appendix 5: Submitted communication – IV Congresso Psi Animal

Comunicação oral, preferencialmente 19 de Outubro

Relação entre cor e mansidão em gatos domésticos

<u>Maria Teresa Umbelino</u>¹, Gonçalo da Graça Pereira², Alfredo Pereira³, Paulo Infante⁴, Theresa DePorter⁵

¹ Universidade de Évora, Évora; ² Faculdade de Medicina Veterinária da Universidade Lusófona de Humanidades e Tecnologias, Lisboa; ³ ICAAM – Instituto Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora; Évora; ⁴ Universidade de Évora, Departamento de Matemática, Évora; ⁵ Oakland Veterinary Referral Services, Animal Behavior Department, Oakland County; teresaumbelino@gmail.com

Existem vários trabalhos que associam a alteração de padrões de cor nas pelagens dos animais com a sua domesticação. Neste estudo, pretendeu-se verificar a existência de relação entre a mansidão dos gatos domésticos e a sua cor ou padrão de cor.

Avaliaram-se 55 gatos em dois abrigos nos Estados Unidos da América, Michigan, recorrendo-se tanto a uma ferramenta já existente, a Cat-Stress-Score (CSS) e a duas ferramentas criadas para esta avaliação, a Oakland Approachability Scale for Cats (OASC) e a Color Scale (CS).

Recolheu-se informação sobre o seu nível de stress quando abordados pelo observador e quão dispostos estavam a estabelecer interações e contacto físico durante o período em que eram avaliados. Os padrões de cor, marcas particulares e tonalidade do pelo foram registados para posterior relacionamento com a mansidão.

A análise estatística inicial não demonstrou qualquer relação entre a cor e a mansidão ou entre a presença de marcas brancas e a mansidão nos gatos em estudo.

No entanto, encontrou-se uma correlação positiva entre a CSS e a OASC. Isto verifica-se pois existe relação entre o stress sentido pelos gatos na antecipação à manipulação e a sua mansidão. Um gato muito manso sentirá um maior nível de stress pela vontade de interagir com o observador, traduzindo-se num maior valor na CSS.

Também foi possível concluir que a posição inicial na jaula, a resposta dada pelo gato à aproximação da mão do observador à jaula e a sua resposta à tentativa de carícia eram fundamentais na determinação do seu nível de mansidão. O modelo que relaciona estas variável com a mansidão, apresentou uma elevada precisão, possibilitando a utilização destas variáveis, como discriminantes. Assim, a continuação deste trabalho poderá utilizar apenas estas variáveis, permitindo a avaliação de um maior número de gatos em menos tempo em futuros trabalhos a desenvolver.