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Behavior and personality analysis in cloned working dog candidates

복제 기능견 후보의 행동과 성격 분석

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최 진

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Behavior and personality analysis in cloned working dog candidates

by Jin Choi

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in

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We accept this thesis as confirming to the required standard

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July 2018

Declaration

This thesis is submitted by the undersigned for examination for the degree of Doctor of Philosophy to the Seoul National University. This thesis has not been submitted for the purposes of obtaining any other degree or qualification from any other academic institution.

I hereby declare that the composition and experiment of this thesis and the work presented in it are entirely my own.

Jin Choi

Behavior and personality analysis in cloned working dog candidates

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ABSTRACT

Cloning specific individuals is the way to produce targeted animals. This makes newborns without surplus animals which should be adapted to new families afterwards or supported by institutions for whole life. Cloning excellent dogs had been started from this point. Many cloned dogs have been produced since the first cloned dog “SNUPPY” was born. Production of seven drug detection dogs (named

“Toppy”) by cloning has been reported in 2009. Cloning rescue dogs, and quarantine dogs were also successful, however their behavioral similarities were not examined yet. Although their genetic identity was confirmed, characteristics in their behavior and detecting ability are the important reason as well. This is the reason why I conducted this research on the cloned puppies. Therefore, this study is the attempt to examine their behavior. I conducted the Puppy Aptitude Test (PAT), which is commonly used to evaluate the tendency of dominance when the puppies are seven weeks old. The Puppy Aptitude Test consists of 10-11 subtests: Social Attraction, Following, Restraint, Social Dominance, Elevation Dominance, Retrieving, Touch Sensitivity, Sound Sensitivity, Sight Sensitivity, Stability, and Structure. The Structure subtest evaluates the puppy’s stance and body balance, and the other 10 subtests are used in this study to score its temperament. The two cloned puppies were classified as being the same type in rescue dog study.

In recent research, personality consistency has become an important characteristic. Diverse traits and human-animal interactions, in particular, are studied in the field of personality consistency in dogs. Here, I investigated the consistency of dominant behaviours in cloned and control groups followed by the modified Puppy Aptitude Test, which consists of ten subtests to ascertain the influence of genetic identity. In this test, puppies are exposed to stranger, restraint, prey-like object, noise, startling object, etc. Six cloned and four control puppies participated and the consistency of responses at ages seven-ten and sixteen weeks in the two groups was compared. The two groups showed different consistencies in

the subtests. While the average scores of the cloned group were consistent (P=0.7991), those of the control group were not (P=0.0089). Scores of Pack Drive and Fight or Flight Drive were consistent in the cloned group, however, those of control group were not. Scores of Prey Drive were not consistent in either the cloned or the control group. Therefore, it suggested that consistency of dominant behaviour was affected by genetic identity and some behaviors could be influenced more than others. My results suggest that cloned dogs could show more consistent traits than non-cloned. This study implies that personality consistency could be one of the ways to analyse traits of puppies.

Key words: canine behavior, cloned dog, puppy aptitude test

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LIST OF ABBREVIATIONS

C-BARQ	**Canine Behavioral Assessment and Research Questionnaire**
Cl-1	**Clone 1**
Cl-2	**Clone 2**
Cl-3	**Clone 3**
Cl-4	**Clone 4**
Cl-5	**Clone 5**
Cl-6	**Clone 6**
Cl-7	**Clone 7**
Co-1	**Control 1**
Co-2	**Control 2**
Co-3	**Control 3**
Co-4	**Control 4**
DMAP	**6-Dimethylaminopyridine**
DNA	**Deoxyribonucleic acid**
ED	**Subtest Elevation Dominance**
F	**Subtest Following**
GFP	**Green fluorescence protein**
PAT	**Puppy Aptitude Test**
PZM-DMAP	**PZM-5 supplemented with 1.9 mM DMAP**

R	**Subtest Restraint**
RFP	**Red fluorescence protein**
Rt	**Subtest Retrieving**
S	**Subtest Stability**
SCNT	**Somatic cell nuclear transfer**
SA	**Subtest Social Attraction**
SD	**Subtest Social Dominance**
SiS	**Subtest Sight Sensitivity**
SOF-DMAP	**mSOF medium supplemented with 1.9 mM DMAP**
SS	**Subtest Sound Sensitivity**
TS	**Subtest Touch Sensitivity**

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PART I

GENERAL INTRODUCTION

1. Literature review

1.1. History of working dogs

1.1.1. Various types of working dogs and their potentials

Various working dogs, including drug detection dogs, quarantine detection dogs, cancer detection dogs, rescue dogs (search and rescue [SAR] dogs), and termite detection dogs, help humans. Drug detection dogs search for illegal drugs at the airport cargo terminal station. This task is difficult to do without working dogs because the staffs cannot check each parcel. SAR dogs save human lives by finding humans under rubble. The ability of detection dogs is more accurate and concise than that of humans or devices. In the assessment for detecting insect pests, the detection dogs showed 100% efficiency in detecting termite colonies, whereas the success rates of devices depended on the operators' skills and sample type [1].

A previous study reported that well-trained cadaver dogs detected the odor of deceased bodies with excellent sensitivity (75—100%) and specificity (91—100%) [2]. Blood and cadaver search dogs detected the remaining scent of two deceased individuals. They were trained to scratch and bark when they find the targets, and show no reaction when there is no human body remains. As a result, the dogs found the contaminated carpets with 94% or 98% accuracy according to the contaminated time. The accuracy was higher with the materials exposed for 10 min

than with those exposed for 2 min. Cadaver dogs are trained to detect the odor from deceased bodies, and this case was a trial to reproduce one of the possible crime scenes. This investigation showed the capability of detection dogs and their superior ability to smell, which surpassed the scientific analysis. Oesterhelweg *et al*. suggested that their reliability and accuracy could be enhanced if they search independently at one crime scene [2].

1.1.2. Demands for service dogs and their characteristics

Dogs have been with humans as companions, guards, guides, and so on [3]. Detection dogs have been trained to search explosives, drugs, human bodies, screw worms, and others [4]. To identify objects related to terrorist threats, the demand for detection dogs has increased [5]. These dogs could save human lives and maintain the efficiency of security systems. Service dogs can help humans as guide dogs, dogs for the deaf, or people in wheelchairs, and as search dogs. The training method for working dogs could differ, and the characteristics required of the dog could vary according to the type of work, which may include endurance, strength, sociability, curiosity, and trainability. Guide dogs have been helping humans navigate through the world since World War I [6]. These dogs need to be large and trainable, but neither highly submissive nor dominant [3]. German Shepherds were usually trained as guide dogs in the early days; however, Labrador Retrievers and Golden Retrievers were more preferred later because of their adaptability to the

lack of activity. Hearing-ear dogs, which assist people with hearing impairment, play a different role [3]. They do not need to be large; but must be have high energy and slight dominance.

1.1.3. Desirable and undesirable traits for detection dogs

Several traits were proposed as important for detection dogs. Detection dogs should be athletic, trainable, and motivated. Speed is suggested as one of the important traits, as it can lead to work efficiency [7]. Working quickly is an important part, however detection dogs should detect target scents without being exhausted [8]. If their findings are not accurate, another screening or detection method would be needed, which defeats their purpose. Stamina is one of the desirable traits because the working environments of detection dogs are not predictable. They should find the intended targets in wild fields, desolate crime scenes, crowded airports, indoors, and so on. Motivation to search is one of the important temperaments for detection dog selection [9], as dogs can be more motivated by play, prey, hunting, or other stimuli. These can be used as rewards after training or finding; therefore, dogs with greater motivation can concentrate more and longer. On the other hand, fear and anxious responses are not desirable for detection dogs [10]. If they are fearful and anxious, various stimuli can distract their work. Therefore, detection dogs with high performances tend to get higher scores for boldness than those with low performances. This personality could vary

according to dog breed. Labrador Retrievers, German Shepherds, Terriers, and English Springer Spaniels are commonly selected as drug detection dogs [8].

1.1.4. What are temperament and personality?

Some researchers have defined temperament as inherited tendencies that continue throughout life in humans [11]. When previous researchers refer to animals aspects, they tend to use temperament to avoid anthropomorphism by using personality. Human-personality psychologists have proposed various ranges and definitions of personality; therefore, with only one definition, the concept of personality cannot be fully grasped. Pervin and John suggested the following definition, that is broad enough to include most aspects of personality: “Personality can be defined as those characteristics of individuals that describe and account for consistent patterns of feeling, thinking, and behaving [12].” Therefore, studies on the temperament or personality of dogs vary in assessment methods and interpretations. The present study was based on these phenomena and identified a more accurate method for evaluating detection dogs. Each test or questionnaire could have its specified aspect, and scoring could be biased according to evaluators.

Temperament influences canine behavior and responses to different environments. These traits could be defined as temperament, personality, or characteristics, and so on. However, the truth that these expressions originate from their genotypes and belong to expressed phenotypes could not be changed.

1.1.5. Temperament test and performance between breeds

Canine behaviors are based on breed-specific and genetic components [10]. Behaviors and their expressions are difficult to define and measure, which make it challenging for researchers, handlers, and trainers. In addition, even if studies could evaluate canine behaviors more accurately, the results would be difficult to apply to other research studies or to combine. For this reason, international standardized testing methods would be ideal for canine temperament tests. If testing can be standardized, scoring the performances of working dogs would be more effective.

Dog breed has typically originated from their working roles. German Shepherds, Belgian Shepherds, and Labrador Retrievers are traditional military-law enforcement working breeds [7]. Beagles and Jack Russell Terriers are used to search invasive animals or agricultural products. They are sometimes ranked by trainability, and the high−rank breeds are the Border Collie, Poodle, German Shepherds, Golden Retriever, Doberman Pinscher, Shetland Sheepdog, Labrador Retriever, Papillon, Rottweiler, and Australian Cattle Dog [7]. The lowest-rank breed overall is the Afghan Hound. Various breeds have their specific abilities and characteristics, however there is not specialist breed for detection work until now, which is one of the challenging aspects. The trainability and intelligence of dogs could be tested according to their definitions, which researchers have tried to evaluate. Trainability can be evaluated on the basis of canine performance and

speed at learning a task, as this can be defined as a canine ability for learning skills or tasks [7].

1.2. Canine behavior test

1.2.1. Early evaluation using behavior testing

For future handlers, trainers, and owners, analysis of canine temperament is important. As raising and training working dog candidates could be a waste of time and effort, the evaluator's role is influential. If the dog fails to be a service dog, finding a new adoptive family for it is difficult. Several researchers tried to evaluate dog temperament by using a puppy aptitude test [13, 14]. This test includes exposing puppies to a set of test situations conducted by a test leader, who should be a stranger to the subjects. The responses are scored according to the degree of dominance. This test allows evaluators to check individuals under the same test situations. Identical factors such as the same test leader, handlers, trainers, evaluators, and age of the subjects are stable. However, early testing could have disadvantages in terms of predicting actual abilities, because animals can change during their development. Thus, the reliability of early age testing is controversial.

The reliability of canine behavior has been tested in behaviors related to rehoming [15]. Moderate levels differ according to the kind of behavior. The responses of approaching kennels were moderate at 2 months' assessment; however, for general handling and grooming, the moderate level was poor. The moderate level could differ according to the behavioral factors. Assessing and predicting dogs' temperaments are important, although the responses are not moderate, as this make them rehomed to their appropriate environment and owners.

Owners' expectations are also one of the successful rehoming factors. The need for standard testing is increasing.

1.2.2. Behavior ratings of the working dog program

A specific subtest procedure could be a behavior rating method [16]. These subtests include affability and handling, leash, tug-of-war, retrieving, dark room, metal stair, unstable table, acoustic startle, visual startle, gradual visual startle, search, and gunfire subtests. Various temperaments can be tested, such as affability, competitiveness, hunting drive, environmental sureness, courage, nerve stability, hardness, liveliness, sharpness, defense drive, co-operation, prey drive, and curiosity of the subtests. The dog is scored under two kinds of situations with strangers and the test leader in the affability and handling subtests. In the leash subtest, the test leader walks the dog while repeatedly changing directions. The test leader invites the dog to a tug-of-war and the dog can bite and pull on the rag for 2 min in the tug-of-war subtest. The dog is allowed to chase a tennis ball and retrieve it in the retrieving subtest, and the chase and retrieval are performed three times. In the dark room subtest, the puppy is tested to find the puppy raiser when the light is turned off, and the puppy raiser crouches at a corner without the puppy seeing. In the metal stair subtest, the dog is let go up a steep metal stairway. Reactions can be scored by different rating methods (behavioral and subjective ratings), although

their predictive validity may not matter [16]. These various subtests could be modified and applied to the evaluation systems regardless of rating methods.

1.2.3. Reliability of assessing working dogs

The predictability of testing working dogs is controversial, and researchers have tried to check the validity of the evaluation methods. Many studies have conducted scent detection tests; therefore, the role of detection dogs seems to be more important for humans. Johnen *et al*. reported the following factors that influence scent detection: target odor, type of scent detection and experimental set-up, samples, test design, dog trainer, and training procedure and dog breeds [17].

Possible bias and recommendations for best practice were suggested [17]. The target odor could come from chemicals or specific objects such as drugs or explosives. The target odor must be defined distinctly and stably, and the testing design should be adapted to the aim of the research. Environmental factors such as temperature and humidity should be standardized for the subjects as much as possible. Targets must be hidden randomly during the scent detection task. The positions of the targets must be randomized. Detailed set-up, including the number of samples, ratio of targets among the whole samples, and allowance of re-check must be described before the assessments. Negative control searches can reduce the motivation of dogs to detect [18]; therefore, careful training must maintain the high motivation status. Positive reinforcement training would be helpful after searching

for repeated non-target samples. The experimental design must be set up to maintain the probability of each sample at 50%, which could be conducted more accurately. These factors must be defined concisely.

No manual has been written about the number samples to be trained to distinguish specific odors; however, Johnen *et al*. suggested that at least 100 target and non-target samples would be needed to train for scent detection tasks [17].

1.2.4. Assessing the behavior and temperament of 1-year-old dogs

Several factors can be scored at 1 year of age [19]. Volunteer puppy raisers scored 1097 prospective guide dogs according to behavioral rating scales. This research showed the potential of reliability of assessing temperaments such as stranger-directed fear/aggression, non-social fear, energy level, owner-directed aggression, chasing, trainability, attachment, and dog-directed fear/aggression. Stranger-directed fear/aggression was scored as degree of fear and barks/growls when approached by unfamiliar children, adult, or visitors. Eight factors were extracted as stable and interpretable as follows: four kinds of fear/aggression, energy level, chasing, trainability, and attachment. Three of the factors were consistent, however, five were not. When standard factors are selected, the reliability of testing can be validated. The guide dog criteria were previously validated, and the researchers confirmed the validity of the questionnaire assessments of the dogs [19].

1.2.5. Assessing dog temperaments using a questionnaire

Determining dog temperaments is valuable for handlers and owners; however, behavioral assessment for all dogs is difficult. The same evaluators, test leaders, environments, and so on must be used in the assessment and maintained throughout the experimental period. Therefore, alternative methods have been attempted, such as a questionnaire survey. The ability or boldness of working dogs was surveyed using a questionnaire for handlers and trainers. C-BARQ consists of owner grading on a 5-point scale [20]. It was developed with 40 and 68 questions, which was increased to 101 in the early 2000s. In the development process, some traits showed correlations with their corresponding component scores, such as playfulness/distance playfulness, curiosity/fearlessness, and sociability. Correlations between C-BARQ scores and outcomes were analyzed [21], and predictability was tested by comparing early-life C-BARQ score and a later-assessed suitability [22].

When C-BARQ was translated into multiple languages, re-examination was recommended because the tendency of the results could be influenced by language or culture. Some differences in fear and aggression components were observed in Taiwanese dogs, and some difference in factor structure was observed in Iranian dogs.

1.2.6. Use of working dogs in detection, search, and aid

Dogs are generally known to have moved from being scavengers nearby human ancestors to being considered human's best friend or even as a family member, and the beginning was about 30,000 years ago [10]. Dogs have played many roles such as companions and working colleagues of humans. The contributions of dogs in human life are varied as follows: pulling sleds, livestock guarding, herding [23], SAR [24], explosive detection [25], military work [26], guiding the blind [6], quarantine detection [27], and human scent identifications in forensic work [28]. Other dogs have shown the ability to detect melanoma [29], estrus in cows [30], microbial growth in buildings [31], insect pests in timber [1], and nematode infections in sheep feces [32]. Many dogs have protected humans from danger, detected forbidden materials, and found wounded people successfully.

Dogs can be trained to find wild animals such as tuataras and geckos, and even could discriminate between different reptile scents [33]. Monitoring populations and locating threatened animals are important parts of conservation research or work. Therefore, various monitoring methods are used to find animals, such as electronic monitoring devices or camera traps. Conservation detection dogs can find individual animals, their feces, and latrines noninvasively. Detection dogs have shown the ability to aid conservation programs and achieve high success rates at detecting target scents, which are 97.8% for tuataras and 86.7% for geckos [33]. These studies suggested that target scents could be expanded more afterward and

the abilities of detection dogs could be improved by conducting various training. The next step would be the development of training methods and their applications.

1.3. Animal cloning by SCNT

Cloning of animals by SCNT has been successful, beginning with the birth of a lamb named Dolly, which was derived using adult somatic cells. Since then, a number of cloned species have been produced using SCNT, including mice [34], sheep [35, 36], goats [37], pigs [38, 39], rabbits [40], cattle [41], dogs [42] and wolves [43], [44].

Although cloned animals are genetically identical, an essential question has been raised regarding their similarities in behavioral patterns. In four cloned Holstein heifers, similar behavior trends were observed, showing higher levels of curiosity, better grooming, and more aggressive nature than controls [45]. While these results showed similarities among cloned animals, Archer *at al*. [46] reported that the behavior of cloned pigs was not consistent with that of naturally bred pigs. Many studies are in progress to discover the influence of genetic identity on behavior. In several species, the behavioral similarity of cloned animals has been reported, however, research on the behavior of cloned dogs has not been conducted yet.

1.4. Canine SCNT

1.4.1. Generation of a cloned dog

A cloned dog was successfully produced in 2005 from a male Afghan hound and was named “Snuppy” (for Seoul National University puppy) [42]. This was the first successful trial of the use of SCNT in dog cloning, and three female cloned Afghan hounds were born the following year [47]. A cloned toy poodle was successfully produced from a 14-year-old donor in 2006 [48], and an endangered gray wolf was cloned by SCNT in 2008 [44]. Wolf cloning was conducted via interspecies SCNT; therefore, this success showed the potential usefulness of the dog cloning technology for the conservation of canine species. Dog cloning was applied to produce working dogs with high performance, and seven cloned drug detection dogs were produced in 2007 [49]. Transgenic dogs were successfully produced using the dog cloning technique, such as the red fluorescent protein (RFP)-expressing dog [50] and green fluorescent protein (GFP) dog [51]. The RFP dog continuously expressed the fluorescent protein and GFP gene was conditionally expressed. Dog cloning by SCNT has been practiced since 2005, and various cloned dogs have been produced since then. This will be continued and tried in expanded fields [52].

1.4.2. Generation of drug detection dogs by SCNT

Drug detection dogs were cloned to produce high-performance dogs without surplus animals [49]. A donor dog was selected by trainers and handlers from the Detection Dog Training Center, and cloned dogs were produced using canine SCNT [49]. The donor cells were obtained from ear skin tissue of an elite drug detection dog (7 years old), injected in the perivitelline space of enucleated *in vivo* matured dog oocytes, fused with electric stimulation using an electro-cell fusion apparatus (Nepa Gene Co., Chiba, Japan), and then activated chemically. *In vivo* matured dog oocytes were collected from bitches. They were not treated with any hormones or drugs for estrus induction during the whole experiment time. By using a microsatellite analysis, the cloned puppies and donor dog were confirmed to be genetically identical [49]. They were trained as drug detection dog candidates at the Dog Training Center, and six of the seven dogs passed the selection test and have become detection dogs.

1.5. Past and present quarantine systems

The word *quarantine* implies its history, which came from the Italian word *quaranta* [53]. It means “forty” and a detention of 40 days to protect against the bubonic plague [54]. The first attempt of systematic application of maritime quarantine was the detention 40 days from arriving at the port of Venice, if pestilence occurred. Today, we have a shorter period such as 5 days for cholera, 5 or 6 days for yellow fever, 7 days for plague, 12 days for typhus fever, and 14 days for smallpox; patients with leprosy are not allowed to land at all [53]. Currently, the quarantine system is expanded to all kinds of travelers and cargo (land, sea, and air). News about the severe acute respiratory syndrome (SARS), a contagious disease with a 10-day quarantine period, easily spread [55]. Quarantine management includes plant and animal diseases, as well as human disease. As invasive alien species could be threats to biosecurity, environment, and food productivity [56], many methods are used to screen passenger baggage for detecting agricultural quarantined materials [57]. Fast and non-invasive inspection methods are used, such as X-ray inspection [57] or detection by working dogs [27], to prevent entry of agricultural materials (e.g., fresh fruits and meat, which can transmit diseases to others). When people return to their countries after going abroad, the Animal and Plant Quarantine Agency requires them to be disinfected at the airport, especially if they work at animal-related institutions, farms, livestock products markets, and so on. Agency clerks check several times whether they show unusual symptoms for a couple of weeks.

2. General objective

The purpose of this study is to analyze the behavior and personality of cloned working dog candidates at different developing stages. This study is focusing on one of the quarantine methods, quarantine detection dogs. Thus, this thesis consists of 5 parts. In part I; as a general introduction, the reason and background of this study design were explained. In part II; general methodology used in this study was described. In part III is explaining analysis of behavior and personality by Puppy Aptitude Test. Modified PAT was used to find out temperaments of the puppies. In part IV, ability of cloned working dog was proven by comparing with naturally bred dogs. Their traits were analyzed by selection test from Quarantine Detection Dog Training Center and the questionnaire survey. Lastly, a final conclusion of this research was described in part V.

PART II

GENERAL METHODOLOGY

1. Care and use of animals

After birth, all of the puppies were monitored for 24 hours as neonatal care until they became one week old. They stayed with their surrogate mothers by the weaning period. They were cared for by the same keepers and handlers. Their living quarters and food were identical. The puppy care system and welfare were in accordance with guidelines of the Quarantine Detector Dog Training Center. Drinkable water was always served and dog food (Royal Canin, U.S.A.) was given once a day and dogs had their own space to eat and sleep which were cleaned daily. All of the puppies could meet and communicate with each other for socialization and do exercises such as running and obstacles at the center. This study was approved by the Institutional Animal Care and Use Committee (IACUC No.; SNU-121130-1) of Seoul National University.

2. PAT

Each puppy stayed alone for about 5 min in the empty test area before the test started, since it needed time to become comfortable. A total of 10 puppies were tested according to Volhard and Bartlett's criteria [58, 59]. The PAT consists of ten subtests as follows:

(1) Social Attraction (SA): After the puppy is placed in the test room, the TL moves in the opposite direction away from the puppy. In a crouched position, he claps his hands to get the puppy's attention. If the puppy comes,

he pets it gently.

(2) Following (F): The puppy is placed in the test room and the TL walks away, making sure he gets the puppy's attention.

(3) Restraint (R): The TL holds the puppy and places it on its back on the floor, holding the puppy down with one hand on its chest for 30 s.

(4) Social Dominance (SD): The TL holds the puppy gently and strokes it from the top of its head to the tail for 30 s.

(5) Elevation Dominance (ED): The TL holds the puppy around its chest with both hands (fingers interlaced) from a short distance above the floor for 30 s.

(6) Retrieving (Rt): The TL crouches beside the puppy and attracts its attention with a crumpled up cloth ball. When the puppy shows interest, the TL tosses the ball 1-2 metres in front of the puppy, encouraging him/her to retrieve it.

(7) Touch Sensitivity (TS): The TL takes the puppy's webbing of one front paw and presses it lightly until the TL gets a response. The TL gradually increases pressure by 1/3 on the whole web and stops as soon as the puppy pulls away or looks uncomfortable.

(8) Sound Sensitivity (SS): The puppy is placed in the centre of the room and an assistant makes a sharp noise at the side of the test room, such as striking a metal pan with another metal object.

(9) Sight Sensitivity (SiS): The TL ties a string around a large towel and jerks

it across the floor 1-2 metres away from the puppy.

(10) Stability (S): The TL opens an umbrella about 2-3 metres from the puppy and places it gently on the ground.

According to PAT [60] the puppy's responses indicate the degree of dominance; There are 6 kinds of types of dominance. If the puppy gets mostly scores of 1, this dog is extremely dominant and has to be trained by an experienced handler. In case of mostly scores of 2, the puppy is dominant and can bite. Firm, consistent, and fair handling is needed. It may be too active for elderly people and too dominant for small children. If the dog gets mostly scores of 3, it is best for the average owner and also good with the elderly and children. A dog with mostly scores of 4 is submissive and slightly less outgoing than a dog that scores mostly 3. This dog gets on famously with children and trains well. Scores of mostly 5 mean that the puppy is extremely submissive and needs special encouragement in handling. It experiences difficulties with changes and frightens easily, so it is not good for a beginner. The puppy that gets mostly scores of 6 is independent and not affectionate. It is difficult to train it as a working dog or make it into a pet.

3. Campbell test

Campbell's criteria [61] were used to evaluate degree of dominance; for the detailed responses the PAT (Puppy Aptitude Test) was used [60]. These responses show the type of puppies. The order of the subtests was conducted as follows: 1) Social Attraction, 2) Following, 3) Restraint, 4) Social Dominance, and 5) Elevation Dominance. A score type of between 1 and 6 was used as a response to each subtest: (1) excessive dominance; (2) dominance; (3) balanced submission; (4) submission; (5) excessive submission; (6) independent. The test consists of 5 subtests as follows:

1) Social Attraction: After the puppy is placed in the test room, the TL moves in the opposite direction away from the puppy. In a crouch position, he claps his hands to get the puppy's attention. If the puppy comes, he pets it gently. The puppy's response is scored from 1 to 6. (1) The puppy came readily, tail up, jumped, bit at hands; (2) the puppy came readily, tail up, pawed, licked at hands; (3) the puppy came readily, tail up; (4) the puppy came readily, tail down; (5) the puppy came hesitantly, tail down; (6) the puppy didn't come at all.

2) Following: The puppy is placed in the test room and the TL walks away to the opposite direction, making sure he gets the puppy's attention. The puppy's response is scored from 1 to 6. (1) The puppy followed the TL readily, tail up, got underfoot, bit at feet; (2) the puppy followed the TL

readily, tail up, got underfoot; (3) the puppy followed the TL readily, tail up; (4) the puppy followed readily, tail down; (5) the puppy followed hesitantly, tail down; (6) the puppy did not follow or went away.

3) Restraint: The TL brings the puppy and places it on its back on the floor, holding the puppy down with one hand on its chest for 30 s. The puppy's response is scored from 1 to 6. (1) The puppy struggled vigorously, flailed, bit at hand; (2) the puppy struggled vigorously, flailed; (3) the puppy settled, struggled, settled with some eye contact; (4) the puppy struggled then settled; (5) the puppy didn't struggle, without eye contact; (6) the puppy didn't struggle, straining to avoid eye contact.

4) Social Dominance: The TL holds the puppy gently and strokes it from the top of its head to the tail for 30 s. The puppy's response is scored from 1 to 6. (1) The puppy jumped, pawed, bit, growled; (2) the puppy jumped, pawed; (3) the puppy cuddled up to TL and tried to lick face; (4) the puppy squirmed, licked at hands; (5) the puppy rolled over, licked at hands; (6) the puppy went away and stayed away.

5) Elevation Dominance: The TL holds the puppy around its chest with both hands(fingers interlaced) a short distance above the floor for 30 s. The puppy's response is scored from 1 to 6. (1) The puppy struggled fiercely, tried to bite; (2) the puppy struggled fiercely; (3) the puppy struggled, settled, struggled, settled; (4) the puppy didn't struggle, relaxed; (5) the

puppy didn’t struggle, its body was stiff; (6) the puppy didn’t struggle, froze.

4. Selection test

To be an excellent working dog is not easy. Researchers have been studying dogs' temperament to select suitable working dogs more efficiently. In drug detection dog candidates, concentration, interest in target, general activity, anxiety and obedience training were highly influential factors [9]. In selection test of assistance dogs fear and submission trait was predictable [3]. Search dog handlers and trainers appeared to think several traits are more important than others and these are as follows: acuity, hunting by smell alone, learning by reward, tendency to be distracted, agility, consistency of behavior, etc [62]. It is reported that the success rate of dog training is approximate 30% [9] or 50% [6]. This means even though candidates participate in the training, 50-70% dogs would be excluded. It can be another problem to find other jobs or family for them except the time and cost of training. If we could estimate temperament and future behavior in advance, would we reduce time and cost to train them? Application of dog cloning to the excellent detection dog production has begun from this idea. The steps of the testing in this study is presented figure 1.

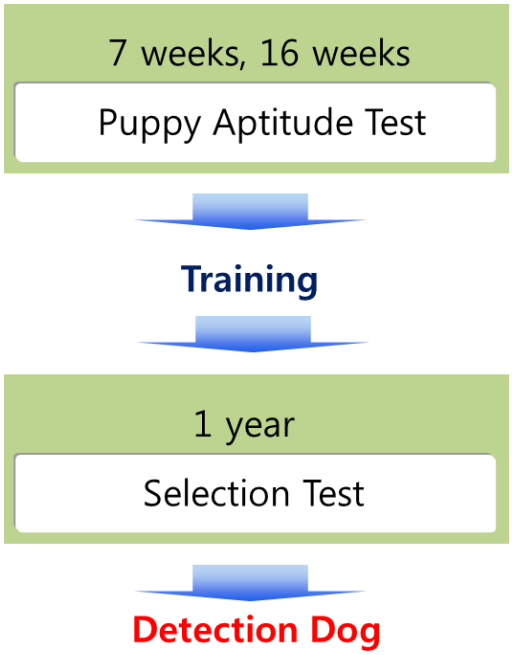

Figure 1. Steps of the testing from the puppies to adult working dogs.

PART III

BEHAVIOR AND PERSONALITY ANALYSIS WITH PAT

Chapter I. Personality consistency analysis in cloned dog

1. Introduction

In recent research, personality consistency has become an important characteristic. Diverse traits and human-animal interactions, in particular, are studied in the field of personality consistency in dogs. Here, I investigated the consistency of dominant behaviours in cloned and control groups followed by the modified Puppy Aptitude Test, which consists of ten subtests to ascertain the influence of genetic identity.

Much effort has been made to assess the personalities of dogs to predict the temperaments of companion animals and to select working dogs more efficiently. Potential dog owners likely want to know their family member's expected characteristics. Bartlett [59] explained how to select a family dog by the Puppy Aptitude Test. Adopting suitable animals could be a beginning of a happy life for both owners and the puppies. Breeders need to check puppies' temperaments for similar reasons to dog owners. Training institutions that produce excellent working dogs focused on selecting candidates at an early age to save money and time. Svartberg et al. investigated the consistency of dogs' behaviours using a standardized behavioural test and could deduce stable traits such as playfulness, chase-proneness and sociability [63]. Substantial consistency of dogs' personalities was reported by Fratkin et al., and they have determined the factors that influence

consistency, which are age, interaction between temperament and age, and length of time interval between tests [64]. These authors did not, however, estimate the influence of genetic background on consistency [64].

Bartlett used ten subtests to analyse puppies' behaviours, including social attraction, dominant or submissive tendency under stress, willingness to work with humans and sensitivities [59]. Volhard divided the subtests into three drives, which are Pack Drive, Fight or Flight Drive, and Prey Drive [58]. Pack Drive consists of three subtests, which are Social Attraction, Following and Social Dominance. These factors seem to be related with living in a group of pack of wolves. Fight or Flight Drive includes the subtests Restraint, Elevation Dominance and Stability. These characteristics are relevant to the ability to struggle or run away. The subtests Retrieving, Sound Sensitivity and Sight Sensitivity belong to Prey Drive, which is related to hunting ability.

Personality consistency is expressed as the degree of repeatability of specific traits at various intervals. Netto et al. reported that attack scores of adult dogs between two aggression tests were correlated [65] and Slabbert et al. found the predictability of behaviour tests targeted for police dogs [14]. They concluded that the most reliable tests were retrieval and aggression tests, and the significance of this research on personality consistency saved unnecessary training efforts and other costs wasted on unsuitable dogs [14]. In contrast, other results showed that fear, anxiety and aggression behaviours were not consistent [66]. The behaviour of cloned dogs has been studied to determine the similarities among animals [67], and

the present study attempted to assess the personality consistency of cloned dogs. This investigation was conducted by comparing cloned and control groups for consistency of their Puppy Aptitude Test (PAT) scores. We supposed that personality consistency of cloned animals could be different from naturally bred ones since their aptitude showed difference in the previous study.

Therefore, in this project I tried to answer this question: Is the personality consistency different between cloned and control groups at 7-10 and at 16 weeks? I used the Puppy Aptitude Test consisting of 10-11 subtests and examined the scores for Pack Drive, Fight or Flight Drive and Prey Drive [58]. This behaviour test has been used at the Quarantine Detector Dog Training Center (Incheon, Korea) to evaluate puppies and improve training methods.

2. Materials and Methods

2.1. Subjects

In this study, six cloned and four control beagle puppies (*Canis familiaris*) were compared by the modified Puppy Aptitude Test [58, 59]. Cloned puppies were produced by somatic cell nuclear transfer (SCNT, [42]). In brief, donor cells were derived from abdominal skin of a 10 year old male beagle. This dog was selected by handlers and trainers from Quarantine Detector Dog Training Center because of his excellent ability in detecting forbidden items at the airport. The role of quarantine detector dog, also called as quarantine dog, is to find forbidden livestock or plant products. This work contributes to disease control by preventing transmission of sources of infection from abroad. Progesterone concentration of oocyte donor dogs was monitored and *in vivo* matured oocytes were collected three days after ovulation. Cells of the excellent dog were injected into enucleated *in vivo* matured dog oocytes, and the couplets were fused by electric stimulation and chemically activated. These embryos were transferred into surrogate mothers. All six puppies were born by caesarean section from different surrogate mothers. All operations such as collecting oocytes and caesarean section were performed under a general anesthesia by isoflurane and lidocaine as pain reliever was injected after operations. Control puppies were produced by artificial insemination. I transferred several embryos into surrogate mothers and four of them were successfully conceived. Procedures to collect *in vivo* matured oocytes were same with the

cloned ones and the male dog was selected randomly as if it occurred in natural breeding. Therefore, control and cloned puppies do not have genetic relations. One of the four control puppies was born by caesarean section and three of them were born by natural birth which was judged by the state of foetuses. These three puppies were littermates and the birthdates of all of the puppies were presented in Table 1. They were raised as candidate for quarantine dogs with other puppies at the Quarantine Detector Dog Training Center. After birth, all of the puppies were monitored for 24 hours as neonatal care until they became one week old. They stayed with their surrogate mothers by the weaning period. The birthdates of the puppies were different as mentioned in Table 1, though they were cared for by the same keepers and handlers. Their living quarters and food were identical. The puppy care system and welfare were in accordance with guidelines of the Quarantine Detector Dog Training Center. Drinkable water was always served and dog food for medium-sized dogs (Royal Canin, U.S.A.) was given once a day and dogs had their own space to eat and sleep which were cleaned daily. All of the puppies could meet and communicate with each other for socialization and do exercises such as running and obstacles at the center. This study was approved by the Institutional Animal Care and Use Committee (IACUC No.; SNU-121130-1) of Seoul National University.

2.2. Testing and comparison between two groups at age 7-10 and 16 weeks

PAT consists of 10-11 subtests [58, 59]: Social Attraction, Following, Restraint, Social Dominance, Elevation Dominance, Retrieving, Touch Sensitivity, Sound Sensitivity, Sight Sensitivity, Stability, and Structure. The Structure subtest evaluates the puppy's stance and body balance, and the other 10 subtests are used in this study to score its temperament. Puppies stayed alone in the test room for 5 min before the test and the duration of each subtest was about 30 sec, so the whole period was about 12 min. A test leader (TL) conducted the PAT and 4 other evaluators including the TL simultaneously scored puppies one at a time. After evaluation, evaluators discussed the exact movements of puppies. Results of the subtests were classified as six responses (scores): (1) excessive dominance; (2) dominance; (3) balanced submission; (4) submission; (5) excessive submission; (6) independent. Both cloned and control puppies were tested at ages 7-10 weeks and again at 16 weeks. Testing at the exact same age would be better, however, the first testing was done on the same day. Therefore the puppies' ages were 7-10 weeks. The proper age for applying the PAT is 49 days [59], and the tests at age 16 weeks were carried out based on the study of Beaudet et al. [13]. The same test was conducted at the age of 7-10 and 16 weeks, and responses of these two kinds of age in each group were compared to find behavioural consistency during the training.

Scores of the ten subtests were classified as three drives according to Volhard's criteria [58]. Pack Drive is related to living in a group, and the subtests Social Attraction, Following and Social Dominance belong to this. Fight or Flight Drive is

a characteristic relevant to ability to struggle or run away. It consists of the subtests Restraint, Elevation Dominance and Stability. Prey Drive has a relationship to hunting ability and includes the subtests Retrieving, Sound Sensitivity and Sight Sensitivity. Correlations between drives were analysed to determine the connections with different temperaments.

2.3. Statistical Analysis

All the PAT scores of six cloned and four control puppies were analysed statistically by the general linear mixed model SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Scores of 10 subtests were used as dependent variables while the independent variable was the age at testing. Four evaluators were calculated as a random effect to reduce the tendency of each evaluator. Pairwise comparison was carried out to analyse differences in the Least Square Post Hoc test. P values were corrected by the Tukey-Kramer adjustment and considered as significant differences when they were less than 0.05.

Correlations were calculated to find out relationships of the Pack Drive, Fight or Flight Drive, and Prey Drive. Scores of the subtests were sorted by drives before analysis. Spearman rank correlations were used and *P* values less than 0.05 were considered as significant.

3. Results

3.1. Consistency between 7-10 weeks and 16 weeks

Scores at 7-10 and 16 weeks old in cloned and control puppies were compared to evaluate the consistency across the training period. Figure 2 shows the average PAT scores at the ages of 7-10 and 16 weeks in cloned and control puppies. Scores of the cloned group were consistent ($F_{1,35}$=0.07, P=0.7991), while those of the control group were not ($F_{1,20}$=8.4, P=0.0089). Average scores of each individual in clone and control puppies are presented in figure 3, figures 4 and 5 show the scores of each subtest in control and cloned groups.

3.2. Consistency of 3 drives

Scores of 3 drives were analysed and are presented in figure 4. Scores of Pack Drive were consistent in cloned puppies ($F_{1,40}$=0.3, P=0.59), but not in control puppies ($F_{1,23}$=7.6, P=0.0112). Scores of Fight or Flight Drive also showed consistency in cloned puppies ($F_{1,40}$=0.11, P=0.7457) while they did not in control puppies ($F_{1,23}$=17.81, P=0.0003). Scores of Prey Drive were not consistent in both cloned ($F_{1,40}$=20.2, P<0.0001) and control puppies ($F_{1,23}$=15, P=0.0008).

Table 1. Birthdate of cloned and control puppies

Individual	Birthdate
Clone 1	2012. 1. 11.
Clone 2	2012. 1. 19.
Clone 3	2012. 10. 23.
Clone 4	2012. 10. 25.
Clone 5	2012. 10. 25.
Clone 6	2012. 10. 25.
Control 1	2011. 12. 27.
Control 2	2012. 1. 5.
Control 3	2012. 1. 5.
Control 4	2012. 1. 5.

Table 2. Spearman rank correlations between drives of PAT in cloned and control puppies

Group and age		Correlations between drives		
		Pack - Fight or Flight	Pack - Prey	Fight or Flight - Prey
Control	7-10 weeks	0.64**	–0.05	–0.02
	16 weeks	0.60*	0.56*	–0.02
Clone	7-10 weeks	–0.12	0.30	0.17
	16 weeks	–0.06	0.19	0.28

*$P < 0.05$; **$P < 0.01$.

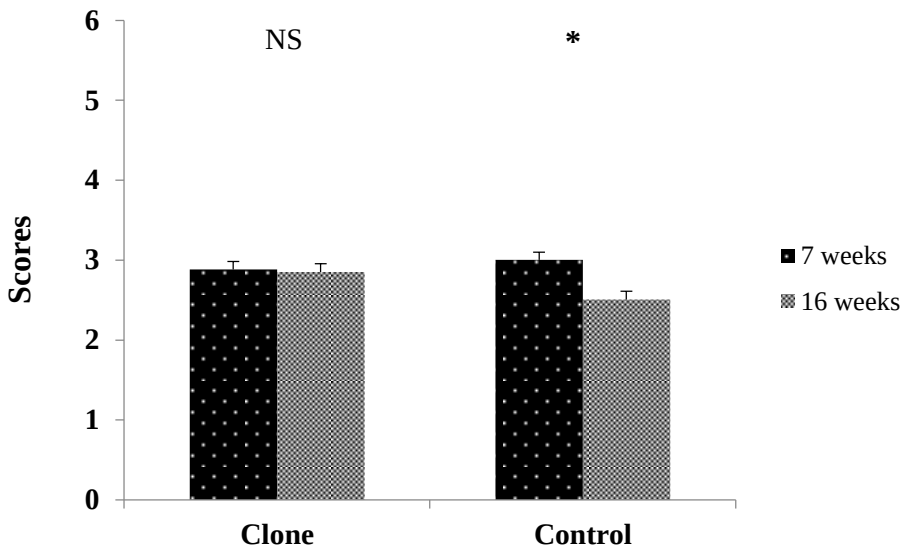

Figure 2. Scores of PAT at age 7-10 weeks and 16 weeks in cloned ($F_{1,35}$=0.07, P=0.7991) and control groups ($F_{1,20}$=8.4, P=0.0089).

NS: not significant, *: P-value < 0.01; general linear mixed model.

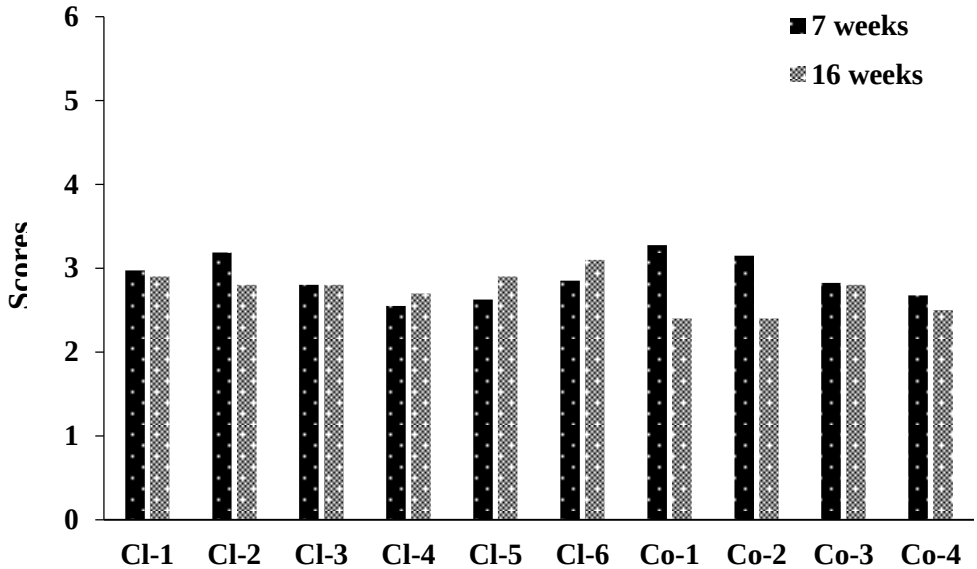

Figure 3. Average scores of each individual of PAT at age 7-10 weeks and 16 weeks in cloned (Cl-1, Cl-2, Cl-3, Cl-4, Cl-5, Cl-6) and control (Co-1, Co-2, Co-3, Co-4) groups.

Cl-1: Clone 1, Cl-2: Clone 2, Cl-3: Clone 3, Cl-4: Clone 4, Cl-5: Clone 5, Cl-6: Clone 6, Co-1: Control 1, Co-2: Control 2, Co-3: Control 3, Co-4: Control 4

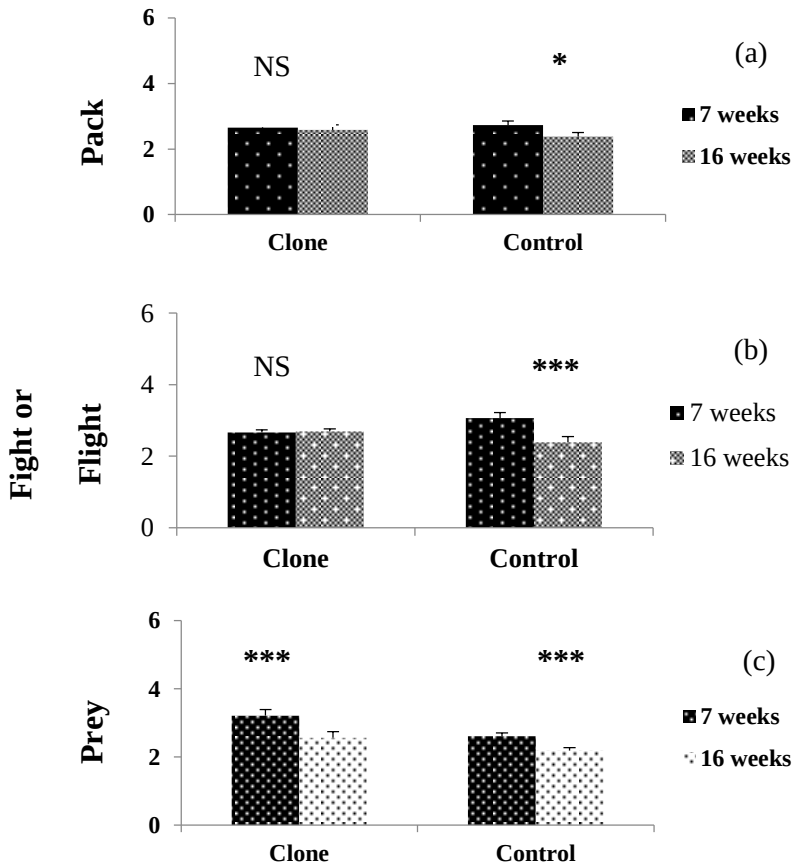

Figure 4. Scores of 3 drives of PAT in cloned and control puppies at age 7-10 and 16 weeks. (a) Pack Drive in cloned ($F_{1,40}$=0.3, P=0.59) and control puppies ($F_{1,23}$=7.6, P=0.0112). (b) Fight or Flight Drive in cloned ($F_{1,40}$=0.11, P=0.7457) and control puppies ($F_{1,23}$=17.81, P=0.0003). (c) Prey Drive in cloned ($F_{1,40}$=20.2, P<0.0001) and control puppies ($F_{1,23}$=15, P=0.0008).

NS: not significant

*: P-value < 0.05; ***: P-value< 0.001;

general linear mixed model.

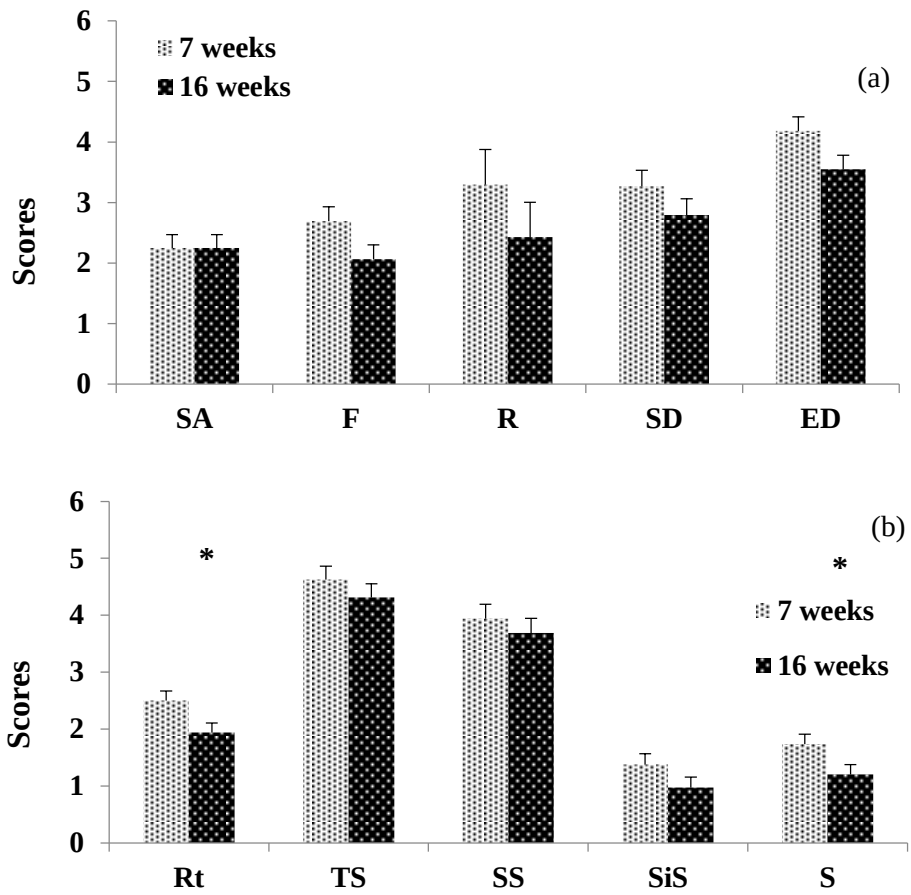

Figure 5. Consistency Scores in PAT at age 7-10 weeks and 16 weeks in control puppies. (a) Subtest Social Attraction (SA), Following (F), Restraint (R), Social Dominance (SD), and Elevation Dominance (ED) were not significantly different between ages. (b) Subtest Retrieving (Rt) and Stability (S) were significantly different between ages. Subtest Touch Sensitivity (TS), Sound Sensitivity (SS) and Sight Sensitivity (SiS) were not significantly different. *$P < 0.05$; general linear mixed model.

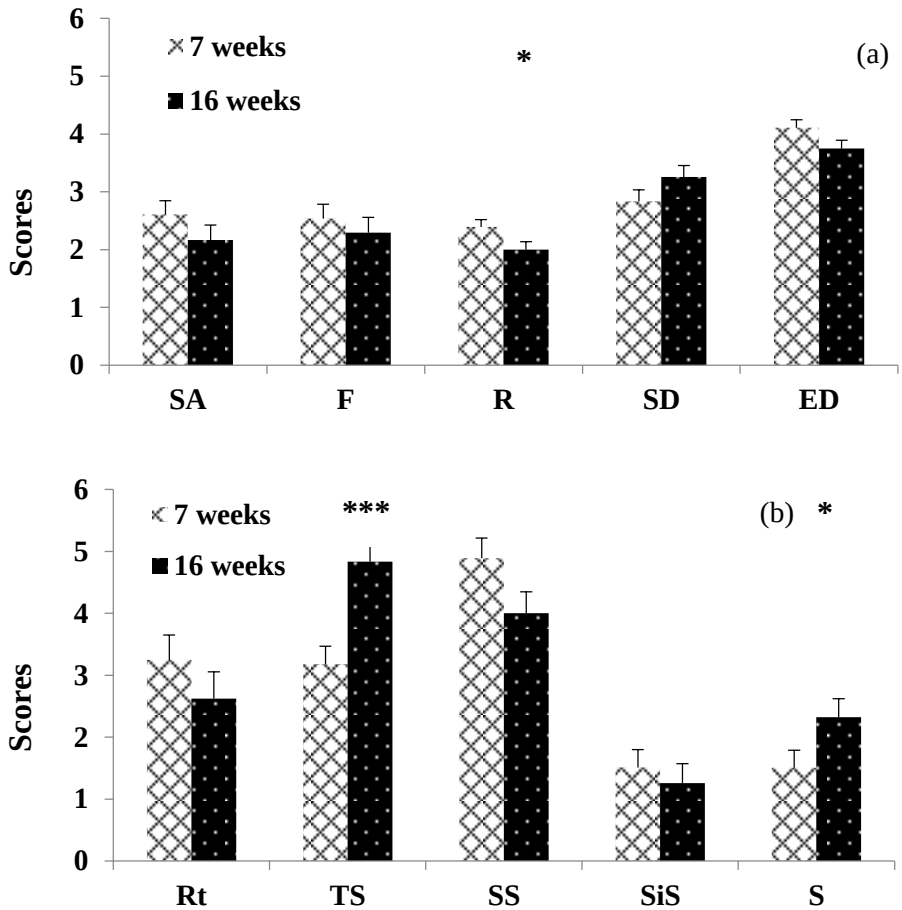

Figure 6. Consistency Scores of PAT at age 7-10 weeks and 16 weeks in cloned puppies. (a) Subtest Restraint (R) showed a significant difference between ages. Subtest Social Attraction (SA), Following (F), Social Dominance (SD), and Elevation Dominance (ED) were not significantly different. (b) Subtest Touch Sensitivity (TS) and Stability (S) were significantly different between ages. Subtest Retrieving (Rt), Sound Sensitivity (SS) and Sight Sensitivity (SiS) were not significantly different. *$P < 0.05$; ***$P < 0.001$; general linear mixed model.

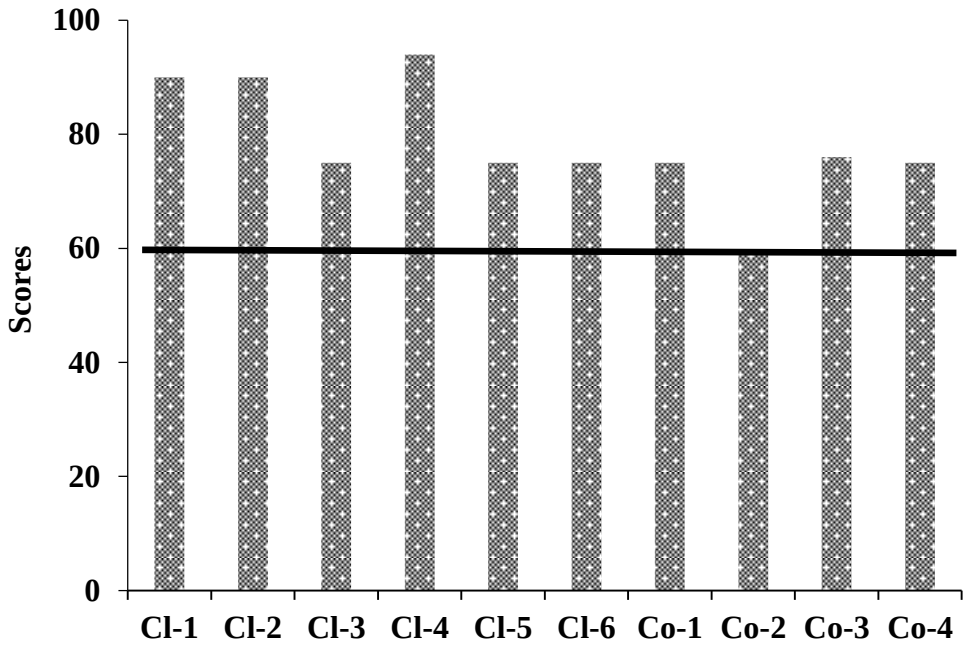

Figure 7. Scores for final selection test in cloned (Cl-1, Cl-2, Cl-3, Cl-4, Cl-5, Cl-6) and control (Co-1, Co-2, Co-3, Co-4) puppies. The solid line indicates the pass mark.

Cl-1: Clone 1, Cl-2: Clone 2, Cl-3: Clone 3, Cl-4: Clone 4, Cl-5: Clone 5, Cl-6: Clone 6, Co-1: Control 1, Co-2: Control 2, Co-3: Control 3, Co-4: Control 4

4. Discussion

In this study, I compared the consistency of personality in cloned and control puppies by the modified Puppy Aptitude Test. The scores of the two groups showed different tendency. Average PAT scores were consistent in the cloned group at 7-10 and 16 weeks, but not in the control group. This indicates that the overall temperament of young dogs could be maintained more consistently in cloned animals. These results will be subjected to further study to validate age of testing data by comparing them with the final results of selection tests for suitability as detection dogs.

My results could be interpreted as cloned dogs exhibiting consistency in degree of socialization with humans, adaptability to the training, and expression of fear. This may suggest that the same training procedures through this period would be suitable for all of them, since it may be necessary to change training styles according to the interpretation of scores [59]. A competent trainer is needed since the extremely dominant dog and the extremely submissive dog needs special handling to instill confidence [59]. In contrast, in the control group all of the drives and the averages were inconsistent. My previous study showed that cloned puppies could be classified into the same group by the Campbell test [67], and the behaviours of the cloned group were more consistent than the control group in this study. While my previous study was conducted with Labrador retrievers, this study was performed with beagles. I proposed to compare these two studies since it could

be more meaningful to analyse the result within breed than between breeds [68]. These two studies were conducted by similar puppy tests which evaluate dominant behaviours and aptitudes of them. The first research focused on the similarity of cloned animals and the second one aimed at the difference between cloned and control groups. Cloned Labrador retrievers were categorized as the same type and cloned beagles showed more consistency in their behaviours. As far we have gotten, cloned dogs seemed to share more similar and consistent behaviours than naturally bred ones, however my further study will be able to present various aspects of cloned animals.

Dog behaviour related to pack could be examined in free-ranging dogs: Cafazzo et al. reported that dominance rank positively affects male copulation and female reproductive success [69]. However, in domesticated environments, instead of mate preferences, dominant individuals tend to be outgoing and active [59]. Therefore, it is supposed that cloned puppies have a same degree of activity at 7-10 and 16 weeks while control puppies do not. The subtests of Pack Drive include social attraction to people, willingness to follow a human, and acceptance of a human's social dominance [58]. The results of cloned dogs were similar to another human-related study by Vas et al., which reported that dogs' responses to human behavioural cues were consistent over repetition [70]. Figures 5 and 6 show more detailed scores for these three subtests (SA, F, and SD), and consistency or inconsistency of each group is presented in figure 3. The control group of my study seemed different from normal in this aspect.

Fight or Flight Drive includes testing the degree of struggling under restrained conditions or situations that cannot be controlled by the puppy, and the startle response [58]. This drive would be related to ability to run away from danger, and calm themselves after startling stimuli. These results could imply that cloned puppies were more consistent in play-fight behaviours than those of control puppies, as presented in figure 4. (Figure 5 and 6 provide the scores of each subtest). Riemer et al. reported that struggling responses varied in potential conflict tests of border collie puppies [71], and playful fighting appeared to be a heritable factor in German shepherd [72]. Therefore, this trait could normally be different among individuals. If so, the reason for the consistency of this response in cloned puppies would be their genetic identity.

The results of Prey Drive showed inconsistency in both cloned and control groups. Therefore, we could suppose that temperaments related to hunting could be influenced by more than other traits such as age or developmental stage. It could be expected that the consistency of each group will become stronger by considering other studies: adult dog personality consistency has been reported to be significantly greater than that of puppies [64, 73] and this tendency was similar in humans [74]. It is also possible that the inconsistencies in Prey Drive were caused by different experiences, since in a study of Swedish Flatcoated Retrievers, almost all traits related to hunting behaviour were affected by test leader, object and previous experience, not by age or sex [75]. The active hunting behaviour can also be related to dominance, because when a wolf pack has a prey, the pack leaders are

allowed to feed first by a hierarchy [76]. Therefore, this suggests that inconsistency in Prey Drive may be derived from changing hierarchies during the period of growth of the puppies. Because of this reason, difference of the first test age as 7-10 weeks could have affected changing hierarchies, however, testing on the same day may reduce the data noise. If these traits could be changed more easily, training focused on for the part related to Prey Drive will be effective. In Prey Drive, the subtest Retrieving showed a different aspect. In the cloned group, scores of Retrieving were consistent from 7-10 weeks to 16 weeks, however, the scores of the control group were not consistent; see figure 4 and 5 for more information. The cloned group maintained the scores of subtest Retrieving as 3, and this score indicates the degree of willingness for human and acceptance of training [58]. These results can provide support for a genetic role in human-animal interaction. Wang et al. [77] insisted parallel selection in humans and dogs, and my results could be considered to be in line with that conclusion.

In the control group, the tendency for consistency was determined in eight subtests except for average and two subtests. These results imply that behaviours of control dogs could be consistent when each factor is analysed separately. The consistent subtests were Social Attraction, Following, Restraint, Social Dominance, Elevation Dominance, Touch sensitivity, Sound sensitivity and Sight sensitivity. Other subtests were consistent although the average was not. However, the overall temperament including three drives were not consistent at all, therefore, it could be deduced that the personality of each individual is not repeatable. It may be

concluded that the factors underlying the dominant behaviours of puppies could usually be maintained, however, overall traits are not consistent.

Correlations between drives suppose that scores of drives cannot be predicted by other drives because they were not correlated with each other in the present study and this is the first trial to compare drives, from best of my knowledge. A correlation coefficient table is provided in Table 2. The cloned group did not show any correlations between drives. Only one cloned group participated in my analysis, and if we could investigate more cloned groups later, the reason for this result could be determined more clearly. However, there are two possible explanations for this: either only this genotype does not show any correlations, or this phenomenon can be observed in most cloned groups. Each drive could be considered as representative of a different temperament, and each of them may be influenced by other genes, since recent studies revealed associations of different genes with behaviour traits (e.g., activity level with *SLC1A2* and COMT [78]; aggression with *DRD1*, *HTR1D*, *HTR2C*, and *SLC6A1* [79]). This can be another crucial research study in the future. Ruefenacht et al. reported that heritability of seven different traits varied in German Shepherd dogs, and analysed traits were self-confidence, nerve stability, defence drive, and so on [80]. It is also possible that some traits are influenced more by genetic background than others (i.e., some genes could be expressed more). Therefore, we have to consider heritability as well as the gene itself.

After training courses lasting 10 months, all of the ten puppies were evaluated as potential quarantine dogs, as presented in figure 7. All of the six cloned puppies passed the selection test, however, only three of the four control puppies were scored to have the appropriate ability. This result may be related to the consistency of PAT scores at 7-10 and 16 weeks, however, in order to determine correlation between the PAT and the selection test, more studies during the whole training courses will be necessary. Until now, it is supposed that clone dogs have less variation during the early training.

My study analysed consistency of behaviour and went a step forward by adding comparisons between cloned and control groups of puppies. Results of this study could be a report that cloned dogs have more similar aptitude at their developing stage. In addition, this research indicates that personality consistency can be used as a part of puppy aptitude analysis as well as dominance and character. Moreover, cloning of an excellent dog by somatic cell nuclear transfer can produce individuals that have less variations than those from natural breeding. In the practical part, earlier selection of cloned dogs for quarantine training will be possible since their traits are consistent.

Chapter II. Puppy Aptitude Test in cloned rescue dogs

1. Introduction

Dogs are man's best friends. Dogs and humans have had a close relationship for thousands of years and during that time their bonds have become greatly strengthened. The dog is one of the most intelligent and thoughtful animals. It also exhibits some outstanding abilities, for example, the sense of smell in dogs is on average 10,000 to 100,000 times better than in humans [81] ; even the best odour detection machines cannot outperform elite dogs. Because of such attributes, dogs have been bred for many useful purposes such as 1) assisting humans in hunting and driving livestock, 2) guarding humans from dangerous predators, 3) being a close companion, and 4) being a valuable tool in society as service animals [82, 83]. Service dogs have special roles to provide security, detect drugs and explosives, assist the blind and save human lies [83-85]. However, it is difficult to produce dogs that are completely suitable as service animals because of the extensive training period and costs, so that the production efficiency of general service dogs is about 30 % [85].

In the Korean National Emergency Management Agency, there is a retired veteran rescue dog named Baekdu that performed lifesaving activities worldwide for six years. Baekdu demonstrated excellent discretion in the International Rescue

Dog Organization as well as in domestic/international disaster situations. Here, we considered canine somatic cell nuclear transfer (SCNT) for the preservation and propagation of Baekdu's abilities. Canine SCNT is a useful assisted reproductive technique for producing pet dogs [48, 86], endangered canids [43, 44] and transgenic dogs [50, 51, 87], but the overall efficacy of the process is still low. In order to improve this low efficiency, two basic activation media used for reconstructed oocytes were compared in terms of *in vivo* development of cloned embryos. The first medium is modified synthetic oviduct fluid (mSOF) which is a culture medium containing BSA (bovine serum albumin) which a protein preparation that has variable, undefined functions and is used in my laboratory for dog cloning [87, 88]. The second medium is porcine zygote medium (PZM-5) which is a chemically-defined medium with a synthetic polymer, PVA (polyvinyl alcohol) [89, 90]. Thus, the purpose of the present study was 1) to clone Baekdu and to compare *in vivo* development of canine cloned embryos produced using two oocyte activation media, and 2) behavioral analysis of Baekdu clones through the puppy aptitude test which is performed primarily to evaluate basic aptitudes of puppies as service dogs [91, 92].

2. Materials and Methods

2.1. Chemicals

Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise stated.

2.2. Subjects

In the study, 30kg mixed origin, large-breed bitches (12 oocyte donor dog and 7 embryo transfer recipients) between 1 and 5 years of age were used. All animal care and experiments carried out in accordance with the Guide for the Care and Use of Laboratory Animals established by the Institutional Animal Care and Use Committee of Seoul National University (Approval number; SNU-121123-13).

2.3. Preparation of donor fibroblasts

Ear skin tissue was collected from a 10-year-old male German shepherd, a veteran rescue dog, Baekdu. The tissue was washed 3 times using PBS (Dulbecco's Phosphate-Buffered Saline; Invitrogen, Carlsbad, CA, USA), minced, cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% (v/v) FBS (Invitrogen) at 38℃ in a humidified atmosphere of 5% CO2 and 95% air. After 7 days of incubation, a fibroblast monolayer was established, passaged, cryopreserved in 10% dimethyl sulfoxide (DMSO) and stored in liquid nitrogen. Cells from passages 3 to 5 were used as donor cells for SCNT.

2.4. SCNT

Canine *in vivo* matured oocytes were recovered by aseptic surgical procedures 70–76 h after the day of ovulation which was considered as the day when the serum progesterone concentration reached 4.0–9.9 ng/ml as described previously [93] . Oocytes surrounded by cumulus cell layers were denuded by repeated pipetting in HEPES-buffered tissue culture medium (TCM)-199 supplemented with 0.1% (w/v) hyaluronidase. The first polar body and metaphase II spindle of denuded oocytes were removed under an inverted microscope equipped with fluorescence. One donor somatic cell was injected into the perivitelline space of each enucleated oocyte, then fused with electric stimulation using two pulses of direct current of 72 V for 15 μsec with an Electro-Cell Fusion apparatus (NEPA GENE Co., Chiba, Japan). The fused couplets were activated by calcium ionophore treatment for 4min, and then cultured in mSOF medium supplemented with 1.9 mM 6-Dimethylaminopyridine (DMAP) (SOF-DMAP), or in PZM-5 supplemented with 1.9 mM DMAP (PZM-DMAP) for 4h.

2.5. Embryo transfer and pregnancy diagnosis

After activation, SCNT couplets were immediately surgically transferred using a 3.5-Fr Tom Cat Catheter (Sherwood, St. Louis, MO, USA) into the ampullary portion of the oviducts of naturally synchronous recipients. Recipients were prepared by predicting ovulation time based on serum progesterone concentrations.

Pregnancy diagnosis was assessed with a SONOACE 9900 ultrasound machine (Medison, Seoul, Korea) approximately 31 days after embryo transfer.

2.6. PAT

The PAT of this experiment used Volhard PAT [94] with modification. The PAT used a scoring system from 1-6 and consisted of ten tests: Social attraction, Following, Restraint, Social Dominance, Elevation Dominance, Retrieving, Touch Sensitivity, Sound Sensitivity, Sight Sensitivity, Stability. The tests were performed sequentially and in the order listed. The tests were carried out in an area (3m x 3 m) unfamiliar to the puppy. At age 7 weeks, a puppy was tested for about 3 min, counting only the first response. There were no other dogs or people, except the tester, in the testing area. The tester was a stranger to the puppy and four scorers evaluated the puppy from outside the test area through a window at the same time. Each test was scored separately, and interpreted on its own merits. In order to preclude the nursing and raising environment, the cloned puppies were brought up suckling breast milk of same recipient (recipient 1) in same condition. The Puppies stayed with recipient 1 till 7 weeks of age when they were weaned.

2.7. Microsatellite analysis for genotyping

Parentage analysis was performed using genomic DNA from the oocyte donor dog, donor cells, cloned puppies and recipients. Genomic DNA was extracted from blood or tissue samples or cell pellets according to the instructions provided with

the G-spin Genomic DNA Extraction Kit (Intron Biotechnology, Seongnam-si, Gyeonggi-do, Korea), and analyzed using microsatellite assays with canine-specific markers [42, 49]. Based on the complete nucleotide sequence of canine mitochondrial DNA (mtDNA; GenBank accession number U96639), oligonucleotide primers were synthesized for the hypervariable region as described in previous studies [42, 49].

2.8. Statistical analysis

Statistical analysis was performed with GraphPad Prism 5 software (GraphPad, San Diego, CA, USA). Statistical significance of i*n vivo* development of canine cloned embryos was analyzed using Column Statistics. Dog behavior data was analyzed by two-way ANOVA with the Bonferroni post-test. Significance level was considered as $P<0.05$.

3. Results

3.1 *In vivo* development of canine cloned embryos using two different activation protocols

A total of 56 and 64 activated, cloned embryos in the PZM-DMAP and SOF-DMAP groups were transferred into three and four female recipient dogs, respectively. Pregnancy was detected in one out of the three surrogate mothers for the PZM-DMAP group (33.3%), and one pregnancy (25%) was detected in the four surrogate mothers of the SOF-DMAP group (Table 3). The two pregnant dogs each gave birth to one healthy cloned puppy by cesarean section (Figure 8). The cloned dogs and the donor cells had identical microsatellite patterns for all loci (Table 4), and the mtDNA sequences of the cloned dogs were identical to those of the oocyte donor dog (Table 5).

3.2 Puppy aptitude test of the two cloned puppies

PAT was conducted to assess each puppy's temperament. A score of 1 to 6 was used to evaluate the response of each subtest. Scores of social dominance (SD) and sound sensitivity (SS) were different between the two cloned dogs ($p < 0.001$), but there was no difference in the other tests (Figure 9). From the interpretation of all 10 subtests, the two cloned puppies were classified as having the same type 3 (dogs of this type accept humans and leaders easily).

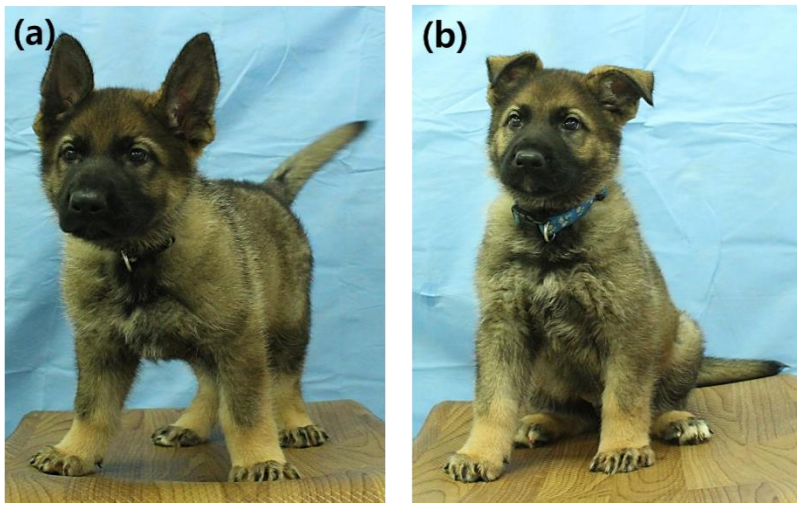

Figure 8. Two cloned puppies derived from Baekdu. (a) 8-week-old first cloned dog (b) 7-week-old second cloned dog derived from cells of Baekdu, an elite rescue dog.

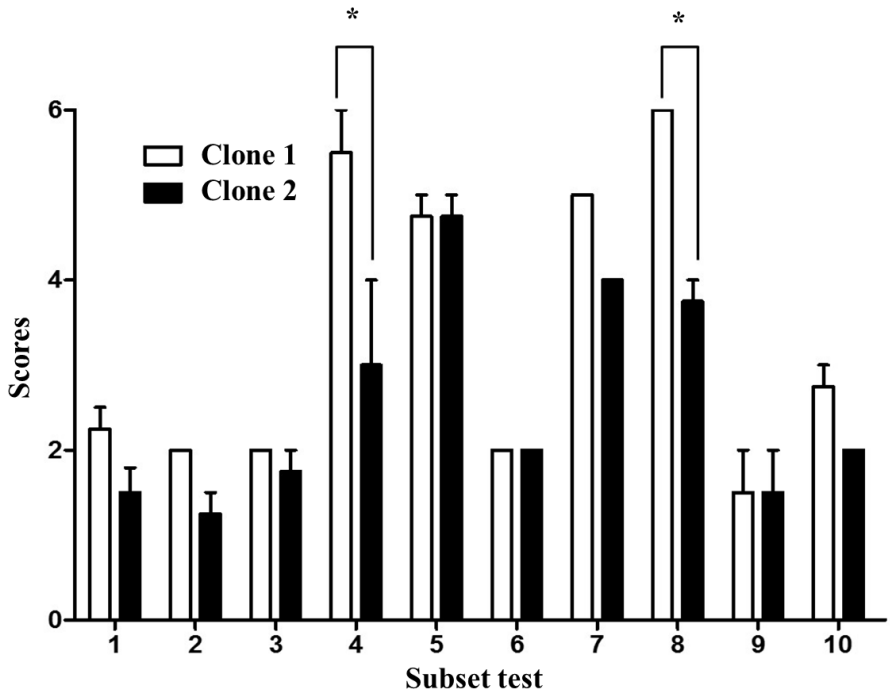

Figure 9. Average scores of two cloned puppies for each subtest (Puppy Aptitude Test). The subtests in numerical order are 1) social attraction (SA), 2) following (F), 3) restraint (R), 4) social dominance (SD), 5) elevation dominance (ED), 6) retrieving (Rt), 7) touch sensitivity (TS), 8) sound sensitivity (SS), 9) sight sensitivity (SiS) and 10) stability (S). *The value is significant when $P<0.001$.

Table 3. *In vivo* development of canine cloned embryos using two different oocyte activation protocols

Group	No. of transferred embryos	No. of recipient females	No. of pregnancies (pregnancies/ recipients)	No. of cloned pups (births/embryos transferred)	Clone ID	Status of cloned pups
SOF-DMAP	64	4	1 (25%)	1 (1.56%)	Clone 1	live
PZM-DMAP	56	3	1 (33%)	1 (1.78%)	Clone 2	live

Table 4. Analysis of genomic DNA testing with canine-specific microsatellite markers in donor cells, cloned dogs, and surrogate females.

Name	Donor cell		Clone 1		Recipient 1		Clone 2		Recipient 2	
PEZ1	123	119	123	119	123	119	123	119	115	115
FHC2054	180	176	180	176	155	155	180	176	151	151
FHC2010	236	224	236	224	232	228	236	224	224	224
PEZ5	103	103	103	103	111	111	103	103	111	111
PEZ20	180	176	180	176	196	188	180	176	177	173
PEZ12	268	268	268	268	283	283	268	268	276	264
PEZ3	128	128	128	128	124	124	128	128	131	124
PEZ6	188	176	188	176	183	183	188	176	184	184
PEZ8	226	226	226	226	238	233	226	226	233	229
FHC2079	274	270	274	270	274	270	274	270	278	274

Table 5. Sequence alignments within 661 bases of the hypervariable region of mtDNA

Sample ID	Nucleotide positions*															
Reference (U96639 v.2)	15435	15518	15526	15595	15612	15632	15639	15643	15652	15800	15814	15815	15912	15955	16003	16083
	G	A	C	C	T	C	T	A	G	T	C	T	C	C	A	A
Donor cell	G	A	C	C	T	C	T	A	G	T	C	T	C	C	A	A
Clone 1	A	C	T	T	C	T	G	G	A	C	T	C	T	T	G	G
Oocyte donor 1	A	C	T	T	C	T	G	G	A	C	T	C	T	T	G	G
Clone 2	G	A	C	C	T	C	T	A	G	T	C	T	C	C	A	A
Oocyte donor 2-1	A	C	T	T	C	T	G	G	A	C	T	C	T	T\	G	G
Oocyte donor 2-2	G	A	C	C	T	C	T	A	G	T	C	T	C	C	A	A

*The nucleotide positions were numbered from those of GenBank accession no U96639 v.2, and 661bases (from 15431 to 16091) were examined.

4. Discussion

Cloning or SCNT is one of the assisted reproductive technologies (ARTs) currently used in animal reproduction. Although SCNT is inefficient compared with other ARTs such as in vitro fertilization or artificial insemination [95], SCNT and banking of somatic cells for SCNT are still utilized to multiply elite animals with desired phenotypic traits and to produce genetically modified animals [96-99].

For animal cloning, embryo activation is a key step in the development of fertilized or cloned embryos [100]. Correct activation is essential to support normal development of cloned embryos. Generally, the combination of ionomycin or ionophore and 6-DMAP is a routine tool for the activation of cloned embryos. Ionomycin or ionophore are widely used to increase intracellular Ca^{2+} levels in activated oocytes [101]. 6-DMAP, a protein serine/threonine kinase inhibitor [102], contributes to DNA synthesis [103] and accelerates pronucleus formation in activated, reconstructed embryos. Many studies have been reported for establishing a species–specific activation protocol because optimal treatment time and concentration of 6-DMAP is critical for this step [104, 105]. However, during 6-DMAP treatment, little attention has been paid to effects of culture medium on embryo development. Therefore, the present study investigated the effects of two different culture media used for 6-DMAP treatment after calcium ionophore treatment of fused couplets on SCNT efficiency. With respect to full-term development of cloned embryos, there was no difference between oocytes activated

in SOF-DMAP and PZM-DMAP. This result shows that the type of medium used in 6-DMAP culture has no effect on dog cloning efficiency.

Next, I investigated whether the resulting cloned dogs have potential as candidate for rescue dogs. My study compared the behavioral patterns of the two cloned dogs using PAT, which can predict the performance of a puppy and its expected temperament they will have in adulthood. Also, specific tests predictive of their future employment as adult service dogs have been developed [106]. The necessity of applying the PAT at an early age has been emphasized because early prediction of a dog's adult behavior could save costs of training and reduce time wasted to train doubtful puppies. The present study was carried out Volhard puppy behavioral test with modification [94, 107]. Although the two cloned dogs did not show a perfectly identical score throughout the 10 subtests, overall they showed a similar tendency. Interpretation of the PAT scores indicated that the two cloned dogs mostly belong to type 3 that the dog accepts human leaders easily, adapts well to new situations and is generally good with children and elderly people as well as making a good obedience prospect, and it usually has a common sense approach to life [94]. Thus, the two cloned dogs were evaluated as possessing the appropriate temperament for rescue training.

Also, I performed PAT test using 7-week-old shepherds (control) born by natural mating. Six controls are littermate, and grew up in the same environment. The result was interesting as follows; two belonged to type 2, the other two belonged to type 3. And the other two control showed a result that is type 4 (data

not shown). In other words, control group derived from natural mating showed various types in PAT test, not the same as the cloned animals.

In conclusion, the present study demonstrates that dogs cloned using donor cells derived from one elite rescue dog have similar behavioral characteristics. The numbers of elite working dogs in diverse fields can be increased by using the SCNT technique with donor cells derived from a small piece of tissue from elite working dogs; this tissue fragment can be collected once only, cultured, passaged, and frozen for many future cloning applications. Further monitoring of the similarities of the performance cloned dogs in lifesaving and security activities is required and is ongoing.

PART IV

ABILITY OF CLONED WORKING DOGS

Chapter I. Are cloned dogs as competent as their donors?

1. Introduction

Working dogs play an active part in detection of human remains, drug, explosives, and quarantine, as well as in guiding and protecting humans. However, production of outstanding dogs is time and cost intensive. In case of assistance dogs, only approximately 50% of dogs were reported to become actual working dogs [3]. Therefore, dog cloning is suggested as a method to produce superior dogs more efficiently without surplus animals. The required traits of service dogs could differ according to their work; trials have been conducted to select appropriate dogs to perform particular tasks [3]. SAR or detection dogs must be energetic, patient, strong, and curious. Some traits could be more heritable than others [80], however, whether suitable temperaments are heritable has not been elucidated yet. Therefore, this study traced one of the origins to identify heritable abilities.

Scent detection testing could be limited by bias. Outcome of scent detection studies could be influenced by several factors such as target odor, type of task and experimental set-up, samples, test design, dog trainer and training procedures, and dog breeds [17]. Odorous objects release various molecules; therefore, whether dogs can recognize all or only components of mixtures is difficult to ascertain. Chemicals interact with each other inside objects; thus, the ability of dogs to detect

them is not certain even if they could distinguish the chemicals from non-target odors. The types of scent detection task could be free search, search along a gradient, and matching to a sample [17]. When these different tasks are evaluated using various methods, the reliability of the results could be biased. Samples themselves can influence the results as a bias factor. In cancer detection, bodily fluids, tissues from young and healthy individuals seem different from those collected from older patients, with a degree of difference higher than the extent between age-matched individuals [108]. Therefore, if the samples are not compared using age-matched methods, dogs can remember only the differences instead of detecting target odors. Even “hospital odors” may influence detection as a confounding factor [109]. When patient samples at hospitals and healthy samples from outside hospitals are collected, “hospital odors” proved to be an influencing factor.

Producing excellent working dogs is difficult. Researchers have been studying dog temperaments to select suitable working dogs more efficiently. In drug detection dog candidates, concentration, interest in the target, general activity, anxiety, and obedience training were highly influential factors [9]. In the selection test of assistance dogs, fear and submission traits were predictable [3]. Search dog handlers and trainers appeared to think that some traits were more important than others, and these are as follows: acuity, hunting by smell alone, learning by reward, tendency to be distracted, agility, and consistency of behavior, among others [62].

The test design is also an important aspect in detection experiments. Dogs can read the body languages of trainers and handlers, but they must be blinded to the order or position of the target samples. Therefore, a blind test is key for successful evaluation. In a similar vein, the process of training is also important. The trainers' emotional status could be reflected on the dogs. In the present study, the same trainers and handlers participated in the training and selection test to reduce their influences.

Various methods can be used to evaluate the abilities in scent detection, however, no qualified standards or guidelines have been established yet [17]. Evaluation by trainers or handlers through a questionnaire survey could be another trial to avoid possible biases. This study was conducted using selection test manuals of the Quarantine Detection Dog Training Center in Korea. These evaluation systems must be validated in the future as well.

2. Materials and Methods

2.1. Subjects

In this study, seven cloned and four control beagle puppies (*Canis familiaris*) were compared using a questionnaire survey. Cloned puppies were produced by somatic cell nuclear transfer (SCNT, [42]) using donor cells. These cells were derived from abdominal skin tissue of a 10-year-old male beagle selected by handlers and trainers from the Quarantine Detector Dog Training Center because of his excellent ability in detecting forbidden items in the airport. All the seven puppies were born by caesarean section from different surrogate mothers. The control puppies were produced by artificial insemination. The control and cloned puppies did not have genetic relations. One of the four control puppies was born by caesarean section, and three were born by natural birth, which was judged on the basis of the state of fetuses. They were raised as candidate quarantine dogs with other puppies at the Quarantine Detector Dog Training Center. The puppy care system and welfare were in accordance with the guidelines of the Quarantine Detector Dog Training Center. This study was approved by the Institutional Animal Care and Use Committee (IACUC No. SNU-121130-1) of Seoul National University.

2.2. Questionnaire survey by handlers and trainers

The subjects of this study were seven cloned dogs and four control dogs. The cloned dogs are shown in figure 10. They were evaluated by handlers and trainers from the Quarantine Detection Dog Training Center. This institute has its own manual and systems for training and selection; therefore, the selection test was conducted in accordance with these indications. The dogs were tested using a questionnaire survey on traits that were selected as important temperaments for working dogs [5].

Important traits include acuity of sense of smell, incentive to find an object that is out of sight, tendency to hunt by smell alone, stamina, health, agility, consistency of behavior from day to day, motivation to chase an object, ability to learn from being rewarded, and tendency to be distracted when searching. Evaluators received explanations about these 10 traits before the survey assessment of the dogs and could discuss their subjects freely. All of them knew the focused dogs; therefore, an accurate evaluation was possible. They scored the control and cloned dogs at the same time to prevent biased examinations. Eight evaluators participated in the questionnaire survey, and this was treated as a random factor in the statistical analysis because each answer could be influenced by individual experiences.

The success rate of dog training was reported to be approximately 30% [9] or 50% [6]. This means that even though candidates participated in the training, 50-70% dogs would be excluded. Finding other jobs or family for the excluded dogs can be another problem besides the time and cost incurred in the training. If the

temperament and future behavior of dogs can be estimated in advance, then the time and cost to train them can be reduced. Application of dog cloning for the production of excellent detection dogs began with this idea.

3. Results

The performance scores of the cloned and control dogs were compared as presented in Table 6. All the cloned candidates and three of the four control puppies passed the standard marks (score of 60). Their success rates were relatively higher at 100% and 75%, respectively, than the reported value of 30% [9] or 50% [6]. For cloned drug detection dogs, the rate was also higher at 85.7% [110]. In addition, the thoughts of the handlers and trainers from the training center could be assessed. A modified questionnaire based on the results of the study of Rooney et al. [62] was used. Eight handlers and trainers answered the questions regarding the behaviors of the puppies. Figure 11 shows the 10 traits investigated and differences between the cloned and control groups. The cloned group had significantly higher scores in seven traits, namely acuity of sense of smell (A), incentive to find an object that is out of sight (I), tendency to hunt by smell alone (T), stamina (S), agility (Ag), consistency of behavior from day to day (C), and motivation to chase an object (M).

Table 6. Selection test scores of the cloned and control dogs

Individual	Group	Scores
1	**Clone**	75
2	**Clone**	90
3	**Clone**	90
4	**Clone**	75
5	**Clone**	94
6	**Clone**	75
7	**Clone**	75
1	**Control**	75
2	**Control**	59
3	**Control**	76
4	**Control**	75

(a)

(b)

Figure 10. Cloned quarantine puppies at ages 5 and 3 months. (a) Middle, clone 1 at 5 months. (b) Left, clones 2 and 3 at 3 months.

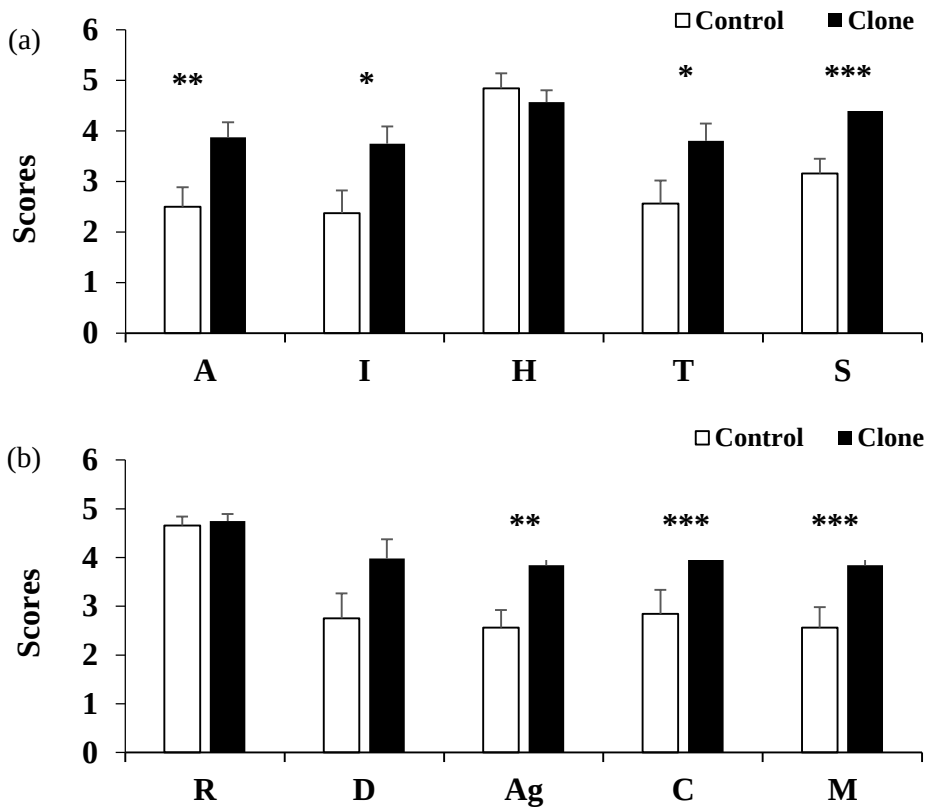

Figure 11. Scores in the questionnaire survey. (a) Trait acuity of sense of smell (A), incentive to find an object that is out of sight (I), tendency to hunt by smell alone, (T) and stamina (S) showed significant differences between the groups. Health (H) was not significantly different. (b) Agility (Ag), consistency of behavior from day to day (C), and motivation to chase an object (M) were significantly different between the clones and controls. On the other hand, ability to learn from being rewarded (R) and tendency to be distracted when searching (D) were not significantly different.

* *P*-value< 0.05; ** *P*-value < 0.01;*** *P*-value < 0.001;

general linear mixed model.

4. Discussion

This study suggests that dog cloning could be an efficient method of producing high-performance dogs. The processes to demonstrate this were cloning outstanding dogs, caring and training them with other dogs, and comparing the two groups. “Danny” was selected as a donor dog, from whom a skin tissue sample for cloning was obtained. He was chosen by handlers and trainers from the Quarantine Detector Dog Training Center because of his remarkable ability to detect forbidden agricultural materials at the airport. He was a 10-years-old beagle and retired from working but still showed excellent detecting ability when visitors came to the center. Seven cloned quarantine dogs were produced through SCNT and raised with four other dogs by the same keepers and handlers. Figure 10 shows three of the cloned dogs when they were puppies. Both the cloned and control puppies participated in training, socialization, and adaptation to the airport as their working place.

Here, the selection test and questionnaire results of the handlers and trainers were compared, and the cloned dogs showed higher scores than the naturally bred dogs. The survey results of the cloned dogs were significantly higher than those of the controls in seven traits, including acuity of sense of smell (A), incentive to find an object that is out of sight (I), tendency to hunt by smell alone (T), stamina (S), agility (Ag), consistency of behavior from day to day (C), and motivation to chase an object (M). Blinding of the dog trainer and the presence of the same people are important when tests are conducted [111]; however, some of the staffs at the center

could recognize individual dogs because they were involved in their training. This was an inevitable process in the training course. The bias was removed when the human participants avoided giving clues by reacting unconsciously, as all the evaluators were blinded to the position of the target odor.

Ten kinds of temperaments were chosen to be investigated in this research, which were surveyed as the most important traits in the questionnaire administered to 244 UK dog handlers and trainers [112]. Acuity of sense of smell refers to the degree of correctness of the dogs' findings. Accuracy could not be 100%, but dogs can work more efficiently when the rate of accuracy is high. Incentive to find an object that is out of sight could be related to the dogs' cheerfulness or passion. This could be encouraged by handlers or trainers, or by even intrinsic motivation. Unhealthy dogs could not participate in activities; therefore, good health is one of the 10 most important traits. Detection dogs usually work without other dogs, which is the reason for the importance of the tendency to hunt by smell alone. Working dogs sometimes have to stay in harsh regions; thus, their stamina is one of their most important characteristics. They must be trainable, so the ability to learn from being rewarded is key for handling. The tendency to be distracted when searching has to be low, which indicates that the dog's concentration is high. The agility of detection dogs is an essential aspect when they search at crowded airports or harbors. Excellent dogs must show consistent performances in their work; therefore, consistent of behavior from day to day is important. Lastly, the motivation to chase an object should be high; this is one of the basic principles in

training search dogs. Eight evaluators received explanations on each trait briefly and given enough time to score the detection dog candidates. They were allowed to discuss freely when they evaluated the 11 dogs. The experience of the evaluators or time spent with dogs could differ and influence the scoring; therefore, eight evaluators were analyzed as a random factor. Cloned and control puppies were evaluated at the same time, as they were considered potential candidates. Their ages were not considered in the scoring.

The 10 important traits are interconnected and manifest organically. The motivation for finding a hidden object is related to trainability, which is essential in dog training. Using olfactory cues is more important than using vision, and this could be explained in an acute sense of smell. Some traits could be ideal for companion dogs but not for working dogs. Friendliness to strangers or adapting to new people could be desirable temperaments for family dogs. However, if working dogs are too friendly to strangers and attract people's interests, this would distract a search by the working dog.

Selecting suitable detection dogs could be expanded to various fields such as explosives detection, wildlife conservation, military work [26], guiding the blind [6], and human scent detection in crime scenes [2]. Detection dogs can be trained to find wild reptiles in the wild [33]. When trained to find tuataras and geckos, dogs could identify their scent with average success rates of 97.8% and 86.7%, respectively. This study suggests that if detection dogs get appropriate training, their detection target can be expanded to various applications. In addition, the kinds

of target odor and animal species can vary. Conservation detector dogs can find individual animals, their feces, and latrines noninvasively. The role of quarantine detector dogs, also called quarantine dogs, is to search for forbidden livestock or plant products. This work contributes to disease control by preventing transmission of sources of infection from abroad.

Testing ideal traits for detection dogs is also an important part in selecting suitable dogs. Motivators are known to be important in dog training [9], and the selection test in this study included motivators such as an incentive to find an object that is out of sight and motivation to chase an object. Motivated dogs can be more trainable and entertained. On the other hand, fearfulness is considered an undesirable trait of detection dogs [10] because they work at crowded and noisy places such as airports or harbors. The question of tendency to be distracted when searching could test fearfulness or boldness.

This study was an attempt to demonstrate the detection ability of dogs cloned from an excellent dog. In this study, the excellent traits of the donor dog were observed in its cloned dogs. Therefore, dog cloning can be an efficient method to produce remarkable detection dogs.

PART V

FINAL CONCLUSION

This research assessed the aptitude that relates to the dominance of cloned dogs derived from donor cells to determine their similarities and differences. Cloned dogs showed more similar dominance behaviors and consistencies. In this research, the reason a puppy test was required was the importance of aptitude in working dogs. Puppy behavior testing has become a tool to select individual dogs for specific tasks [14]. By using the puppy test, the present research performed a comparative study of cloned dogs. The results of the comparison indicated that dogs cloned by SCNT could have more-similar behavioral patterns.

This study analyzed the consistency of behavior and went one step further by adding comparisons between cloned and control puppies. This study indicates that personality consistency, dominance, and character can be used as part of the puppy aptitude analysis, and cloning of an excellent dog by SCNT can produce individual dogs with less variations than those from natural breeding. In addition, earlier selection of cloned dogs for quarantine training will be possible because of their consistent traits.

The purpose of this research was to identify similarities of cloned dogs, and the validation of the PAT would be the next step of the study. Whether puppy testing outcome can be predicted is controversial; however, in this study, the puppy test results seemed related to adult selection testing because all the cloned puppies passed the selection test after they achieved type 1 or 2 in the Campbell test. However, in the control puppies, correlation was difficult to find. The Campbell test is generally conducted at about 6-8 weeks of age, and the selection test is

performed at about 1 year old. Wilsson and Sundren revealed that the relationship between puppy test results and the performance of adults was negligible [113]. The validation of the reliability of the Campbell test was limited because of the insufficient sample size in the present study. Therefore, the purpose of this study was to identify the influence of genotype on puppy behavior, not to establish a selection method for working dogs, although it is hope that this may eventually prove satisfactory. The puppy aptitude test was used to analyze similarities and differences between the cloned and control animals.

This study was conducted with Labrador Retrievers and Beagles; therefore, this research would be one of the important studies if the performance differences among dog breeds can be determined in more breeds. In addition, if a future study could identify which characteristics are more suitable for detection dogs, this would improve the detection dog program because little study has presented traits to be selected or avoided for detection dogs. Relevance between various traits and the abilities in the detection field would play a key role in evaluation.

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국문초록

복제 기능견 후보의 행동과 성격 분석

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수의학과 수의산과·생물공학 전공

동물 복제는 유전적으로 동일한 동물을 생산하는 효율적인 방법으로, 기능견 연구 및 실제 훈련에서 우수한 개체를 생산할 수 있게 한다. 이러한 목적과 필요성으로 우수한 기능견을 복제하는 것이 시작되었으며 최초 복제견 “스너피” 이후 많은 복제견들이 생산되었다. 2009 년에 복제 기술로 7 두의 마약탐지견(투피)을 생산하였으며, 그 이후에도 마약탐지견, 검역탐지견을 생산하고 있다. 복제견에서 유전적 동일성은 확인되었지만 행동의 유사성과 그 탐지 능력의 발현 정도를

비교한 결과는 보고되지 않았다. 따라서, 이 연구의 목적은 복제견의 행동을 분석하는 것이며, 유견 적성 평가(PAT) 방법을 이용하였다. 이것은 유견이 7 주령일 때 우위성 정도 평가를 위해 주로 사용하는 방법으로 사회적인 친화도, 따르기, 행동의 제한, 사회적 지배, 거상 지배, 물체 가져오기, 조작 민감도, 소리 민감도, 시력 민감도, 안정성 및 구조 등 10-11 개의 하위 검사로 구성된다. 하위 검사 중 구조는 유견의 자세와 몸의 균형을 평가하고 다른 10 개의 하위 검사는 기질을 평가하기 위한 것으로 본 연구에서는 적성 하위 검사 위주로 진행하였다. 복제 구조견 2 두의 분석 결과에서는 유견 적성 검사에서 같은 유형으로 분류되었다.

성격의 일관성은 최근 성격 분석의 중요한 특징 중 하나로 이용되고 있으며, 다양한 특성과 인간 - 동물 상호 작용은 특히 유견의 성격 일관성 분야에서 연구되고 있다. 여기에서 복제견과 대조군에서 우위성 행동의 일관성을 조사하였으며 유전적 동일성의 영향을 확인하기 위한 유견 적성 검사 중 10 개의 하위 검사를 실시했다. 이 검사에서 유견은 낯선 사람, 행동의 제한, 먹이 같은 물체, 사람이 잡는 것, 놀라게 하는 물체 등에

노출되었다. 6 두의 복제견과 4 두의 대조군을 대상으로 7-10 주 및 16 주의 반응에 대한 일관성을 비교분석하였다. 두 그룹은 하위 검사에서 서로 다른 일관성을 보였으며, 복제견의 평균 점수는 일관적이었지만 (P = 0.7991), 일반견의 평균 점수는 그렇지 않았다 (P = 0.0089). 무리 행동 및 위급 상황과 관련한 행동 결과에서 복제견은 일관성을 보였지만 대조군의 점수는 그렇지 않았다. 먹이 사냥과 관련한 행동의 점수는 두 그룹 모두에서 일관성을 보이지 않았다. 따라서 행동 일관성의 경향은 유전자의 동일성 여부의 영향을 받으며 일부 행동이 다른 행동보다 더 영향을 받을 수 있다고 볼 수 있었다. 본 연구의 결과는 복제견이 자연적으로 번식한 견보다 유견, 자견, 성견의 각 발달단계에서 더 일관된 형질을 나타낼 수 있음을 시사하며, 또한 성격에 대한 일관성이 유견의 특성을 분석하는 방법 중 하나로 쓰일 수 있음에 대한 가능성을 보여준다.

주요어: 개 행동, 복제견, 유견 적성 검사

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