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Personality consistency analysis in cloned quarantine dog candidates

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Abstract

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, we 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 7–10 and 16 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 the control group were not. Scores of Prey Drive were not consistent in either the cloned or the control group. Therefore, it is suggested that consistency of dominant behaviour is affected by genetic identity and some behaviours can be influenced more than others. Our 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.

Canine behaviour, cloned dog, puppy aptitude test

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 (1979) explained how to select a family dog using the Puppy Aptitude Test. Adopting suitable animals could be the beginning of a happy life for both owners and the dogs. Breeders need to check the puppies' temperaments for similar reasons for their future 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. (2005) investigated the consistency of dogs' behaviours using a standardized behavioural test and could deduce stable traits such as playfulness, chase-proneness and sociability. Substantial consistency of dogs' personalities was reported by Fratkin et al. (2013), who determined the factors that influence consistency as the age, interaction between temperament and age, and length of time interval between tests. These authors did not, however, estimate the influence of the genetic background on consistency (Fratkin et al. 2013).

Bartlett (1979) used 10 subtests to analyse puppies' behaviours, including social attraction, dominant or submissive tendency under stress, willingness to work with humans and sensitivities. Volhard and Volhard (2001) divided the subtests into three drives,

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namely the Pack Drive, the Fight or Flight Drive, and the Prey Drive. The 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 the Prey Drive, which is related to the hunting ability.

Personality consistency is expressed as the degree of repeatability of specific traits at various intervals. Netto and Planta (1997) reported that attack scores of adult dogs between two aggression tests were correlated, and Slabbert and Odendaal (1999) found the predictability of behaviour tests targeted for police dogs. 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 (Slabbert and Odendaal 1999). In contrast, other results showed that fear, anxiety and aggression behaviours were not consistent (Stephen and Ledger 2007). The behaviour of cloned dogs has been studied to determine the similarities among animals (Choi et al. 2014), 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 differences in the previous study.

Therefore, in this project we tried to answer the question: Is personality consistency different between cloned and control groups at 7–10 and at 16 weeks? We used the Puppy Aptitude Test consisting of 10–11 subtests and examined the scores for the Pack Drive, Fight or Flight Drive and Prey Drive (Volhard and Volhard 2001). This behaviour test has been used at the Quarantine Detector Dog Training Centre (Incheon, Korea) to evaluate puppies and improve training methods.

This study is the first attempt to assess personality consistency in cloned working dog candidates.

Materials and Methods

Subjects

In this study, six cloned and four control Beagle puppies (*Canis familiaris*) were compared by the modified Puppy Aptitude Test (Bartlett 1979; Volhard and Volhard 2001). Cloned puppies were produced by somatic cell nuclear transfer (SCNT) (Lee et al. 2005). In brief, donor cells were derived from the abdominal skin of a 10-year-old male Beagle. This dog was selected by handlers and trainers from the Quarantine Detector Dog Training Centre because of his excellent ability in detecting forbidden items at the airport. The role of a quarantine detector dog, also called 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 general anaesthesia and pain reliever was injected after operations. Control puppies were produced by artificial insemination. We transferred several embryos into surrogate mothers and four of them were successfully conceived. Procedures to collect *in vivo* matured oocytes were the same as 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 are presented in Table 1. They were raised as candidates for quarantine dogs with other puppies at the Quarantine Detector Dog Training Centre. After birth, all of the puppies were monitored for 24 h at 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 Centre. Drinking water was always served and dog food of the Royal Canin brand was given once a day. Dogs had their own space to eat and sleep which was 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 centre. This study was approved by the Institutional Biosafety Committee (IBC No.; SNUIBC-P130218-1-1) and the Institutional Animal Care and Use Committee (IACUC No.; SNU-121130-1) of the Seoul National University.

Table 1. Birth dates of cloned and control puppies.

Puppy	Birth date
Clone 1	Jan 11, 2012
Clone 2	Jan 19, 2012
Clone 3	Oct 23, 2012
Clone 4	Oct 25, 2012
Clone 5	Oct 25, 2012
Clone 6	Oct 25, 2012
Control 1	Dec 27, 2011
Control 2	Jan 5, 2012
Control 3	Jan 5, 2012
Control 4	Jan 5, 2012

Testing and comparison between the two groups at ages 7–10 and 16 weeks

The Puppy Aptitude Test (PAT) consists of 10–11 subtests (Bartlett 1979; Volhard and Volhard 2001): 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 s, 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 (Bartlett 1979), and the tests at the age of 16 weeks were carried out based on the study of Beaudet et al. (1994). The same test was conducted at the age of 7–10 and 16 weeks, and the responses of these two ages in each group were compared to find behavioural consistency during the training.

Scores of the ten subtests were classified as the three drives according to Volhard's criteria (Volhard and Volhard 2001). Pack Drive is related to living in a group, and the subtests Social Attraction, Following, and Social Dominance are part of it. The Fight or Flight Drive is a characteristic relevant to the ability to struggle or run away. It consists of the subtests Restraint, Elevation Dominance, and Stability. The Prey Drive relates to the hunting ability and includes the subtests Retrieving, Sound Sensitivity, and Sight Sensitivity. Correlations between drives were analysed to determine the connections with different temperaments.

Table 2. Spearman rank correlations between drives of the Puppy Aptitude Test 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

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

Description of the Puppy Aptitude Test

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 Bartlett's and Volhards' criteria (Bartlett 1979; Volhard and Volhard 2001). 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 or she claps his or her hands to get the puppy's attention. If the puppy comes, he or she pets it gently.

(2) Following (F): The puppy is placed in the test room and the TL walks away, making sure he or she 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.

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 Least Square Post Hoc test. *P* values were corrected by the Tukey-Kramer adjustment and considered as significant differences when they were lower than 0.05.

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

Results

Consistency between 7–10 weeks and 16 weeks

Scores at the ages of 7–10 and 16 weeks in cloned and control puppies were compared to evaluate the consistency across the training period. Fig. 1 shows the mean 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 ($F_{1.20} = 8.4$, $P = 0.0089$). Mean scores of each individual in clone and control puppies are presented in Fig. 2. Figs 4 and 5 show the scores of each subtest in control and cloned groups.

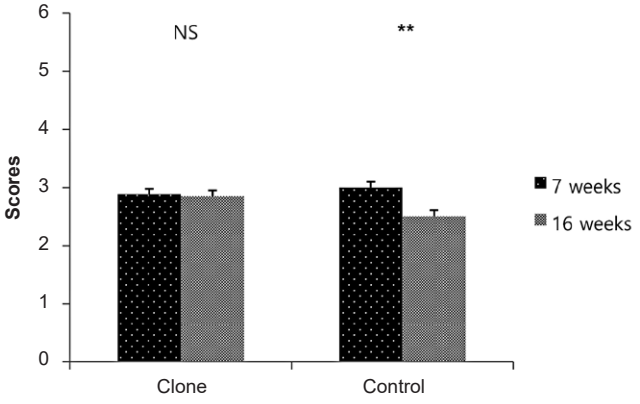

Fig. 1. Scores of Puppy Aptitude Test (PAT) at age 7–10 weeks and 16 weeks in cloned ($F_{1.35} = 0.07$, $P = 0.7991$) and control groups ($F_{1.20} = 8.4$, $P = 0.0089$). $^{**}P < 0.01$; general linear mixed model.

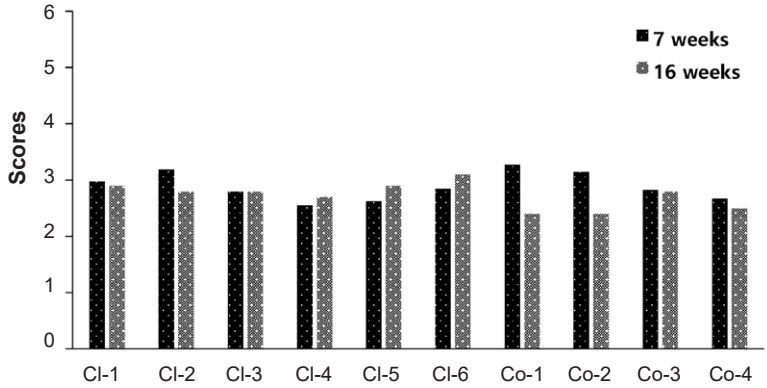

Fig.2. Mean scores of Puppy Aptitude Test (PAT) of each puppy at the ages of 7–10 weeks and 16 weeks in cloned (Cl-1, Cl-2, Cl-3, Cl-4, Cl-5, Cl-6) and control (Co-1, Co-2, Co-3, Co-4) groups.

Consistency of the three drives

Scores of the three drives were analysed and are presented in Fig. 3. Scores of the 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 the Fight or Flight Drive also showed consistency in cloned puppies ($F_{1,40} = 0.11$, $P = 0.7457$), while they did not do so in control puppies ($F_{1.23} = 17.81$, $P = 0.0003$). Scores of the Prey Drive were not consistent either in cloned ($F_{1.40} = 20.2$, $P < 0.0001$) or control puppies ($F_{1.23} = 15$, $P = 0.0008$).

Discussion

In this study, we compared the consistency of personality in cloned and control puppies by the modified Puppy Aptitude Test. The scores of the two groups showed different tendencies. The mean 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.

Our results could be interpreted as cloned dogs exhibiting consistency in the degree of socialization with humans, adaptability to training, and expression of fear. This may suggest that the same training procedures throughout this period would be suitable for all of them, since it may be necessary to change the training styles according to the interpretation of scores (Bartlett 1979). A competent trainer is needed since an extremely dominant dog and an extremely submissive dog need special handling to instil confidence (Bartlett 1979). In contrast, in the control group all of the drives and the means were inconsistent. Our previous study showed that cloned puppies could be classified into the same group by the Campbell test (Choi et al. 2014), and the behaviours of the cloned group were more consistent than those of the control group in this study. While our previous study was conducted with Labrador Retrievers, this study was performed with Beagles. We proposed to compare these two studies since it could be more meaningful to analyse the result within a breed than between breeds (Mehrkam and Wynne 2014). These two studies were conducted by similar puppy tests that evaluate dominant behaviours and aptitudes. 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. Based on our existing findings, cloned dogs seemed to share more similar and consistent behaviours than naturally bred ones, however, our further study will be able to present various aspects of cloned animals.

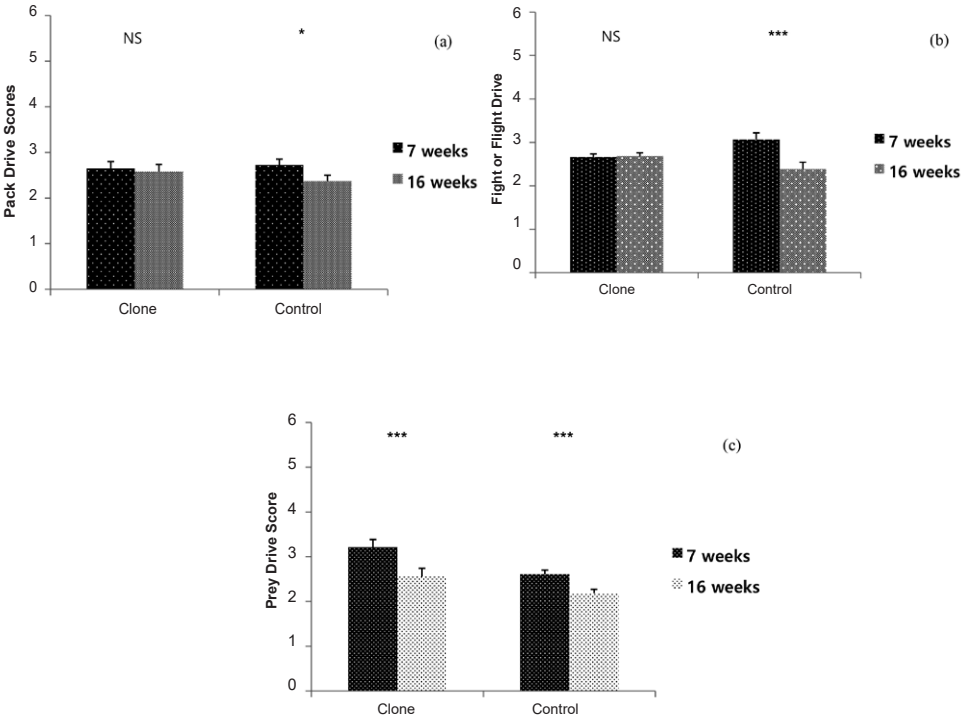

Fig. 3. Scores of the three drives of the Puppy Aptitude Test in cloned and control puppies at the ages of 7–10 weeks and 16 weeks. (a) Pack Drive in cloned ($F_{1.40} = 0.3$, $P = 0.59$) and control puppies ($F_{1.23} = 7.6$, $P = 0.0112$). (b) Fight or Flight Drive in cloned ($F_{1.40} = 0.11$, $P = 0.7457$) 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 ($F_{1.23} = 15$, $P = 0.0008$).
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; general linear mixed model.

Pack-related dog behaviour could be examined in free-ranging dogs: Cafazzo et al. (2014) reported that the dominance rank positively affects male copulation and female reproductive success. In domesticated environments, however, instead of mate preferences, dominant individuals tend to be outgoing and active (Bartlett 1979). Therefore, it is supposed that cloned puppies have the same degree of activity at 7–10 and 16 weeks while control puppies do not. The subtests of the Pack Drive include social attraction to people, willingness to follow a human, and acceptance of a human's social dominance (Volhard and Volhard 2001). The results in cloned dogs were similar to another human-related study by Vas et al. (2008), which reported repeatedly consistent dogs' responses to human behavioural cues. Figs 4 and 5 show more detailed scores for these three subtests (SA, F,

and SD), and the consistency or inconsistency of each group is presented in Fig. 3. The control group in our study seemed different from normal in this aspect.

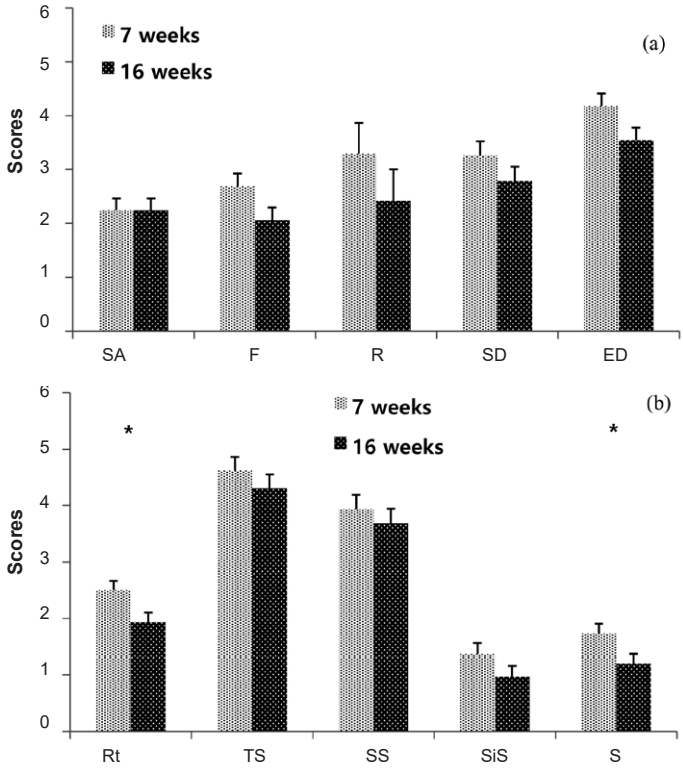

Fig. 4. Consistency Scores in the Puppy Aptitude Test (PAT) at the ages of 7–10 weeks and 16 weeks in control puppies. (a) Subtest Social Attraction (SA), Following (F), Restraint (R), Social Dominance (SD), and Elevation Dominance (ED) were not significantly different between ages. (b) Subtest Retrieving (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.

The 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 (Volhard and Volhard 2001). This drive would be related to the ability to run away from danger, and calm down 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 Fig. 3. (Fig. 4 and 5 provide the scores of each subtest). Riemer et al. (2013) reported that struggling responses varied in potential conflict tests of Border Collie puppies, and playful fighting appeared to be a heritable factor in the German Shepherd (Reuterwall and Ryman 1973). 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 the Prey Drive showed inconsistency in both the 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 (Goddard and Beilharz 1986; Fratkin et al. 2013) and this tendency was similar in humans (Srivastava et al. 2003). It is also possible that the inconsistencies in the 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 the test leader, object and previous experience, and not by age or sex (Lindberg et al. 2004). The active hunting behaviour can also be related to dominance, because when a wolf pack has a prey, by hierarchy the pack leaders are allowed to feed first (Bradshaw 2006). Therefore, this suggests that inconsistency in the Prey Drive may be derived from changing hierarchies during the period of the puppies' growth. Because of this reason, the difference of the first test age at 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 the part related to the Prey Drive will be effective. In the 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 (Figs 4 and 5). The cloned group maintained the scores of subtest Retrieving at 3, and this score indicates the degree of willingness for and acceptance of human training (Volhard and Volhard 2001). These results can provide the support for a genetic role in human-animal interaction. Wang et al. (2013) insisted parallel selection in humans and dogs, and our results could be considered in line with that conclusion.

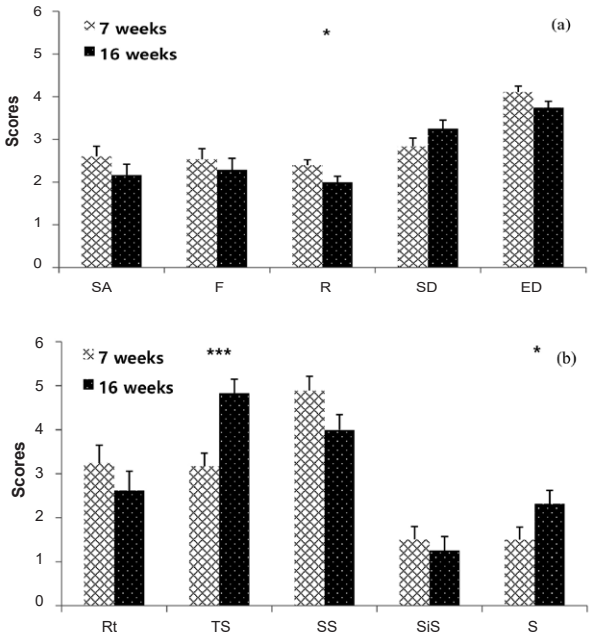

Fig. 5. Consistency Scores of the Puppy Aptitude Test (PAT) at the ages of 7–10 weeks and 16 weeks in cloned puppies. (a) Subtest Restraint (R) showed a significant difference between ages. Subtest Social Attraction (SA), Following (F), Social Dominance (SD), and Elevation Dominance (ED) were not significantly 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. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; general linear mixed model.

In the control group, the tendency for consistency was determined in eight subtests except for the mean 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 mean was not. However, the overall temperament including the 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.

To the best of our knowledge, this study is the first trial to compare drives and the correlation results show that the drive scores cannot be predicted based on other drives because they were not found to be correlated with each other. 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 our 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 (Takeuchi et al. 2009); aggression with *DRD1*, *HTR1D*, *HTR2C*, and *SLC6A1* (Vage et al. 2010)). This can be another crucial research study in the future. Ruefenacht et al. (2002) reported that heritability of seven different traits such as self-confidence, nerve stability, the defence drive and others varied in German Shepherd dogs. It is also possible that some traits are influenced more by the 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 the 10 months of training courses, all of the ten puppies were evaluated as potential quarantine dogs, as presented in Fig. 6. 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 PAT and the selection test, more studies during the whole training course will be necessary. Until now, it is supposed that cloned dogs have less variation during early training.

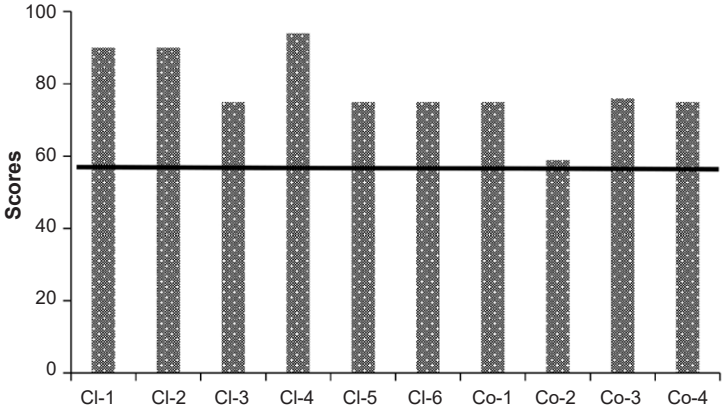

Fig. 6. Scores for the final selection test in cloned (Cl-1, Cl-2, Cl-3, Cl-4, Cl-5, Cl-6) and control (Co-1, Co-2, Co-3, Co-4) puppies. The solid line indicates the pass mark.

Our study analysed the consistency of behaviour and went a step forward by adding comparisons between cloned and control groups of puppies. The results of this study suggest 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, the 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.

Acknowledgements

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