

Behavioral Analysis of Cloned Puppies Derived from an Elite Drug-Detection Dog

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Abstract Since the first cloned dog “Snuppy” was born, many cloned dogs have been produced by somatic cell nuclear transfer (SCNT) technology. We reported the production of seven cloned drug detection dogs (named “Toppies”) in 2009. Although their genetic identity was confirmed, similarities in behavior and the drug-detecting ability were not examined. Therefore, this study is the first attempt to examine their behavior. We conducted the Campbell test which is commonly used to evaluate the tendency of dominance. Data were analyzed by the general linear mixed model. The scores among seven cloned puppies and four naturally-bred controls were significantly different ($P < 0.0001$). After the test, cloned and control puppies were trained according to the Korea Customs Detector Dog Training Center’s manual. The selection rate for detector dog in the cloned puppies was higher (86 %) than that of naturally-bred dogs (30 %). Therefore, it can be concluded that drug detection dogs with high performance can be propagated more efficiently using SCNT.

Keywords Campbell test · Canine behavior · Cloned dog · Drug detection dog · Puppy aptitude test

Introduction

Dogs are generally superior to other animals and as technological devices in scent detection. The dogs that are especially proficient in this regard are often used to detect targets by scent and, are called detector or search dogs. Detector dogs are used for detecting dangerous materials such as explosives or drugs (narcotics) (Williams and Johnston 2002; Lit and Crawford 2006). For example, drug detecting dogs are employed in airports and prisons and are trained to scan large numbers of people for the presence of narcotics (Rooney et al. 2004). The use of such dogs has increased in recent years, because of modern phenomena such as drug trafficking and terrorist threats. Although selection procedures for producing drug detection dogs are established within individual organizations, only a small minority of animals can be successfully trained for their specific roles. Using this approach, there is a very low probability of finding the best or “elite” detector dogs. Although there have been several attempts to establish breeding programs for propagation of the specialist detection dogs, there is an insufficient supply of elite dogs (Rooney et al. 2004). Alternative ways of providing such animals are needed, and in the present study, the somatic cell nuclear transfer (SCNT) technique was examined as a method to produce elite drug-detecting dogs.

SCNT is a unique reproductive engineering technology that can yield a newborn that is virtually identical to the somatic cell donor. Since the birth of Dolly, the cloned sheep, SCNT has become a reliable method for several species cloning (Wakayama et al. 1998; Baguisi et al.

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1999; Onishi et al. 2000; Chesné et al. 2002; Lee et al. 2005). In our previous study, seven cloned puppies derived from somatic cells of an elite drug-detecting dog were produced via SCNT (Oh et al. 2009). We demonstrated that the seven cloned puppies have the same genotype as the donor dog, but that study did not examine their behavior patterns or drug-detecting potential. Therefore, the present study performed through using Campbell test for behavioral analysis for the dogs and compared the outcomes with their selection results as drug detection dogs.

The Campbell test consists of five separate subtests that are scored separately: Social Attraction, Following, Restraint, Social Dominance and Elevation Dominance (Pérez-Guisado et al. 2008). The tests were performed primarily to evaluate underlying aptitudes of puppies for drug detection (Detector Dog Test Manual, 2009, Customs Detector Dog Training Center, Customs Border Control Training Center, Korea Customs Service). By better predicting which dogs can be successfully trained, burdens on trainers and costs can be reduced. The training course consists of subjecting the dogs to various environments, motivation by reward, improving concentration, distinguishing between drugs and other chemicals, etc. In this study, during standard training procedures, judgments on the relative ability of the cloned dogs, and whether they should continue training or be rejected, were made by experienced trainers.

Therefore, the aim of this study was to determine whether cloning by SCNT can affect the behavioral patterns of cloned dogs, with a special emphasis on cloned drug-detecting dogs.

Methods

Study sample

In order to compare the behaviors using Campbell test, we used cloned dogs and control dogs. Cloned dogs were produced by SCNT (Oh et al. 2009). In brief, ear skin fibroblasts from 7-year-old adult male elite drug detection dog were isolated and cultured in vitro as donor cells. For SCNT, a total of 544 in vivo matured dog oocytes obtained by flushing the uterine tubes of 51 oocyte donor dogs. The oocytes were enucleated, injected with donor cells, fused by electrical stimulation using an Electro-Cell Fusion apparatus (NEPA GENE Co., Chiba, Japan) and activated chemically. A total of 400 activated couplets were transferred into oviduct of naturally synchronous 18 recipient dogs. Four bitches were pregnant, pregnant females delivered eight live pups either naturally or by cesarean section. For SNCT, oocyte donors and recipients were not treated by any hormones or drugs. All cloned pups were genetically identical to the donor dog and their mitochondrial DNA was from their oocyte donor dogs

(Oh et al. 2009). Four control puppies were naturally bred by an elite drug detection dog (male) and a breeding dog (female). The litter size was eight; however we have scored data from only four puppies among them. The somatic cell donor dog and the parents of the controls have no genetic relationship. The parents of control puppies and the donor dog were not tested because we systematized this evaluation course after they were grown up. All the dogs in these experiments are labrador retrievers, and the ages of the puppies at testing time were between 9 and 13 weeks old in cloned and control dogs. After the tests they experienced more evaluations and training courses at similar age according to the manual. Their ages of starting various activities were similar also. All of the puppies were cared by the same persons and their living quarters were identical. Puppy care system was followed by guidelines of Customs Detector Dog Training Center.

Testing and evaluation for 8 weeks old puppy

Puppies were evaluated individually at the Customs Detector Dog Training Center using the same test area (10 m × 4.4 m) for each animal. The test was conducted by only one test leader (TL) in the absence any other object, animal or person that could attract the puppies' attention. The duration of the test for each puppy was the same: each subtest lasted 30 s so the whole test period was about 3 min. The TL was a stranger to the puppies and 4 people (TL and 3 handlers) evaluated the all puppies at the same time. Only the TL directly watched the puppies during each test; the other three evaluators observed them from outside the test area through a window so as not to be seen by the puppies, and they also checked the results after the test with a video recording and discussed the exact responses of the tested individuals. The evaluators classified the results according to the responses defined by Campbell (1972). This test was supposed to be conducted at age 8 weeks; however it was carried out at 9–13 weeks old because of movement procedure of puppies.

Types of responses in the Campbell test

Each puppy stayed alone for about 3 min in the empty test area before the test started, to allow time for it to be comfortable. A total of 11 puppies were tested according to Campbell's test (Campbell 1972). The test consists of 5 subtests and the order of the subtests conducted as follows: (1) Social Attraction, (2) Following, (3) Restraint, (4) Social Dominance, (5) Elevation Dominance. Bartlett (1979) described the test in more detail so we applied her description of the response interpretation.

According to Bartlett's (1979) interpretation, the puppy's responses indicate the degree of dominance: If the puppy gets score 1 (type 1 dog), this dog is extremely dominant and has to be trained by an experienced handler

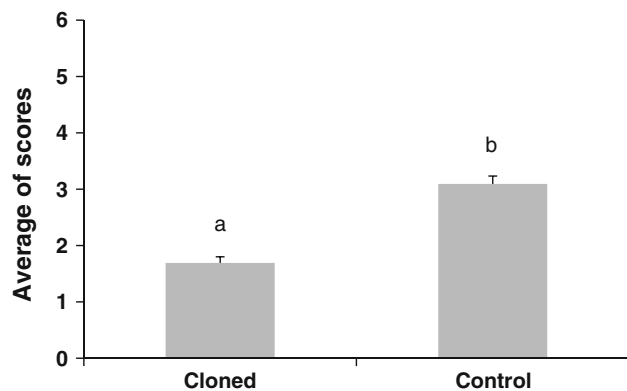


Fig. 1 Average scores of Campbell tests in cloned and control puppies. Eleven puppies from cloned and control groups were compared, and four observers were statistically processed as a random effect. Five subtest scores were used as dependent variables. Independent variables were individuals and groups. The average score of the cloned puppies is 1.6929 (standard error = 0.1073) and that of controls is 3.0937 (standard error = 0.1400). Average scores between cloned and control puppies were significantly different ($F_{1,30} = 66.11$, $P < 0.0001$)

Table 1 Factors and least squares means of average scores of Campbell tests in cloned and control puppies

Type 3 tests of fixed effects						
Effect	Num DF	Den DF	F value	P > F		
Group	1	30	66.11	<.0001		
Least squares means						
Effect	Group	Estimate	Standard error	DF	t Value	P > t
Group	Cloned	1.6929	0.1073	30	15.78	<.0001
Group	Control	3.0937	0.1400	30	22.10	<.0001

because of extreme dominance and aggressive tendencies. This dog can be a good detection dog with him or her. In case of score 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. Type 2 is generally regarded as the most appropriate aptitude for working dogs at the Korea Customs Detector Dog Training Center because this kind of dog has an active and outgoing temperament. If the dog gets score 3, it is best for the average owner and also good with the elderly and children. Type 3 is regarded as a working dog as well. This dog adapts well to changes. A dog with score 4 is submissive and slightly less outgoing than the score 3 dog. This dog gets on famously with children and trains well. However, types 4 and 5 dogs are not supposed to be good prospects for working dogs since they are submissive and less active. Score 5 means 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 score 6 is independent and not affectionate. It is difficult to train as a working dog as well as a pet.

Training and evaluation for selecting drug detection dog

After Campbell classification the puppies were trained for about a year according to the Detector Dog Test Manual. Selection tests were conducted when the training course ended. The test areas were at the building used for training and at Incheon airport. There were five evaluation items: boldness, concentration, detecting process, response of detection and possessiveness. Each item was scored as 4 (poor), 8 (fair), 12 (average), 16 (good), and excellent (Weiss 2002) and the full score was 100. When the puppies were tested, more than 4 kinds of narcotics were used; hemp, MDMA, Philopon and cocaine. These were hidden in glass or vinyl bottles and concealed in cloths, boxes, hard cases and a person's forearm. The aim of this final test was the selection of drug detection dogs that will work successfully in the field.

Statistical analysis

Individual scores were compared between and within groups: cloned or control puppies. To analyze differences among the puppies, the general linear mixed model was used with SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Differences between cloned and control puppies were analyzed. A score of between 1 and 6 was used as a response to each Campbell subtest: (1) excessive dominance; (2) dominance; (3) balanced submission; (4) submission; (5) excessive submission; (6) independent. Five kinds of subtest scores were used as dependent variables. Independent variables were individuals and groups. Evaluators were calculated as a random factor to reduce their effect on the data since they can be different from each other. By pairwise comparison each individual dog was analyzed to find if anyone was significantly different from another. The same procedure was carried out for all five Campbell subtests. The sum of scores for each type was divided by the total number of individuals to determine the frequency distribution of scores of each type. To determine the average number of scores, a method for obtaining average values (Pérez-Guisado et al. 2008) was used with modification. Pairwise comparison was used to analyze the differences in Least Square Post Hoc test. *P* values were corrected by Tukey–Kramer adjustment.

Results

Comparison between cloned and control puppies

Eleven puppies from cloned and control groups were compared, and four observers were statistically processed as a random effect. This procedure is to reduce the bias recorded by the observers. Five subtest scores were used as dependent variables. Independent variables were individuals and groups. Figure 1 presents the differences of the average test scores ($F_{1,30} = 66.11$, $P < 0.0001$), Table 1 shows the factors and least squares means. The scores were significantly different between the experimental groups. The average score of control puppies was 3.0937 (standard error = 0.1400) while that of cloned puppies was 1.6929 (standard error = 0.1073). The scores of all five subtests were significantly different between the two groups (Fig. 2). The factors and least squares means are presented in Tables 2, 3, 4, 5, and 6. For the subtest Social

Attraction, the score for controls was 3.5626 and for cloned it was 1.2857 ($F_{1,30} = 121.74$, $P < 0.0001$); for the subtest Following, the control score was 2.1875 versus 1.4286 for cloned ($F_{1,30} = 14.89$, $P = 0.0006$); for Restraint, the control score was 3.3229 for cloned ($F_{1,29} = 53.70$, $P < 0.0001$); for Social Dominance, the controls scored 2.9369 versus 1.9643 for cloned ($F_{1,29} = 10.23$, $P = 0.0033$); and for Elevation Dominance, the scores were 3.5795 for control and 2.0357 for cloned ($F_{1,29} = 22.30$, $P < 0.0001$).

These results show that cloned puppies achieved more dominant scores than control puppies in the five subtests. In addition, we confirmed that there were also differences in distribution frequency of scores between cloned and control puppies. Table 7 shows that the cloned group achieved (2) type most frequently, while the control group achieved (3) type most frequently in the Campbell test. Variation of assessed types was higher in the control than in the cloned group, since the former obtained 4 kinds of type 6—(2),

Fig. 2 (a) Scores of the Social Attraction subtest in cloned and control puppies. Five subtest scores were used as dependent variables. Independent variables were individuals and groups. Four observers were statistically processed as a random effect. The scores were significantly different ($F_{1,30} = 121.74$, $P < 0.0001$). (b) Scores of the Following subtest in cloned and control puppies. The scores were significantly different ($F_{1,30} = 14.89$, $P = 0.0006$). (c) Scores of the Restraint subtest in cloned and control puppies. The scores were significantly different ($F_{1,29} = 53.7$, $P < 0.0001$). (d) Scores of the Social Dominance subtest in cloned and control puppies. The scores were significantly different ($F_{1,29} = 10.23$, $P = 0.0033$). (e) Scores of the Elevation Dominance subtest in cloned and control puppies. The scores were significantly different ($F_{1,29} = 22.3$, $P < 0.0001$)

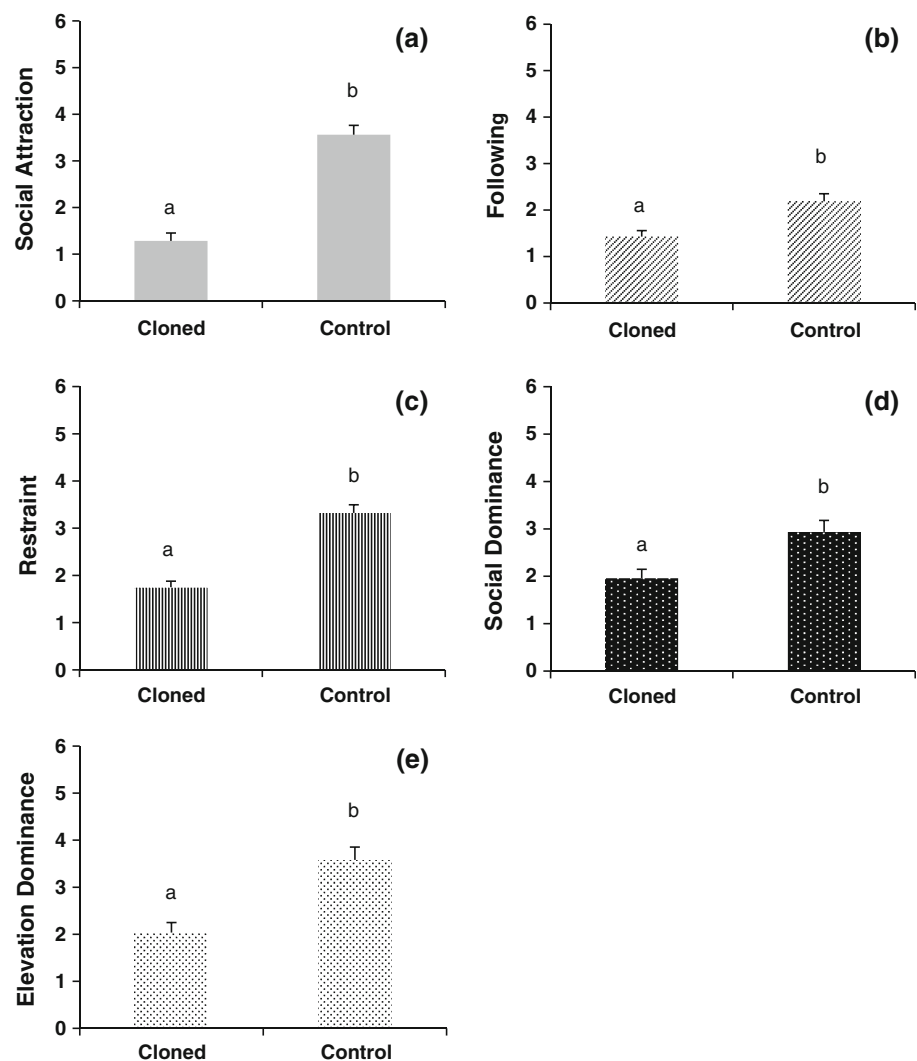


Table 2 Factors and least squares means of scores of the Social Attraction subtest in cloned and control puppies

Type 3 tests of fixed effects						
Effect	Num DF	Den DF	F value	P > F		
Group	1	30	121.74	<.0001		
Least squares means						
Effect	Group	Estimate	Standard error	DF	t Value	P > t
Group	Cloned	1.2857	0.1684	30	7.63	<.0001
Group	Control	3.5625	0.1999	30	17.82	<.0001

Table 3 Factors and least squares means of scores of the Following subtest in cloned and control puppies

Type 3 tests of fixed effects						
Effect	Num DF	Den DF	F Value	P > F		
Group	1	30	14.89	0.0006		
Least squares means						
Effect	Group	Estimate	Standard error	DF	t Value	P > t
Group	Cloned	1.4286	0.1278	30	11.18	<.0001
Group	Control	2.1875	0.1640	30	13.34	<.0001

(3), (4), (5), while the latter scored 3 kinds of the type—(1), (2), (3) though the sample size of controls was smaller than that of cloned. In comparing individuals within the cloned group, only To-Wedn was significantly different from To-Mon and To-Thur.

Evaluation of score frequency of each type of the Campbell test

We compared the number of the subtest type score obtained between cloned and control puppies. Scores were graded by the same four evaluators for all of the puppies. Table 7 shows the average value of scores frequency of each type and the averages of cloned and control animals. Range of these values is from 0 to 1 because this table presents the rate of the whole number of times. The type of the highest average of cloned dogs was type (2) (0.5500) and that of control dogs was type (3) (0.4581); the highest average in total was type (2) (0.4334). In case of type (4) submission and type (5) excessive submission, control puppies scored 0.2875 (4) and 0.0250 (5), however cloned puppies did not obtain (4) or (5). Variation of assessed types was higher in the control than in the cloned group, since the former obtained 4 kinds of type 6—(2), (3), (4), (5) while the latter scored 3 kinds of the type—(1), (2), (3) although the sample size of control is smaller than that of cloned.

Table 4 Factors and least squares means of scores of the Restraint subtest in cloned and control puppies

Type 3 tests of fixed effects						
Effect	Num DF	Den DF	F value	P > F		
Group	1	29	53.70	<.0001		
Least squares means						
Effect	Group	Estimate	Standard error	DF	t Value	P > t
Group	Cloned	1.7500	0.1281	29	13.66	<.0001
Group	Control	3.3229	0.1722	29	19.29	<.0001

Table 5 Factors and least squares means of scores of the Social Dominance subtest in cloned and control puppies

Type 3 tests of fixed effects						
Effect	Num DF	Den DF	F value	P > F		
Group	1	29	10.23	0.0033		
Least squares means						
Effect	Group	Estimate	Standard error	DF	t Value	P > t
Group	Cloned	1.9643	0.1829	29	10.74	<.0001
Group	Control	2.9369	0.2430	29	12.08	<.0001

Comparison within cloned group

Seven puppies of cloned group were compared by general linear mixed model, as four observers were considered as a random effect. Five kinds of subtest scores were used as dependent variables. Independent variables were the cloned individuals. There were differences within the cloned group. Differences between To-Wedn and To-Mon ($P = 0.0031$), To-Wedn and To-Thur ($P = 0.0098$) were significant. Figure 3 shows the differences ($F_{6,18} = 4.81$, $P = 0.0043$). P values were corrected by Tukey–Kramer adjustment. The factors and least squares means are presented in Table 8.

Final selection test of cloned and control puppies

Six cloned dogs that finished the training course were evaluated by a final drug-detection dog selection test and all of them passed. The pass level was a score of 60. To-Tue was graded as Excellent (score 90) and the remaining five dogs were evaluated as Good. In age matched-controls, seven puppies finished the training course and one of them passed the test. One of the eight puppies died before the training course was over. The pass rate of cloned dogs was 86 % since six puppies passed among seven cloned ones. That of controls was 13 % in the aggregate since one

Table 6 Factors and least squares means of scores of the Elevation Dominance subtest in cloned and control puppies

Type 3 tests of fixed effects						
Effect	Num DF	Den DF	F value	P > F		
Group	1	29	22.30	<.0001		
Least squares means						
Effect	Group	Estimate	Standard error	DF	t Value	P > t
Group	Cloned	2.0357	0.2123	29	9.59	<.0001
Group	Control	3.5795	0.2742	29	13.06	<.0001

passed among eight control ones. This value was lower than generally found as 30 % (Maejima et al. 2007) or 50 % (Weiss and Greenberg 1997).

Discussion

The present study investigated for the first time the behavior of cloned dogs derived from a somatic cell of an elite drug detection dog. Although seven dogs is a small number, the present study has a relatively large significance because the dogs are genetically identical. All the clones and age-matched-controls were born within a short time period, making behavioral comparisons possible.

First, we attempted to determine whether any differences in behavioral trends existed among the cloned dogs with the same genotype. Secondly, the Campbell test was performed to evaluate the importance of prior puppy behavior in adult working dogs. Puppy behavior testing has become a valuable tool to select individuals for specific tasks (Slabbert and Odendaal 1999). The puppies are classified into six kinds of aptitude types by the Campbell test scores.

Among 6 kinds of aptitude type, the cloned puppies belonged to type 2 (dominance), while the control puppies

Table 7 Frequency distribution of scores of each type of the Campbell test in cloned and control dogs

Type	1	2	3	4	5	6
Cloned	0.3786	0.5500	0.0714	0.0000	0.0000	0.0000
Control	0.0000	0.2294	0.4581	0.2875	0.0250	0.0000
Individual						
	1	2	3	4	5	6
To-Sun	0.1500	0.8500	0.0000	0.0000	0.0000	0.0000
To-Mon	0.7000	0.3000	0.0000	0.0000	0.0000	0.0000
To-Tue	0.3500	0.5500	0.1000	0.0000	0.0000	0.0000
To-Wedn	0.0500	0.7500	0.2000	0.0000	0.0000	0.0000
To-Thur	0.6000	0.4000	0.0000	0.0000	0.0000	0.0000
To-Fri	0.4000	0.5500	0.0500	0.0000	0.0000	0.0000
To-Sat	0.4000	0.4500	0.1500	0.0000	0.0000	0.0000
C-1	0.0000	0.1177	0.8824	0.0000	0.0000	0.0000
C-2	0.0000	0.2000	0.4000	0.4000	0.0000	0.0000
C-3	0.0000	0.2000	0.2000	0.6000	0.0000	0.0000
C-4	0.0000	0.4000	0.3500	0.1500	0.1000	0.0000
Average	0.2409	0.4334	0.2120	0.1046	0.0091	0.0000

Cloned is the average value of cloned puppies (To-Sun, To-Mon, To-Tue, To-Wedn, To-Thur, To-Fri, To-Sat), Control is the average value of control puppies (C-1, C-2, C-3, C-4). Control puppies achieved (3) type most frequently (average 0.4581), however, cloned puppies achieved (2) type most frequently (0.5500)

1 excessive dominance, 2 dominance, 3 balanced submission, 4 submission, 5 excessive submission, 6 independent

belonged to type 3 (balanced submission). It is believed that dominance behavior of all clones might be a heritable characteristic derived from the genetics of an elite drug detecting dog by SCNT. In English cocker spaniels, the Campbell test showed an association between dominance behavioral patterns and genetic factors (Pérez-Guisado et al. 2006). A study in mice reported that genotype might significantly affect the aggressive behavior (Palmour 1983). Consistent with the aptitude types results, all of the five subtest type scores were significantly different between

Fig. 3 Scores of five subtests in seven cloned puppies ($F_{6,18} = 4.81$, $P = 0.0043$). Seven puppies of cloned group were compared by general linear mixed model, as four observers were considered as a random effect. Five kinds of subtest scores were used as dependent variables. Independent variables were cloned individuals. To-Wedn is significantly different from To-Mon ($P = 0.0031$) and To-Thur ($P = 0.0098$). P values were corrected by Tukey–Kramer adjustment (* $P < 0.05$)

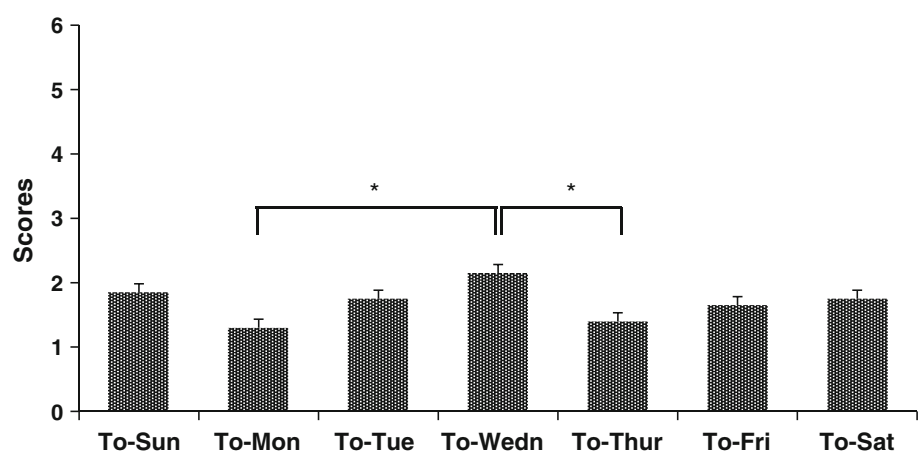


Table 8 Factors and least squares means of scores of five subtests in seven cloned puppies

Type 3 tests of fixed effects									
Effect	Num DF		Den DF		F value		P > F		
ID	6		18		4.81		0.0043		
Least squares means									
Effect	ID	Estimate	Standard error	DF	<i>t</i> Value	Pr > <i>t</i>			
ID	To-Fri	1.65	0.1323	18	12.47	<.0001			
ID	To-Mon	1.30	0.1323	18	9.83	<.0001			
ID	To-Sat	1.75	0.1323	18	13.23	<.0001			
ID	To-Sun	1.85	0.1323	18	13.98	<.0001			
ID	To-Thur	1.40	0.1323	18	10.58	<.0001			
ID	To-Tue	1.75	0.1323	18	13.23	<.0001			
ID	To-Wedn	2.15	0.1323	18	16.25	<.0001			
Differences of least squares means									
Effect	ID	ID	Estimate	Standard error	DF	<i>t</i> Value	P > <i>t</i>	Adjustment	Adjusted <i>P</i>
ID	To-Fri	To-Mon	0.3500	0.1828	18	1.91	0.0716	Tukey–Kramer	0.4962
ID	To-Fri	To-Sat	−0.1000	0.1828	18	−0.55	0.5910	Tukey–Kramer	0.9976
ID	To-Fri	To-Sun	−0.2000	0.1828	18	−1.09	0.2883	Tukey–Kramer	0.9219
ID	To-Fri	To-Thur	0.2500	0.1828	18	1.37	0.1882	Tukey–Kramer	0.8112
ID	To-Fri	To-Tue	−0.1000	0.1828	18	−0.55	0.5910	Tukey–Kramer	0.9976
ID	To-Fri	To-Wedn	−0.5000	0.1828	18	−2.74	0.0136	Tukey–Kramer	0.1453
ID	To-Mon	To-Sat	−0.4500	0.1828	18	−2.46	0.0241	Tukey–Kramer	0.2302
ID	To-Mon	To-Sun	−0.5500	0.1828	18	−3.01	0.0075	Tukey–Kramer	0.0883
ID	To-Mon	To-Thur	−0.1000	0.1828	18	−0.55	0.5910	Tukey–Kramer	0.9976
ID	To-Mon	To-Tue	−0.4500	0.1828	18	−2.46	0.0241	Tukey–Kramer	0.2302
ID	To-Mon	To-Wedn	−0.8500	0.1828	18	−4.65	0.0002	Tukey–Kramer	0.0031
ID	To-Sat	To-Sun	−0.1000	0.1828	18	−0.55	0.5910	Tukey–Kramer	0.9976
ID	To-Sat	To-Thur	0.3500	0.1828	18	1.91	0.0716	Tukey–Kramer	0.4962
ID	To-Sat	To-Tue	0	0.1828	18	0.00	1.0000	Tukey–Kramer	1.0000
ID	To-Sat	To-Wedn	−0.4000	0.1828	18	−2.19	0.0421	Tukey–Kramer	0.3481
ID	To-Sun	To-Thur	0.4500	0.1828	18	2.46	0.0241	Tukey–Kramer	0.2302
ID	To-Sun	To-Tue	0.1000	0.1828	18	0.55	0.5910	Tukey–Kramer	0.9976
ID	To-Sun	To-Wedn	−0.3000	0.1828	18	−1.64	0.1181	Tukey–Kramer	0.6597
ID	To-Thur	To-Tue	−0.3500	0.1828	18	−1.91	0.0716	Tukey–Kramer	0.4962
ID	To-Thur	To-Wedn	−0.7500	0.1828	18	−4.10	0.0007	Tukey–Kramer	0.0098
ID	To-Tue	To-Wedn	−0.4000	0.1828	18	−2.19	0.0421	Tukey–Kramer	0.3481

the two groups. This may be the less variation in the cloned group compared to control group. This observation indicates the possibility of a correlation between genotype and dominance behavior. In agreement with our results, cloned cattle exhibited behavioral trends that indicated a genetic influence. Similar behavioral trends were observed in grooming, curiosity, win–loss interaction, dominance and aggressiveness, as well as inter-suckling and front mounting (Savage et al. 2003).

Based on the current results, we hypothesized that the cloned dogs would be selected as drug detection dogs

through training and the selection test. This possibility was verified in this study using the selection test of the Korea Customs Detector Dog Training Center. Here, we demonstrated the ability of the cloned dogs as drug detectors. Six trained cloned puppies successfully completed the drug detection dog selection test (Fig 4). We have the data of success or failure of all of the eight control dogs, however precise figures of four of them could not be obtained. Therefore we indicated only the accurate marks in Fig. 4. In the Korea Customs Detector Dog Training Center, the general pass mark of the selection test is a score of 60, and

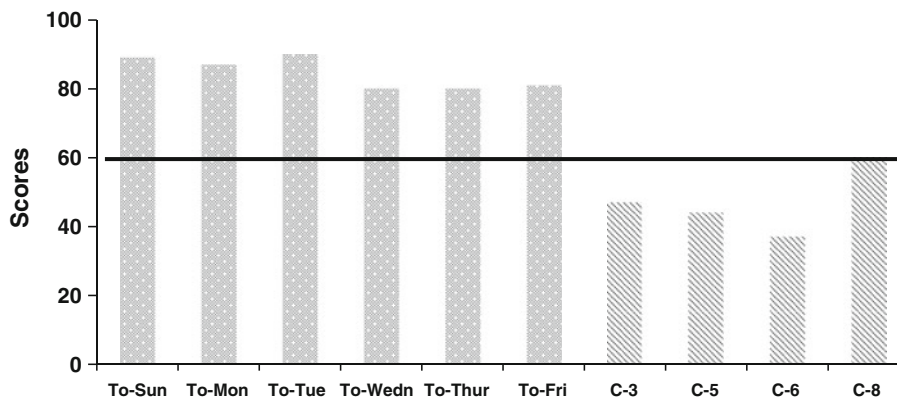


Fig. 4 Result of the detector dog selection test in six cloned dogs and four control dogs. All of the six trained cloned dogs passed the test. One puppy of seven controls (C-8) also passed the test. We have the data of success or failure of all of eight control dogs, however precise figures of four of them (C-1, C-2, C-4, C-7) could not be obtained.

six of seven cloned dogs exceeded the pass mark. The failed one was To-Sat, who had an appropriate attributes for detection; nevertheless he could not complete the training course because of a leg fracture from an accident. In contrast, only one of eight control dogs passed the selection test, a very low rate (13 %) compared to that of the cloned dogs. In addition to the present study, other studies (Campbell 1972; Bartlett 1979) have reported a low selection rate of 30 % up to 50 % with dogs produced from natural breeding (Maejima et al. 2007; Weiss and Greenberg 1997; Weiss 2002). The Korea Customs Detector Dog Training Center was adopted as an efficient financial management institute because it saved five hundreds million Won for promoting drug detecting dog in 2010 through utilizing cloned dogs (<http://www.joseilbo.com/news/htmls/2010/07/20100713102231.html>). Therefore it was proved that cloning of elite service dogs can be an economic way to produce excellent dogs.

In this study the results of puppy testing appear to be related to adult selection testing since all the cloned puppies passed the selection test after they achieved type 1 or 2 in Campbell test. However, in the control puppies there was no correlation between Campbell test and the selection test. Therefore, we propose that cloning of a detection dog with high performance can be a better way to produce outstanding working dogs. After finishing the experiment, test-failed dogs can be a matter because it is not easy to find foster families even though they are good dogs as a pet.

In conclusion, the present study demonstrated that genetically identical clones are more consistent in their behavior than naturally bred animals, and that cloned dogs can be classified into the same behavioral groups by Campbell test. In addition, another important outcome was to verify the ability as drug detection dogs of cloned dogs

Therefore we indicated only the accurate marks in this figure. The *solid line* is the pass mark. To-Sun, To-Mon, To-Tue, To-Wedn, To-Thur, and To-Fri are cloned dogs and C-3, C-5, C-6, and C-8 are control dogs

derived from a donor detection dog with excellent ability, and one cloned dog revealed excellent performance in final selection test. At this moment, drug detecting cloned dogs are five years old and doing outstanding works at Incheon airport and the harbor in South Korea. They do not show any quick aging and subjected to further studies.

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References

- Baguisi A, Behboodi E, Melican DT, Pollock JS, Destrempes MM, Cammuso C et al (1999) Production of goats by somatic cell nuclear transfer. *Nat Biotechnol* 17(5):456–461
- Bartlett M (1979) A novice looks at puppy aptitude testing. *Am Kennel Gazette*. 1–14
- Campbell WE (1972) A behavior test for puppy selection. *Mod Vet Pract* 12:29–33
- Chesné P, Adenot PG, Viglietta C, Baratte M, Boulanger L, Renard J-P (2002) Cloned rabbits produced by nuclear transfer from adult somatic cells. *Nat Biotechnol* 20(4):366–369
- Lee BC, Kim MK, Jang G, Oh HJ, Yuda F, Kim HJ et al (2005) Dogs cloned from adult somatic cells. *Nature* 436(7051):641
- Lit L, Crawford CA (2006) Effects of training paradigms on search dog performance. *Appl Anim Behav Sci* 98(3–4):277–292
- Maejima M, Inoue-Murayama M, Tonosaki K, Matsuura N, Kato S, Saito Y et al (2007) Traits and genotypes may predict the successful training of drug detection dogs. *Appl Anim Behav Sci* 107(3–4):287–298
- Oh HJ, Hong SG, Park JE, Kang JT, Kim MJ, Kim MK et al (2009) Improved efficiency of canine nucleus transfer using roscovitine-treated canine fibroblasts. *Theriogenology* 72(4):461–470
- Onishi A, Iwamoto M, Akita T, Mikawa S, Takeda K, Awata T et al (2000) Pig cloning by microinjection of fetal fibroblast nuclei. *Science* 289(5482):1188–1190

- Palmour RM (1983) Genetic models for the study of aggressive behavior. *Prog Neuropsychopharmacol Biol Psychiatry* 7(4–6):513–517
- Pérez-Guisado J, Lopez-Rodríguez R, Muñoz-Serrano A (2006) Heritability of dominant-aggressive behaviour in English Cocker Spaniels. *Appl Anim Behav Sci* 100(3–4):219–227
- Pérez-Guisado J, Muñoz-Serrano A, López-Rodríguez R (2008) Evaluation of the Campbell test and the influence of age, sex, breed, and coat color on puppy behavioral responses. *Can J Vet Res* 72:269–277
- Rooney NJ, Bradshaw JWS, Almey H (2004) Attributes of specialist search dogs—a questionnaire survey of UK dog handlers and trainers. *J Forensic Sci* 49(2):300–306
- Savage AF, Maull J, Tian XC, Taneja M, Katz L, Darre M et al (2003) Behavioral observations of adolescent Holstein heifers cloned from adult somatic cells. *Theriogenology* 60(6):1097–1110
- Slabbert JM, Odendaal JSJ (1999) Early prediction of adult police dog efficiency—a longitudinal study. *Appl Anim Behav Sci* 64:269–288
- Wakayama T, Perry ACF, Zuccotti M, Johnson KR, Yanagimachi R (1998) Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* 394(6691):369–374
- Weiss E (2002) Selecting shelter dogs for service dog training. *J Appl Anim Welf Sci* 5(1):43–62
- Weiss E, Greenberg G (1997) Service dog selection tests: effectiveness for dogs from animal shelters. *Appl Anim Behav Sci* 53:297–308
- Williams M, Johnston JM (2002) Training and maintaining the performance of dogs (*Canis familiaris*) on an increasing number of odor discriminations in a controlled setting. *Appl Anim Behav Sci* 78:55–65