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Assessment of personality traits in cloned minipigs using three different behavioral tests

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Abstract

Identifying and grouping personality types in pigs may be important for their management and welfare. This study assessed the within-individual consistency and between-group differences in activity level, boldness, and exploration in 12 two-year-old male miniature pigs from three clone groups using three behavioral tests: the open field test, the human approach test, and the novel object test, each repeated three times. The study examines the consistency of individual behaviors over time and potential differences between clone groups. Activity levels were consistent within individual pigs in the open field test. Conversely, the time spent close to the stimulus in the human approach test, and the novel object test was not consistent within individual pigs. Clone groups showed different activity levels in the open field test but did not differ in the time spent near the person or balloon in the human approach test and the novel object test, suggesting a genetic influence on activity levels but not on boldness or exploration in male miniature pigs.

Keywords: pig, genetics, animal behavior, personality.

Introduction

The term personality is used to describe individual behaviors that remain consistent over time and across contexts (Reale et al., 2007; Gosling, 2008). In recent years, understanding personality in livestock and selecting animals based on desirable personality traits has become increasingly important to improve welfare, management and physiological measures, namely growth, feed intake, immune function, and meat quality (Scheffler et al., 2014; O'Malley et al., 2019) to improve commercial production (Hayne and Gonyou, 2003). For example, it was recognized that environmental changes and stressors (e.g., group changes or mixing) affect animals differently based on certain individual behavioral characteristics and that grouping animals with behavioral heterogeneity resulted in better growth performance (Hayne and Gonyou, 2003). Therefore, the use of personality as an animal-based measure (ABM) to evaluate and select animals could benefit group formation and management, with a reduction in aggression-related problems (e.g., tail biting), an improvement in weight gain, and a better quality of life, with a positive impact on animals' welfare (O'Malley et al., 2019).

Despite the importance of selection in maintaining certain phenotypic traits, whether genetic selection allows for behaviorally standardized animals is still a matter of debate (O'Malley et al., 2019). Scheffler et al. (2014) reported some heritable behavioral parameters in response to a back test in pigs (i.e., a test in which the pigs were taken out of their pen and placed on their backs in a special V-shaped device), such as the latency to the first escape attempt and the number of escape attempts. Conversely, Archer et al. (2003) found equal or greater behavioral variation in cloned pigs compared to their naturally bred counterparts when examined in a variety of behavioral tests (i.e., food preference, towel test, back test, pick-up test). Therefore, although the inclusion of certain personality traits in pig selection programs may help to achieve desired phenotypes, the outcome of an individual's personality is likely determined by the interaction between genes, environment, and experience.

The mechanisms of personality development are complex and not yet fully understood (Stamps and Groothuis, 2010). Using cloned animals to understand the extent to which personality traits are heritable could provide some insights. Cloned animals make it possible to study personality by separating genetic influences from environmental factors. Several behavioral tests are used to assess the most studied personality traits of shyness/boldness, exploration, activity, aggressiveness, and sociability in pigs (Gosling and John, 1999; O'Malley et al., 2019). Among them, the open field test (OFT), the human approach test (HAT), and the novel object test (NOT) are some of the most widely used to assess personality traits (Forkman et al., 1995; Hayne and Gonyou, 2003; Janczak et al., 2003; Scheffler et al., 2014; Friel et al., 2016). The OFT evaluates pigs' behavior when brought into an unfamiliar experimental area. The HAT is used to evaluate the reaction of pigs to humans, whether familiar or unfamiliar and the NOT to evaluate pigs' response to and interaction with an unfamiliar object. All three tests can be used to assess multiple personality traits, including activity, boldness and exploration (O'Malley et al., 2019).

To the authors' knowledge, only one study has assessed the personality of cloned pigs (Archer et al., 2003). However, this study did not assess the within-individual consistency of personality traits across multiple contexts, nor did it include the behavioral tests most used in pig personality research. Our study built on the work of Archer et al. (2003) by incorporating three widely used behavioral tests - the Open Field Test (OFT), the Human Approach Test (HAT), and the Novel Object Test (NOT) - to assess within-individual consistency and between-group differences in personality traits in three different groups of cloned minipigs. Our study used minipigs instead of the "classic" commercial pig breed because minipigs are becoming increasingly important in the experimental field. Hypothesizing that the selected behavioral tests would detect consistent personality traits of individual subjects and that there would be differences in personality between different clone lines, our study aimed to provide new insights into the genetic contributions to personality development in cloned minipigs. Therefore, the present work allowed us not only to investigate the influence of genetics on the personality in minipigs, but also to determine which behavioral tests were most consistent in identifying personality traits in minipigs. The results could have a positive impact on the evaluation of the animals and, consequently, on the improvement of their management.

Materials and Methods

The study was conducted on 12 two-year-old intact male miniature pigs, henceforth minipigs. Three cloned populations were obtained by cloning fibroblasts from three different donors (intact, clinically healthy male minipigs) via somatic cell nuclear transfer and labelled groups A, B and C, consisting of 6, 4 and 2 animals, respectively. The 12 minipigs were housed in three boxes (365 x 446 cm) in groups of four, sorted based on weight and not genetic line. Inside each box, the animals had access to an enriched environment (i.e., metal chains, plastic balls, straws, chewable wooden sleepers), a resting area with solid floor and straw bedding, a feeding/walking area, and an external paddock. The minipigs were housed under natural lighting conditions. The minipigs were fed with 420 g/day of specific porcine pellets (mix of Progeo M2 pellet and Progeo Stalla fibra pellet, Italy) in bowls, 210 g twice a day (i.e., 8:00 AM and 4:00 PM) and had ad libitum access to water through specific drinking nipples for pigs. Prior to the study, the animals had only interacted with animal care staff and the veterinarian for routine checks and had not undergone any specific habituation sessions in the presence of humans. The three genetic lines of minipigs underwent the same type of management, namely the same environmental conditions and the same kind of social interaction with humans.

A single empty box, of the same size as the housing boxes, was set up and dedicated to the experimental procedures. This box had never been used to house other animals, and the minipigs were only brought in for experimental testing. The experimental box was divided into 6 quadrants by drawing lines, each quadrant measuring 182.5x148.7 cm (Figure 1). In all three tests performed (i.e., OFT, HAT, NOT), the animals were led freely inside the experimental box, without any type of restraint. The same procedure was followed for the exit and return of the animal to the housing box. All the tests were recorded with two fixed cameras (Figure 1).

The OFT test started when the minipig entered the experimental box and the door was closed. From that moment on, the animal was free to explore the environment. After 5 minutes, the test was terminated, the door was opened and the minipig was allowed to exit. The OFT was performed three times with a period of one week in between trials. The parameter evaluated was the number of passages made by the animal from one quadrant to another (i.e., the number of times the minipig crossed a line drawn to divide the experimental box into 6 quadrants) (Figure 1).

In the HAT, the minipig entered the experimental box, and the experimenter entered the box after the minipig. The experimenter then sat on the central line near the box entrance door and remained motionless until the door was closed and the test began. The minipig was filmed for 5 minutes, during which the animal was free to interact with the experimenter, but the experimenter did not interact with the animal in any way. The experimenter was always the same person for all test repetitions and was a person the minipig had never seen prior to the first trial. The HAT was performed three times with a period of one week between trials. The parameter evaluated was the amount of time the subject spent in the two quadrants nearest to the person (Figure 1). The threshold for considering a minipig-human interaction was the boundaries of the two quadrants nearest to the person (each quadrant measuring 182.5x148.7 cm), where the pig had to remain in these quadrants with at least its two forelimbs for more than 10 seconds and may or may not touch the human.

The NOT apparatus was placed in the experimental box before the minipig entered. The NOT apparatus has been previously described by others (Baragli et al., 2021). The apparatus consisted of a metal panel with a 3 cm diameter hole in the center, covered by two small flaps. One end of a compressed air hose was inserted into the hole and an inflatable balloon was attached to it, while the other end was connected to a compressed air cylinder (Baragli et al., 2016; Scopa et al., 2018; Baragli et al., 2021). The airflow was regulated by a tap that, when opened by the experimenter, inflated the balloon, and opened the flaps. In the first phase (i.e., pre-test), the minipig was left alone for 5 minutes to get used to the presence of the apparatus in the experimental box without inflating the balloon. The experimenter then opened the air valve to inflate the balloon, which opened the flaps on the device. The balloon suddenly appeared and remained inflated for 5 minutes. Video recording began at the beginning of the pre-test and continued throughout the NOT (10 minutes total). During the test, the experimenter opened the air valve to inflate the balloon from outside the experimental box without interacting with the pig. The minipig was filmed for 10 minutes, during which the animal was free to interact with the object. The NOT was repeated three times, one week apart, for each subject. The parameter evaluated was the amount of time the subject spent in the two quadrants closest to the balloon (Figure 1). The threshold for considering a minipig-object interaction was the boundaries of the two quadrants nearest to the object, where the pig had to remain in these quadrants with at least its two forelimbs for more than 10 seconds and may or may not touch the object.

The decision to use a longitudinal repeated measures design (i.e., three behavioral tests repeated over three trials) was made to reduce random variability by distinguishing consistent behavioral traits from transient fluctuations. Measuring the same individuals across multiple trials controlled for individual differences, minimized the influence of outliers, and increased statistical power (Anderson, 2019). The consistency of responses across trials increased confidence in the observed patterns, ensuring that they reflected stable characteristics rather than transient fluctuations or external factors. In addition, the use of multiple tests to assess the personality traits in pigs strengthened the robustness of the assessment, as suggested in the literature (Gosling and John, 1999; Janczak et al., 2003; Réale et al, 2007; O'Malley et al., 2019). The video recording was performed using two cameras mounted above the experimental box doors (i.e., entrance door and paddock door) and positioned in front of each other to avoid blind spots. The paddock door was always closed during the tests. The tests were continuously recorded. The video recordings were analyzed by two observers who were blind to the identity of the minipigs. The observers were trained by ARR to ensure consistency in the recorded position of the minipigs. For all measures, the scores of the two observers were highly correlated (all $r > 0.84$; all $p < 0.001$), and the data recorded by one observer (TC) were used for all further analyses. Camera footage was analyzed using VLC media player (version 3.0).

Descriptive statistics of the number of passages made by the animal from one quadrant to another in the OFT, and the times spent close to the person and the balloon in the HAT, and NOT respectively, were performed using a commercial software (Microsoft Excel, Microsoft 365 MSO, Version 2306 Build 16.0.16529.20100). Data are expressed as mean ± standard deviation. A one-way ANOVA was used to evaluate differences in minipigs weight across clone groups.

To examine the consistency of each animal within each test over time, we calculated the intraclass correlation coefficient (ICC), using a two-way random model, for each animal's scores in the three trials of the OFT, HAT, and NOT. To determine whether the three clone groups differed in their average behavior across the three trials for each of the three tests, we constructed a linear mixed model for each test with the behavior of each animal in each trial as the outcome, the clone group (i.e., A, B, C) as a between-subjects factor, and the trial number and the interaction between clone group and trial number as within-subjects factors. Individual identity was included and a random factor. Assumptions for each model (homogeneity of variance and normally distributed residuals) were examined by visual inspection of residuals with Q-Q plots and scatterplots of residuals against predicted values. All inferential statistics were done using SPSS (IBM) v.28.0 for Macintosh.

Results

Means, standard deviations and confidence intervals of the number of passages made by the animal from one quadrant to another (open field test, OFT) and the time spent close to the person or balloon (novel object test, NOT, and human approach test, HAT) in the three trials are reported in Table 1. Three clone groups did not differ in weight ($F_{2,11} = 1.33$, $p = 0.30$). Figure 2 illustrates within individual consistency and between groups differences across the three trials and the three tests. Activity level in terms of the number of passages made by the animal from one quadrant to another in each trial in the OFT was repeatable within individual minipigs (ICC = 0.63 [0.30,0.86], $F_{11,22} = 6.07$, $p < 0.001$, Figure 2A). The time spent close to the stimulus in the HAT and NOT was not repeatable within individual animals across trials (HAT: ICC = 0.26 [-0.09,0.65], $F_{11,22} = 2.03$, p=0.08, Figure 2B; NOT: ICC = 0.04 [-0.24,0.47], $F_{11,22}$ =1.13, p=0.39, Figure 2C).

The three clone groups differed in their activity level in the OFT (Table 2, Figure 2A) but did not significantly differ across trials (Table 2, Figure 2A) nor was there any significant clone group by trial number interaction (Table 2, Figure 2A). In the HAT and NOT, there was no significant effect of clone group, trial number, nor any clone group by trial number interaction on the time spent near the person or balloon (Table 2, Figures 2B and 2C).

Discussion

Summary of key findings

This study examined both within-individual consistency and between-group differences in open field activity level, and boldness and exploration towards humans or novel objects in three different groups of cloned minipigs using three different behavioral tests (i.e., OFT, HAT, and NOT). We found that activity level was consistent within minipigs and differed among clone groups, whereas the response to a novel human or object was not repeatable and did not significantly differ between groups.

Genetic vs. environmental influences on minipigs personality traits

Activity level, measured by the number of passages the animal made from one quadrant to another in the OFT, showed significant within-individual consistency. Within-individual consistency in activity level during the OFT has often been reported in the literature (Réale et al., 2007; Watters and Powell, 2011; Horback and Parsons, 2016), supporting the use of the OFT in assessing activity levels as a personality trait in minipigs (Finkemeier et al., 2018; O'Malley et al., 2019). Unlike the OFT, the HAT and NOT did not show within-individual consistency across repeated measurments, suggesting that these tests may not have captured a consistent personality trait. This result may due to a previously reported observation (O'Malley et al., 2019) that pigs can quickly habituate to the presence of humans or objects. It is possible that motivation to do the test and/or test novelty wore off throughout the trials once the human or object was more familiar. However, Friel et al. (2016) reported significant repeatability in the HAT and NOT. The lack of within-individual consistency found may be related to the novel object used or a response influenced by the human involved in the test. In addition, the lack of consistency within-individual could be explained by the small sample size, which increased the likelihood of a type II error.

The between-group differences we found in the OFT suggest that genetic background may play a significant role in determining activity levels in minipigs. Examining activity level as a personality trait, previous studies have reported two distinct categories of pigs: proactive and reactive (O'Malley et al., 2019). Proactive pigs, sometimes referred to as high-resisting pigs, have been reported to be more active and bolder than reactive pigs, also known as low-resisting pigs (O'Malley et al., 2019). If, as our results suggest, activity levels are at least partly genetically based, then selection for activity levels in pigs may be feasible, which could lead to better management and welfare. Other factors, such as early-life experiences and hierarchical dynamics within the groups may have influenced our results. Indeed, personality is influenced by both genetic and epigenetic factors that allow individuals to adapt to their environment and group dynamics, especially in gregarious species (O'Malley et al., 2019). However, the fact that the three groups of clones were mixed in the boxes according to their weight and still retained a difference in the activity levels reinforces the idea that the personality differences detected are at least partly genetic. In contrast to the OFT, we did not find any differences between different clone lines for HAT and NOT matching a previous report on the cognitive abilities of cloned minipigs (Paganelli et al., 2023) and a low reported heritability of the latency of the pigs to touch humans during HAT (Scheffler et al., 2014). However, the lack of between-group differences we found could also be because the HAT and NOT were conducted in a novel arena, which would have limited the experimenters' ability to detect shyness/boldness independently of exploration, as previously reported (O'Malley et al., 2019). Last, the lack of differences between groups could be due, as with the lack of within-individual consistency, to the small sample available.

Limitations and future research directions

There are some limitations of our study which means that our results should be interpreted with caution. The number of groups and the number of pigs within groups was small. This may have affected the power of our statistical analysis, both for within-individual and between-group comparisons. However, this limitation should be considered in the context of the uniqueness of the population, which consisted entirely of cloned minipigs from three different cell lines. The small number of animals is linked to the complexity of the cloning procedure and the resulting costs. Moreover, we accounted for the small sample size by using a longitudinal repeated measures design and multiple tests to assess personality. Our minipig population consisted only of males. This was a technical limitation due to the management requirements of the animals, but it would be desirable to sample both sexes and mixed-sex groups, as personality can be influenced by sex (Camerlink et al., 2022), and female pigs appeared to be more stress-prone than their male counterparts when exposed to novelty (Adcock et al., 2015). The decision to house the minipigs in three groups of four individuals based on weight may have influenced the hierarchical dynamics within the groups and, thus, the responses of individuals to the behavioral tests. In our study, we employed the commonly used OFT, HAT and NOT to assess personality traits in the minipigs, but we did not consider other potentially useful behavioral tests. Finally, we do not know whether it is possible to generalize our results to non-cloned pigs, as cloned pigs may have different behavioral patterns than their non-cloned counterparts, and only cloned subjects were included in our study.

Despite its limitations, our study investigated personality traits in cloned minipigs, a sample that provides a rare opportunity to isolate genetic influences from environmental variability. Activity level seems to be a promising personality trait in the selection of proactive animals, more resistant to stress and less influenced by the external environment, and in general, knowledge of personality traits could help in the formation of groups, with a reduction in intraspecific aggression and a consequent improvement in welfare. This pilot study has, therefore, provided a starting point for future studies that aim to investigate the genetic basis of personality to potentially select animals and improve their welfare.

In future studies, it would be desirable to increase the sample size to more confidently verify the existence of within-individual consistency and between groups differences in the personality of cloned minipigs. Moreover, it would be useful to test minipigs with other behavioral tests namely, for example, food competition test (i.e., a group of pigs is fed at the same time and then aggression, success in obtaining food, or order of feeding is recorded), food motivation test (i.e., pigs are fasted for some time, then fed and their behavior recorded), and social challenge test (i.e., pigs are introduced into a neutral space and their interactions are recorded) (Bell et al., 2009; Camerlink et al., 2016) to obtain a more complete assessment of minipig personality. Both sexes, male and female, should be included in future studies, and an assessment of hierarchy within the groups to contextualize behavioral responses would certainly be desirable. If our findings are confirmed and certain personality traits are found to be genetically based, breeding efforts could be directed toward obtaining more active (i.e., proactive) pigs that are less affected by the housing environment, have stronger immune responses, and are less susceptible to stress-related pathologies.

Conclusion

Our study is among the first to assess personality in cloned minipigs. Our results are consistent with some, but not all previously published findings in minipigs. In our study, activity levels assessed by the OFT showed within-individual consistency and an influence of genetic background. In contrast, boldness and exploration, assessed by the HAT and the NOT, showed neither individual consistency nor significant differences between clone lines. The relatively small number of subjects included in the study limits the statistical power, but the use of a longitudinal repeated measures design and multiple tests to assess personality reduced random variability, controlled for individual differences, minimized the influence of outliers, and strengthened the robustness of the assessment, providing a basis for further studies. Nevertheless, our study provides a first insight into the genetic underpinnings of personality traits in minipigs. Based on our preliminary results, future studies can verify if activity level can be one of the parameters to consider in the selection and management of pigs. A better understanding of pig personality traits may make it possible to select animals that are more resistant to environmental stressors and to better organize groups, with less aggression, better growth and improved welfare.

Ethical Considerations

This was an observational study. No ethical approval was required. The animals involved in this observational study were part of a project approved by the University of Pisa's Animal Welfare Committee (OPBA) with resolution n° 28/21.

Declaration of Interest Statement

The authors declare no conflicts of interest.

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Author contributions

Martina Felici: conceptualization, data curation, investigation, methodology, validation, writing – original draft, review and editing. **Aurora Paganelli**: conceptualization, data curation, formal analysis, investigation, methodology, validation, writing – original draft, review and editing. **Tammi Cottrell**: data curation, formal analysis, writing – original draft, review and editing. **Micaela Sgorbini**: conceptualization, funding acquisition, project administration, resources, supervision, writing – original draft, review and editing. **Paolo Baragli**: conceptualization, writing – original draft, review and editing. **Adam Reddon**: conceptualization, formal analysis, methodology, software, validation, Writing – original draft, review and editing.

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Conflict of Interest

The authors declare no conflicts of interest.

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Table caption

Table 1. Results of descriptive statistics of the number of passages made by the animal from one quadrant to another (open field test, OFT) and the time spent close to the person or balloon (novel object test, NOT, and human approach test, HAT) in the three trials. Values are expressed as mean ± standard deviation and confidence interval (CI). Passages from one quadrant to another are expressed in numbers (n°) and times in seconds (sec).

	Clone group A			**Clone group B**			**Clone group C**		
	Trial 1	*Trial 2*	*Trial 3*	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 1*	*Trial 2*	*Trial 3*
OFT (CI)	13.00±8.02 (7.90, 18.10)	11.67±6.92 (7.27, 16.07)	14.00±5.22 (10.68, 17.32)	17.00±10.80 (10.14, 23.86)	21.75±10.87 (14.84, 28.66)	15.75±8.81 (10.15, 21.35)	23.50±4.95 (20.35, 26.65)	43.50±6.36 (39.46, 47.54)	25.00±5.66 (21.40, 28.60)
HAT (CI)	35.63±58.25 (-1.38, 72.64)	34.56±53.55 (0.54, 68.58)	19.12±32.86 (-1.76, 40.00)	27.96±29.48 (9.23, 46.69)	37.15±41.09 (11.04, 63.26)	28.82±40.39 (3.16, 54.48)	9.68±13.69 (0.98, 18.38)	55.02±57.29 (18.62, 91.42)	52.51±23.46 (37.60, 67.42)
NOT (CI)	40.16±56.37 (4.34, 75.98)	29.95±36.08 (7.03, 52.87)	25.00±34.09 (3.34, 46.66)	73.19±72.16 (27.34, 119.04)	26.98±25.10 (11.03, 42.93)	37.77±46.34 (8.33, 67.21)	63.78±48.75 (32.81, 94.75)	35.07±29.44 (16.36, 53.78)	91.09±72.47 (45.04, 137.14)

Legend: OFT = Open Field Test; HAT = Human Approach Test; NOT = Novel Object Test; CI = confidence interval; Clone group A = 6 minipigs; Clone group B = 4 minipigs; Clone group C = 2 minipigs

Table 2. Output of Linear Mixed Models examining minipig behavior as a function of trial number, clone group, and the interaction between trial number and clone group.

		F	**df**	**p**
OFT	Trial number	2.83	2,19.96	0.08
	Clone group	**11.13**	**2,25.75**	**<0.001**
	Trial*Clone	1.74	4,19.96	0.18
HAT	Trial number	0.33	2,20.45	0.72
	Clone group	0.10	2,24.99	0.91
	Trial*Clone	0.40	4,20.45	0.81
NOT	Trial number	1.20	2,21.36	0.32
	Clone group	1.07	2,21.50	0.36
	Trial*Clone	0.62	4,21.36	0.66

Legend: OFT = Open Field Test; HAT = Human Approach Test; NOT = Novel Object Test

Figure caption

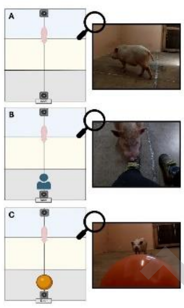

Figure 1. Schematic representation of the experimental box in which the tests were performed. A) open field test; B) human approach test; C) novel object test. Minipigs were considered far away when they were in the blue quadrants and close when they were in the grey quadrants. Components of the tests are not drawn to scale.

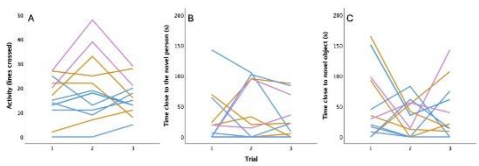

Figure 2. Plots of the 12 minipigs' behavior in each of the three tests: A) open field test; B) human approach test; and C) novel object test, with each line within each plot representing a single animal. The three clone groups are represented as blue (clone group A), orange (clone group B), and purple (clone group C) lines. Activity level was significantly repeatable across the three trials, while the time close to the person or balloon was not. The three clone groups significantly differed in activity level (A) but not in time close to the person (B) or balloon (C).

Highlights

- Minipigs were tested using the Open Field, Human Approach, and Novel Object tests
- Activity was repeatable within individual pigs and differed between clone groups
- Activity resulted partly genetically based and potentially selectable
- Boldness and exploration were neither repeatable nor different between groups
- Selection of personality traits would improve minipig welfare and management