

# Selection for reluctance to avoid humans during the domestication of mice

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**Many animal species have been domesticated over the course of human history and became tame as a result of domestication. Tamelessness is a behavioral characteristic with 2 potential components: (1) reluctance to avoid humans and (2) motivation to approach humans. However, the specific behavioral characteristics selected during domestication processes remain to be clarified for many species. To quantify these 2 different components of tamelessness separately, we established 3 behavioral tests: the ‘active tame’, ‘passive tame’ and ‘stay-on-hand’ tests. We subjected genetically diverse mouse strains to these tests, including 10 wild strains (BFM/2Ms, PGN2/Ms, HMI/Ms, BLG2/Ms, NJL/Ms, KJR/Ms, SWN/Ms, CHD/Ms, MSM/Ms and CAST/Ei), a fancy strain (JF1/Ms) and 6 standard laboratory strains (C3H/HeNjcl, CBA/J, BALB/cAnNCrCrlj, DBA/2Jjcl, 129<sup>+Ter</sup>/SvJcl and C57BL/6Jjcl). To analyze the effects of domestication, these 17 strains were divided into 2 groups: domesticated strains (fancy and laboratory strains) and wild strains. Significant differences between strains were observed in all traits, and the calculated estimates of broad-sense heritability were 0.15–0.72. These results illustrate that tamelessness in mice is significantly influenced by genetic background. In addition, they clearly show the differences in the features of tamelessness in domesticated and wild strains. Most of the domesticated strains showed significantly greater reluctance to avoid humans than wild strains, whereas there was no significant difference in the level of motivation to approach humans between these 2 groups. These results might help to clarify the genetic basis of tamelessness in mice.**

Keywords: Domestication, handling test, heritability, inbred strains, tame behavior, wild mice

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Humans have developed many domestic animals for various purposes. These include companion animals (e.g. dogs and cats), farm animals (e.g. cattle, pigs and chickens) and experimental animals (e.g. mice and rats). Domesticated animals commonly have characteristics of tamelessness that are absent from their wild ancestors. Tamelessness is defined as increased interaction of an animal with humans, and is ‘a measure of the extent to which an individual is reluctant to avoid or motivated to approach humans’ (Price 2002). Tamelessness is negatively correlated with levels of anxiety and fear toward humans or environmental situations associated with humans (Albert *et al.* 2008; Price 2002). The capacity for habituation to interactions with human, a tendency to explore novel situations and altered functions of sensory systems are also associated with tamelessness. It seems that the wild ancestors of domesticated animals displayed a genetic variation for tamelessness, which was subjected to natural or artificial selection to produce tamelessness in domestic animals through the domestication process (Darwin 1861; Driscoll *et al.* 2009).

To understand the influence of genetics on tamelessness, long-term behavioral selection experiments on foxes and rats have been performed in Russia (Belyaev 1978; Trut 1998, 1999). In the case of foxes, 30 males and 100 vixens, most of them from a commercial fur farm, were used as founder animals. Initially, Belyaev and coworkers developed behavioral tests to evaluate tamelessness in foxes. Using the results of these tests, they crossed the 4–5% tamest females with the 20% tamest males to produce the next generation, by maintaining genetic heterogeneity to some extent. The selection was successful, and the proportion of male and female foxes showing a high score of tamelessness increased from 18% to 35% from the 10th to the 20th generation.

In another set of experiments, tame and aggressive rats were selected from a wild-caught population of *Rattus norvegicus* (Albert *et al.* 2011; Belyaev & Borodin 1982). The researchers selected 30% of animals that exhibited the highest scores or the lowest scores of aggression toward an approaching human hand, and allowed them to mate within each population to produce successive generations. This selection revealed a behavioral difference by the 10th to 12th generation, and generated two lines, one of which was aggressive and the other of which was tame. These results indicate that tame behavior is at least sometimes influenced by genetic factors, and might be used as a behavioral parameter to study the domestication process in animal species.

In mice, laboratory strains have been established from a group of fancy mice that originated mostly from European wild mice, *Mus musculus domesticus* (Bonhomme & Guénet 1996; Ferris *et al.* 1983; Yonekawa *et al.* 1980). On the other hand, many mouse fanciers in Japan in the late 18th century bred a variety of mutant mice, such as the albino, agouti,

pink-eyed dilution, dwarf, waltzer and piebald strains (Tokuda 1935). Some of these fancy mice were later introduced into Europe, and subsequently into North America, and their phenotypes and the genetic bases of some of these phenotypes were reported (Darbishire 1902a, 1902b, 1903, 1904; Gates 1926; Koide *et al.* 1998; Little & Tyzzer 1915; So & Imai 1920; Tyzzer 1915; Yerks 1907). However, it is unclear how these European and Japanese fancy mice were produced from the ancestral wild mice.

The *M. musculus* species in the wild is divided into three major subspecies groups: Domesticus, Musculus and Castaneus (Bonhomme & Guénet 1996; Moriwaki *et al.* 1994; Silver 1995). These three groups differ genetically, as shown by the diversity of their biochemical markers and mitochondrial DNA, and polymorphisms in their nuclear genes. Several research groups have developed a variety of inbred strains from wild mice captured in different countries (Bonhomme & Guénet 1996; Gregorova & Forejt 2000; Moriwaki *et al.* 1994, 2009). The high frequency of genetic polymorphism among these genetically defined wild strains has proven to be useful for many genetic and behavioral studies (Koide *et al.* 2000). The wild strains exhibited large phenotypic differences in a variety of behavioral tests (Furuse *et al.* 2002; Koide *et al.* 2000, 2011; Sugimoto *et al.* 2011, Takahashi *et al.* 2006). Moreover, these strains generally showed behavioral phenotypes characteristic of wild mice, namely, rapid movement, excessive responses to handling and higher anxiety-like behavior than tame strains, almost certainly because the wild strains had never been subjected to deliberate attempts to domesticate them (Koide *et al.* 2000, 2011; Takahashi *et al.* 2006). In spite of the characteristics of wildness observed in these wild strains, they may to some extent show the behavioral repertoire for potential tameness owing to the substantial genetic diversity among the strains. Furthermore, it is important to determine how tameness in wild strains differs from that in

domesticated strains. Therefore, we aimed to characterize tame behavior using 17 inbred mouse strains: 10 wild strains, 1 Japanese fancy-mouse strain and 6 laboratory strains.

Several handling tests were previously reported to evaluate tameness in deer mice and rats (Albert *et al.* 2009; Cottle & Price 1987; Haysen 1997). In this study, on the basis of these previous reports, we developed three behavioral tests for measuring tameness in mice: the 'active tame', 'passive tame' and 'stay-on-hand' tests. The three types of tame test could characterize the levels of 'reluctance to avoid humans' and 'motivation to approach humans' separately. In the active tame test, we placed one hand in an open-field arena and waited for the animal to move toward it; this enabled measurement of the animal's motivation to approach the hand. In contrast, in the case of the passive tame test, we put one hand in the open-field arena and then pursued the mouse slowly until the hand touched it. Thus, the passive tame test measures the level of reluctance to avoid the hand. The stay-on-hand test measures an aspect of tameness similar to that of the passive tame test, but it involves subjecting the mouse to more tight contact with human hand insofar as the mouse is forcibly placed on the hand and then stroked gently with a thumb. These tests enable the assessment of various aspects of tame behavior in mice.

## Materials and methods

### Animals

A full list of the 17 inbred strains used in the present study and their origins is shown in Table 1, which includes definitions of all abbreviations. The 10 strains – BFM/2, PGN2, CAST, HMI, BLG2, NJL, KJR, SWN, CHD and MSM – were derived from wild mice (Furuse *et al.* 2002; Koide *et al.* 2000; Moriwaki *et al.* 2009). The CAST strain was obtained from The Jackson Laboratory (Bar Harbor, ME, USA), and the other nine wild strains were established at the National Institute of Genetics (NIG), Mishima, Japan. An inbred strain,

**Table 1:** Mouse strains used in this study

Origin	Strain	Abbr.	Subspecies group	Subspecies	Place of collection	Year of capture	Current generations
Laboratory mice	C3H/HeNjcl	C3H	Domesticus				
	CBA/J	CBA	Domesticus				
	BALB/cAnNCrIcrlj	BALB	Domesticus				
	DBA/2Jjcl	DBA/2	Domesticus				
	129+Ter/SvJcl	129	Domesticus				
	C57BL/6J	B6	Domesticus				
Fancy mouse	JF1/Ms	JF1	Musculus	<i>M. m. molossinus</i>	Denmark*	1987	F35
Wild mice	PGN2/Ms	PGN2	Domesticus	<i>M. m. domesticus</i>	Ontario, Canada	1979	F77
	BFM/2Ms	BFM/2	Domesticus	<i>M. m. brevisrostris</i>	Montpellier, France	1976	F108
	CAST/EiJ	CAST	Castaneus	<i>M. m. castaneus</i>	Thailand	1971	F96
	HMI/Ms	HMI	Castaneus	<i>M. m. castaneus</i>	Heimei, Taiwan	1986	F64
	NJL/Ms	NJL	Musculus	<i>M. m. musculus</i>	Northern Jutland, Denmark	1980	F76
	BLG2/Ms	BLG2	Musculus	<i>M. m. musculus</i>	General Toshevo, Bulgaria	1980	F89
	CHD/Ms	CHD	Musculus	<i>M. m. gansuensis</i>	Chendu, China	1981	F76
	SWN/Ms	SWN	Musculus	<i>M. m. yamasinai</i>	Suwon, Korea	1984	F65
	KJR/Ms	KJR	Musculus	<i>M. m. yamasinai</i>	Kojuri, Korea	1984	F79
	MSM/Ms	MSM	Musculus	<i>M. m. molossinus</i>	Mishima, Japan	1978	F84

\*JF1 was found in Denmark but characterized as a Japanese fancy mouse by genetic study (Koide *et al.* 1998).

JF1, was established from Japanese fancy mice at NIG (Koide *et al.* 1998). In addition to JF1, six laboratory strains – C3H, CBA, BALB, DBA/2, 129 and B6 – were used as domesticated strains. Mice of four laboratory strains (C3H, DBA/2, 129 and B6) and two laboratory strains (CBA and BALB) were obtained from CLEA Japan Inc. (Tokyo, Japan) and Charles River Japan Inc. (Yokohama, Japan), respectively. All strains were kept under specific-pathogen-free (SPF) conditions at the NIG in a 12-h/12-h light/dark cycle with food and water available *ad libitum*. After weaning, we used large tweezers covered with silicon tubing to handle the mice when changing bedding. Naive males ( $n=12$ ) from each strain were used in phenotypic analyses. All procedures were carried out with approval (permit number 22–12) from the Committee for Animal Care and Use of the NIG.

### Phenotypic measurements

Three different tame tests were established and performed in order to measure the degree of tameness in mice. All tests were carried out during the light period using an open-field arena (40 × 40 × 40 cm) made of grayish polyvinylchloride (O'Hara & Co. Ltd., Tokyo, Japan) and illuminated with 100lux at the center of the field. Animals were monitored by video recording using a commercially available CX5 digital camera (Ricoh Company, Ltd., Tokyo, Japan). The three tame tests – the (1) active tame test, (2) passive tame test and (3) stay-on-hand test – were applied sequentially for each mouse. The active tame test was conducted first because this test involves less contact of the mouse with a human hand than the other two tests; this reduces the likelihood of habituation to the human hand. The mice were 42–48 days (henceforth referred to as 6 weeks) of age when they experienced the open-field arena for the first time. After undergoing this series of tame tests at 6 weeks, the mice were individually identified by the ear-punch method. All the mice were then returned to the original group-housing cage and kept until 56–62 days (8 weeks) of age in order to undergo a second round of the same tame tests. We chose 6 and 8 weeks for behavioral tests to investigate tame behavior in both young and adult mice. The mice were group-housed, except when undergoing the behavioral tests, and kept with the same mice until all of the behavioral tests were completed. Each mouse was subjected to all three behavioral tests at both 6 and 8 weeks. Once all three tests had been completed for each mouse, the test arena was wiped with cloth impregnated with 0.2% Mikro Klene disinfectant (Ecolab G.K., Tokyo Japan), followed by wiping with a dry paper towel to ensure that the test field was clean and dry. The mice used in the study were familiar with the disinfectant used, because it was routinely used to clean the instruments used when changing the beddings in their cages. The gloves that were worn during the tame tests were cleaned in the same manner as the test arena after testing each mouse. The next mouse was then evaluated using the same series of tame

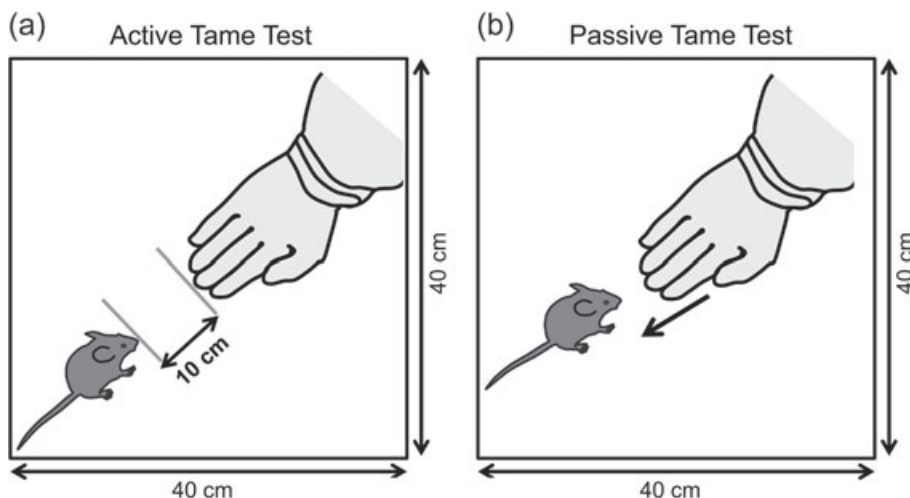
tests. All behavioral traits were measured in terms of the duration of events at a resolution of 0.1 seconds using tanaMove software (version 0.01), which is freely available from the website of the NIG (<http://www.nig.ac.jp/labs/MGRL/tanaMove.html>). All the behavioral tests were conducted by the same experimenter; besides the hand of the experimenter, mice were able to see the face and upper body of the experimenter by looking upward.

### Active tame test

The active tame test was designed to evaluate an animal's active responses to a human hand. Two examples of this test, in which high and low levels of tameness were exhibited, are shown in Movies S1 and S2, Supporting Information, respectively. Each mouse was gently caught by its tail using tweezers covered with silicon tubing, in order to reduce pain, and placed gently in the center of the field. An experimenter placed his hand at the bottom of the test field and attempted to keep his hand approximately 10 cm away from the mouse during the 1-min trial (Fig. 1a). At the same time, the experimenter moved his fingers slightly but continuously to ensure that the mouse realizes that hand covered with the plastic glove was part of a human body. When the mouse moved away, the hand followed it, while maintaining the same distance. However, when the mouse headed toward or contacted the hand, the hand was kept in the same position with slight movement of the fingers. If a mouse attempted to escape by climbing up the experimenter's arm, it was placed back in the field using the other hand. The durations of three behavioral traits – heading toward the hand, contacting the hand and jumping – were measured (Movies S1 and S2). In addition, locomotion was measured as the duration of traveling, including the heading and jumping behavior described above.

### Passive tame test

The passive tame test was designed to evaluate an animal's passive responses to a human hand. Two examples of this test, in which high and low levels of tameness were exhibited, are shown in Movies S3 and S4, respectively. We conducted the passive tame test directly after the active tame test. An experimenter again placed his hand at the bottom of a test field without moving his fingers during the 1-min trial. The hand continually pursued the mouse slowly until it touched the body of the mouse (Fig. 1b). If the mouse accepted being touched, the experimenter attempted to touch the mouse for as long as possible. If the mouse climbed up the experimenter's arm, it was placed back in the field in the same manner as in the active tame test. The durations of three behavioral traits (heading toward the hand, accepting touching by the hand and jumping) were measured for each trial (Movies S3 and S4). Heading toward the hand, jumping and locomotion are defined in the same manner as in the active tame test.



**Figure 1: Schematic representations of the active and passive tame tests.** (a) Active tame test. (b) Passive tame test. Detailed procedures are described in *Materials and methods*.

### Stay-on-hand test

The stay-on-hand test was used to quantify behavioral responses to forced stimulation by a human. Two examples of this test, in which high and low scores were exhibited, are shown in Movies S5 and S6, respectively. This test was conducted directly after the passive tame test. An experimenter picked up the mouse by its tail using tweezers and placed it on his hand three times (three trials). While the mouse remained on the hand, the thumb was continually used to stroke the back of the mouse softly at a frequency of once a second. The maximum time was set to 10 seconds for each trial. The lengths of time that the mouse remained on the hand for the three trials were counted from each movie and their median was treated as the trait data (Movies S5 and S6).

### Statistics

One-way analysis of variance (ANOVA) was performed to compare factors in terms of 'strain' (17 strains) for all behavioral traits except for jumping. If there was a significant difference ( $P < 0.05$ ), a *post hoc* test was conducted using Tukey's honestly significant difference test with StatView ver. 5 (SAS Institute Inc., Cary, NC). Jumping behavior was treated as categorical data, and was compared with the effect of the strain of animal involved by using Kruskal–Wallis one-way ANOVA.

We compared all behavioral indices for the effect of domestication. We also divided the 17 strains into two groups: domesticated (six laboratory strains and a fancy strain) and wild strains (10 wild strains). Thereafter, the mean values of the 17 strains were analyzed by unpaired two-tailed Student's *t*-test ( $P < 0.05$ ) for comparison between domesticated and wild groups.

Genetic correlations were analyzed for all possible combinations of behavioral traits. Pearson's correlation coefficients were determined using StatView. Mean scores of the strains ( $n = 17$ ) were used for the analysis. We adjusted the *P* value for multiple comparisons (Bonferroni,  $N = 36$ ), and the significant threshold was set at  $P = 0.00139$ .

To compare the results of the three tame tests between 6 and 8 weeks, Pearson's correlation coefficients were calculated and their significance was assessed by Fisher's *z*-conversion test. Mean values of the strains ( $n = 17$ ) for each behavioral trait at both ages were used for the genetic correlation analyses.

Broad-sense heritability was estimated from the intraclass correlation between members of the same inbred strain ( $r_1$ ), following the method of Festing (1979):

$$r_1 = (MS_B - MS_W) / [MS_B - (n - 1) MS_W],$$

where  $MS_B$  is the mean square of the between strain,  $MS_W$  is the mean square of the within strain and  $n$  is the number of individuals tested per strain. These values were obtained in one-way ANOVA for each phenotype.

## Results

### The active tame test

The active tame test aims to quantify an animal's tendency to head actively toward and/or contact a human hand (Fig. 1a). We examined the tame behavior of the strains at 6 weeks of age. In terms of the amount of time spent heading toward a human hand, ANOVA showed significant differences between the strains ( $F_{16,187} = 11.020$ ,  $P < 0.0001$ ). The 129, CAST and BLG2 strains spent a significantly longer amount of times actively heading toward a human hand than the other strains, whereas PGN2, SWN and MSM spent significantly shorter times actively heading toward a human hand than the other strains tested ( $P < 0.05$ ) (Fig. 2a, Table S1).

In terms of the duration of locomotion, ANOVA showed significant differences between the strains ( $F_{16,187} = 33.094$ ,

$P < 0.0001$ ). The PGN2, HMI, CHD, SWN and MSM strains showed significantly shorter times than the other strains ( $P < 0.05$ ) (Fig. 2a, Table S1).

ANOVA also showed significant strain differences ( $F_{16,187} = 8.341$ ,  $P < 0.0001$ ) in terms of the duration of contacting a human hand. The 129 and CAST strains exhibited significantly longer time in contact with a human hand than the other 15 strains ( $P < 0.05$ ) (Fig. 2c, Table S1).

The duration of jumping behavior also differed significantly between strains (Kruskal–Wallis test,  $P = 0.0053$ ). The C3H, CBA, BALB, DBA/2, 129, B6, JF1, BLG2 and CHD strains never showed jumping behavior (Fig. 2e).

To investigate the phenotypic differences between domesticated and wild groups, we tested the amount of time spent heading toward a human hand, the amount of time spent contacting a human hand and the time spent jumping. However, there were no significant differences between the two groups in these three traits (Fig. 2b,f,h). In contrast, locomotion of the domesticated group in active tame test proceeded for a significantly longer duration than for the wild group ( $P = 0.0128$ ; Fig. 2d).

### The passive tame test

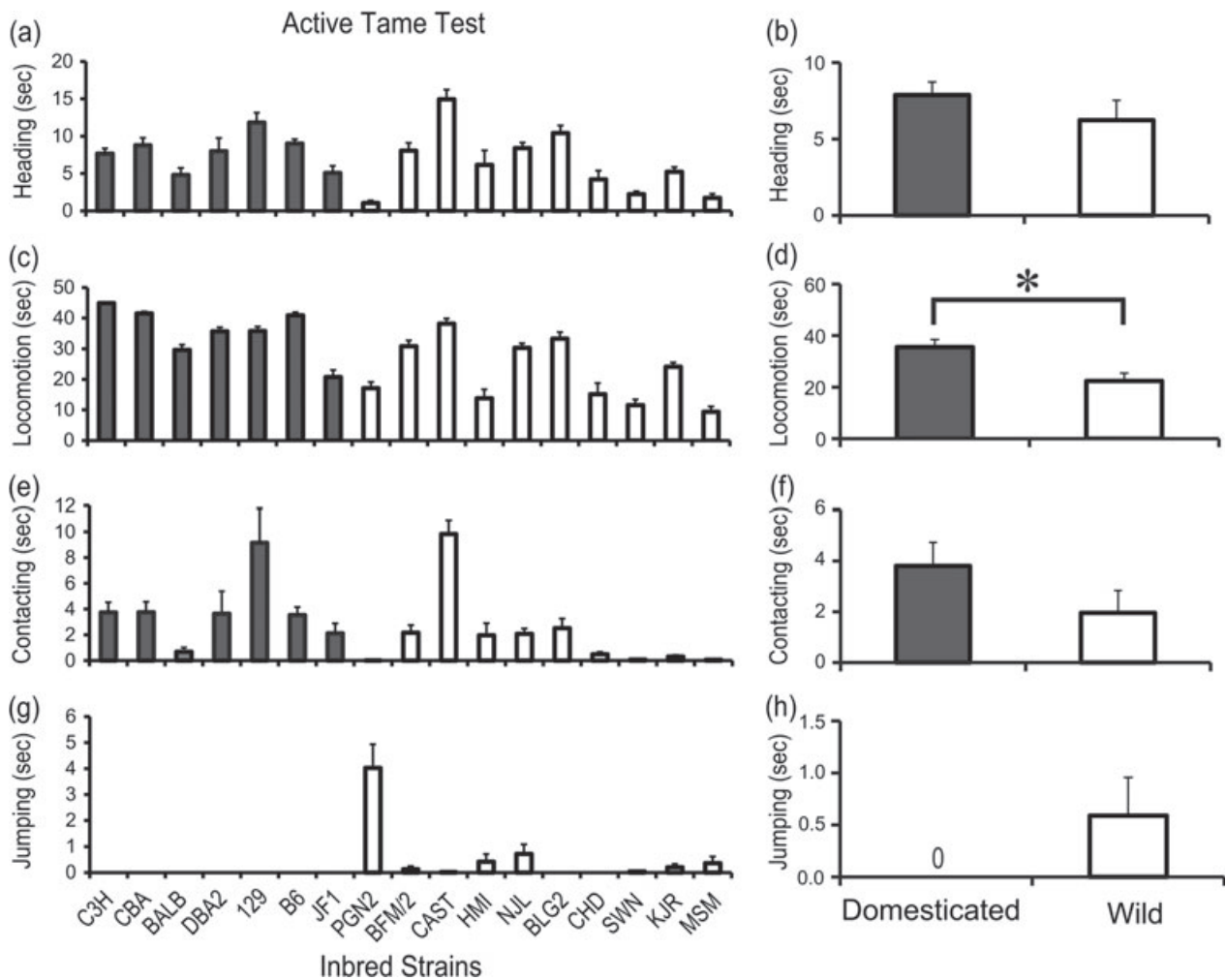
The passive tame test aims to quantify an animal's response when it is continuously pursued by a human hand (Fig. 1b). In the results of this test at 6 weeks of age, ANOVA showed significant differences between the strains in terms of the amount of time spent heading toward a human hand that is pursuing them ( $F_{16,187} = 31.588$ ,  $P < 0.0001$ ). The C3H, CBA, BALB, DBA/2 and 129 strains exhibited a significantly longer amount of time spent heading toward a human hand that is pursuing them than the other strains tested ( $P < 0.05$ ; Fig. 3a, Table S1). The domesticated group showed a significantly longer amount of time spent heading toward a pursuing hand than the wild group (Fig. 3b,  $P = 0.0006$ ).

ANOVA showed significant strain differences ( $F_{16,187} = 12.496$ ,  $P < 0.0001$ ) in terms of the duration of locomotion. The JF1, BLG2, HMI and SWN strains showed significantly shorter times than the other strains ( $P < 0.05$ ; Fig. 3c, Table S1). The domesticated group showed a significantly longer duration of locomotion than the wild group ( $P = 0.0259$ ; Fig. 3d).

There was a significant difference between strains ( $F_{16,187} = 18.913$ ,  $P < 0.0001$ ) in the duration of accepting being touched by a human hand without fleeing. The C3H, CBA, BALB, DBA/2, 129 and JF1 strains showed significantly longer durations of this characteristic among the 17 strains ( $P < 0.05$ ), whereas PGN2 and MSM showed shorter durations ( $P < 0.05$ ; Fig. 3e, Table S1). The domesticated group showed higher values than the wild group ( $P < 0.0001$ ; Fig. 3d).

In terms of the duration of jumping upon the approach of a human hand, significant differences were found between strains (Kruskal–Wallis test,  $P < 0.0001$ ). The PGN2 and NJL strains exhibited more jumping, whereas C3H, CBA, BALB, DBA/2, 129, B6, JF1 and CAST strains never showed jumping behavior (Fig. 3e). As shown in Fig. 3f, the wild group exhibited significantly more jumping behavior than the domesticated group, which did not jump at all ( $P = 0.0485$ ).





**Figure 2: Comparison of heading toward the hand, locomotion, contacting the hand and jumping in the active tame test among the different strains at 6 weeks of age.** (a, c, e and g) Differences among the 17 strains. (b, d, f and h) Differences between domesticated and wild-derived mice. Bars indicate standard error of the mean (SEM).

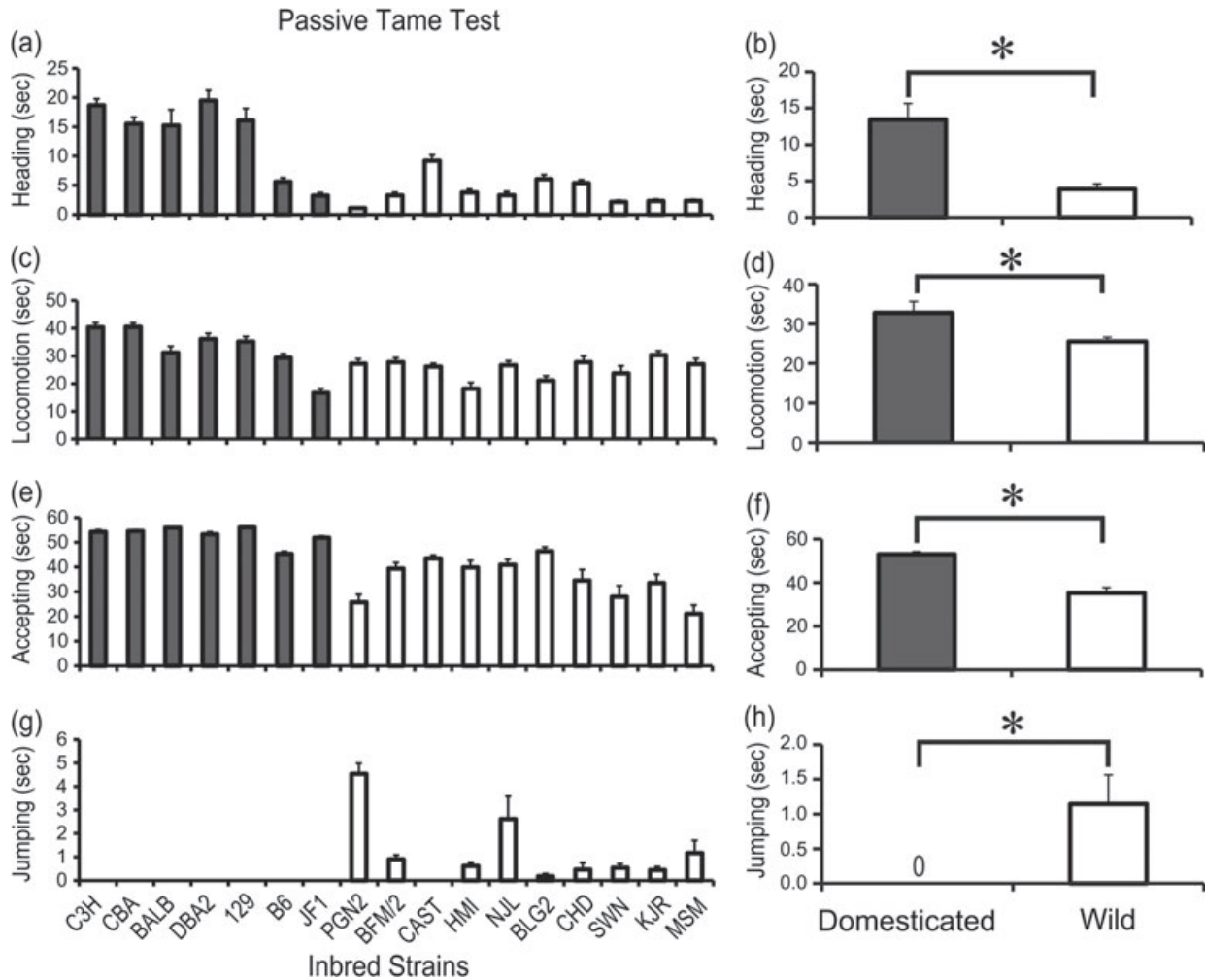
### The stay-on-hand test

The stay-on-hand test aims to quantify an animal's response to forced handling by a human. ANOVA indicated a significant difference between strains ( $F_{16,187} = 23.956$ ,  $P < 0.0001$ ) in terms of time remaining on a hand (staying time) in the stay-on-hand test at 6 weeks of age. As shown in Fig. 4a, the 129 strain showed a significantly longer staying time on a hand than any of the 16 strains tested ( $P < 0.05$ ; Table S1). The other domesticated mice also showed significantly longer staying times ( $P < 0.05$ ; Table S1). There was a significant difference between the domesticated and wild groups ( $P = 0.0127$ ; Fig. 4b).

### Correlations between behavioral traits

To compare the relationships between the different behavioral traits, genetic correlations between all combinations

of traits were analyzed (Fig. 5). 'Heading toward' and 'contacting' the hand in the active tame test showed a strong correlation ( $r = 0.88$ ), which indicated that these two traits in this tame test reflect similar characteristics. 'Locomotion' in the active tame test showed significant correlations with 'heading toward' in both active and passive tame tests and 'accepting' in the passive tame test. 'Heading toward' the hand in the passive tame test showed a strong correlation with 'locomotion' and 'accepting' in the passive tame test. The strong correlation between 'jumping' behavior in the active and passive tame tests ( $r = 0.92$ ) suggested that jumping is caused independently of the specific situation in the tame tests. Several combinations of behavior showed distributions with an 'L' shape. 'Jumping' in the passive tame test showed these types of distribution when plotted against 'contacting' a human hand in the active tame test, 'heading toward' a pursuing human hand in the passive tame test and 'staying time' in the stay-on-hand test. Similar tendencies



**Figure 3: Comparison of heading toward the hand, locomotion, accepting touching by the hand and jumping in the passive tame test among the different strains at 6 weeks of age.** (a, c, e and g) Differences among the 17 strains. (b, d, f and h) Differences between domesticated and wild-derived mice. Bars indicate standard error of the mean (SEM).

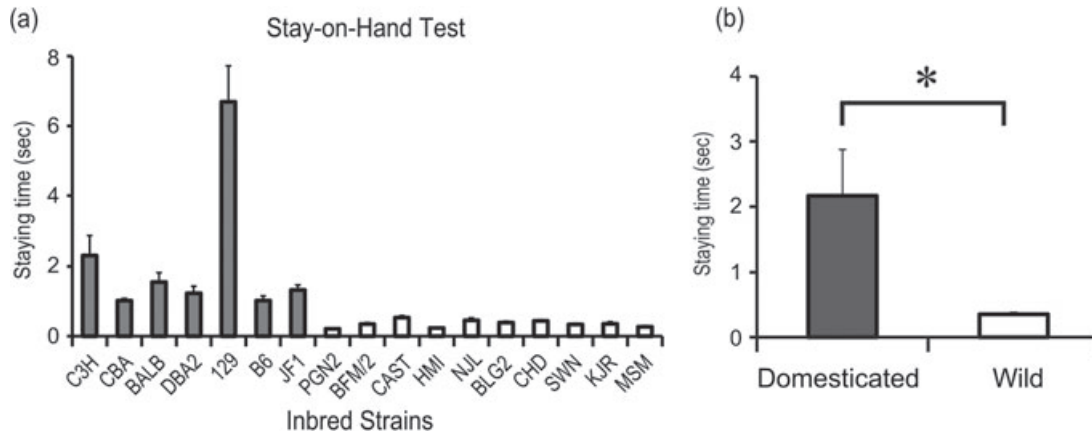
were observed when 'jumping' in the passive tame test was plotted against 'contacting' in the active tame test, and 'heading toward' a pursuing human hand in the passive tame test was plotted against 'staying time' in the stay-on-hand test. These 'L'-type distribution patterns suggested an association between lacking one type of behavior and accentuation of the contrasting trait.

#### Correlations of traits between animals of 6 and 8 weeks of age

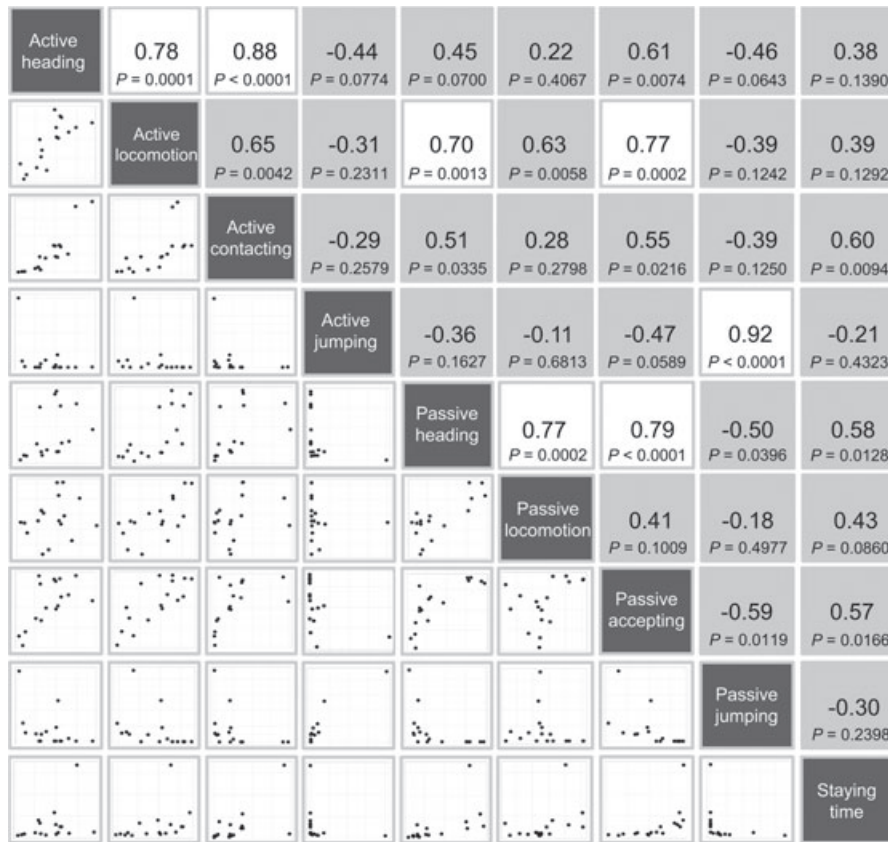
We conducted the same set of tame tests at 8 weeks of age as had been done at 6 weeks (Fig. S1). To determine behavioral changes between 6 and 8 weeks, we determined genetic correlations of trait data between these two ages (Table 2). High correlations ( $r = 0.52$ – $0.99$ ) were found in all behavioral traits. Moreover, the pattern of strain differences at 8 weeks was similar to that at 6 weeks.

#### Estimation of heritability

Broad-sense heritability estimates for all traits were subdivided by age and are shown in Table 3. Heritability estimates at 6 weeks for the amount of time spent heading toward a pursuing human hand and the duration of accepting being touched by a human hand in the passive tame test and staying time in the stay-on-hand test were relatively high (0.60–0.72). At 8 weeks of age, these heritability estimates were slightly higher (0.66–0.79). On the other hand, heritability estimates at 6 weeks of age for 'heading toward' and 'contacting' a human hand, 'locomotion' as well as 'jumping' in the active tame test and 'jumping' in the passive tame test (0.38–0.73) had decreased by 8 weeks of age (0.15–0.63). The decreased heritability in the 8-week-old mice relative to the 6-week-old mice can be attributed to decreased interstrain variance, except for the case of 'contacting in active tame test' for which increased intrastrain variance was observed (Table S2).



**Figure 4: Comparison of staying time in the stay-on-hand test among the different strains at 6 weeks of age.** (a) Differences among the 17 strains. (b) Differences between domesticated and wild strains. Bars indicate standard error of the mean (SEM).



**Figure 5: Scatter diagrams and Pearson's correlation coefficients between the different traits for all combinations of behaviors at 6 weeks of age.** The mean scores for 12 mice for each strain (a total of 17 strains) are plotted on each panel in the lower left side. Pearson's correlation coefficients between two different traits are indicated in the upper right side. The significant *P*-value threshold for multiple comparisons was adjusted with Bonferroni correction (*N* = 36), and set at 0.00139. The cells showing no significance are indicated in gray background.

### Discussion

This study assessed differences between mice strains in their tame behaviors toward humans. We evaluated different aspects of tameness using three different behavioral tests: the active tame, passive tame and stay-on-hand tests. The results of these three tests helped to categorize the features associated with tameness, 'reluctance to avoid humans'

and 'motivation to approach humans' in laboratory and wild strains. However, the tame tests applied to mice in this study involve several issues that need to be considered further. Given that tameness is defined as increased interaction of animals with humans, and involves many factors (such as anxiety, fear, novelty-seeking behavior, the ability to habituate to interaction with human and the functions of sensory systems), the results in this study are unable

**Table 2:** Genetic correlation of the data between 6 and 8 weeks in all traits

Trait measurement	Pearson's correlation	Fisher's z-conversion test
Heading in active tame test	0.79	$P < 0.0001$
Locomotion in active tame test	0.87	$P < 0.0001$
Contacting in active tame test	0.52	$P = 0.0294$
Jumping in active tame test	0.99	$P < 0.0001$
Heading in passive tame test	0.97	$P < 0.0001$
Locomotion in passive tame test	0.84	$P < 0.0001$
Accepting in passive tame test	0.85	$P < 0.0001$
Jumping in passive tame test	0.97	$P < 0.0001$
Staying in stay-on-hand test	0.85	$P < 0.0001$

**Table 3:** Broad-sense estimates of the heritability of all traits

Trait measurement	Intraclass correlation ( $r_1$ )	
	6 weeks	8 weeks
Heading in active tame test	0.46	0.31
Locomotion in active tame test	0.73	0.63
Contacting in active tame test	0.38	0.15
Jumping in active tame test	0.49	0.35
Heading in passive tame test	0.72	0.79
Locomotion in passive tame test	0.49	0.60
Accepting in passive tame test	0.60	0.66
Jumping in passive tame test	0.51	0.29
Staying in stay-on-hand test	0.66	0.69

to clarify the behavioral basis of tameness. Given that we applied the mice to the open-field test arena without acclimation, the test results are influenced by the level of anxiety induced in the mice by this novel environment. This possibility is supported by results that show that wild strains exhibited significantly lower locomotion than domesticated strains in both active and passive tame tests at 6 weeks of age, but that differences in the locomotion of the two groups were not significantly different in the second test at 8 weeks. The mice tested at 6 weeks would have been influenced by their anxiety over the novel test conditions, which might have been diminished in the same animals tested at 8 weeks, in addition to the difference in age. Furthermore, different levels of experience of contacting with human hand during the first tests (at 6 weeks) may have influenced the response to the hand during the second tests (at 8 weeks). Therefore, the difference of locomotion in both active and passive tame tests observed between the 6- and 8-week tests might be caused by one or a combination of these effects. However, there were similar tendencies in terms of the strain distribution patterns between 6 and 8 weeks (Table 2), and it is likely that age and test experience had much less of an influence on the characteristics of tameness than genetic background. In addition to the possibility of an association of anxiety with tameness, it is also possible that novelty seeking is a factor associated with tameness. Regarding the

above explanation related to anxiety, a change in the features of novelty-seeking behavior from the time of the first test (at 6 weeks of age) to that at the time of the second test (at 8 weeks) seems unlikely to have a drastic influence on the observed behaviors; the two sets of tests showed similar tendencies.

Our results clearly show differences in the characteristics of tameness in domesticated and wild strains. Compared with the wild strains tested, most of the domesticated strains showed longer amount of time spent heading toward a human hand and accepting touching by the hand in the passive tame test, as well as a longer staying time in the stay-on-hand test. These results suggest that domesticated mice have been selected for reluctance to avoid humans. In contrast, it is likely that these strains were never selected for higher motivation to approach humans, given that domesticated and wild strains did not show clear differences in terms of the durations of 'heading toward' and 'contacting' a human hand in the active tame test.

The results obtained in this study have some similarity to a previous study that investigated wildness in 21 inbred strains, including 4 wild strains (Wahlsten *et al.* 2003). Among the 21 strains, 6 strains used in that study were also used in our study, including a wild strain CAST. The study also showed that wild strains are more difficult to capture than most laboratory strains studied. Among the wild strains, CAST is more difficult to capture than B6, DBA/2, BALB/cByJ, C3H/HeJ and 129S1/SvImJ strains (Wahlsten *et al.* 2003); this is consistent with the results of the stay-on-hand test in this study. Comparison of these two studies indicated that decreased wildness has similarity to increased reluctance to avoid humans, although further investigation is needed to clarify this point. Given that a higher motivation to approach humans was not investigated in the previous study, we are unable to compare the results of active tame test to the results of the study by Wahlsten *et al.* (2003).

The JF1 strain was established from Japanese fancy mice (Koide *et al.* 1998). Its fancy-mouse ancestors may have been developed in Japan, as reported in 1787 (Tokuda 1935). They had been intensively selected for tameness, and expressed extremely tame characteristics, such as slow movement and limited responses to handling. However, the short duration of contact by JF1 in the active tame test suggests that the fancy-mouse ancestors of JF1 were not selected for a greater motivation to approach humans, as occurred for other European domesticated mice.

Among the 17 strains studied, several strains exhibited interesting behaviors. Most wild strains exhibited jumping in the passive tame test, although none of the laboratory strains showed this behavior. In particular, PGN2 and NJL strains showed a higher incidence of jumping than other wild strains. Given that jumping is characteristic of wild strains (Fernandes *et al.* 2004; Holmes *et al.* 2000), PGN2 and NJL strains have retained this characteristic behavior of wild mice. The genetic correlation analyses between several behavioral traits showed an 'L'-type distribution pattern (Fig. 5). The results suggest that longer duration of 'contacting' the hand in the active tame test, 'heading toward'



a pursuing human hand in the passive tame test and 'staying time' in stay-on-hand test may be associated with lack of 'jumping' behavior. This suggests that PGN2 and NJL strains should be considered to be less tamed than the other wild strains.

The 129 and CAST strains also displayed unusual traits related to tameness. In the active tame test, 129 exhibited long durations of heading toward and contacting a human hand. Therefore, 129 has the unusual characteristic of a higher motivation to approach humans actively. Furthermore, this strain showed an extremely long staying time in the stay-on-hand test, even when the experimenter stroked the mouse gently with his thumb. Thus, 129 showed tame behavior in terms of both 'reluctance to avoid humans' and 'motivation to approach humans', and turned out to be an extremely tame strain. The CAST strain also spent a considerable amount of time heading toward and contacting a human hand in the active tame test. In contrast, CAST did not show particularly long durations of accepting being touched by the hand in the passive tame test and staying time in the stay-on-hand test. These results indicate that CAST has characteristics of tameness in terms of 'motivation to approach humans' but not for 'reluctance to avoid humans'. It is noteworthy that CAST never showed jumping behavior in the passive tame test, which was unique among the wild strains. These results indicate that CAST has characteristics of tameness despite having been classified as a wild strain. Accordingly, these results are different to the previous report in that CAST was rated as one of four strains that exhibited the highest level of wildness among 21 inbred strains (Wahlsten *et al.* 2003).

Heritability of characteristics related to tameness from the tests was estimated to be in the range of 0.15–0.79 at both 6 and 8 weeks of age in the 17 strains. On the basis of studies of classical inbred strains, behavioral heritability estimates were reported to be approximately 0.2–0.7 for open-field activity, wheel running, running speed and locomotor behaviors (Billat *et al.* 2005; Festing 1979; Lightfoot *et al.* 2004, 2010; Mhyre *et al.* 2005). Although it should be noted that these studies used different sets of inbred strains, these results suggest that genetic factors play a role in determining tameness as evaluated by handling tests, as for other behavioral traits. As a next step, genetic mapping studies for tame behaviors should be performed to reveal genetic loci that contribute to the phenotypic variance. The clear segregation of the traits 'motivation to approach humans' and 'reluctance to avoid humans' when comparing the strains suggests that these two types of tameness are controlled by different genetic factors. Given that heterogeneous stock established from eight different inbred strains has been successfully used for the genetic mapping of complex traits (Demarest *et al.* 2001; Valdar *et al.* 2006), it would be useful to apply heterogeneous stocks (Koide *et al.* 2012) or the Diversity Outbred cross (Svenson *et al.* 2012) involving genomes of wild strains to address genetic factors that are associated with tameness in mice.

In conclusion, this study illustrates that tame behavior in mice is significantly influenced by genetic background. In addition, we report novel handling tests that can be used to evaluate the traits of both 'reluctance to avoid

humans' and 'motivation to approach humans'. Most of the domesticated strains showed significantly greater reluctance to avoid humans than wild strains, whereas there was no significant difference in the level of motivation to approach humans between these two groups. The results may shed light on the history of domestication of mice, and provide a starting point for further analysis of the genetic basis of tameness in mice.

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## Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

**Movie S1:** Example movie file for the active tame test. The movie file shows low tameness in this test. Timing and durations of three behavioral traits, 'heading toward' and 'contacting' a human hand as well as 'jumping', are indicated in the movie.

**Movie S2:** Example movie file for the active tame test. The movie file shows high tameness in this test. Timing and durations of three behavioral traits, 'heading toward' and 'contacting' a human hand as well as 'jumping', are indicated in the movie.

**Movie S3:** Example movie file for the passive tame test. The movie file shows low tameness in this test. Timing and durations of three behavioral traits, 'heading toward' a pursuing human hand and 'accepting' being touched by the hand as well as 'jumping', are indicated in the movie.

**Movie S4:** Example movie file for the passive tame test. The movie file shows high tameness in this test. Timing

and durations of three behavioral traits, 'heading toward' a pursuing human hand and 'accepting' being touched by the hand as well as 'jumping', are indicated in the movie.

**Movie S5:** Example movie file for the stay-on-hand test. The movie file shows low tameness in this test. Timing and durations of staying time are indicated in the movie.

**Movie S6:** Example movie file for the stay-on-hand test. The movie file illustrates the suitability of this test for demonstrating a high level of tameness. Timing and durations of staying time are indicated in the movie.

**Fig. S1:** Behavioral phenotype of the three tame tests at 8 weeks of age. The results showed high similarity of behavioral traits to those observed at 6 weeks of age (Figs. 2–4).

**Table S1:** Results of *post hoc* test following the one-way ANOVA for behavioral phenotype.

**Table S2:** Mean square of the between- and within-strain variance for behavioral phenotype.