Behavioral and adrenal responses to various stressors in mule ducks from different commercial genetic selection schemes and their respective parental genotypes

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ABSTRACT The mule duck, a hybrid produced by crossing a Muscovy drake and a Pekin female, is reported to express inappropriate behavior such as collective avoidance of people, the resulting distress and physical consequences potentially compromising their welfare. The present study was carried out to characterize the responses of mule duck strains from different commercial selection schemes to various stressful conditions and to confirm previous data on the genetic cross effects observed in a specific genotype. Three independent experiments were conducted with ducks from 3 French breeding companies (A, B, and C). Each experiment compared 2 mule genotypes sharing one common parental origin (paternal for ducks from company A or maternal for ducks from companies B and C). Mule duck males from the 2 genotypes and their respective parental genotypes (Pekin and Muscovy) were subjected to a set of social and stressful physiological and behavioral tests. Previously reported differences in genetic cross effects on fear responses between the parental genotypes and the corresponding hybrid were confirmed in these commercial crosses. Both mule duck and Pekin genotypes showed more active physiological and behavioral responses to stress than Muscovy genotypes. The new finding of this study is that mule genotypes appear to be more sensitive to the social environment than both respective parental genotypes. Few differences were observed between the 2 mule genotypes from A and C. On the other hand, several traits of the 2 mule genotypes from B differed. In addition, A and C mule genotypes were characterized by the same adrenal and behavioral traits but contrasting responses. The B mule genotypes were characterized by a different set of behavioral traits, and only 1 of the 2 B mule ducks was characterized by a group of adrenal traits.

Key words: corticosterone, fear, sociability, duck, genetic effect

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INTRODUCTION

The mule duck, which is the most widely used duck genotype in French production systems, is reported to express a high level of fear of humans. Avoidance behavior appears between 5 and 6 wk of age and results in sudden, intense, and collective motor activity (Guémené et al., 2006). They also crowd together at the end of the pen furthest from the human. This distress and the potential physical consequences such as suffocation and injury can seriously affect their welfare. In the production sector, animal welfare is a major issue for farmers because, in addition to ethical issues, it has direct consequences on productivity (Rushen et al., 1999). The Council of Europe (1999, T-AP: [95/20]) has specifically recommended that duck genotypes should be selected to avoid health and welfare problems (article 11) and that scientific studies on welfare should be carried out before modified genotypes are used for production (article 21).

Animal welfare must be improved in livestock farming by using less stressful systems or more appropriate strains. Response to stress and adaptation result from evolutionary processes that induce genetic changes over the generations (Price, 1984). Genetic influences on fear-related responses have been demonstrated by comparing different genotypes or specific crosses in rats (Stöhr et al., 1999), hens (Craig et al., 1983), lambs (Boissy et al., 2005), cattle (Morris et al., 1994), rainbow trout (Woodward and Strange, 1987), red jungle fowl (Håkansson et al., 2007), and ducks (Desforges and Wood-Gush, 1975). These genetic influences should be taken into account, particularly in livestock systems in

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which current strains result from very intensive genetic selection.

The mule duck is a sterile hybrid from the interspecific cross of a Muscovy drake and a common female duck. Many different phenotypes are offered by different commercial breeders according to their specific selection traits. Farmers report that the fearfulness of mule ducks varies with genotype and phenotype. This concern was raised concomitantly with the loss of coloration in common duck strains: mule ducks with white or light-colored plumage are considered to be more high-strung than others. It has been shown that 2 different mule genotypes express contrasted fear-related responses, whereas within a single mule genotype, ducks with white or colored plumage show similar fear-related responses (Guémené et al., 2006). In this context, the first aim of the present study was to compare how mule ducks from different breeders and with different genotypes and contrasting phenotypes respond to various stressful conditions. The second aim was to compare the genetic cross effects observed within these different commercial crosses with those described previously for other specific genotypes (Faure et al., 2003; Arnaud et al., 2008).

Three independent experiments were performed on mule genotypes provided by 3 French duck-breeding companies (A, B, and C). Males from 2 mule genotypes and from their respective parental genotypes (Muscovy and Pekin) produced by each breeder were subjected to a set of tests to compare their responses. To take into account the multidimensional aspect of the response to stress (Dantzer and Mormède, 1979), different physiological and behavioral tests were used to assess corticotropic axis functionality and reactivity, fear of an unknown environment, fear of humans, and social motivation, which could all play a part in the collective expression of inappropriate responses.

MATERIALS AND METHODS

The experimental procedure was carried out in compliance with the ethical principles for the use of experimental animals by authorized people (authorization numbers 06-255 and 37-138) in an officially authorized experimental structure (Unité Expérimentale des Palmipèdes à Foie Gras, INRA-UE89, Benquet, France; approval number A40-624) and was approved by a committee on animal care in research (CL2007-37/CREEA Centre-Limousin, France).

Birds

Each of the 3 experiments involved male ducks from 2 mule genotypes (n = 60 per genotype) and from their respective parental genotypes (n = 30 per genotype) provided by 3 French breeding companies (SEPALM: Route de Meilhan, Souprosse, France; Gourmaud Sélection, Saint André Treize Voies, France; Grimaud Frères Sélection, Roussay, France). The 2 mule genotypes from each breeder had a common maternal or paternal genetic origin and phenotypical differences as described below.

Breeder A. There were 2 Muscovy genotypes (*aM1* and *aM2*), 1 Pekin genotype (*aP0*), and 2 mule genotypes (*aM1P0* from the A_1 cross between *aM1* and *aP0* and *aM2P0* from the A_2 cross between *aM2* and *aP0*). The mule genotypes differed phenotypically in BW, *aM1P0* being heavier than *aM2P0*.

Breeder B. There were 2 Pekin genotypes (*bP1* and *bP2*), 1 Muscovy genotype (*bM0*), and 2 mule genotypes (*bM0P1* from the B_1 cross between *bM0* and *bP1* and *bM0P2* from the B_2 cross between *bM0* and *bP2*). These mule genotypes differed phenotypically in plumage color, *bM0P1* being white and *bM0P2* colored.

Breeder C. There were 2 Pekin genotypes (*cP1* and *cP2*), 1 Muscovy genotype (*cM0*), and 2 mule genotypes (*cM0P1* from the cross C_1 between *cM0* and *cP1* and *cM0P2* from the cross C_2 between *cM0* and *cP2*). These mule genotypes differed phenotypically in plumage pattern and activity, *cM0P1* being "piebald" and reported by farmers to be more active than *cM0P2*, which was "blue-barred."

Rearing Conditions

All ducklings were supplied on the day of hatching (February 2006 for genotypes from origins A and B and April 2006 for genotypes from origin C). They were given a standard feeding diet ad libitum for 8 wk and then rationed up to 11 wk of age. The ducks were raised in collective floor pens (n = 30) measuring 6 × 2 m and maintained under natural photoperiod in a windowed barn (latitude 43°53′N), apart from the first 3 d of life when they were kept under continuous artificial lighting. Birds were kept indoors until the end of the experimental period.

Experimental Measures During the Rearing Period

Ducks were tested during the seventh week of age because it has previously been shown that fear responses are overexpressed in mule ducks between the fifth and sixth week of age (Guémené et al., 2006). All of the same ducks were submitted to the following tests and measures performed in a separate barn following the same sequence. Adrenal measures were all done on the same day and behavioral tests were realized during the 3 following days in the order of description as follows. Ducks from the different genotypes and pens were tested alternately to avoid any possible rank effect.

Corticosterone Measures. To assess corticosterone (**CORT**) levels, blood samples (3 mL) were collected from the occipital sinus (Vuillaume, 1983) under 3 different experimental conditions. On average, it took 2 min to catch a duck in its pen and to transfer it to the testing area; then, the blood sampling procedure took

around 1 min. Plasma was separated by centrifugation and kept at −20°C before analysis. Corticosterone levels were measured using the previously described specific RIA (Etches, 1976), and a series of assays was performed for each experiment. Samples from a specific bird and test were assayed within a single assay, and ducks from different genotypes and pens were tested alternately.

To assess levels before any experimental treatment (initial level, **INI**), the first blood sample was collected just after capture. A second sample was collected after the duck had been hung by the feet for 10 min to assess the consequence of physical restraint (response to restraint, **REST**). Hanging by the legs is similar to restraint applied on the shackle line before stunning at slaughtering. This treatment has been reported to be more stressful in laying hens and broilers than simple restraint (Jones, 1992). It is likely to be as stressful as being caught in a net, which has been shown to induce a very high CORT response in ducks (Guémené et al., 1998).

An i.m. injection of a high dose (10 μg/kg of BW) of adrenocorticotropic hormone (Synacthène Immédiat, Novartis Pharma, Rueil-Malmaison, France) was administered just after the second sample had been taken, and ducks were placed in individual transport cages for 10 min. This dose and time period were chosen because they have previously been shown to allow the maximal CORT level to be reached in ducks (Noirault et al., 1999). At the end of the 10-min period, a third sample was collected for pharmacological assessment of the maximum capacity of the adrenal gland (**MAX**; Guémené et al., 2001).

The ratio of REST:CORT to MAX:CORT was calculated to assess the relative amplitude of the REST-CORT response (**REST:MAX**). The latter involves not only the hypothalamic-pituitary-adrenal axis but also central sensorial and cognitive mechanisms, whereas the response to the adrenocorticotropic hormone challenge provides specific information about adrenal gland reactivity or maximum response capacity (Arnaud et al., 2008).

Behavioral Observations. The 1-min period of the social motivation (**SOC**) test and the response-to-human (**RH**) test was videorecorded and analyzed by focal sampling using the Observer 3.0 program (Noldus Information Technology, Wageningen, the Netherlands).

The experimental arena of the SOC test presented in Figure 1a was an adaptation of a previously described procedure (Faure et al., 2003). In brief, it consisted of a corridor measuring 12 × 1 m, subdivided longitudinally into 7 equal zones numbered 1 to 7. Three naïve ducks from an independent Pekin genotype (different from the tested genotypes) were placed near the zone 1 end of the corridor as a social stimulus. The tested ducks were introduced into the corridor one by one through a trap door in zone 4 of the corridor and could see their congeners in zone 1 but could not have physical contact with them. During the 1-min observation period, several parameters were monitored: direction of first movement (toward or away from the congeners), latency to first immobilization (**IMB**), number of lines crossed (**LINES**), total ambulation time (**AMB**), and time spent in each zone. A distance index (**DI**) was calculated using the following formula: $DI = \Sigma(z_i \times t_i)$, where z_i is the zone identification number ($i = 1$ to 7) and t_i is the time in seconds spent in the ith zone (Faure et al., 2003). Thus, DI could theoretically range from 60 (whole time spent in zone 1, 60 s × 1) to 480 (whole time spent in zone 7, 60 s × 7). These conditions represented an unknown environment, which is considered to be a source of fear for ducks (Ossenkopp, 1980; Suarez and Gallup, 1980). At the same time, their motivation to reestablish social contact could be tested through the presence of congeners (Mills and Faure, 1990).

The experimental procedure presented in Figure 1b was similar to the SOC procedure described above, and the same parameters were monitored, except that the congeners were replaced by a human near the zone 1 end of the corridor. In this test, which has previously been described and validated (Faure et al., 2003; Arnaud et al., 2008), ducks were subjected to 3 sources of fear: an unknown environment, being isolated from counterparts as in the open-field test (Ossenkopp, 1980; Suarez and Gallup, 1980), and a human presence (Boissy et al., 2005).

The tonic immobility (TI) test involves placing the bird on its back in a cradle and holding it in this position for 10s. Tonic immobility duration (**DUR**) was assessed by measuring the time before the bird tried to right itself. If it did not remain immobile for at least 10 s after release, the TI attempt was considered to be unsuccessful and was repeated. The number of attempts (**ATT**) was recorded. After 5 unsuccessful attempts, DUR was scored 0 s and ATT was considered equal to 5. For technical reasons, if the duck was still in TI after 8 min, the test was stopped and the maximum duration

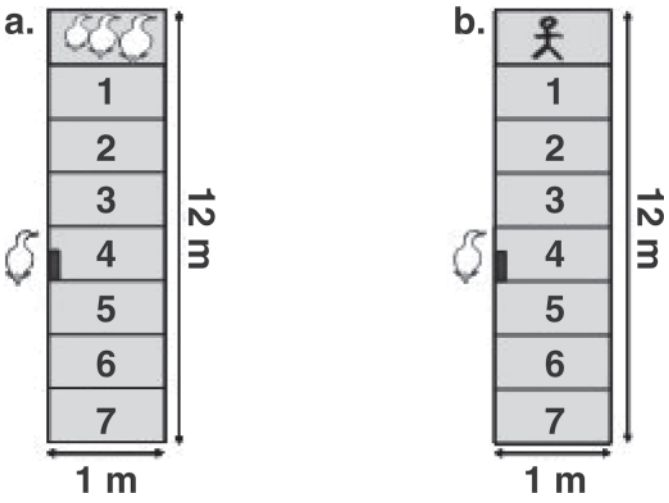

Figure 1. Experimental arena of (a) the social motivation test and (b) the response-to-human test lasting 1 min.

(480 s) was attributed. The number of interrupted tests (**INT**) was noted. The innate behavior induced by the initial physical restraint corresponds to a catatonic-like state that is supposed to decrease the predator's attention (Jones and Faure, 1981); the lower the number of attempts to induce it and the longer the DUR, the higher the level of fear (Gallup, 1977).

Experimental Measures During Force-Feeding

All of the same mule genotype ducks were subjected to force-feeding and to the experimental measures described below. In this aim, at 12 wk of age, they were transferred into a force-feeding room for the whole period and placed in collective cages measuring 0.85 × 0.85 m, with 4 ducks per cage. In each experiment, ducks from the 2 different genotypes were caged separately in alternate cages. During the 12.5-d force-feeding period, ducks received 25 meals [i.e., 1 meal in the evening after the transfer, which occurred in the morning, then 2 meals per day at 12-h intervals (from 0700 to 0800 h and from 1900 to 2000 h for all ducks)]. The meal consisted of a mix of corn mash with warm water and was gradually increased from 250 to 450 g.

CORT Measures. Blood was collected following a previously described procedure to assess CORT related to force-feeding. The assessment was therefore carried out 10 min after transfer to the force-feeding cage (**TRANSF**), 1 h before (**BEF**) and 10 min after (**AFT**) the 1st (**FF1**) and the 23rd force-fed meal (**FF23**).

Behavioral Observations. To assess resistance to force-feeding, the force-feeder attributed a subjective score of resistance (**RESI**) to each duck during the 1st (FF1), 12th (**FF12**), and 23rd (FF23) force-fed meal. The scores illustrated the physical reactivity to the restraint and the force-feeding act and thus were defined as follows: 1 = no resistance (no movement); 2 = light (few movements); 3 = moderate (a lot of movements); and 4 = high (too many movements to allow the realization of the act, the restraint had to be renewed).

Statistical Analysis

Physiological data followed a normal distribution pattern, but behavioral data did not (Kolmogorov-Smirnov test). When more than 2 groups were compared, overall comparisons were made using ANOVA tests (physiological data) or Kruskal-Wallis tests (behavioral data). When these were significant ($P < 0.05$), multiple pair comparisons were carried out using the Student *t*-test (physiological data) and the Mann-Whitney test (behavioral data). To assess the effect of treatment on adrenal levels and to compare the corresponding variables measured in the SOC test and the RH test within each genotype, Student *t*-tests for matched data and Wilcoxon tests were used, respectively. A Bonferroni sequentially rejective multiple test procedure (Holm, 1979) was used in multiple pair comparisons to determine the corrected significance threshold corresponding to $P < 0.05$. All of these analyses were performed using the StatView program (SAS 5.0, SAS Institute Inc., Cary, NC).

Comparison Between a Mule Genotype and Its Respective Parental Genotypes. Data of each mule genotype and its respective parental genotypes were statistically compared. The cross effects were assessed (6 comparisons: *aM1P0* vs. *aM1* vs. *aP0*, *aM2P0* vs. *aM2* vs. *aP0*, *bM0P1* vs. *bM0* vs. *bP1*, *bM0P2* vs. *bM0* vs. *bP2*, *cM0P1* vs. *cM0* vs. *cP1*, and *cM0P2* vs. *cM0* vs. *cP2*).

Comparison of 2 Distinct Paternal or Maternal Genotypes from the Same Breeder. The 2 different paternal or maternal genotypes from each breeder were compared. The phenotypical differences were assessed to help understand the mule effects (*aM1* vs. *aM2*, *bP1* vs. *bP2*, and *cP1* vs. *cP2*).

Comparison of 2 Mule Ducks from the Same Breeder. Data of 2 mule genotypes from the same breeder were compared. This was done to assess the genotype effect when the mule genotype had one common genetic parental origin out of 2 (3 comparisons: *aM1P0* vs. *aM2P0*, *bM0P1* vs. *bM0P2*, and *cM0P1* vs. *cM0P2*).

Comparison of Mule Ducks from Different Breeders. Responses of the mule genotypes from the 3 breeders were compared to assess the effect of large genetic differences resulting from independent selection schemes (1 comparison: *aM1P0* vs. *aM2P0* vs. *bM0P1* vs. *bM0P2* vs. *cM0P1* vs. *cM0P2*). To do this, factor analyses were performed using the SPAD 3.0 program (CISIA, St. Mandé, France). Two separate analyses were performed for physiological and behavioral data because these were not correlated (data not shown) and only physiological data followed a normal distribution pattern. The physiological data was analyzed using a principal components analysis (**PCA**) with the physiological criteria as active variables. The behavioral data were analyzed using a factorial correspondence analysis (**FCA**) with the behavioral criteria as active variables. The mule genotype was used as an illustrative variable in both analyses. For the FCA, each behavioral variable included in the analysis was divided into 2 or 3 subgroups according to the nature of the variable and data to have approximate equivalent frequency whenever possible. All 8 physiological criteria were included in the PCA (INI, REST, MAX, TRANSF, BEFFF1, AFTFF1, BEFFF23, and AFTFF23), whereas, to increase the test strength, only 13 out of the 17 behavioral criteria were included in the FCA (SOC/RH-AMB, -IMB, -LINES, and -DI; TI-ATT and -DUR; and RESI-FF1, -FF12, and -FF23), zone 1 and zone 7 being included in DI. For the sake of clarity, only the first 2 axes will be considered for each analysis in the present paper.

Comparison of Responses for the Same Variable Assessed in Different Situations. Adrenal levels

Table 1. Mean values (±SD) for genotypes from breeder A (Muscovy genotypes *aM1* and *aM2*, Pekin genotype *aP0*, and their hybrid *aM1P0* from the A_1 cross and *aM2P0* from the A_2 cross)[1]

Genotype	*aM1*	*aM1P0*	*aP0*	*aM2P0*	*aM2*
CORT					
INI (ng/mL of plasma)	5.6 ± 0.9^{uv}	7.7 ± 0.8^{u} (+42)	4.8 ± 0.5^{v}	6.3 ± 0.7 (+25)	5.3 ± 1.2
REST (ng/mL of plasma)	38.8 ± 4.6	42.7 ± 5.0 (−10)	56.3 ± 7.3	41.8 ± 4.6 (−22)	51.3 ± 3.9
MAX (ng/mL of plasma)	73.5 ± 6.2^{w}	93.2 ± 3.4^{v} (−14)	$141.9 \pm 7.4^{u,x}$	91.5 ± 4.6^{y} (−20)	85.8 ± 5.1^{y}
REST:MAX	0.55 ± 0.07	0.46 ± 0.05 (−2)	0.39 ± 0.05^{y}	0.49 ± 0.05^{xy} (−5)	0.64 ± 0.06^{x}
SOC					
AMB (s)	26.2 ± 2.6^{w}	44.3 ± 1.5^{u} (+47)	$34.1 \pm 2.4^{v,y}$	42.3 ± 1.6^{x} (+46)	23.8 ± 2.0^{z}
IMB (s)	5.8 ± 1.1^{v}	14.8 ± 2.0^{u} (+77)	10.9 ± 1.3^{u}	2.1 ± 1.4 (−78)	8.4 ± 1.1
LINES	4.4 ± 0.3^{v}	5.7 ± 0.2^{u} (+15)	$5.5 \pm 0.5^{uv,x}$	5.3 ± 0.2^{x} (+11)	4.0 ± 0.3^{y}
Z1 (s)	13.7 ± 3.8^{v}	31.3 ± 2.5^{u} (+139)	$12.5 \pm 3.4^{v,y}$	30.1 ± 2.7^{x} (+190)	8.2 ± 3.0^{y}
Z7 (s)	6.8 ± 2.7	5.1 ± 1.6 (−30)	7.7 ± 2.7	6.1 ± 1.8 (−45)	14.8 ± 3.5
DI	217.0 ± 22.1^{u}	157.0 ± 13.2^{v} (−28)	$218.6 \pm 20.5^{u,x}$	169.7 ± 14.8^{y} (−31)	274.8 ± 21.3^{x}
RH					
AMB (s)	11.8 ± 1.6^{v}	26.4 ± 1.7^{u} (+26)	$30.1 \pm 2.8^{u,x}$	28.5 ± 1.5^{x} (+32)	13.0 ± 1.4^{y}
IMB (s)	6.0 ± 0.8	7.8 ± 0.6 (+13)	7.8 ± 0.9^{x}	8.1 ± 0.6^{x} (+30)	4.6 ± 0.4^{y}
LINES	3.1 ± 0.2^{v}	4.3 ± 0.2^{u} (+6)	$5.0 \pm 0.6^{uv,x}$	4.6 ± 0.2^{x} (+15)	3.0 ± 0.2^{y}
Z1 (s)	1.5 ± 1.4	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.5	0.0 ± 0.0
Z7 (s)	14.4 ± 4.6	21.5 ± 3.0 (+10)	24.5 ± 4.1	21.1 ± 2.8 (−22)	29.9 ± 4.6
DI	278.2 ± 20.3	301.6 ± 13.2 (0)	324.9 ± 15.8	298.7 ± 13.6 (−12)	351.5 ± 14.0
TI					
ATT	1.2 ± 0.1	1.4 ± 0.1 (+22)	1.1 ± 0.1	1.4 ± 0.1 (+16)	1.3 ± 0.1
DUR (s)	344.6 ± 36.9^{uv}	357.5 ± 19.1^{u} (−7)	$421.7 \pm 22.0^{v,x}$	396.0 ± 18.2^{x} (+19)	243.8 ± 33.4^{y}
INT (%)	43.5^{v}	39.6^{v} (−33)	$75.0^{u,x}$	65.5^{x} (+33)	23.1^{y}
CORT					
TRANSF (ng/mL of plasma)		42.9 ± 2.9		36.4 ± 2.2	
BEFFF1 (ng/mL of plasma)		29.6 ± 2.1		25.3 ± 1.7	
AFTFF1 (ng/mL of plasma)		17.4 ± 1.5		18.4 ± 1.4	
BEFFF23 (ng/mL of plasma)		22.5 ± 1.7		23.1 ± 1.8	
AFTFF23 (ng/mL of plasma)		21.4 ± 1.7		13.3 ± 1.3	
RESI					
FF1		2.5 ± 0.2		2.5 ± 0.2	
FF12		2.5 ± 0.2		2.5 ± 0.1	
FF23		2.5 ± 0.2		2.5 ± 0.1	

[u–w]When $P < 0.05$ for overall comparison within a row, A_1 genotypes (aM1, aM1P0, aP0) with different superscripts differ significantly ($P < 0.05$).

[x–z]When $P < 0.05$ for overall comparison within a row, A_2 genotypes (aP0, aM2P0, aM2) with different superscripts differ significantly ($P < 0.05$).

[1]For levels of corticosterone (CORT) at initial stage (INI), after restraint for 10 min (REST), 10 min after injection of a high dose of adrenocorticotropic hormone (MAX), and ratio between level after restraint and after the injection of adrenocorticotropic hormone (REST:MAX); duration of ambulation (AMB), latency to first immobilization (IMB), number of lines crossed (LINES), time spent in zone 1 (Z1) and zone 7 (Z7), and distance index from stimulus (DI) during the social motivation (SOC) test and the response-to-human (RH) test; the number of attempts (ATT), duration (DUR), and number of interrupted trials (INT) for the tonic immobility (TI) test; level of CORT after the transfer into force-feeding cages (TRANSF), 1 h before and 10 min after the 1st and 23rd force-fed meal (BEFFF1, AFTFF1, BEFFF23, and AFTFF23, respectively); and score of resistance behavior to force-feeding (RESI) at the 1st, 12th, and 23rd force-fed meal (FF1, FF12, and FF23, respectively). Heterosis percentages for the hybrids are presented in parentheses

measured in the different situations together with corresponding behavioral variables measured in the SOC test and the RH were compared to assess the respective effects of the different treatments within each mule genotype. For these comparisons, significant differences were considered at the $P = 0.05$ statistical threshold.

RESULTS

Genetic Cross Effects

Physiological and behavioral data, together with the results of statistical comparisons between genotypes in the 6 A, B, and C crosses are shown in Tables 1, 2, and 3, respectively. With regard to the maternal or paternal genotypes of the same species from each breeder, very few significant differences were observed in A and C: *aM1* showed lower SOC-AMB ($P < 0.05$) and RH-DI ($P < 0.01$) and higher RH-zone 7 ($P < 0.05$) than *aM2*, and *cP1* showed higher TI-DUR ($P < 0.01$) than *cP2*. No difference was observed in B ducks: *bP1* and *bP2* were similar for all traits ($P > 0.05$ for all comparisons). With regard to each mule genotype and its respective parental genotypes, significant differences between genotypes were observed for half of the traits observed for each cross on average.

Physiological Data. Differences for INI were observed in 3 out of the 6 crosses: in the A_1 cross, the mule genotype showed a similar level with the Muscovy genotype, but a higher level than the Pekin genotype; in the B_1 cross, it had a similar level with the Pekin genotype but a higher level than the Muscovy genotype; and in the B_2 cross, it was between parental genotypes. No differences between genotypes were observed for REST in the 6 crosses. Differences for MAX were observed in 4 out of the 6 crosses: in A_1, C_1, and C_2 crosses, the Pekin genotype always had a higher level than the Muscovy genotype; the mule genotype had an

intermediate level between parental genotypes; and in the A_2 cross, the mule genotype had a similar level with the Muscovy genotype and a lower level than the Pekin genotype. The REST:MAX differed between genotypes in 4 out of the 6 crosses: in B_1, B_2, C_1, and C_2 crosses, the mule genotype showed a ratio similar to that of the Pekin genotype and lower than the Muscovy genotype.

Behavioral Data. Only traits for which significant differences were found are reported below.

In the SOC test, the mule duck genotypes showed a higher AMB than their respective parental genotypes in all 6 crosses. Differences in IMB were observed in 3 out of the 6 crosses: in A_1, C_1, and C_2 crosses, the mule genotype showed a similar response to the Pekin genotype and a higher response than the Muscovy genotype. Differences in LINES were observed in 4 out of the 6 crosses: in A_1, A_2, C_1, and C_2 crosses, the mule genotype showed a similar response to the Pekin genotype and a higher response than the Muscovy genotype. Differences in zone 1 were observed in 4 out of the 6 crosses: in A_1, A_2, and C_2 crosses, the mule genotype showed a higher response than both parental genotypes, and in the C_1 cross, it had a similar response to the Pekin genotype and a higher response than the Muscovy genotype. Differences in DI were observed in 4 out of the 6 crosses: in A_1, A_2, and C_1 crosses, the mule genotype showed a lower response than both parental genotypes, and in the C_2 cross, its response was similar to that of the Muscovy genotype and lower than the Pekin genotype.

In the RH test, differences in AMB were observed in all 6 crosses: in A_1, A_2, B_1, and C_2 crosses, the mule genotype showed a similar response to the Pekin genotype and a higher response than the Muscovy genotype, and in B_2 and C_1 crosses, it had a higher response than both parental genotypes. Differences in IMB were ob-

Table 2. Mean values (±SD) for genotypes from breeder B (Muscovy genotype *bM0*, Pekin genotypes *bP1* and *bP2*, and their hybrid *bM0P1* from the B_1 cross and *bM0P2* from the B_2 cross)[1]

Genotype	*bP1*	*bM0P1*	*bM0*	*bM0P2*	*bP2*
CORT					
INI (ng/mL of plasma)	4.3 ± 0.6^{u}	7.2 ± 1.1^{u} (+136)	$1.8 \pm 0.3^{v,z}$	3.5 ± 0.4^{y} (−6)	5.7 ± 1.4^{x}
REST (ng/mL of plasma)	70.0 ± 10.1	54.1 ± 4.8 (−21)	67.7 ± 5.8	55.4 ± 4.8 (−9)	54.0 ± 6.6
MAX (ng/mL of plasma)	109.7 ± 10.0	106.6 ± 6.7 (+7)	88.8 ± 6.5	104.1 ± 5.0 (+5)	110.1 ± 11.7
REST:MAX	0.60 ± 0.07^{v}	0.54 ± 0.05^{v} (−24)	$0.82 \pm 0.06^{u,x}$	0.56 ± 0.04^{y} (−21)	0.60 ± 0.08^{xy}
SOC					
AMB (s)	40.1 ± 3.6^{v}	49.7 ± 1.5^{u} (+46)	$27.9 \pm 2.6^{w,y}$	38.6 ± 2.2^{x} (+32)	30.7 ± 3.4^{xy}
IMB (s)	8.4 ± 1.1	14.8 ± 2.0 (+108)	5.8 ± 1.1	12.1 ± 1.4 (+45)	10.9 ± 1.3
LINES	5.2 ± 0.4	5.5 ± 0.2 (+11)	4.7 ± 0.2	5.4 ± 0.2 (−3)	6.5 ± 0.6
Z1 (s)	24.4 ± 4.8	35.5 ± 2.6 (+49)	23.2 ± 4.0	27.5 ± 2.9 (+36)	17.3 ± 4.9
Z7 (s)	6.6 ± 2.8	4.6 ± 1.6 (−36)	7.9 ± 3.3	7.2 ± 2.0 (+14)	4.7 ± 2.7
DI	170.2 ± 21.9	147.4 ± 12.9 (−17)	187.3 ± 21.6	64.2 ± 14.8 (−18)	211.6 ± 21.6
RH					
AMB (s)	33.2 ± 2.6^{u}	39.8 ± 2.1^{u} (+44)	$22.1 \pm 2.8^{v,z}$	40.1 ± 1.9^{x} (+48)	32.0 ± 3.1^{y}
IMB (s)	6.7 ± 1.4^{uv}	8.7 ± 1.2^{u} (+51)	4.8 ± 0.9^{v}	7.1 ± 1.2 (+15)	7.5 ± 2.4
LINES	6.0 ± 0.4^{u}	5.1 ± 0.3^{uv} (−1)	$4.3 \pm 0.4^{v,y}$	5.9 ± 0.3^{x} (+15)	5.9 ± 0.6^{x}
Z1 (s)	4.4 ± 2.3^{u}	0.5 ± 0.5^{v} (−77)	0.0 ± 0.0^{v}	1.0 ± 0.9 (+122)	0.9 ± 0.9
Z7 (s)	19.7 ± 3.9	27.9 ± 2.8 (+27)	24.2 ± 4.3	26.0 ± 2.7 (+7)	24.4 ± 4.4
DI	281.9 ± 21.3	332.6 ± 11.3 (+10)	323.3 ± 16.9	321.3 ± 10.9 (+1)	313.9 ± 16.5
TI					
ATT	1.4 ± 0.1	1.3 ± 0.1 (0)	1.2 ± 0.1	1.6 ± 0.1 (+14)	1.6 ± 0.2
DUR (s)	382.0 ± 29.5^{u}	373.3 ± 17.9^{u} (+13)	276.6 ± 32.2^{v}	347.6 ± 21.0 (+12)	341.8 ± 38.5
INT (%)	60.0^{u}	46.5^{u} (+8)	$25.9^{v,y}$	41.5^{xy} (−5)	61.5^{x}
CORT					
TRANSF (ng/mL of plasma)		55.3 ± 6.6		49.7 ± 4.4	
BEFFF1 (ng/mL of plasma)		30.1 ± 4.6		20.6 ± 2.1	
AFTFF1 (ng/mL of plasma)		15.2 ± 1.8		12.3 ± 1.1	
BEFFF23 (ng/mL of plasma)		19.9 ± 2.2		13.0 ± 1.3	
AFTFF23 (ng/mL of plasma)		12.7 ± 1.9		13.2 ± 2.2	
RESI					
FF1		1.9 ± 0.1		2.2 ± 0.2	
FF12		1.4 ± 0.1		1.5 ± 0.1	
FF23		1.1 ± 0.1		1.2 ± 0.1	

[u–w]When $P < 0.05$ for overall comparison within a row, A_1 genotypes (aM1, aM1P0, aP0) with different superscripts differ significantly ($P < 0.05$).

[x–z]When $P < 0.05$ for overall comparison within a row, A_2 genotypes (aP0, aM2P0, aM2) with different superscripts differ significantly ($P < 0.05$).

[1]For levels of corticosterone (CORT) at initial stage (INI), after restraint for 10 min (REST), 10 min after injection of a high dose of adrenocorticotropic hormone (MAX), and ratio between level after restraint and after the injection of adrenocorticotropic hormone (REST:MAX); duration of ambulation (AMB), latency to first immobilization (IMB), number of lines crossed (LINES), time spent in zone 1 (Z1) and zone 7 (Z7), and distance index from stimulus (DI) during the social motivation (SOC) test and the response-to-human (RH) test; the number of attempts (ATT), duration (DUR), and number of interrupted trials (INT) for the tonic immobility (TI) test; level of CORT after the transfer into force-feeding cages (TRANSF), 1 h before and 10 min after the 1st and 23rd force-fed meal (BEFFF1, AFTFF1, BEFFF23, and AFTFF23, respectively); and score of resistance behavior to force-feeding (RESI) at the 1st, 12th, and 23rd force-fed meal (FF1, FF12, and FF23, respectively). Heterosis percentages for the hybrids are presented in parentheses.

served in 2 out of the 6 crosses: in A_2 and B_1 crosses, the mule genotype showed a similar response to the Pekin genotype and a higher response than the Muscovy genotype. Differences in LINES were observed in 4 out of the 6 crosses: in A_1, A_2, B_2, and C_1 crosses, the mule genotype showed a similar response to the Pekin genotype and a higher response than the Muscovy genotype. Differences in zone 1 were observed only in the B_1 cross: the mule genotype and the Muscovy genotype showed lower responses than the Pekin genotype.

In the TI test, mean INT was around 30% for the Muscovy, 60% for the Pekin, and 50% for mule genotypes. Differences in DUR and INT were observed in half of the crosses: in A_2 and B_1 crosses, the mule genotype showed a similar response to the Pekin genotype and a higher response than the Muscovy genotype, and in the A_1 cross, it had a similar response to the Muscovy genotype and a higher response than the Pekin genotype.

Mule Genotype Effects

Mule Genotypes from the Same Breeder and with One Common Parent. Mule genotypes from breeder A differed in 2 out of the 18 traits: *aM1P0* showed lower TI-DUR and higher AFTFF23-CORT than mule genotype *aM2P0* ($P < 0.05$ for all comparisons). Mule genotypes from breeder B differed in 6 out the 18 traits: *bM0P1* showed a significant higher INI-CORT, SOC-AMB, RH-IMB, BEFFF1-CORT, and BEFFF23-CORT and lower TI-ATT than *bM0P2* ($P < 0.05$ for all comparisons). Mule genotypes from breeder C differed in only 1 out of the 18 traits: *cM0P1* showed higher TI-ATT than *cM0P2* ($P < 0.05$ for all comparisons).

Mule Genotypes from Different Breeders. Multivariate analyses were carried out to obtain an overall characterization of the 6 mule genotypes and to understand the respective adrenal and behavioral response profiles.

Table 3. Mean values (±SD) for genotypes from breeder C (Muscovy genotype *cM0*, Pekin genotypes *cP1* and *cP2*, and their hybrid *cM0P1* from the C_1 cross and *cM0P2* from the C_2 cross)[1]

Genotype	*cP1*	*cM0P1*	*cM0*	*cM0P2*	*cP2*
CORT					
INI (ng/mL of plasma)	10.0 ± 1.5	8.3 ± 1.3 (+10)	5.1 ± 1.5	8.4 ± 1.3 (+33)	7.5 ± 2.1
REST (ng/mL of plasma)	62.6 ± 7.0	67.4 ± 5.0 (−2)	74.6 ± 6.1	72.3 ± 5.3 (−2)	72.5 ± 7.8
MAX (ng/mL of plasma)	155.0 ± 9.5^{u}	132.9 ± 6.2^{uv} (0)	111.4 ± 8.2^{v,y}	133.8 ± 7.0^{xy} (−1)	159.7 ± 7.9^{x}
REST:MAX	0.40 ± 0.04^{v}	0.52 ± 0.04^{v} (−7)	0.72 ± 0.05^{u,x}	0.57 ± 0.05^{y} (−2)	0.45 ± 0.05^{y}
SOC					
AMB (s)	26.9 ± 1.8^{v}	33.9 ± 1.5^{u} (+42)	20.7 ± 2.0^{v,z}	35.8 ± 1.2^{x} (+44)	29.1 ± 1.7^{y}
IMB (s)	6.9 ± 0.8^{u}	6.1 ± 0.6^{u} (+15)	3.7 ± 0.7^{v,y}	6.6 ± 0.5^{x} (+39)	5.8 ± 0.6^{x}
LINES	5.9 ± 0.5^{u}	5.5 ± 0.3^{u} (+16)	3.6 ± 0.3^{v,y}	5.6 ± 0.2^{x} (+12)	6.4 ± 0.5^{x}
Z1 (s)	13.1 ± 4.1^{uv}	22.7 ± 0.6^{u} (+102)	9.3 ± 2.8^{v,y}	22.6 ± 2.4^{x} (+188)	6.4 ± 2.2^{y}
Z7 (s)	22.0 ± 4.4^{u}	6.5 ± 1.8^{v} (−52)	5.4 ± 2.4^{v,y}	10.8 ± 2.3^{y} (−5)	17.4 ± 3.4^{x}
DI	277.9 ± 26.5^{u}	196.1 ± 13.9^{v} (−25)	247.8 ± 17.5^{u,xy}	201.3 ± 14.4^{y} (−25)	293.4 ± 18.8^{x}
RH					
AMB (s)	26.3 ± 2.6^{uv}	32.1 ± 1.2^{u} (+42)	18.9 ± 1.7^{v,y}	31.0 ± 1.2^{x} (+28)	29.5 ± 1.8^{x}
IMB (s)	5.5 ± 0.4	7.9 ± 0.8 (+36)	6.1 ± 0.8	6.7 ± 0.5 (+6)	6.5 ± 0.5
LINES	5.5 ± 0.6^{u}	4.5 ± 0.3^{u} (+4)	3.1 ± 0.3^{v,z}	4.0 ± 0.2^{y} (−13)	6.1 ± 0.5^{x}
Z1 (s)	0.6 ± 0.6	0.0 ± 0.0	1.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
Z7 (s)	32.5 ± 4.1	38.6 ± 2.3 (+30)	26.7 ± 4.2	34.4 ± 2.7 (+25)	28.1 ± 3.5
DI	357.0 ± 13.3	382.0 ± 5.5 (+9)	339.8 ± 15.6	371.9 ± 8.1 (+8)	349.7 ± 13.1
TI					
ATT	1.2 ± 0.1	1.4 ± 0.1 (+8)	1.4 ± 0.1	1.7 ± 0.1 (+10)	1.7 ± 0.2
DUR (s)	402.2 ± 28.9^{u}	376.9 ± 18.5^{uv} (+6)	306.0 ± 29.8^{v}	333.9 ± 19.9 (+13)	282.4 ± 38.5
INT (%)	72.0^{u}	53.6^{uv} (+3)	32.1^{v}	41.7 (+8)	44.8
CORT					
TRANSF (ng/mL of plasma)		36.4 ± 4.2		35.1 ± 3.2	
BEFFF1 (ng/mL of plasma)		27.6 ± 2.6		27.3 ± 2.3	
AFTFF1 (ng/mL of plasma)		16.6 ± 2.1		20.0 ± 2.2	
BEFFF23 (ng/mL of plasma)		15.2 ± 1.6		18.2 ± 2.1	
AFTFF23 (ng/mL of plasma)		11.2 ± 1.6		10.9 ± 1.9	
RESI					
FF1		2.1 ± 0.1		1.9 ± 0.1	
FF12		1.3 ± 0.1		1.1 ± 0.0	
FF23		1.4 ± 0.1		1.2 ± 0.1	

^{u,v}When $P < 0.05$ for overall comparison within a row, A_1 genotypes (aM1, aM1P0, aP0) with different superscripts differ significantly ($P < 0.05$).

$^{x–z}$When $P < 0.05$ for overall comparison within a row, A_2 genotypes (aP0, aM2P0, aM2) with different superscripts differ significantly ($P < 0.05$).

[1]For levels of corticosterone (CORT) at initial stage (INI), after restraint for 10 min (REST), 10 min after injection of a high dose of adrenocorticotropic hormone (MAX), and ratio between level after restraint and after the injection of ACTH (REST:MAX); duration of ambulation (AMB), latency to first immobilization (IMB), number of lines crossed (LINES), time spent in zone 1 (Z1) and zone 7 (Z7), and distance index from stimulus (DI) during the social motivation (SOC) test and the response-to-human (RH) test; the number of attempts (ATT), duration (DUR), and number of interrupted trials (INT) for the tonic immobility (TI) test; level of CORT after the transfer into force-feeding cages (TRANSF), 1 h before and 10 min after the 1st and 23rd force-fed meal (BEFFF1, AFTFF1, BEFFF23, and AFTFF23, respectively); and score of resistance behavior to force-feeding (RESI) at the 1st, 12th, and 23rd force-fed meal (FF1, FF12, and FF23, respectively). Heterosis percentages for the hybrids are presented in parentheses.

Table 4. Contributions on the first and second factors of the principal components analysis analysis[1]

	Contribution (%)	
Variable	First factor	Second factor
CORT		
INI	14.3[2]	0.0
REST	11.9	26.9[2]
MAX	9.7	30.6[2]
TRANSF	9.7	0.0
BEFFF1	14.8[2]	3.9
AFTFF1	18.7[2]	0.0
BEFFF23	11.4	19.4[2]
AFTFF23	9.6	19.4[2]
Genotype		
aM1P0	0	36.1[2]
aM2P0	6.5	28.7[2]
bM0P1	0.9	9.1
bM0P2	18.7[2]	0
cM0P1	1.4	21.8[2]
cM0P2	8.4	25.1[2]

[1]With levels of corticosterone (CORT) at initial stage (INI), after restraint during 10 min (REST), after injection of a high dose of adrenocorticotropic hormone (MAX), after the transfer into force-feeding cages (TRANSF), and before and after the 1st and the 23rd force-fed meal (BEFFF1, AFTFF1, BEFFF23, and AFTFF23, respectively) as active variables, with mule genotypes as illustrative variables.

[2]Values considered significant ($P < 0.05$).

A graph showing the physiological variables and the 6 mule genotypes in the 1–2 factorial plan of the PCA analysis is presented in Figure 2. The first 2 factors of the PCA explain 44.1% of the whole variability: 27.4% by the first eigenvalue (2.19) and 16.7% by the second eigenvalue (1.34). The mean contribution on the axes was 12%; therefore, only traits showing a contribution of at least 14% will be examined. Contributions on the physiological traits axis are presented in Table 4. The variability of the first factor is mainly explained by FF1 and INI, which showed significant negative values on this factor. The variability of the second factor is mainly explained by FF23, which showed significant positive values, and by MAX and REST which showed significant negative values on this factor.

For mule genotypes from breeder A, *aM1P0* and *aM2P0* showed significant positive values on the second factor and are thus characterized by low REST and MAX and high FF23. For mule genotypes from breeder B, although *bM0P1* did not show significant values on the 1–2 factor plan, *bM0P2* showed a significant positive value on the first factor and is thus characterized by low INI and FF1. For mule genotypes from breeder C, *cM0P1* and *cM0P2* showed significant negative values on the second factor and are thus characterized by high REST and MAX and low FF23.

Categorization of behavioral variables is shown in Table 5. A graph showing the behavioral variables and the 6 mule genotypes in the 1–2 factorial plan of the FCA analysis is presented in Figure 3. The first 2 axes of the FCA explain 19.7% of the whole variability: 11.5% by the first eigenvalue (0.20) and 8.2% by the second eigenvalue (0.14). The mean contribution on the axes was 7.2%, and therefore only traits showing a contribution of at least 11% on the axes will be examined. Contributions on the axis for behavioral traits are presented in Table 6. The first factor variability is mainly explained by DI and LINES: positive values correspond to high DI, and low LINES and negative values correspond to low DI and high LINES. The second factor variability is mainly explained by RESI and SOC-IMB:

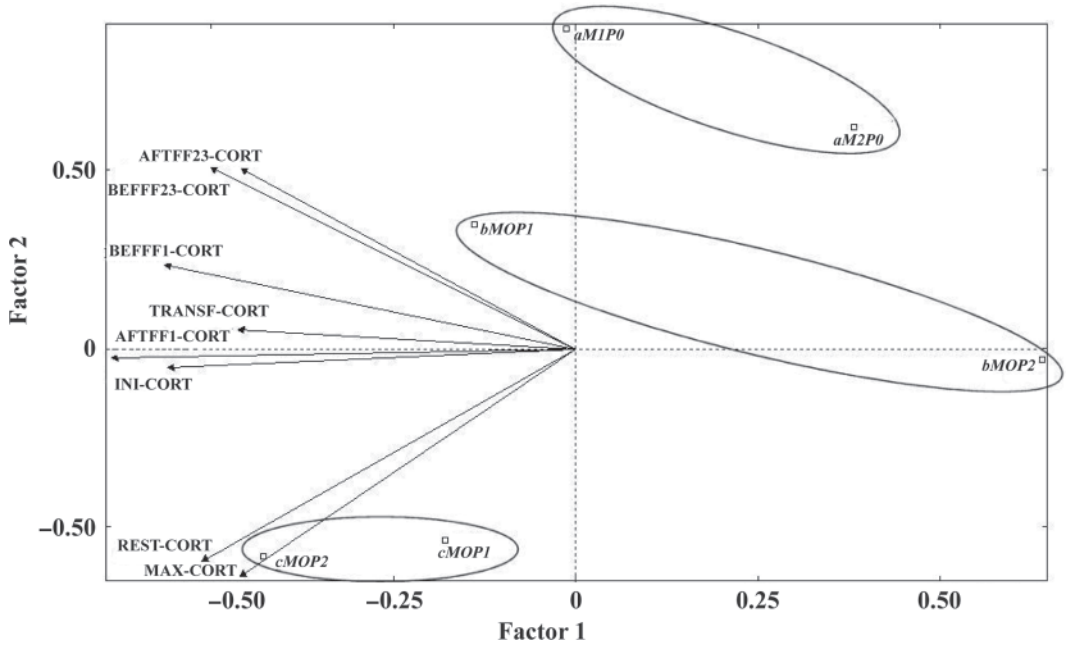

Figure 2. Graph showing the 1–2 factorial plan of the principal components analysis for the active variables: initial corticosterone level (INI-CORT), corticosterone level after restraint (REST-CORT), after injection of a high dose of adrenocorticotropic hormone (MAX-CORT), after transfer to the force-feeding cage (TRANSF-CORT), and before and after the 1st (BEFFF1-CORT and AFTFF1-CORT, respectively) and 23rd (BEFFF23-CORT and AFTFF23-CORT, respectively) force-fed meal. The mule genotypes from the different breeders A (*aM1P0* and *aM2P0*), B (*bM0P1* and *bM0P2*), and C (*cM0P1* and *cM0P2*) were included as illustrative variables.

positive values correspond to low RESI and SOC-IMB, and negative values correspond to high RESI and SOC-IMB.

For mule genotypes from breeder A, *aM1P0* and *aM2P0* showed significant negative values on the second factor and are thus characterized by high RES and high SOC-IMB. For mule genotypes from breeder B, *bM0P1* and *bM0P2* showed significant positive values on the first factor and are thus characterized by low DI and high LINES. For mule genotypes from breeder C, *cM0P1* and *cM0P2* showed significant positive values on the second factor and are thus characterized by low RES and low SOC-IMB.

Treatment Effects on Mule Genotype Responses

With regard to adrenal levels, MAX was the highest level measured in all mule genotypes. For 3 mule genotypes (*aM1P0*, *aM2P0*, and *bM0P2*), INI was the lowest, but it did not significantly differ from AFTFF23 for the other 3 genotypes (*bM0P1*, *cM0P1*, and *cM0P2*). It was found that REST did not significantly differ from TRANSF for 4 mule genotypes (*aM1P0*, *aM2P0*, *bM0P1*, and *bM0P2*), but it was higher for the other 2 genotypes (*cM0P1* and *cM0P2*). We also found that TRANSF was significantly higher than BEFFF1 for 3 mule genotypes (*aM2P0*, *bM0P1*, and *bM0P2*) but did not significantly differ for the other 3 genotypes (*aM1P0*, *cM0P1*, and *cM0P2*). In all mule genotypes, BEFFF1 was significantly higher than AFTFF1. For 4 mule genotypes (*aM1P0*, *bM0P1*, *bM0P2*, and *cM0P1*), BEFFF23 did not differ from AFTFF23, but it was significantly higher for the other 2 genotypes (*aM2P0* and *cM0P2*). For 4 mule genotypes (*aM1P0*, *bM0P2*, *cM0P1*, and *cM0P2*), BEFFF1 was significantly higher than BEFFF23 but did not significantly differ for the other 2 genotypes (*aM2P0* and *bM0P1*). It was found that AFTFF1 did not significantly differ from AFTFF23 for 4 mule genotypes (*aM1P0*, *bM0P1*, *bM0P2*,

Table 5. Categorization of behavioral variables[1]

Code	Class	N	Range
SOC			
SOC-AMB = 1	Low	66	$3.50 \leq x < 33$
SOC-AMB = 2	Mean	70	$33 \leq x < 46$
SOC-AMB = 3	High	66	$46 \leq x \leq 61.03$
SOC-IMB = 1	Low	66	$0.80 \leq x < 4.50$
SOC-IMB = 2	Mean	74	$4.50 \leq x < 8.50$
SOC-IMB = 3	High	62	$8.50 \leq x \leq 57.29$
SOC-LINES = 1	Low	34	$1 \leq x < 5$
SOC-LINES = 2	Mean	111	$x = 5$
SOC-LINES = 3	High	57	$5 < x \leq 13$
SOC-DI = 1	Low	63	$67.07 \leq x < 100$
SOC-DI = 2	Mean	74	$100 \leq x < 200$
SOC-DI = 3	High	64	$200 \leq x \leq 419.12$
RH			
RH-AMB = 1	Low	64	$3.18 \leq x < 27$
RH-AMB = 2	Mean	69	$27 \leq x < 37$
RH-AMB = 3	High	69	$37 \leq x \leq 60.80$
RH-IMB = 1	Low	70	$0.88 \leq x < 4$
RH-IMB = 2	Mean	71	$4 \leq x < 9$
RH-IMB = 3	High	61	$9 \leq x \leq 53.88$
RH-LINES = 1	Low	92	$1 \leq x \leq 3$
RH-LINES = 2	High	110	$4 \leq x \leq 13$
RH-DI = 1	Low	77	$70.02 \leq x < 350$
RH-DI = 2	Mean	64	$350 \leq x < 405$
RH-DI = 3	High	61	$405 \leq x \leq 422.78$
TI			
TI-ATT = 1	Low	124	$x = 1$
TI-ATT = 2	High	78	$2 \leq x \leq 5$
TI-DUR = 1	Low	54	$0 \leq x < 260$
TI-DUR = 2	Mean	53	$260 \leq x < 480$
TI-DUR = 3	High	95	$x = 480$
RESI			
RESI-FF1 = 1	Low	69	$x = 1$
RESI-FF1 = 2	Mean	60	$x = 2$
RESI-FF1 = 3	High	73	$3 \leq x \leq 4$
RESI-FF12 = 1	Low	123	$x = 1$
RESI-FF12 = 2	High	79	$2 \leq x \leq 4$
RESI-FF23 = 1	Low	129	$x = 1$
RESI-FF23 = 2	High	73	$2 \leq x \leq 4$

[1]Classes used in the factorial analysis components for duration of ambulation (AMB), latency to first immobilization (IMB), number of lines crossed (LINES), and distance index from stimulus (DI) during the social motivation (SOC) test and the response-to-human (RH) test; the number of attempts (ATT) and the duration (DUR) of tonic immobility (TI); and the score of resistance behavior to force-feeding (RESI) at the 1st, 12th, and 23rd force-fed meal (FF1, FF12, and FF23, respectively).

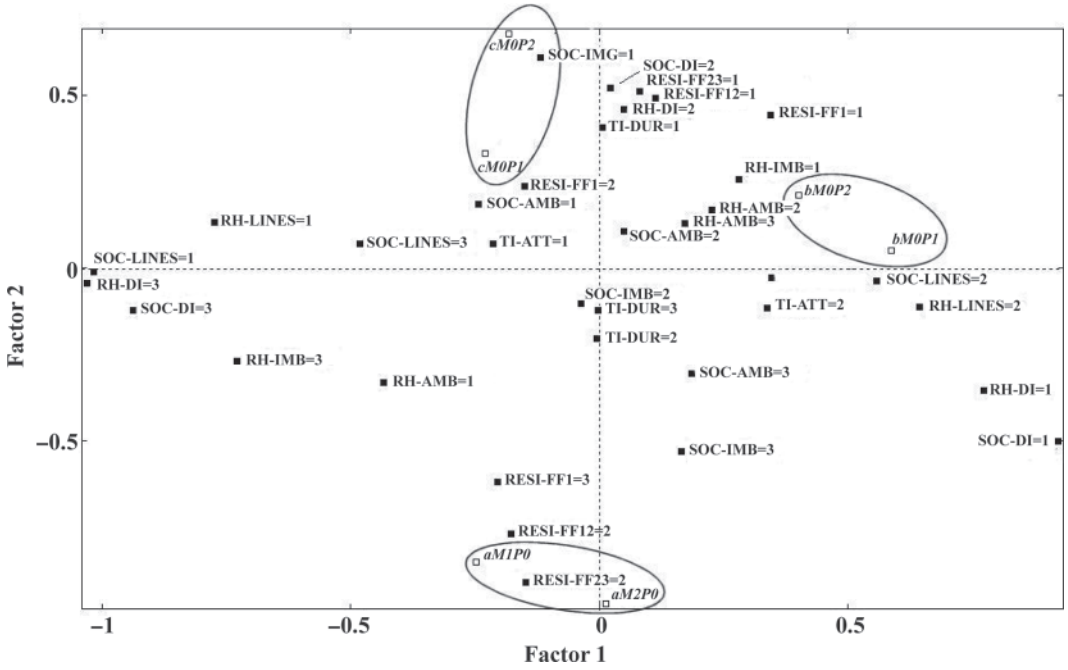

Figure 3. Graph showing the 1–2 factorial plan of factorial correspondence analysis for the active behavioral variables: duration of ambulation (AMB), latency to first immobilization (IMB), number of lines crossed (LINES) and distance index (DI) during the social motivation (SOC) test and the response-to-human (RH) test; the number of attempts (ATT) and duration (DUR) of tonic immobility (TI); and the score of resistance behavior to force-feeding (RESI) at the 1st, 12th, and 23rd force-fed meal (FF1, FF12, and FF23). The mule genotypes from the different breeders A (*aM1P0* and *aM2P0*), B (*bM0P1* and *bM0P2*), and C (*cM0P1* and *cM0P2*) were included as illustrative variables.

and *cM0P1*), but it was significantly higher for the other 2 genotypes (*aM2P0* and *cM0P2*).

With regard to the behavioral data, SOC-DI was higher and RH-DI was lower than the theoretical mean DI in all mule genotypes; SOC-DI was significantly higher than RH-DI for all of the mule genotypes. For 4 mule genotypes (*aM1P0*, *aM2P0*, *bM0P1*, and *cM0P2*), SOC-AMB was significantly higher than RH-AMB but did not significantly differ for the other 2 genotypes (*bM0P2* and *cM0P1*). It was found that SOC-IMB was significantly higher than RH-IMB for 4 mule genotypes (*aM1P0*, *aM2P0*, *bM0P1*, and *bM0P2*), but it was significantly lower for 1 mule genotype (*cM0P1*) and did not significantly differ for the sixth genotype (*cM0P2*). In 4 mule genotypes (*aM1P0*, *aM2P0*, *cM0P1*, and *cM0P2*), SOC-LINES was significantly higher than RH-LINES but did not significantly differ for the other 2 genotypes (*bM0P1* and *bM0P2*).

DISCUSSION

Inappropriate behavioral expression and fear response in mule ducks has been reported concomitantly with loss of color in common duck strains, but variability in intensity depends on the genetic selection scheme. The present study thus had 2 main objectives: (1) to characterize different commercial mule duck genotypes for fear and sociability traits and (2) to characterize the genetic cross effects in these different commercial genotypes to validate previous findings in an experimental mule duck genotype (Arnaud et al., 2008).

First of all, several common features were observed in the overall characterization, all genotypes showing high

Table 6. Contributions on the axis for behavioral traits[1]

	Contribution (%)	
Variable	First factor	Second factor
SOC		
AMB	1.2	2.5
IMB	0.5	11.7[2]
LINES	16.2[2]	0.10
DI	21.5[2]	10.20
RH		
AMB	3.5	2.8
MB	9.1	2.5
LINES	19.7[2]	0.8
DI	21.7[2]	6.4
TI		
ATT	2.8	0.5
DUR	0.0	3.4
RESI		
FF1	2.5	12.3[2]
FF12	0.3	20.9[2]
FF23	0.5	25.9[2]
Genotype		
aM1P0	4.4	71.2[2]
aM2P0	0	71.9[2]
bM0P1	19.0[2]	0.2
bM0P2	11.8[2]	4.6
cM0P1	5.9	18.4[2]
cM0P2	3.7	75.3[2]

[1]Contributions on the first and second factors of the principal components analysis for duration of ambulation (AMB), latency to first immobilization (IMB), number of lines crossed (LINES), and distance index from stimulus (DI) during the social motivation (SOC) test and the response-to-human (RH) test; the number of attempts (ATT) and the duration (DUR) of tonic immobility (TI); and the score of resistance behavior to force-feeding (RESI) at the 1st, 12th, and 23rd force-fed meal (FF1, FF12, and FF23, respectively), with mule genotypes as illustrative variables.

[2]Values considered significant ($P < 0.05$).

avoidance of humans, with a higher DI in the presence of a human than congeners. Furthermore, the calculated index in the presence of a human was always higher than the theoretical mean. Likewise, Pekin and mule genotypes were very sensitive to the TI test, around 50% of the mule ducks and 60% of the Pekin ducks remaining in TI at the end of the 8-min test period. Mule genotypes were also found to be very sociable (attracted by congeners), as clearly illustrated by the fact that the DI values in the presence of congeners was one-third of the maximum theoretical DI. From the physiological point of view, the corticotropic axis was functional in all ducks, as indicated by the fact that it was responsive to physical and pharmacological challenges. Interestingly, the adrenal response level measured after the physical restraint was relatively high, corresponding to at least 50% of the maximum adrenal response capacity for all genotypes. The adrenal response level measured after transfer into the force-feeding barn was largely similar to the level measured after restraint. This result indicates that mule ducks perceived this procedure as very stressful. Adrenal response levels measured during the force-feeding period were always significantly lower than those measured after the various other challenges. Surprisingly, adrenal response levels measured just after the first force-fed meal were lower than those measured 1 h before, whereas the levels before and after the last force-fed meal of the period were generally similar. This result supports earlier findings that force-feeding does not elicit an adrenal response and is not therefore a source of acute stress (Guémené et al., 2001).

Mule Genotype Effects

Mule genotypes from the same breeder were compared first to assess the genotype effects when they had a common parental origin. Few differences were observed in these paired comparisons, especially among A and C genotypes. These pairs of mule genotypes, whose common parental origins differed (maternal for A and paternal for C), thus showed mainly similar fear and sociability traits. Indeed, the 2 distinct paternal genotypes from breeder A and the 2 distinct maternal genotypes from breeder C showed few trait differences. The relationship between BW or feather color and fear responses has been widely discussed in the literature. However, in ducks, the genetic relationship with plumage color has been rejected (Guémené et al., 2006) and these results did not support either of these hypotheses because the 2 mule genotypes from breeder A differed in live BW and the 2 mule genotypes from breeder C differed in plumage color. However, the 2 mule genotypes from breeder B, which also differed in feather color and had a common paternal origin like those from breeder C, showed some contrasting fear-related responses in some tests, whereas there was no difference in any of the traits studied in the 2 distinct maternal genotypes from breeder B. If the relationship with color phenotype is ruled out, 3 hypotheses could be made: 1) an indirect relationship between fear responses and feather color, probably due to a founder effect; 2) the fear profile could be influenced by a difference in just one parent with the same commercial origin; and 3) the fear profile could be dependent on strong genetic-environment interactions.

Some interesting relationships between traits studied in mule genotypes were highlighted by the multivariate analyses. The first factor of the factorial analysis on adrenal responses corresponds to the initial CORT level and the levels measured before and after the first force-feeding meal, whereas the second factor corresponds to the level measured after the restraint, before and after the last force-feeding meal, and the maximum adrenal response capacity. Interestingly, initial adrenal levels were independent of adrenal response to physical challenges or the maximum adrenal response capacity, whereas these 2 traits were positively correlated. Thus, the first axis could be interpreted as the adrenal sensitivity of the ducks, whereas the second axis could be link to the adrenal response to more acute stress. These interpretations may confirm the hypothesis that the force-feeding act was not associated with an acute stress in an adrenal point of view because, at the beginning of the period, it was associated only with sensitivity response and, at the end of the period, the response was negatively correlated with response to acute stress. Interestingly, there was no correlation between the adrenal levels related to the first and last force-fed meals. This could be related to a substantial change of the physiological and metabolic status of the ducks between these 2 moments.

The first factor of the factorial analysis on behavioral responses corresponds to DI and LINES in an unfamiliar environment in the presence of either congeners or a human, whereas the second factor corresponds to RESI and IMB in an unfamiliar environment in the presence of congeners. For each factor, the 2 traits correspond to very distinct features of the responses. Authors do not have any clear interpretation of the association of these 2 traits for each factor and thus cannot propose satisfactory short labeling. Indeed, both factors are described by social and response-to-human components as well as by locomotor behavior in an unfamiliar environment. Interestingly, LINES in the 2 different test conditions (i.e., congeners or a human) was the same and the highest motivation to reestablish social contact was associated with the lowest avoidance of humans and vice versa. These observations could indicate either that humans and congeners represent a similar feature or that the fear of humans is correlated with low sociability. This result appears to contradict previous findings in Japanese quail, in which 2 lines divergently selected for contrasted social motivation showed no significant difference for fear of humans when assessed using a TI test (Mills and Faure, 1991). However, in the present study, TI variables were not correlated in the factorial plan with the DI during the RH test. We can thus hypothesize that DUR and the DI during the

RH test corresponded to 2 distinct components of a multidimensional fear response. Surprisingly, resistance to force-feeding measures was intercorrelated but overall was independent of the other fear-related traits observed during the rearing period. Again, this trait seems to illustrate an independent feature of the overall fear profile.

The multivariate analyses confirmed that mule genotypes within their specific A and C origins had very similar adrenal and behavioral sociability and fear-related response profiles, whereas the 2 mule genotypes from breeder B had similar behavioral profiles but contrasting adrenal profiles. In addition, mule genotypes from breeder A and breeder C were characterized by contrasting profiles on the same adrenal and behavioral traits, whereas the mule genotypes from breeder B were characterized by different adrenal and behavioral profiles to those describing the A and C mule genotypes. The 3 independent breeding schemes thus resulted in 4 different mule genotype profiles characterized by specific fear-related and sociability traits.

Genetic Cross Effects

Although similarities were observed, some values obtained in this study differ from those reported in a previous study (Arnaud et al., 2008). In fact, the mule genotype described in that study has been withdrawn from the market due to its extreme panic response to farm workers. Nevertheless, the previously reported differences between parental genotypes and their corresponding hybrids were mostly confirmed in the 6 crosses from the 3 different breeders. The genetic cross effects observed for a given trait varied, but this variation did not depend on whether the distinct parental component was Pekin or Muscovy. Thus, both parental genetic backgrounds influence the cross effects for these traits in ducks, as previously shown for reproductive traits in rabbit (Baselga et al., 2003) and in cattle (Kahi et al., 1995) and for growth traits in fish (Maluwa and Gjerde, 2006). The selection criteria used in the Pekin and Muscovy lines for several traits (production, reproduction, and health) seem, directly or indirectly (co-selection), to influence the observed cross effects.

From a physiological perspective, the differences observed in the maximum adrenal response capacity of mule and parental genotypes support the existence of an additive effect, with a superiority of the Pekin genotype in the different genetic selection backgrounds (Faure et al., 2003; Arnaud et al., 2008). On the other hand, we did not observe the difference in the adrenal response to restraint that was found in a previous study. However, different types of restraint were used, the earlier study involving restraint in a net. This could be considered to be less stressful than hanging by the feet (Jones, 1992). We cannot therefore exclude the possibility that different traits were involved. Interestingly, the ratio of adrenal response to restraint to maximum adrenal capacity, which indicates the biological response to stress relative to overall physiological capacity, was higher in Muscovy genotypes than in mule and Pekin genotypes, which is consistent with a previously reported observation (Arnaud et al., 2008) and highlights some species differences in the responsiveness of the corticotropic axis. On the other hand, it is well known that the initial level of CORT in ducks is very sensitive to environmental conditions and that variation can indicate a difference in the level of sensitivity to capture and handling (Noirault et al., 1999). The genetic cross-breeding effects for this measure varied in the crosses used in the present study, indicating that this sensitivity is also very dependent on parental genetic differences.

Some previous findings for genetic cross effects were confirmed regarding behavioral responses in stressful conditions for the different crosses. The heterosis effect previously observed on displacement (i.e., AMB, IMB, or LINES), interpreted as a panic response of the hybrid to stressful environments (Arnaud et al., 2008), was also observed in these commercial mule genotypes. On the other hand, the higher avoidance of humans by the hybrid genotype than by the Pekin genotype reported in previous studies (Faure et al., 2003; Arnaud et al., 2008) was only observed in 1 of the 3 commercial breeds. This suggests that this fear-related trait is particularly dependent on differences in genetic origin.

Differences between mule and parental genotypes were also observed in sociability responses, suggesting that the mule genotypes had higher levels of motivation to reestablish social contact than their respective parental genotypes. This trait is usually a good indicator of sociability (Mills and Faure, 1990). The observation of a heterosis effect on this trait is a new finding and supports the hypothesis that in stressful conditions, such as an open-field test involving an unfamiliar environment and social isolation (Faure et al., 1983), the social component (i.e., motivation to reestablish social contact) overcomes the fear component of the global response in mule genotypes but not in Pekin and Muscovy genotypes (Arnaud et al., 2008). To our knowledge, no data regarding the relationship between sociability and emotivity in ducks have been published, and it would be interesting to investigate this question further because it is a key component of adaptability to rearing conditions. Indeed, although gregariousness is an important factor in the collective life of livestock, it has been shown that Japanese quails selected for high sociability are more sensitive to social stress, such as social isolation, than quails selected for low sociability and could potentially be more sensitive to group mixing or rearing in large groups (Mills et al., 1993). It is likely that the high level of sociability observed in all of the commercial strains of mule ducks observed exacerbate the panic or aversion-like behavior observed in collective rearing conditions.

In conclusion, the independent commercial breeding schemes resulted in different mule genotypes characterized by specific patterns of both fear and sociability

traits. Nevertheless, all of the mule ducks showed an exacerbate response of fear. This overall response was higher than that shown by the Muscovy but similar to that of the Pekin genotypes. Moreover, all of the mule genotypes appeared to be more sociable than either the Pekin or the Muscovy genotypes.

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