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Artificial Selection for Increased Maternal Defense Behavior in Mice

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Abstract Maternal aggression is directed towards intruders by lactating females and is critical for defense of offspring. Within-family selection for increased maternal defense in outbred house mice (*Mus domesticus*; Hsd:ICR strain) was applied to one selected (S) line, using total duration of attacks in a 3-min test as the selection criterion. One control (C) line was maintained and both lines were propagated by 13 families in each generation. Prior to selection, heritability of maternal aggression was estimated to be 0.61 based on mother-offspring regression. Duration of attacks responded to selection with a mean realized heritability of 0.40 (corrected for within-family selection) after eight generations. At generation 5, the S and C line also differed significantly for litter size at birth and at mid-lactation (both lower in S), average individual pup mass at mid-lactation (higher in S), and pup retrieval latency (longer in S), but not for other maternal measures that we studied (e.g., dam mass). Additionally, number of entries to middle and closed plus maze compartments was significantly higher in S mice in Generation 5. This is the first study to select for high maternal defense and these mice will be made available as a tool for understanding the genetic and neural basis of maternal aggression.

Keywords Aggression · Anxiety · Fear · Maternal aggression · Maternal defense · Mice · Selective breeding

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Introduction

Maternal defense of offspring (also termed maternal aggression) is a key characteristic of extant birds and mammals, and is widely presumed to be a highly adaptive behavior. In rodents, maternal aggression typically includes fierce attacks against intruders by lactating females and is thought to play a critical role in keeping offspring safe from potential attackers (Agrell et al. 1998; Parmigiani et al. 1999; Wolff 1985; Wolff 1993). Various strains of laboratory house mice exhibit maternal aggression (Gammie et al. 2003; Gammie and Nelson 2001; Svare et al. 1981; Svare and Gandelman 1973), indicating that this is a robust behavior.

Many studies have examined the basis of maternal defense, but to date only a few genetic approaches have been used. For example, studies of maternal aggression in knockout mice have been useful in identifying genes that may contribute to the behavior (Del Punta et al. 2002; Gammie et al. 2005; Gammie and Nelson, 1999; Stowers et al. 2002). Also, one study of quantitative trait loci identified chromosomal regions that corresponded with levels of maternal behavior, including aggression (Peripato et al. 2002), but the actual genes contributing to the phenotype have not yet been isolated.

Selective breeding has been used in a number of studies to help elucidate the genetic basis of aggressive

behaviors. For example, mice have been selected for high intermale (Sandnabba 1996; van Oortmerssen and Bakker 1981) and high interfemale (not maternal) aggression (Hyde and Sawyer 1980). However, to date, no selection study has targeted maternal defense. The aim of these experiments was to use a within-family selection design to increase levels of maternal aggression in outbred mice. We present mother-offspring regression to estimate heritability of maternal aggression in the base population, and also realized heritability after eight generations of selective breeding. Further, we examined traits that might be hypothesized to correlate (positively or negatively) with high aggression, including pup number (litter size), pup mass, pup retrieval, dam mass, and levels of anxiety in the dam using one indirect measure, the elevated plus maze. An important goal of this study was to produce a line a mice that would continue to provide a unique opportunity for examining the genetic and neural basis of maternal aggression.

Materials and methods

Animal husbandry

Outbred Hsd:ICR (also known as CD-1) (Harlan, Madison, WI) strain house mice (*Mus domesticus*) were used as the founder population and for intruder males. All focal mice were given ad lib access to Harlan Teklad Mouse Breeder Diet 7004 (Harlan) and tap water. For breeding in all generations (see additional selection details below), females were singly housed with a male mouse from the same group. Following impregnation (~2 weeks), each female mouse was housed individually for the remainder of the study. Just prior to parturition, dams were given precut squares of compressed paper for nesting material. Polypropylene cages were changed once weekly, but after parturition cages were not changed until maternal aggression or elevated plus maze testing was complete. Mice were weaned at 21 days of age and same-sex siblings were group housed (4/cage) until pairing occurred between 40 and 60 days of age. Intruder male mice (~2-months old) used in aggression testing were sexually naïve, group-housed (4/cage), and not directly related to test mice. Intruder mice were purchased from vendor (Harlan) in separate batches from original focal mice used for selection or control groups. Intruder males from the same cage were used to test both S and C mice. Intruder males were given ad lib access to regular chow. All animals were housed on a 14:10 light/dark cycle with lights on at 0600 CST. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin.

Artificial selection

Twenty-six male and twenty-six female outbred Hsd:ICR mice were purchased from Harlan with the stipulation that no two mice be siblings. For Generation −1, an equal number of mice were randomly assigned to either the control (C) or selected (S) line (Fig. 1). For Generation −1, thirteen females from each group were then assigned a specific family number (1–13), so that within-family selection could be conducted (Fig. 1). Within-family selection began for S mice beginning in Generation 0. For a given family (e.g., family #1 of S mice), all sibling dams were tested in Generation 0, but only the daughters from the one sister with the highest levels of maternal aggression were mated and tested for aggression in the next generation. Although three measures of maternal aggression were determined (described below), total duration of attacks (also termed time aggressive) was used as the selection criterion. (Number of attacks correlates highly with duration of attacks and hence could have been used as the selection criterion. However, latency to attack as a selection criterion could have allowed for selection of traits not specific to overall levels of aggression per se, such as locomotor quickness. Also, for a subset of mice, a quick attack can be followed by relative quiescence. Therefore, selecting for quick-attacking mice in many cases would not have resulted in selection of mice with the highest duration of attacks.) The daughters of the selected mouse then became the test females for Generation 1 for a given family. For C mice, each generation a dam was randomly chosen and her offspring were paired to be examined in the subsequent generation.

For Generation 0 and subsequent generations, the following stipulations were also used. Pairings only occurred between mice from the same group (e.g., S), but were done such that all the sisters from a given family (e.g., family #1) were paired with brothers from a different family (e.g., family #7). No sibling–sibling pairings ever occurred. The males for all pairings were brothers of the sisters that were assigned to the next generation. For this design, for each generation, the pairings between members of different families (in both S and C groups) were randomly chosen.

Maternal aggression testing

On either postpartum Day 4, 5 or 6, each dam was exposed to a novel unrelated intruder male for 3 min in her home cage between 0900 and 1400 h. The pups were removed from the cage just prior to the behavioral test. Removal of the pups from a dam just before an aggressive test does not diminish the expression of maternal aggression in mice (Svare et al. 1981). The days of testing (postpartum Day 4–6) occurred within the window of peak maternal aggression that occurs from postpartum Day 4 though 10 in mice (Svare 1990).

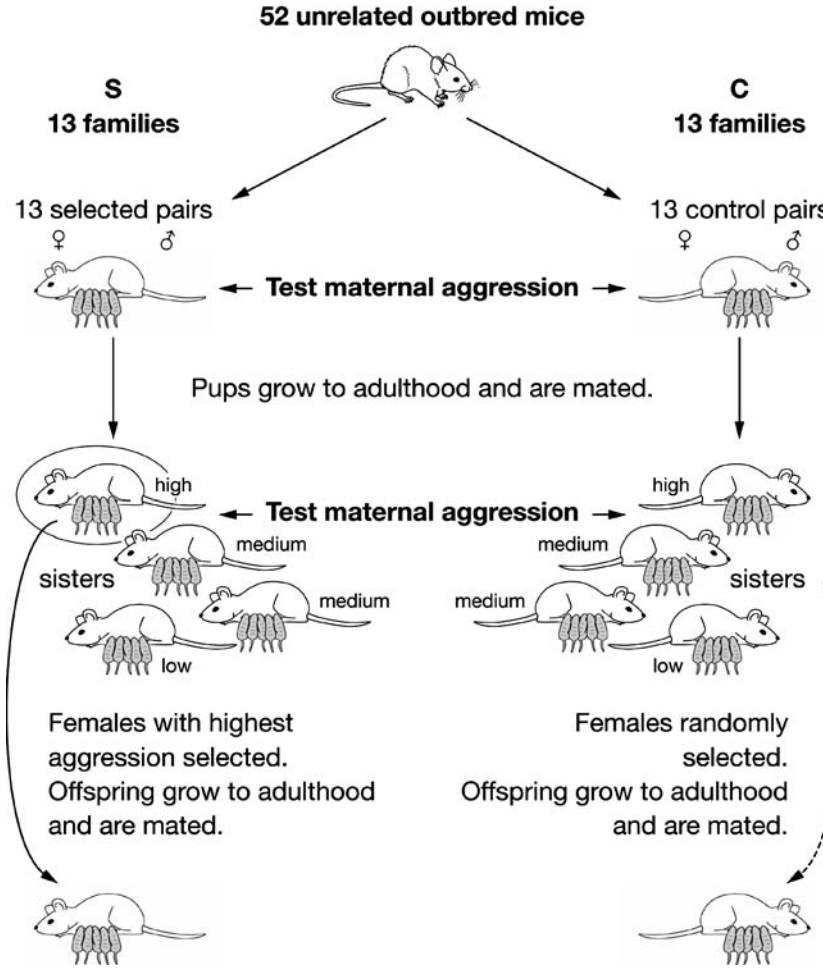

Fig. 1 Diagram of within-family selection paradigm used to select for high maternal aggression. In some generations, only a subset of all offspring C mice were tested

An intruder male mouse (unrelated to focal mice) was placed in the dam's home cage and each test session was recorded on videotape and subsequently analyzed off-line to quantify maternal aggression. Intruder males were never used more than once per day and were used for ~3 tests each. Maternal aggression scoring was conducted by individuals blind to experimental conditions and treatments. For quantification of maternal aggression the following features were measured: latency to first attack, number of attacks, and total duration of attacks (Gammie et al. 2000; Gammie and Nelson 1999). For Generation 5, the amount of time attacking different regions of the male (including head/neck, flank/back, or a combination of these two general regions) was also recorded. At the completion of each test in Generation 5, the pups were randomly scattered in the home cage and the time to retrieve the first and fourth pup to the nest site was recorded. Pup retrieval testing lasted for a maximum of 2 min.

Elevated plus maze testing

The plus-maze apparatus was made of black Plexiglas and had two open arms (35 × 5 cm) and two enclosed arms of the same size with walls 15 cm high. The apparatus was elevated 70 cm above the ground. The arms were connected by a central square (5 × 5 cm). Indirect lighting from a 25 W incandescent bulb was used in an otherwise dark room. All testing for Generation 5 was conducted on the day following the aggression test between 1200 and 1500 h. Mice were tested individually in 5 min sessions. Each mouse was removed from the home cage (pups were left in the cage) and placed on the center platform facing an open arm to initiate the test session. Behaviors scored were latency to enter open arm; number of entries to open arm, closed arm and middle square; and time spent in open arms, closed arms, and middle square of the plus maze. Arm entries were defined as entry of all four paws into the arm. All tests were videotaped and scored off-line by individuals blind to treatment.

Data analysis

In the case of time to first attack (latency), if an animal was not aggressive (no aggression shown during the test period), then a time of 180 s was assigned (the maximum

possible for the test). For mice that did not retrieve, a score of 120 s was assigned (the maximum possible for the test). A nested analysis of variance (ANOVA) using Type 3 Tests of Fixed Effects in the SAS (SAS Institute, Cary, NC) MIXED procedure (version 8) was used to compare S and C lines. Family was considered as a random effect nested within line (S versus C), and so the denominator degrees of freedom for determining significance levels (P values) from the F statistics were based on the number of families tested rather than the total number of individuals. All traits were transformed as needed to improve normality of residuals from the nested ANOVAs. Because pup number varied among individuals, it was also included as a continuous covariate in analyses of aggressive behaviors. Although culling of pups in these studies could have been employed to minimize effects of litter size on aggression, removal of a subset of pups would have directly affected the within-family selection paradigm, so this approach was not used. Sex ratios did not differ between S and C mice and sex ratio did not correlate with aggressiveness in either group, so this variable was not used as covariate in analyses.

Initial and realized heritability

To estimate heritability in the base population, the average values of maternal aggression in offspring of Generation 0 were regressed on the values for their mothers from generation −1, and the slope was doubled. To determined realized heritability across generations 1–8, the selection differential was first determined for each generation. This differential was the difference between the aggression score for the individual selected for the next generation versus the mean from that given family for the same generation. This number was then divided by two because only selection on females occurred. The differential mean was then determined using data points from each of the 13 S families and then divided by the response to selection. Response to selection was defined as the actual difference in mean duration of attacks between S and C mice for the generation following selection.

Using an established approach (Lynch 1980; Swallow et al. 1998), the estimate of realized heritability from regression was also adjusted for within-family selection by the following formula:

$$h^2 = h^2_{\mathrm{R}}(1-t)/(1-r),$$

where h^2 is that expected from mass selection or a randomly mating population, $h^2{}_{\mathrm{R}}$ is the realized heritability obtained from a within-family selection protocol, r is the coefficient of relationship of full sibs (0.5), and t is the intraclass correlation of full sibs for maternal aggression. The intraclass correlation for duration of attacks (untransformed) was calculated as the among-family component of variance divided by the sum of the among-family plus the within-family components of variance. Variance components were estimated with procedure VARCOMP (REML estimation) in SPSS for Windows version 11.5, based on generation 0 values for the 13 families designated to become the selected line (number of sibs tested per family ranged from 3 to 9, with a mean of 5.6). From this set of animals, the intraclass correlation was estimated as 0.12246.

Results

Response to selection

For Generations −1 and 0, no significant differences existed between S and C mice in terms of mean duration of attacks, $F(1,24) = 0.01$, $P = 0.94$ and $F(1,24) = 0.42$, $P = 0.52$, respectively (Fig. 2A). For other measures of maternal defense, including time to first attack and number of attacks, no differences existed between groups for Generations −1 and 0 (Fig. 2B–C).

The change in mean duration of attacks (the dependent measure used for selection), number of attacks and time to first attack across 8 generations of selection are shown in Fig. 2. Significantly higher levels of mean duration of attacks (time aggressive) for S versus C mice were first detected in Generation 2, $F(1,24) = 7.27$, $P = 0.012$. These differences were not significant in Generation 3, $F(1,23) = 2.14$, $P = 0.15$, but again reached significance for Generation 4, $F(1,24) = 29.24$, $P = 0.001$, and Generations 5–8 (Fig. 2A; Table 1). Likewise, number of attacks was significantly higher in S mice in Generation 2, $F(1,24) = 7.79$, $P = 0.01$, Generation 4, $F(1,24) = 29.26$, $P = 0.001$, and Generations 5–8 (Fig. 2B; Table 1). Latency to first attack was significantly shorter for S mice in Generations 4, $F(1,24) = 23.81$, $P = 0.001$, and 5–8 (Fig. 2C; Table 1).

When pup number was used as a covariate in analysis, it had little effect on differences observed. For example, for Generations 5 and 8, for total duration of attacks the P-values for comparing the Selected and Control line were: 0.0021 (unadjusted nested ANOVA) versus 0.0025 (with pups as covariate, $P = 0.9500$) (Gen. 5) and 0.0005 (unadjusted nested ANOVA) versus 0.0006 (with pups as covariate, $P = 0.9308$) (Gen. 8).

In terms of sites of attacks, no differences were observed between groups (data not shown). Overall, for both S and C mice, the breakdown of attacks was approximately as follows: 12% to the belly, 62% to the flank/back, 22% to the head/neck region, and 3% lunges.

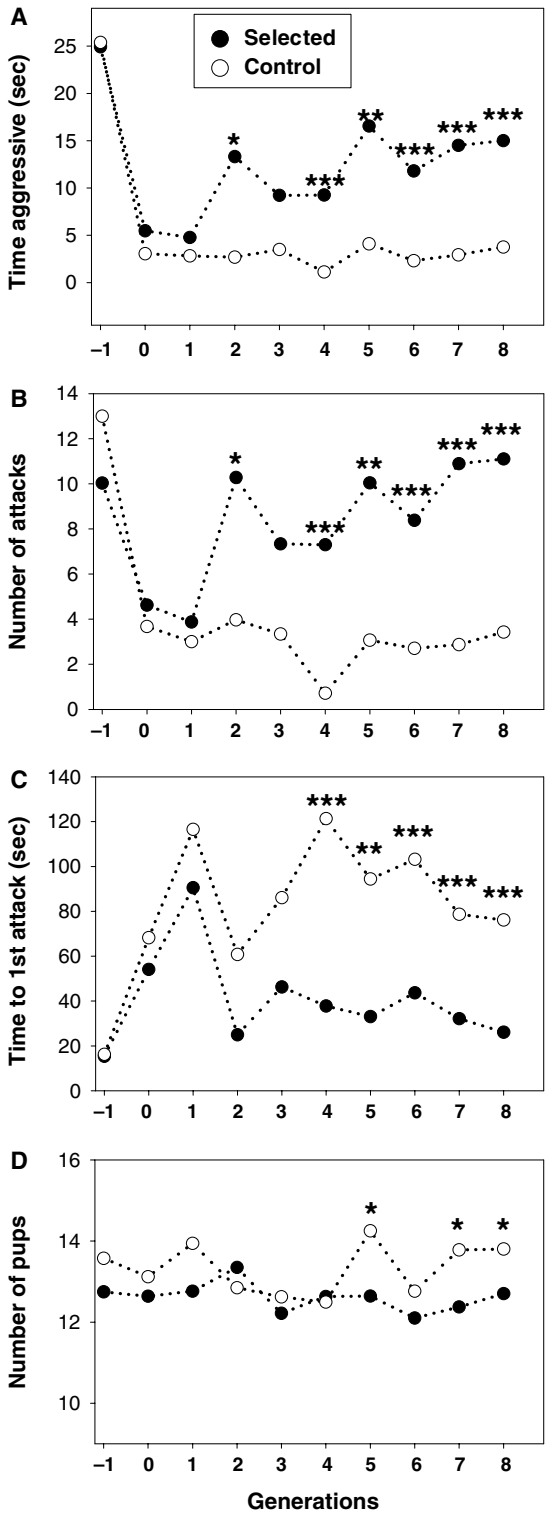

Fig. 2 Mean levels of aggression in S (black circles) and C (white circles) mice over 8 generations of selection. (A) mean duration of attacks (time aggressive) in a 3 min test. (B) mean number of attacks. (C) mean latency to first attack. (D) mean number of pups per dam per generation. Significant differences between the S and C lines are indicated by asterisks. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (see Methods for statistical methods used). All means were back-transformed from LSMEANS computed by SAS

Pup numbers, pup retrieval, pup and dam mass, and latency to give birth following pairing with male

As shown in Fig. 2D, pup number (litter size at birth and mid-lactation) differed between S and C mice in Generations 5, 7, and 8 (Fig. 2D and Table 1). Litter sizes were constant for both S and C mice from birth through mid-lactation.

In terms of time to retrieve first pup, S mice exhibited significantly longer latencies relative to C mice (Table 2). Because almost no S or C mice had retrieved the fourth pup within the 2 min window, this measure was not analyzed. Mean individual pup mass was significantly higher in S relative to C mice (Table 2). For Table 2, the transformation used to improve normality is indicate and both transformed means (± SE) and back-transformed means (shown in parenthesis) are provided.

In terms of latency from pairing with a male to birth of offspring, a significant difference was found between S and C mice in Generations 1, $F(1,24) = 5.55$, $P = 0.027$, and 2, $F(1,24) = 6.44$, $P = 0.018$ with C mice showing a shorter latency. However, differences were not detected in subsequent generations, including Generation 5 (Table 2). In no other reproductive measure were differences found between groups (Table 2).

Elevated plus maze

In terms of number of entries to both middle square and closed arms, S mice showed significantly more entries relative to C mice (Table 3). For all other measures, no significant differences between groups were found.

Heritability

The duration of attacks (time aggressive) for all dams in Generation −1 is plotted against mean duration of attacks of offspring for that dam in Generation 0 in Fig. 3. The slope of the regression line for parent-offspring regression is 0.305. When this slope is doubled to account for only a single parent contributing to the regression, an estimate of heritability of 0.610 is provided. The average realized heritability for duration of attacks was 0.23 (Table 4), or 0.403 when corrected for within-family selection. A plot of the cumulative response to selection is provided in Fig. 4.

Discussion

Our estimates of heritability for maternal aggression based on mother-offspring regression ($h^2 = 0.601$) and realized across eight generations of selection for high levels of aggression ($h^2 = 0.403$ when corrected for within-family

Table 1 Statistical comparison of aggression profiles (and litter size) for dams in the Selected and Control lines from Generations 5–8

Trait examined	Transform	Control	Selected	$F_{1,23}$	P
Time aggressive (seconds) (Gen 5)	Power 0.7	2.68±0.94	7.12±0.86	11.9	0.002
Time aggressive (seconds) (Gen 6)	Power 0.4	1.41±0.25	2.69±0.22	14	0.001
Time aggressive (seconds) (Gen 7)	Power 0.5	1.71±0.32	3.81±0.29	23	<0.001
Time aggressive (seconds) (Gen 8)	Power 0.5	2.68±0.97	3.87±0.31	15.9	<0.001
Number of attacks (Gen 5)	Power 0.7	1.93±0.36	5.02±0.61	9.6	0.005
Number of attacks (Gen 6)	Power 0.6	1.81±0.33	3.58±0.28	16.1	<0.001
Number of attacks (Gen 7)	Power 0.7	2.09±0.45	5.32±0.39	28.6	<0.001
Number of attacks (Gen 8)	Power 0.7	2.36±0.47	5.40±0.39	24	<0.001
Attack latency (seconds) (Gen 5)	Power 0.2	2.48±0.10	2.01±0.09	10.9	0.003
Attack latency (seconds) (Gen 6)	Power 0.5	10.1±0.69	6.60±0.63	14.3	<0.001
Attack latency (seconds) (Gen 7)	Power 0.3	3.70±0.16	2.83±0.14	15.7	<0.001
Attack latency (seconds) (Gen 8)	Power 0.3	3.66±0.18	2.66±0.15	17.4	<0.001
Number of pups (Gen 5)	Power 2.0	202.9±12.5	159.7±11.3	6.52	0.017
Number of pups (Gen. 6)	Power 1.5	45.5±2.38	42.10±2.21	1.15	0.294
Number of pups (Gen. 7)	Power 1.8	112.5±6.70	92.5±5.8	5.04	0.034
Number of pups (Gen. 8)	Power 3	2638±152	2089±131	7.43	0.018

Transformations used to improve normality are indicated. Values are least squares means and corresponding standard errors as computed in SAS using a nested ANOVA (see Methods for more details), and are for transformed data. See Fig. 2 for back-transformed means

selection) are relatively high. Because we do not have replicated selected lines (see Swallow et al. 1998; Swallow and Garland 2005), one caveat for the high heritability is that this one line may not be representative of the realized heritability of the trait (Konarzewski et al. 2005). The decreasing response to selection with subsequent generations (Fig. 4) suggests the possibility that we are approaching a selection limit. With these caveats, though, the heritability of maternal aggression found here is within the range for other social and reproductive behaviors in rodents. For example, selection for high female–female (not maternal) aggression in wild mice resulted in a realized heritability of 0.13 (Hyde and Sawyer 1980) and selection for short attack latency in male mice resulted in a realized heritability of 0.30 (van Oortmerssen and Bakker 1981). High maternal care in mice had a realized heritability of 0.24 (Chiang et al. 2002). Litter size in mice had an estimated heritability of 0.18 (Clutter et al. 1990) and a realized heritability of 0.19 examining response to selection over 122 generations using a residual maximum likelihood analysis (Holt et al. 2005). Quantity of nest building in mice had a realized heritability of 0.18 (unadjusted) and 0.28 (adjusted for within-family selection) when examined over 15 generations (Lynch 1980).

Differences in maternal aggression between the S and C lines were first evident in Generation 2 and were consistently observed from Generations 4–8. In terms of total duration of attacks, C mice aggression was fairly stable from Generations 0–8. A notable exception is Generation −1, where both C and S mice show higher levels of aggression relative to Generation 0. Why aggression levels differ between the first two generations is unclear, but prepartum stress has been found to elevate maternal aggression in mice in some (Kinsley and Svare 1988; Meek et al. 2001) but not all (Maestripieri et al. 1991; Pardon et al. 2000) studies, so stress of shipping could have altered aggression in Generation −1 mice.

Uterine and postnatal maternal effects from the dam can contribute to the phenotype of offspring (Rhees et al. 1999). Thus, although the genetics of the offspring may contribute largely to the phenotype, maternal effects still need to be considered. Although sex ratios could have influenced aggression (Svare 1990), in this study sex ratios were almost identical between S and C mice and sex ratio of a litter was not predictive of aggression. Hence, it is not thought that sex ratios affected phenotype in this study.

In rats and mice, epigenetic effects have been shown to affect behavioral phenotypes. Levels of licking and grooming of pups are epigenetic in that pups exposed to high licking and grooming themselves exhibit higher levels of licking and grooming of their offspring (Caldji et al. 2000a, b; Caldji et al. 1998; Francis et al. 2002). Because

Table 2 Statistical comparison of dam and pup profiles for Selected and Control lines from Generation 5

Trait examined	Transform	Control	Selected	$F_{1,23}$	P
Dam mass Day 1 (g)	Power 1.5	(39.3) 246.5±6.29	(38.7) 241.3±5.8	0.38	0.545
Dam mass Day 6 (g)	Power 1.5	(43.0) 281.9±8.49	(42.2) 274.2±7.86	0.43	0.516
Pup mass Day 1 (g)	Log 10	(1.81) 0.26±0.009	(1.86) 0.27±0.008	0.7	0.410
Pup mass Day 6 (g)	Log 10	(3.98) 0.60±0.011	(4.26) 0.63±0.010	5.22	0.031
Latency from pairing to birth (days)	Log 10	(21.8) 1.34±0.00	(20.8) 1.32±0.00	3.11	0.091
Retrieval latency 1st pup (seconds)	Power 3.0	(87.7) 675923±136993	(103.4) 1107271±1117846	5.7	0.025

Table 3 Elevated Pus Maze Behavior in S and C mice at Generation 5. The back-transformed means are provided in parentheses prior to least squares means±SEs for the transformed data. A nested ANOVA was used for analysis (see Methods for more details)

Trait examined	Transform	Control	Selected	$F_{1,23}$	P
Latency to open arm (seconds)	None	155.9±20.1	166.6±17.31	0.16	0.692
Number of entries to open arm	Power 0.4	(0.6) 0.85±0.12	(0.8) 0.92±0.11	0.19	0.668
Time on open arm (seconds)	Power 0.4	(7.26) 2.21±0.35	(8.7) 2.38±0.31	0.14	0.717
Number of entries to middle square	Power 0.8	(13.3) 7.96±0.43	(16.3) 9.36±0.40	5.57	0.027
Time on middle square (seconds)	Power 0.4	(109) 6.54±0.17	(124) 6.89±0.15	2.19	0.152
Number of entries to closed arm	Power 0.8	(11.5) 7.09±0.365	(14.3) 8.41±0.33	7.00	0.014
Time on closed arm (seconds)	Power 1.4	(159) 1208±101	(148) 1096±94	0.66	0.426

The back-transformed means are provided in parentheses prior to least squares means ± SEs for the transformed data. A nested ANOVA was used for analysis (see Methods for more details)

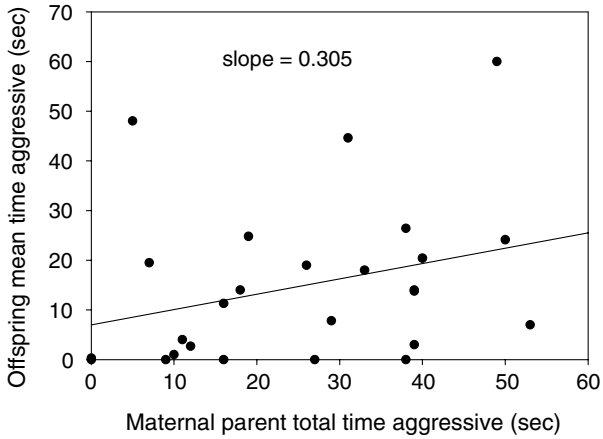

Fig. 3 Plot of levels of total duration of attacks (time aggressive) for all dams (S and C groups) in Generation −1 against mean duration of attacks of their daughters (siblings combined) in Generation 0. Slope of least-squares linear regression = 0.305, but provides an estimate of heritability of 0.610 when the slope is doubled to account for only one parent being used in the equation

Table 4 Realized heritability (unadjusted for within-family selection) through Generation 8

Generation	Realized h^2
1	0.14
2	0.52
3	0.19
4	0.21
5	0.27
6	0.19
7	0.18
8	0.16
Mean	0.23

early life events can alter stress reactivity epigenetically, with some strains showing greater responses to early life events (Anisman et al. 1998), an examination of the contribution of epigenetic effects in S and C mice would be valuable in future studies. Our comparisons of the S and C lines reared under identical conditions (except for their maternal ''environments''), though, demonstrates a genetic basis for the divergence in maternal aggression phenotype.

In the present selection experiment, an important caveat regarding any apparent correlated response is that we do not have replicate lines (Swallow et al. 1998; Swallow and Garland 2005). Thus, it is possible that some of the correlated changes may not reflect changes in allele frequencies at loci that actually responded to the selective-breeding protocol. Recent work suggests that with strong separation of the dependent trait (as seen here), correlated traits in selection studies not involving replicated lines can be identified (Konarzewski et al. 2005). However, correlated traits seen here should be viewed as possibilities, but not as necessities. Examinations of correlated traits, such as plus maze performance, when greater separation between groups has occurred should help to clarify possible genetic correlations between different behaviors.

Notwithstanding caveats involving the lack of replication in this selection experiment, the finding of significantly lower number of pups in S mice in Generations 5, 7, and 8 (Table 1) suggests the intriguing possibility that there is a genetic relationship between pup number and maternal aggression. In a previous study in mice, pup number and maternal aggression were significantly related to one another using a first order polynomial fit (an inverted U-shaped response) with peak aggression around a mean of 11 pups and decreasing aggression with both higher and lower numbers (Gammie et al. 2003). Other studies have found a simple positive relation between litter size and maternal aggression in voles (Koskela et al. 2000) and mice (Maestripieri 1990). Interestingly, for the latter study increasing litter size to 8 and 12 relative to 4 conferred increased aggression, but larger litter sizes were not examined. Thus, our finding of about 12 pups per litter for S mice may reflect in part an ''optimal'' litter size for the production of maternal aggression. The low variance in mean pup number in S mice from generation 3 through 8 (range 12.1 to 12.8) could reflect a stabilizing of pup number that supports peak aggressive output. Additional

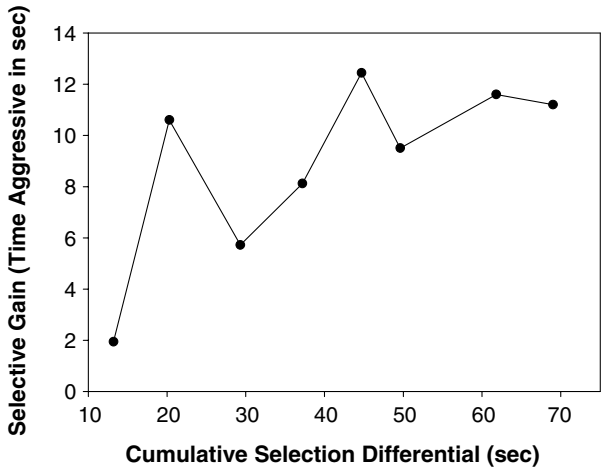

Fig. 4 Plot of the response to selection, measured as the deviation between S and C mice against the cumulative selection differential in S mice

analysis and studies would be needed, though, to address this issue.

Consistent with other studies (Falconer 1960), the lower pup number in S mice was associated with a higher per pup size relative to C mice (Table 2). Another confound is that, in mice, smaller litters yield larger-sized pups that as adults tend to have larger litters with smaller pups (Falconer 1960). As a consequence, cyclic changes in pup number and individual body mass may occur.

Previous work has indicated that the neural circuitries controlling pup retrieval and maternal aggression are mostly distinct, with some exceptions: for review, see (Gammie 2005). Thus, the evolution of longer time to retrieve 1st pup by S mice (Table 2) was unexpected. This result could reflect alterations in brain regions that support maternal aggression and that either directly or indirectly negatively impact retrieval behavior. If so, then a ''trade-off'' between maternal defense performance and other maternal behaviors may exist. On the other hand, some other maternal indices (see Table 2) did not change in the S line relative to the C line, indicating that maternal defense can be modified without affecting all maternal profiles. In future studies it would be valuable to reexamine retrieval profiles and to examine additional maternal behaviors, such as time licking and grooming pups, time on nest, time arch back nursing, or time passive nursing; for example, see (Girard et al. 2002).

The lack of a difference in time on the open arm in the plus maze between the two groups is interesting because two previous studies found increased open arm time (thought to reflect decreased anxiety) to be associated with higher levels of maternal aggression (Maestripieri and D'Amato 1991; Parmigiani et al. 1999). In recent work, we found that open arm time could be increased (via environmental enrichment) without triggering concomitant increases in maternal aggression (Friske and Gammie 2005), suggesting that open arm and maternal aggression performance can be disassociated. Recent work suggests plus maze performance can reflect both exploratory as well as anxiety levels (Rodgers and Dalvi 1997; Wall and Messier 2001; Weiss et al. 1998), and that some aspects of maze performance may largely reflect novelty seeking (Roy et al. 2001). Increased entries by S mice to both the middle square and closed arm (Table 3) suggests the possibility that novelty seeking and/or exploratory behaviors differs between S and C mice. Additional tests of novelty seeking and anxiety, such as such as acoustic startle, defensive burying, or the punished drinking paradigm (that have less of an exploratory component) would help address whether anxiety differences exist between groups.

Future studies examining intermale aggression in S mice will be valuable. In previous studies that involved selection for high intermale aggression, maternal aggression was found to be higher in selected mice in two studies (Hood and Cairns 1988; Sandnabba, 1993), but not another (Benus 2001). A range of studies suggest both overlapping and unique components of maternal and intermale aggression (Gammie et al. 2003; Gammie et al. 2000; Gammie and Nelson 1999; Hasen and Gammie 2005; Lonstein and Gammie 2002). Hence, if higher levels of intermale aggression are found in S mice, then it is likely that the neural substrate upon which selection for high maternal aggression occurred contributes to production of intermale aggression. We do not feel, though, that we have selected for overall increases in aggression in a generic sense because in periodic testing of S virgin females we have never found aggression.

The development of mice bred for high maternal aggression allows for unique approaches to examine the neural and genetic basis of maternal aggression. Previous selection studies on levels of nest building in mice allowed for identification of differences in levels of the neuropeptide, vasopressin, as a key contributor to this behavior (Bult et al. 1992). Although a number of signaling molecules have been implicated in the control of maternal aggression, such as corticotropin releasing factor (Gammie et al. 2004), urocortin 1 and 3 (D'Anna et al. 2005), nitric oxide (Gammie and Nelson 1999), and GABA (Mos and Olivier 1989) (for review, see (Lonstein and Gammie 2002), what modulations to the CNS occurred in the S mice still needs to be determined. One promising approach for identifying neural changes with selection is to use high density gene arrays, as has recently been done for mice selected for high wheel-running behavior (Bronikowski et al. 2004) and for short attack latency in males (Feldker et al. 2003a; Feldker et al. 2003b).

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