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ORIGINAL RESEARCH

Natural Genetic Variation Underlying Differences in *Peromyscus* Repetitive and Social/Aggressive Behaviors

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Abstract *Peromyscus maniculatus* (BW) and *P. polionotus* (PO) are interfertile North American species that differ in many characteristics. For example, PO exhibit monogamy and BW animals are susceptible to repetitive behaviors and thus a model for neurobehavioral disorders such as Autism. We analyzed these two stocks as well as their hybrids, a BW Y^{PO} consomic line (previously shown to alter glucose homeostasis) and a natural *P. maniculatus* agouti variant (A^{Nb} = wide band agouti). We show that PO animals engage in far less repetitive behavior than BW animals, that this trait is dominant, and that trait distribution in both species is bi-modal. The A^{Nb} allele also reduces such behaviors, particularly in females. PO, F1, and A^{Nb} animals all dig significantly more than BW. Increased self-grooming is also a PO dominant trait, and there is a bimodal trait distribution in all groups except BW. The inter-stock differences in self-grooming are greater between males, and the consomic data suggest the Y chromosome plays a role. The monogamous PO animals engage in more social behavior than BW; hybrid animals exhibit intermediate levels. Surprisingly, A^{Nb} animals are also more social than BW animals, although A^{Nb} interactions led to aggressive interactions at higher levels than any other group. PO animals exhibited the lowest incidence of aggressive behaviors, while the hybrids exhibited BW levels. Thus this group exhibits natural, genetically tractable variation in several biomedically relevant traits.

Keywords Stereotypies · Aggression · Social interaction · Y chromosome · *Peromyscus*

Introduction

Peromyscus (deer and white-footed mice) offer rare opportunities to identify alleles underlying natural variation in biomedically relevant behaviors. These common

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North American mammals are adapted to nearly every terrestrial habitat (Dewey and Dawson 2001). Accordingly, they present a wide variety of natural genetic variation affecting numerous characteristics (Vrana et al. 2013). The *P. maniculatus* species complex is particularly widespread, variable, and amenable to genetic analyses. Wild-derived stocks of a number of species and populations are maintained at the *Peromyscus* Genetic Stock Center (http://stkctr.biol.sc.edu/index.html). These stocks differ from most other commonly used rodent strains in having truly wild-type genomes and not having been deliberately subjected to artificial selection in captivity.

Several *Peromyscus* species have been used extensively in behavioral research, largely with a focus on the effects of environmental/hormonal variables (Martin et al. 2007; Glasper et al. 2011; De Jong et al. 2013; Walton et al. 2013; Williams et al. 2013). However, there has been relatively little investigation into the genetic basis of *Peromyscus* behaviors. The BW stock of *P. maniculatus bairdii* (tall grass prairie subspecies, derived from 40 wild caught ancestors in Washtenaw Co MI) and the PO stock of *P. polionotus subgriseus* (derived from 21 animals caught in Ocala National Forest, FL) have proven fruitful in genetic analyses and differ in a number of biomedically and evolutionarily relevant traits. BW females mated to PO males (BW × PO) yield slightly undersized but healthy and fertile offspring. The reciprocal cross—PO female crossed to BW males—has been extensively studied for its severe developmental/epigenetic effects) (Dawson et al. 1993; Duselis and Vrana 2007; Wiley et al. 2008; Shorter et al. 2012).

These two species have been shown to differ in numerous behavioral and physiological characteristics. Notable among these are social behaviors: *P. polionotus* is among the few monogamous mammalian species, (Blair 1951; Foltz 1981), while multiple paternity has been demonstrated within wild BW litters (Birdsall and Nash 1973). *We hypothesize that many of the interspecific differences may be linked to the differing social behaviors of the two species.*

For example, PO and BW have been shown to differ in aggressiveness towards conspecifics in the resident intruder test, with PO males consistently exhibiting more aggressive behaviors (Trainor et al. 2007b).

Glucose homeostasis is much more stable in PO animals of both sexes relative to BWs, although the effect is more pronounced in males. The difference in males appears to be due to PO Y chromosome sequences (Oriel et al. 2008). This hypothesis was tested via a consomic animal line that has a BW genome except for the Y chromosome (BW Y^{PO}). Several lines of evidence suggest that these differences in regulating blood sugar levels are due to a superior ability of the PO animals to buffer stress.

Importantly, BW animals have also been well studied for their tendency to engage in repetitive behaviors (jumps, backflips, etc.) (Powell et al. 1999; Hadley et al. 2006; Korff et al. 2009; Tanimura et al. 2010; Güldenpfennig et al. 2011). They are therefore potential models for behavioral/neurological disorders characterized by stereotypies (repetitive behaviors that lack function or purpose), e.g. Autism Spectrum Disorders (ASD) and Obsessive Compulsive Disorder (OCD) (Lewis et al. 2007). BW animals are variable in their repetitive behavior at a frequency suggestive of a genetic polymorphism within the stock. Anecdotal observations suggest that PO animals engage in far less such behavior. As PO animals exhibit much less sexual dimorphism in body size and parental behavior (PO males aid in pup-rearing), we hypothesized that BW animals would also be more dimorphic in other measures.

Recent sequencing of both the BW and PO genomes makes identification of the polymorphisms underlying these behavioral differences feasible. Thus, genetic studies of mammalian systems that naturally exhibit variations in social and repetitive behaviors could lead to discovery of causative alleles and subsequent development of natural disease models (e.g. ASD, OCD, ADHD). Simple assessment of whether there are shared genetic components between these characteristics may be relevant to understanding disease etiology.

We therefore tested BW, PO, (BW × PO) F1 hybrids and BW Y^{PO} consomic animals as an initial assessment of the genetic underpinnings of the interspecific behavioral differences.

We also tested animals heterozygous for wide-band agouti (A^{Nb}). The A^{Nb} allele is a natural variant of the agouti (*a*) locus that has been bred onto a BW genetic background (Robinson 1981). This allele overexpresses the agouti gene, resulting in a more yellow coat color. This allele is thought to be adaptive, as animals carrying A^{Nb} live in a sandy habitat (Linnen et al. 2009). We are also using A^{Nb} as a biomarker for epigenetic effects, similar to the lab mouse viable yellow allele of agouti (Shorter et al. 2012). *Peromyscus* lacking agouti expression (black or non-agouti) have been shown to be less aggressive and groom more than their wild-type agouti counterparts (Hayssen 1997); these differences are thought to be due to the agouti protein's function as a melanocortin receptor antagonist (Versteeg et al. 1993). We therefore expected the opposite trend from A^{Nb} animals (i.e. more aggressive, less grooming). Moreover, as PO animals are lighter colored than BW, we hypothesized that A^{Nb} behaviors might be more similar to PO animals in some aspects of social behavior.

As an initial step towards these goals, we employed a simple behavioral test battery that can be employed on hundreds of back- or inter- cross animals as initial assessment of these species differences. Thus, we used an open field test and a novel individual/social interaction test in this study. Major goals of this study were to (1) quantitate basic interspecific differences; (2) assess whether these simple tests would uncover sufficient variation to undertake back and/or intercross tests and (3) assess basic inheritance patterns of the interspecific differences.

Materials and Methods

Animals

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of South Carolina. Animals were taken from the stocks maintained at the *Peromyscus* Genetic Stock Center. Animals were kept on a 16:8 h light–dark cycle and were given food and water ad libitum. All animals tested were 4–6 month old (young adult; both species live 4+ years) virgins. All animals had been housed with other same-sex animals post-weaning, and were tested in the middle of the light period (>4 h from both lights on/off). We bred BW females to PO males to obtain F1 hybrids. We bred BW females to homozygous A^{Nb} males to generate A^{Nb} heterozygotes. Apart from breeding records and coat-color, A^{Nb} genotype was also determined by several SNPs (Linnen et al. 2009). PCR primers to generate a ~200 bp amplicon for sequencing were: Agouti F gggattcgtttttccaggtt and Agouti R aacgctgtgggttcagactc. These A^{Nb} heterozygotes, BW, PO, (BW × PO) F1 hybrids BW Y^{PO} consomic (15th generation backcross, as previously described (Oriel et al. 2008) were all tested.

Behavioral Testing

We tested twelve males and twelve females of BW, PO, F1, and A^{Nb} stock and twelve males from the Y consomic stock (which are only male). Each open field test consisted of first placing a single animal into a standard rat (10.25″W × 1″L × 8″H) opaque polycarbonate cage with ~0.75 inches of aspen shavings and a ventilated transparent cover. After 5 min of observation, we introduced a novel animal of the same sex and species. The subsequent 5-min period was the social interaction test. The novel animal's tail was marked with a non-toxic marker to distinguish it from the animal being tested. The cage was cleaned between each animal tested (including replacement of bedding).

Video Analysis

All behaviors were recorded with a digital camcorder. We used the Noldus Observer XT software (http://www.noldus.com/) to score behaviors from the video data. For the open field test, we scored the following behaviors: burrowing, freezing, jumping, back-flipping, running in circles, and grooming. See the video for examples of each (Supplementary material). Based on these videos, we considered straight vertical jumping, back-flipping, and running circles as repetitive behaviors. We also scored exploratory behaviors (e.g. walking the cage perimeter) and instances where the animal remained stationary, but these were not included in the analyses as they did not appear informative.

For the social interaction test videos, we scored the same behaviors as in the open field test with the addition of social and aggressive behaviors. General social behaviors included sniffing, following, and allogrooming. Aggressive behaviors included biting, chasing, boxing, and mounting. Many of these had been described by Eisenberg in the "Behavior Patterns" chapter of the first comprehensive *Peromyscus* compilation (Eisenberg 1968).

All behaviors were scored by incidence; we assessed behavior type at five second intervals throughout the video. Two people scored each video; overall inter-rater reliability was at least 95 %. At least one scorer was blind to the genotype of the animals being scored. When specific behavioral assessments disagreed, we alternated accepting the assessment of scorer 1 versus scorer 2. The data collected by scoring videos were graphed with Microsoft Excel. Behaviors are reported as percentage of incidence of behavior. Statistics were calculated using the Minitab and SPSS software packages. Note that we used Kruskal–Wallis one-way analysis of variance in cases where there was clearly a non-normal distribution in one or more of the groups being compared, and ANOVA in other instances.

Results

Differences in Repetitive Jumping Behavior Between Stocks and Sexes

Because the data did not meet the assumption of normality for analyses of variance, the data were analyzed using the non-parametric Kruskal–Wallis test. As predicted, BW animals engaged in more repetitive behavior than other stocks in combined sex analyses (Fig. 1a). BW animals exhibited significantly higher amounts of repetitive behaviors when compared to PO, F1, A^{Nb} and BW Y^{PO} animals ($p \leq 0.008$, Kruskal–Wallis test). The difference with the latter two categories is most surprising as both stocks have a genetic make-up that is almost entirely BW. The differences between BW and the (BW × PO) hybrids also suggest dominant PO sequences in suppressing such behavior. We also assessed sexual dimorphism of repetitive

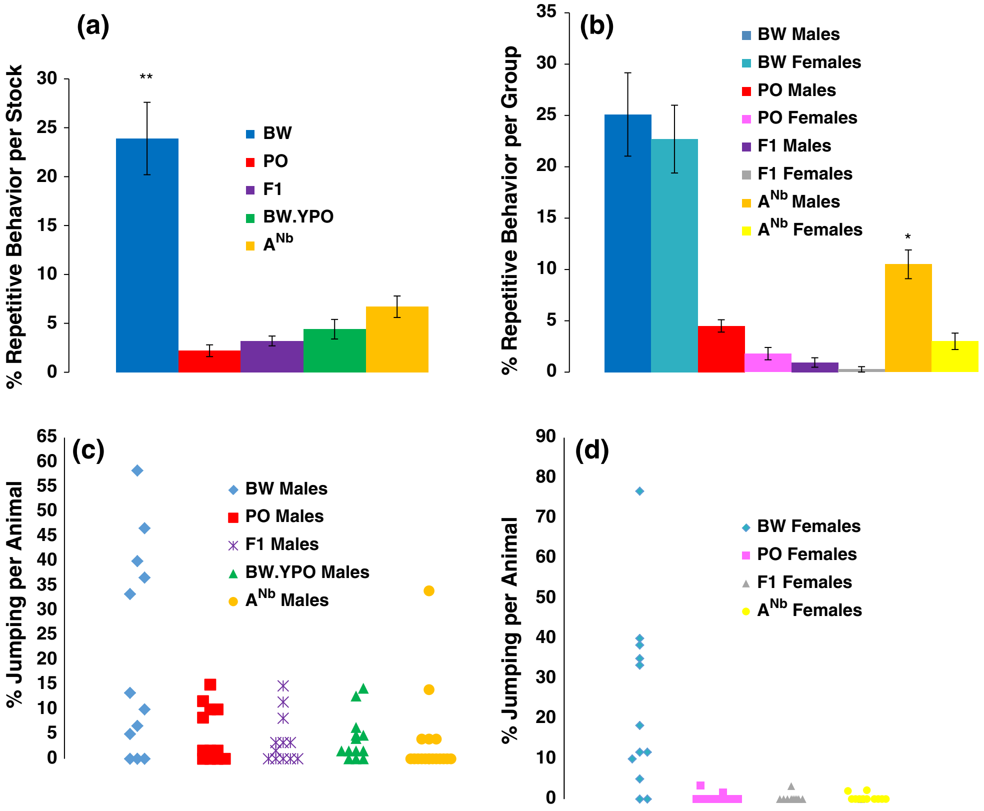

Fig. 1 **a** Frequency of repetitive behaviors (various kinds of jumps, circle running) in each stock tested as a percentage of total behaviors. Mean values with standard error (*bars*) are shown. BW values are significant when compared to each of the other stocks (p <=0.008, Kruskal–Wallis test). Other stocks are not significant compared to each. *Double asterisk* indicates $p \leq 0.01$ comparing BW to other stocks. **b** Repetitive behaviors between sexes; Mean values with standard error (**bars**) are shown. A^{Nb} males perform repetitive behaviors significantly more than A^{Nb} females ($p = 0.049$, Kruskal–Wallis test; $p = 0.041$, 1-way ANOVA). BW and PO males perform repetitive behaviors more than the females of their respective stocks, but these differences are not statistically significant according to a 1-way ANOVA. A *single asterisk* indicates $p < 0.05$ between the sexes of a given stock. **c** Potential bimodal distribution of male jumping behaviors. High jumping groups were compared to low jumping groups in the same stock using an unpaired (independent) *t* test: BW male high versus low jump t = 8.16, $p < 0.001$, DF = 10. PO male high verus low jump t = 6.6, $p < 0.001$, DF = 10. F1 male high versus low jump t = 7.45, $p < 0.001$, DF = 10. Y consomic male high versus low jump t = 6.49, $p < 0.001$, DF = 10. A^{Nb} male high versus low jump t = 5.21, $p < 0.001$, DF = 16. **d** Distribution of female jumping behaviors. The two groups (high & low) were again tested for significance using the *t* test. A bimodal distribution is evident only in BW females: high versus low jump t = 5.27, $p < 0.001$, DF = 10. Divisions between high and low jump are not apparent for other females of the PO, F1, and A^{Nb} stocks

behaviors within each stock (Fig. 1b). While males of each stock had higher levels of repetitive behavior, the difference was only significant in the A^{Nb} stock ($p = 0.049$, Kruskal–Wallis test).

As noted, previous studies have shown that BW animals fall into at least two groups based on jumping frequency (i.e. high-frequency vs. low-frequency jumpers). Such a pattern is evident in males of the BW, PO, and Y consomic stocks (Fig. 1c). Significance could not be calculated for A^{Nb} males as only one high jumper was recorded. Surprisingly, the BW × PO hybrids did not have two apparent groups; this may be due to the limited number of parents we employed to generate F1 animals used in this study.

A bimodal jumping distribution is also evident in BW female animals, but not females of other stocks (Fig. 1d). This finding may be attributed to the low average amount of jumps in females within stocks other than BW, at least during the short interval we observed.

Differences in Burrowing Between Stocks and Sexes

The open field tests yielded only one significant difference between stocks in digging/burrowing behavior: A^{Nb} animals dug more than BW animals ($p = 0.017$, Kruskal–Wallis test; data not shown). In social interaction tests, however, digging is significantly higher in PO, F1, and A^{Nb} animals as compared to BW animals ($p \leq 0.014$, Kruskal–Wallis test; Fig. 2a). This suggests that PO alleles are dominant in inducing a predisposition to digging, and that variation at the agouti locus may be a major contributor to these differences. Consistent with this hypothesis, BW Y^{PO} consomic males are similar to BW males in digging incidence (Fig. 2a).

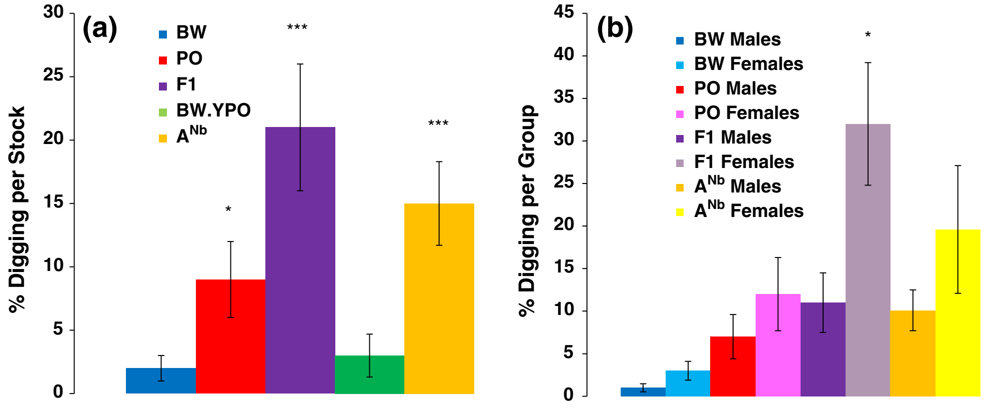

Fig. 2 **a** Digging/burrowing between different stocks in the social interaction test. Mean values with standard error (bars) are shown. Burrowing is significantly higher in PO, F1, and A^{Nb} animals than in BW and BW Y^{PO} animals ($p \leq 0.014$, Kruskal–Wallis test). *Asterisks* indicate significance compared to BW (* indicates $p <= 0.05$, ** indicates $p <= 0.01$, and *** indicates $p \leq 0.001$). **b** Sexual dimorphism in digging. Difference is deemed significant between F1 males and females ($p = 0.026$, Kruskal–Wallis test; $p = 0.013$, 1-way ANOVA). Females of each stock burrow more than males but are not statistically significant by 1-way ANOVA. A *single asterisk* indicates $p < 0.05$ between sexes of a given stock

Sex differences in digging incidence were apparent across all groups, with females always having a greater propensity to dig/burrow. However, only the difference between female and male F1 animals was found to be significant ($p = 0.026$, Kruskal–Wallis test; Fig. 2b).

Grooming Differences Between Stocks and Sexes

BW animals (combined sexes) self-groom significantly less than PO and F1 animals ($p \leq 0.043$, Kruskal–Wallis test; Fig. 3a). This again suggests dominant PO alleles that mediate such behavior. These inter-stock differences are more pronounced in males: BW males groom significantly less than PO males and Y consomics ($p \leq 0.019$, Kruskal–Wallis test) (Fig. 3b).

In contrast, females of each stock tested perform self-grooming behaviors in similar amounts (Fig. 3c). Sexual dimorphism in grooming behaviors was most noticeable in PO animals: Male PO animals groom significantly more than female PO animals ($p = 0.025$, data not shown). This sexual dimorphism is not evident in any of the other stocks tested.

Surprisingly, PO, F1, and BW Y^{PO} males exhibit an apparent bimodal distribution for grooming behavior. This pattern is not evident in BW or A^{Nb} males (Fig. 3d), and thus consistent with being influenced by PO alleles of Y chromosome sequences. Similar to jumping, there appear to be high grooming, low grooming, and no grooming categories. The differences between high versus low/no grooming groups in males of stocks noted above were confirmed as significant using *t* tests.

Comparisons of Social Behaviors Between Stocks and Sexes

BW animals engaged in significantly less general social behavior (as noted- allogrooming, sniffing, following) than animals of the PO, F1, and A^{Nb} stocks ($p \leq 0.002$, Kruskal–Wallis test; Fig. 4a). Only BW Y^{PO} consomic males registered levels of social behavior similar to BW males (i.e. indicating no role of the Y chromosome in underlying these species differences). PO animals also exhibited more social behavior than both A^{Nb} and F1 animals ($p \leq 0.001$, Kruskal–Wallis test). Thus the higher levels of PO social behavior are consistent with a single semi-dominant locus or perhaps several loci (e.g. one dominant, one recessive). The A^{Nb} stock animals exhibit these behaviors at the same levels as the F1 animals, suggesting a role for the agouti gene in mediating such behaviors.

Intra-stock sexual dimorphism in these general social interactions is evident in several stocks. Male F1 animals are more social than female F1 animals ($p = 0.024$, Kruskal–Wallis test) and male A^{Nb} animals are more social than female A^{Nb} animals ($p = 0.006$, Kruskal–Wallis test; Fig. 4b).

Differences in Aggressive Behaviors Between Stocks

The general social behaviors observed in A^{Nb} animals appeared to frequently lead to aggressive encounters. This hypothesis is supported by data showing that the incidence of aggressive behaviors (biting, boxing, chasing, mounting) was significantly higher in A^{Nb} animals than any other stock ($p \leq 0.022$, Kruskal–Wallis test; Fig. 4c).

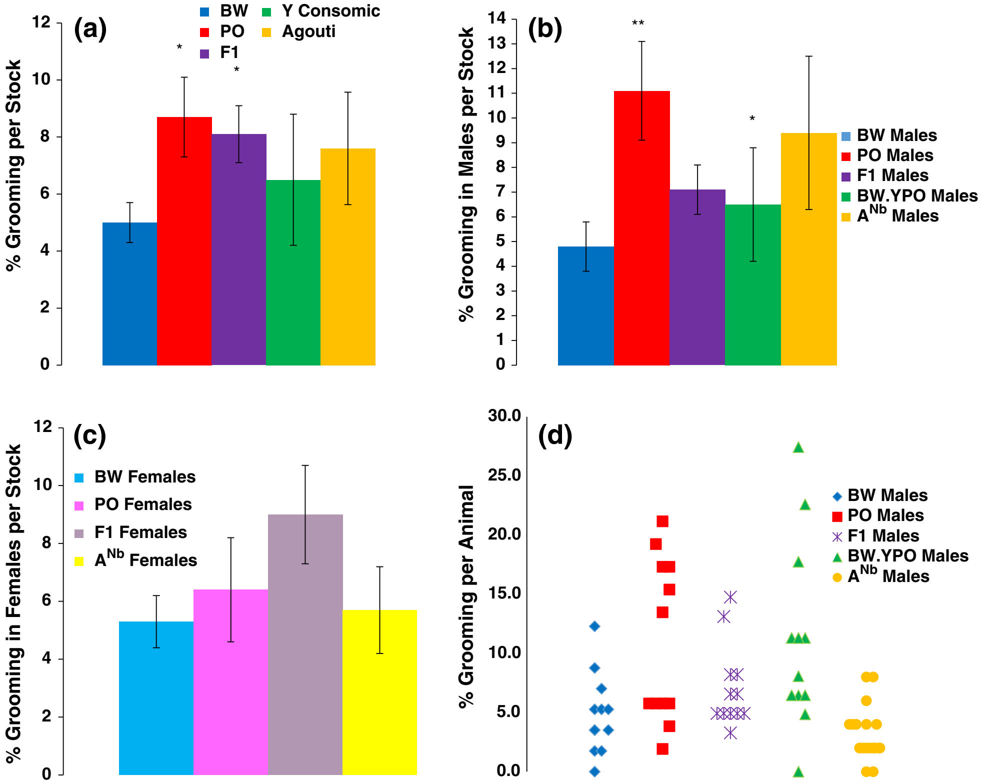

Fig. 3 **a** Self-grooming in each stock. Mean values with standard error (*bars*) are shown Self-grooming is higher in PO and F1 animals than in BW animals ($p \leq 0.043$, Kruskal–Wallis test). A *single asterisk* indicates $p < 0.05$ in comparison to BW. **b** Differences between stocks for self-grooming are more evident in males. This is particularly true for PO males versus BW males ($p = 0.009$, Kruskal–Wallis test). BW Y^{PO} animals groom significantly more than BW males ($p = 0.019$, Kruskal–Wallis test). Asterisks signify significance in comparisons to BW males (* indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicates $p < 0.001$). **c** There is no significant difference between females of stocks in self-grooming. **d** Self-grooming shows a bimodal distribution in PO, F1, and BW Y^{PO} male animals but not in BW or A^{Nb} animals. High grooming groups were compared to low grooming groups in each stock using an unpaired *t* test: PO male high versus low groom $t = 9.66$, $p < 0.001$, $DF = 10$. F1 male high versus low groom $t = 4.72$, $p = 0.001$, $DF = 10$. Y consomic high versus low groom $t = 3.79$, $p = 0.004$, $DF = 10$. Divisions between high and low grooming in males of BW and A^{Nb} stocks are not apparent

The greatest contrast was with the PO animals, for which we did not record any aggressive behaviors. However, the BW, F1 and Y consomic lines were intermediate between the PO and A^{Nb} lines (though the BW animals had much less variability than the latter two lines). Thus these data suggest a combination of BW and the A^{Nb} (or a tightly linked) alleles results in the most aggressive behavior. In this case, the BW alleles appear to be dominant to those of PO, and the PO Y chromosome does not appear to play a role.

Discussion

These data indicate the great potential of using this *Peromyscus* species group to elucidate the genetic (& epigenetic) basis of mammalian behaviors. The data presented here show that multiple genetic modules underlie the complex behavioral differences between the monogamous species *P. polionotus* and the polygamous *P. maniculatus* as well as their variants (e.g. the wide band agouti stock, A^{Nb}). In combination with the nascent resources (genome sequences and a genetic map of the BW and PO stocks), back- or intercrosses may be used to discover the genetic architecture underlying several important traits (Kenney-Hunt et al. 2014).

The pathways underlying BW repetitive behaviors (jumps flips, circle running) appear to be affected by variation at multiple loci. First, we hypothesize that an ancestral polymorphism underlies the bimodal distribution observed within both the BW and PO stocks (i.e. a single locus with two additive alleles; for example, $HH > Hh > hh$). An additional locus or loci must therefore underlie the significant differences in repetitive behaviors between the two populations. In males, the Y chromosome must play a role, as the BW Y^{PO} consomic animals are not distinguishable from their PO male ancestors in the incidence of repetitive behavior. It is possible that epigenetic variation also plays a

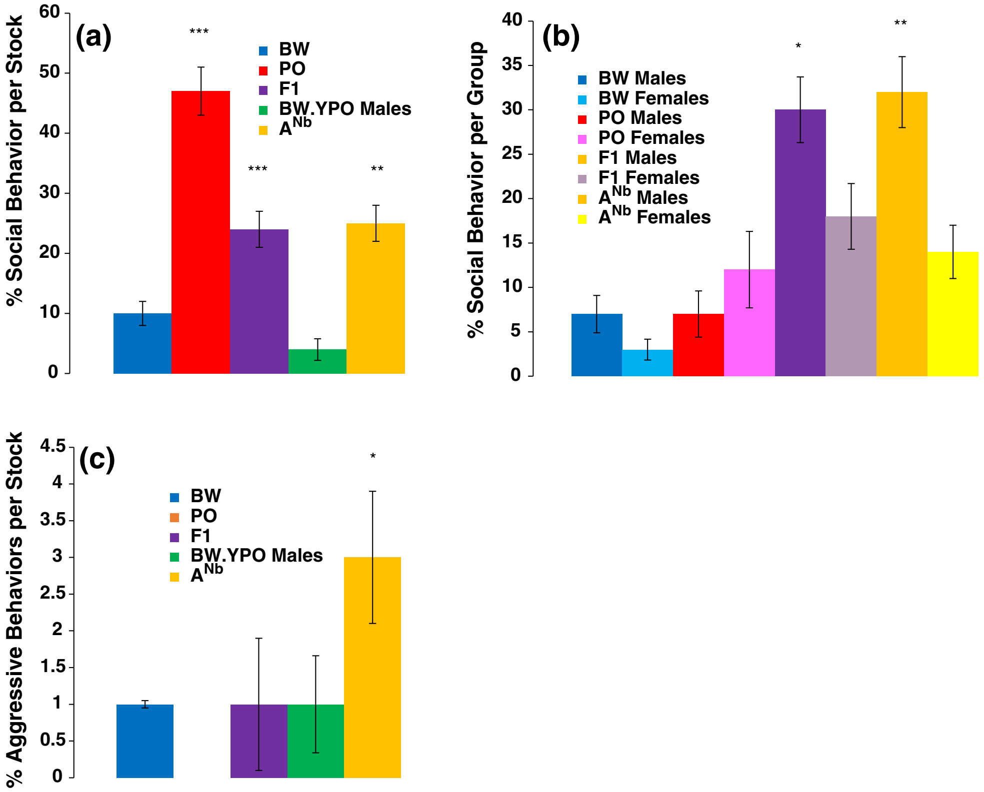

Fig. 4 **a** Social behaviors occur more frequently in PO animals ($p < 0.001$, Kruskal–Wallis test). Mean values with standard error (*bars*) are shown. F1 animals are significantly different from both BW ($p = 0.001$, Kruskal–Wallis test) and PO ($p < 0.001$, Kruskal–Wallis test), indicating an incomplete dominance mode of inheritance. A^{Nb} animals are also more social than BW animals ($p = 0.002$, Kruskal–Wallis test). *Asterisks* indicate significance when compared to BW (* indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicates $p <= 0.001$). **b** Sexual dimorphism in social behaviors is significant only in F1 animals ($p = 0.024$, Kruskal–Wallis test) and A^{Nb} ($p = 0.006$, Kruskal–Wallis test) stocks. *Asterisks* indicate significance differences between the males and females of a given stock (* indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicates $p < 0.001$). **c** Aggressive behaviors in social-interaction tests. A^{Nb} animals exhibit higher amounts of aggressive behaviors than other stocks tested. Importantly, A^{Nb} animals are significantly more aggressive than BW animals ($p = 0.022$, Kruskal–Wallis test). A single asterisk indicates $p < 0.05$. Note that PO animals performed no aggressive behaviors during the test

role in etiology of these stereotypies, as environmental factors reduce the incidence later in life (Hadley et al. 2006). Definitive genetic tests must be performed to determine the genetic versus epigenetic contribution to the BW distribution (e.g. mating high incidence animals and assessing repetitive behaviors in the offspring).

We suggest that the agouti gene (*a*) may be also involved, given the reduced jumping in the A^{Nb} animals and potential pleiotropic effects of this hormone pathway. However, while the A^{Nb} has been bred onto the BW background for decades, it is possible that genes tightly linked to agouti have not recombined. If so, they may have non-BW alleles which are the source of differences in the A^{Nb} line. There are several loci (largely with unknown function) that overlap the large *a* locus and thus necessarily cannot recombine when selecting for the A^{Nb} allele.

While the PO allele(s) of the loci affecting the intra-specific differences in repetitive behavior must be dominant, it is not necessarily clear which is the derived (vs. ancestral) condition. There is variation even within *P. maniculatus* in such behaviors: a forest subspecies, *P.m. gracilis*, jumps and freezes less than *P.m. bairdii* (e.g. BW) (Foster 1959).

The deeper, more elaborate burrows built by PO animals are influenced by a major and several minor autosomal loci (Dawson et al. 1988; Weber et al. 2013). The distinct nesting styles may be indicated by the differences in digging activity we observed even in these short duration tests. In this case, the PO alleles underlying this difference appear clearly dominant, as shown by the burrowing activity of the hybrids. The PO Y chromosome clearly does not play a role, as evidenced by the similar profiles of BW and Y consomic animals. However, the agouti locus again is a suspect in these differences, as the A^{Nb} animals are similar in profile to the PO stock. This raises the possibility that the A^{Nb} or a tightly-linked allele was selected for behavior in addition to the cryptic coloration.

There is some indication of an ancestral sexual dimorphism in burrowing, as females in every stock had a higher percentage of burrowing activity. While this difference only achieved statistical significance in the BW × PO hybrids, we suggest that testing additional animals may resolve this issue. It seems possible that the differential burrowing activity is related to greater nest-building by females.

Self-grooming behaviors are more complicated; Females of each stock self-groom in near-equal amounts, but males differ significantly with PO males grooming much more than BW males. Again the PO alleles are at least semi-dominant, as reflected by increased (relative to BW) self-grooming in both the hybrid and Y consomic lines. However, PO males also have an apparent bimodal distribution in terms of self-grooming levels- the apparent presence of two such groups in both the F1 and Y consomic lines is consistent with an effect of Y chromosome sequences. How the PO Y chromosome would induce such a distribution in a line (lacking a bimodal distribution) is less clear. Our hypothesis that A^{Nb} animals would groom less was clearly contradicted, nor is there convincing evidence from these studies that this locus is involved in the interspecific differences in self-grooming.

In *Mus*, self-grooming is considered an anxiety behavior (Kalueff and Tuohimaa 2004; Kalueff and Tuohimaa 2005). This interpretation is intriguing given that PO animals have significantly higher levels of the stress hormone corticosterone than BW animals, but appear able to buffer its effects better as reflected by their ability to regulate blood glucose levels (Oriel et al. 2008). Interestingly, the Y consomic animals exhibited significantly lower corticosterone levels than either stock, and had blood glucose drop to very low levels when challenged. The hypothesis that PO Y chromosome sequences affect self-grooming is also supported by PO males grooming significantly more than PO females. Thus it is possible that the PO Y chromosome is the sole determinant of the inter-specific and male intra-specific differences, but interactions with autosomal loci seems likely.

Apart from the susceptibility of BW to stereotypies, perhaps the most intriguing differences between these two species are those involving social behaviors. Indeed we hypothesize that the greater social interactions frequently seen in monogamous species requires greater stress buffering in order to engage in these behaviors (as observed in PO). As hypothesized, PO animals engage in such behaviors significantly more than BW animals. The intermediate status of the F1 animals suggests the PO trait is semi-dominant, or affected by multiple loci. The presence of significant sexual dimorphism in the F1s (but not in PO) is more consistent with the latter.

Despite greater amount of these interactions in male hybrids, the Y chromosome appears to play no role in these behaviors: BW Y^{PO} males were indistinguishable from standard BW animals. The agouti locus, however, is again a candidate, as the A^{Nb} animals (1) exhibit comparable levels of social interaction to the F1 hybrids and (2) have a similar sexual dimorphism in those behaviors (with males engaging in more interactions).

A major difference in A^{Nb} social encounters is that they led to aggressive behaviors at twice the frequency of any other stock; note that this supports the hypothesis that levels of the *a* protein are causal to aggressiveness (Hayssen 1997). The multiple behavioral effects (burrowing and aggression) of A^{Nb} raises the question of whether the lighter color it confers (i.e. cryptic coloration) is the only cause for selection of this allele (Linnen et al. 2009; Pennisi 2013).

The A^{Nb} aggression frequency is most divergent from the PO animals, for which we did not record any aggressive behaviors. While PO males have been documented as being more aggressive than BW, this was in a resident intruder test wherein the first male had been housed alone for several weeks before introduction of the second male (i.e. allowing establishment of a territory (Trainor et al. 2007b)). Also, animals in the present study were housed under long day (16 h light) conditions, and aggression is maximized under short days (Trainor et al. 2007a, b) as well as using unfamiliar animals (Dewsbury 1988). For aggressive behaviors under these conditions (meeting of an unfamiliar animal in an open neutral space), the BW alleles appear dominant, as the F1 (and Y consomic) exhibit similar frequencies. The latter is surprising, as the Y chromosome has extensive documentation as contributing to differential aggression in (inbred) *Mus* lines (Maxson et al. 1989; Maxson 1996; Miczek et al. 2001). However, the Y chromosome and testosterone are generally considered to be more involved in territorial aggression while the current study would likely measure what would be considered defensive aggression (Albert et al. 1993).

Unlike other more commonly used mammalian models, *Peromyscus* offer the opportunity to assess the effects of natural genetic variation on disease/disorder predisposition. Moreover, their behavioral repertoire offers opportunities not present in laboratory mice or rats. These initial studies suggest that a number of important characteristics (e.g. repetitive behavior susceptibility, social interaction tendencies) are tractable through genetic studies via these simple behavioral assays. In addition to straightforward back or intercrosses, these analyses show that consomic or variants at individual loci may also be informative. For example, assessing the male offspring of homozygous A^{Nb} females bred to BW Y^{PO} males may yield further insights into the genetic basis of the behaviors described here.

Thus, further behavioral genetic studies of these *Peromyscus* stocks may lead to novel and more natural biomedical models for conditions such as ASD, anxiety-related disorders, and those related to impaired social interactions. For example, a number of *Mus* inbred strains have been extensively characterized for social and repetitive behaviors (Moy et al. 2007, 2008). Of these, the C58 strain has evolved as an ASD model (Ryan et al. 2010; Muehlmann et al. 2012). Behavioral variation in these *Peromyscus* lines appears to compare favorably to the *Mus* lines; more extensive testing (e.g. elevated plus maze, Barnes Maze) will aid further comparisons. While these animals do not yet have the molecular tools available to in Mus, the *Peromyscus* lines offer several advantages. These include their wild-derived genomes, outbred status (e.g. natural heterogeneity in repetitive behavior exhibited by the BW animals) and social behaviors not seen in *Mus* (pair-bonding). Additionally, this system has a unique potential for understanding the evolution of monogamy and co-selected traits.

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