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Wheat grain consumption and selection by inbred and outbred strains of mice☆

Lottes O.C. [a], Kiszonas A.M. [b], Fuerst E.P. [c,b], Morris C.F. [b,*]

[a] Formerly with the Dept. of Animal Science, Honors College, Washington State University, Pullman, WA 99164-6394, USA
[b] USDA-ARS Western Wheat Quality Laboratory, E-202 Food Quality Bldg., Washington State University, Pullman, WA 99164-6394, USA
[c] Dept. of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6394, USA

HIGHLIGHTS

- Strains of mice were evaluated for selection and consumption of wheat seeds.
- All strains exhibited a similar pattern of preferred seed selection.
- Consumption of grain was most influenced by the mouse strain due to animal size.
- C57BL/6J was confirmed to be a highly effective strain to be used as a model system.

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ABSTRACT

Food selection and avoidance are driven primarily by orosensory cues. Previous studies with C57BL/6J mice indicated marked differences in selection and consumption of individual grains of different wheat varieties when presented in binary mixtures. The present study examined the patterns of mouse grain selection across four strains of laboratory mice: two inbred, BALB/c and C57BL/6J, and two outbred, Swiss-Webster and CD1. Four pairs of wheat varieties that were known to vary a priori for consumption preference or seed coat ('bran') color were tested. Two variety pairs were near-isogenic (>98% similar) with contrasting red and white seed coat coloration/pigmentation. All four mice strains exhibited similar preferences between wheat variety pairs, whereas consumption was not highly related to mouse body weight. This result indicates a more generalized phenomenon regarding how mice select and then consume individual wheat grains. The study supported the continued use of C57BL/6J as an effective strain model system to study food perception.

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1. Introduction

Taste and palatability are key determinants of food acceptance or aversion. In mice, we have observed marked differences in selection and consumption of individual grains of different wheat (*Triticum aestivum*) varieties [1–4]. In those studies, C57BL/6J mice showed a strong consumption preference for softer texture, that is, for soft wheat grains over hard grains, and genetically white grains over red (those with reddish brown pigmentation in the outer 'bran' tissues). Using the C57BL/6J strain, we further identified contrasting wheat varieties within the same seed hardness/seed color class that were highly different in consumption preference [2]. In these studies, somewhat like "two-bottle tests", grains of two wheat varieties were mixed and provided to mice over multiple 24-h periods. Mice selected and consumed grains of the variety that they preferred. Our hypothesis is that this preference/aversion is driven by orosensory compounds that are perceived by mice and that vary among different wheat varieties (not unlike, say, the selection of apple varieties by humans), and that the underlying genetic control of the compounds can be identified. Our longer-term goal is to relate food perception by mice (as a model system) to that of human subjects. In this context, some whole grain foods are objectionable due to their bitter flavor profiles, especially among children [5,6].

The house mouse (*Mus musculus*) evolved as a commensal [7–9], and is thus well adapted to the consumption of cereal grains. However, ecological adaptability with its inherent genetic variation can hinder experimental advances. Clearly, the field of physiology and behavior has

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* Corresponding author at: USDA-ARS WWQL, Washington State University, E-202 Food Quality Bldg., Pullman, WA 99164-6394, USA.
E-mail address: craig.morris@ars.usda.gov (M. C.F.).

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benefitted greatly from the use of highly inbred mouse strains due to their within-strain genetic similarity. In this regard, it has been repeatedly shown that different mouse strains differ in their perception of various compounds including salt [10,16–18], sucrose [11,12], Intralipid [13–15], amino acids and carbohydrates [19], and macronutrients [20,21].

Consequently, it is useful to gain a greater appreciation for the consumption discrimination of the inbred C57BL/6J strain compared to other commonly used inbred and outbred strains. A concern a priori was that C57BL/6J mice were possibly either less effective for perceiving differences among wheat grains, or were uniquely suited (in a genetic sense) to discriminate orosensory compounds. Consequently, we compared C57BL/6J to BALB/c (inbred), CD-1 (outbred), and Swiss-Webster (outbred) strains using four contrasting pairs of wheat varieties, either known a priori to differ in mouse consumption preference, or selected because they differed iso-genetically for white vs. red bran color.

2. Methods

2.1. Subjects

All animal experiment protocols were approved and determined to be ethically sound by the Washington State University Institutional Animal Care and Use Committee (ASAF#03964-001). Four different mice strains or stocks were utilized; two inbred strains: C57BL/6J (Jackson Laboratory, Sacramento, CA, stock #00064), and BALB/c (Jackson Laboratory, stock #00651); and two outbred stocks: CD-1 (Jackson Laboratory, stock #00646), and Swiss-Webster (Charles River Laboratory, Wilmington, MA, stock #024).

Ten female mice of approximately the same age (six weeks old) were randomly selected from each strain or stock, totaling 40 subjects. Females of the same age were chosen to prevent confounding due to sex and age. All mice had a training period of three to five weeks wherein they were provided a blend of hard, soft, white and red wheat grains before initiating these studies. Mice, in general, are avid consumers of wheat grains; during this period the mice became accustomed to having a variety of grain in their environment as a food source. Mice were provided standard chow and water *ad libitum* and maintained individually in standard housing cages (Harlan 70-L paper bedding, Harlan '2018' chow containing 18% protein, 14-h light:10-h dark schedule, temperature 20–22 °C, 20–30% relative humidity). The main ingredients in this chow were wheat, corn, and soy meals and other fractions of grains. Regardless of training period or feeding trial, mice always had both chow and some type of wheat grain continuously available. Mouse cages were 28 cm (length) × 17 cm (width) × 11 cm (height). Cages were filled with approximately 1800 cm^3 of paper bedding with an average size of 1 cm.

2.2. Grain samples

'Vida-White', 'Vida-Red', 'Choteau-White', and 'Choteau-Red' were obtained from Dr. Luther Talbert, Montana State University, Plant Sciences & Plant Pathology Department [6]. Both Vida and Choteau possess three red (*R*) grain color genes. They were crossed to 'Clear White' hard white spring wheat variety, and subsequently backcrossed to their red-seed parent to produce BC_5 (back-cross-5) NILs (near-isogenic lines), being more than 98% genetically identical to the original Vida and Choteau parent varieties, respectively. The Vida-White and Choteau-White samples used in this study had the three non-functional alleles conferring white seed coat ('bran') color at the *R* loci (*rrr*), whereas the Vida-Red and Choteau-Red possessed three functional *R* genes (*RRR*). Grain of 'Dayn', hard white, 'Clear White' hard white, 'Hollis', hard red, and 'WB-Fuzion' hard red (hereafter 'Fuzion') wheat varieties were all obtained from Dr. Stephen Guy, Washington State University.

2.3. Experimental protocol

The protocol emulates the 'two-bottle test' and was based on a prior study optimizing the design for these binary grain comparisons [3]. Grains of the two selected varieties for each paired trial (2.5 or 3.0 g each, depending on mouse strain/mouse size) were blended and poured onto the bedding of the cage at the same time each day, giving a total of 5.0 or 6.0 g of grain. Sufficient grain was provided so that neither variety was completely consumed. Grains were blended so that mice would encounter individual grains of ether variety with approximately the same frequency. Trials were conducted across two sequential 24-h periods (Monday through Thursday). The first 24-h period was to introduce the mice to the specific wheat mixture and allow them to acclimate to the varieties being used. The second 24-h period was the experimental day wherein the collected grain was sorted and weighed as described below. At the end of each 24-h period, the entire bedding contents of the cage were recovered. New bedding was immediately introduced and the mouse was returned to its cage. Monday through Thursday, a new sample of mixed grains of two varieties was introduced at this time. Uneaten grains were recovered from the bedding by sifting and manual sorting. Individual grains were further sorted as described below.

When the grains of two of the variety pairs were of similar color (i.e. two "red" or two "white" wheats) they could not be readily identified and sorted according to the original grain lot after collection from the mouse cage. Consequently, a marking technique developed in our earlier study [1] was employed wherein a small (ca. 1 mm) dot was placed on the dorsal side of each wheat grain using "fine point permanent" markers (Sharpie, Sanford L.P, Oak Brook, IL). Previous studies [1] have demonstrated that there is no significant difference in consumption based on ink color; marked kernels were allowed to set at room temperature at least overnight to dissipate any marker solvent. All grains were marked, red ink on one variety and black ink on the other. In the two studies with mixed color class varieties (red and white), no marks were necessary. The uneaten grains were weighed and consumption was calculated on a daily basis by subtraction.

2.4. Data analysis

Individual mice were the experimental units and provided replication within each mouse strain. Analysis of variance (ANOVA) was conducted using Proc GLM to obtain *F*-values. The ANOVA was performed using a randomized complete block design with 'split-plots'. There were four blocks, strains were the 'plots', and diets the 'sub-plots'. Consequently, the ANOVA for strain differences were tested using the block x strain interaction term as the error. Diet was tested using the residual error, and the strain x diet interaction term was tested using the block x strain x diet interaction.

Student's *t*-test was conducted using the following SAS code (SAS v. 9.3; SAS Institute, Cary, NC): proc mixed; model consumption = strain diet strain*diet; random mouse mouse*strain mouse*diet; lsmeans strain/adjust = tukey pdiff; lsmeans diet/adjust = tukey pdiff. In this code, "consumption" = grams of each wheat variety consumed, "diet" = the two varieties being tested and "mouse" = the specific strain of mice. This analysis provided the Least Squares (LS) mean consumption for each variety. The *P*-value for each LS mean difference was computed, the latter using Tukey's "honestly significant difference" adjustment of Student's *t*. H_o: (LS mean 1) − (LS mean 2) = 0. The difference in the two LS means was tested using Tukey's adjustment to Student's *t*-test. Data were also analyzed by calculating a consumption ratio (CR), wherein [Variety 1/(Variety 1 + Variety 2)] * 100, and [Variety 2/(Variety 1 + Variety 2)] * 100. Ranges were calculated as two standard deviations away from the mean. Data were presented with mean ± standard deviation. Box and whisker plots were examined for skewness and normality. Outliers were identified as the most extreme values in each set of strain-variety combinations. The 1% most extreme