INTRODUCTION

It is estimated that by 2025 more than two thirds of the world's population will be living in a water-scarce environment (UN-Water, 2013). The issue is intensified by increased demands for water due to a growing population, changing diets and development (de Fraiture & Wichelns, 2010; Godfray et al., 2016; Schlink, Nguyen, & Viljoen, 2010). This shortage demands innovative techniques for reducing water usage. While direct consumption of water by livestock only accounts for about 1% of water usage (Maupin et al., 2010) there is certainly merit in its reduction. This is particularly true in developing countries, such as those in northern Africa, where water is scarce and demand for animal products is on the rise (Allan, 2001). These areas need animals that can consume limited amounts of water whilst obtaining maximal growth for meat production. There is also a need for animals to perform draught work as crop production increases in developing countries (Schlink et al., 2010). Again, animals that are less affected by water scarcity could be beneficial.

Many animals already show adaptations that make them more suitable to these challenging environments. In fact, adaptations of “tropical” cattle are very well characterized. However, the genetic controls of many of these adaptations are unknown (Barendse, 2017).

ORIGINAL ARTICLE

Genetic basis of voluntary water consumption in two divergently selected strains of inbred mice

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Abstract

Background: Inbred mouse strains with normal renal function show a substantial difference in daily water consumption across strains. This study uses two strains of inbred mice C57BR/CDJ (BR), which are high consumers, and C57BL/10J (BL), which are low consumers, their reciprocal F₁ crosses, inter se bred F₂s and backcrosses produced by breeding high consuming F₂ animals to the low consumer parent strain and low consuming F₂ animals to the high consuming parent strain. Consumption was corrected for body weight prior to analysis.

Methods: The effective number of genes controlling water consumption was estimated using the Castle–Wright estimator. Additive and dominance genotypic values as well as the degree of dominance were calculated using estimated strain means.

Results: According to Castle–Wright, a minimum of 10 factors were estimated to affect the difference in consumption across the two strains. Between seven and eight are expected to be high effect factors. Using the Zeng adjustment, it was determined that 30–40 factors potentially affect the difference in consumption.

Conclusions: These numbers were surprising but may be related to several sources of variation present in the BR strain. A negative degree of dominance indicated the BL strain has more dominant factors.

KEYWORDS

genetics, mice, water consumption
Under the premise of genetic control being conserved across species, a long-term backcross study to isolate genes that control water consumption in two divergently selected strains of mice was designed.

In standard laboratory conditions, inbred mouse strains with an altered kidney function show about a fourfold range of daily water consumption (Tordoff, Bachmanov, & Reed, 2007). Using this information, a high consuming strain and a low consuming strain were selected. The objective of the present study is to estimate the effective gene number controlling the difference in consumption between these two strains and estimate additive and genotypic values for the trait.

2 | METHODS

2.1 | Experimental animals

Foundation C57BL/10J (Black) and C57BR/CDJ (Brown) were purchased from Jackson Laboratories (Bar Harbor, ME). These strains were chosen based on previously quantified difference (Blacks: 0.35 mL/g/0.667/d; Browns: 0.80 mL/g/0.667/d) in water consumption (Bachmanov, et al., 2007). The strains show no difference in renal function (Thaisz et al., 2012). These animals were bred to establish two single-strain colonies at the University of Missouri, Columbia. In accordance with the suggested design for diallel crosses (Griffing, 1956), females from each strain were bred to males from the opposite parental strain; high consumers to Blacks and low consumers to Browns. Backcrosses produced from the high consumer were referred to as High Backcross 1s (HB1s). Those from the low consumer were referred to as Low Backcross 1s (LB1s).

Consumption and weight data were collected on 848 animals: 68 Black, 81 Brown, 117 F2, 338 F2, 129 HB1 and 115 LB1. Animals were housed in plastic tub containers with corn cob bedding per ACUC approved protocol 8565. All males were individually housed, breeder females were group housed and experimental animals were individually housed during water consumption measurements. The temperature was maintained at 24 ± 1°C.

2.2 | Weight and consumption measurement

Animals were weaned at 4 weeks, weighed, and separated into individual cages with custom-built 25-ml serological pipette water bottles based upon a previous design (Bachmanov, Tordoff, & Beauchamp, 1996). To see a more specific description of data collection methods, refer to Haag, Wells, and Lamberson (2018).

2.3 | Statistical analysis and selection

Regression analysis of measured water intake on body weight, strain and sex was conducted prior to other analyses.

To estimate means, variances and standard errors mixed model analysis of adjusted consumption were conducted using PROC MIXED in SAS software. Adjusted consumption was designated as the dependent variables and fitted to a linear mixed model in the analysis:

\[ N_{ij} = \mu + \text{strain}_i + \text{sex}_j + \text{ sire}_k \times \text{strain}_i + \text{ sex}_j + \epsilon_{ijkl} \]

In this model, \( N_{ij} \) is the dependent variable, adjusted consumption, \( \mu \) is the mean, strain, \( \text{sex} \) and strain*sex are the designated fixed effects for strain, sex and the strain*sex interaction, respectively, \( \epsilon_{ijkl} \) is the sire within strain random effect and, finally, \( \epsilon_{ijkl} \) is the error term.

Effective gene number was estimated using the Castle–Wright estimator (Castle, 1921; Cockerham, 1986; Lande, 1981; Wright, 1968):

\[ n_e = \frac{(z(P_1) - z(P_2))^2 - \text{Var}[z(P_1)] - \text{Var}[z(P_2)]}{8 \times \sigma_s^2} \]

In this equation, \( n_e \) is the estimated number of factors affecting a trait of interest, \( zP_1 \) and \( zP_2 \) are the observed means of the trait for each parental strain, \( \text{Var}[zP_1] \) and \( \text{Var}[zP_2] \) are the sampling variances for each parental strain estimated by squaring observed standard errors and \( \sigma_s^2 \) is the estimated segregation variance for each trait. Segregation variance estimation is described later in this section. The number of effective factors was estimated using the square root of the variance of \( n_e \).

To yield a less biased estimation, Zeng’s adjustment equation (Zeng, 1992) was used to calculate \( n_e \):

\[ n_e = \frac{2m_e + c_n(n_e - 1)}{1 - n_e(1 - 2c)} \]

To evaluate assumptions associated with the Castle–Wright estimator, adjusted consumption data were evaluated for epistasis and additivity. Epistasis testing was completed using the equation (Lynch & Walsh, 1998):

\[ \Delta = z(F_2) - \left( \frac{z(P_1) + z(P_2)}{4} + \frac{z(F_1)}{2} \right) \]

where \( \Delta \) represents the epistatic estimate, \( z(F_2) \), \( z(P_1) \), \( z(P_2) \) and \( z(F_1) \) represent the observed line means for the \( F_2 \), Black, Brown and \( F_1 \) animals, respectively. As the observed \( \Delta \) was not zero for the dataset, a sampling variance of \( \Delta \) was estimated using the following equation:

\[ \text{Var}(\Delta) = \text{Var}[z(F_2)] + \frac{\text{Var}[z(P_1)]}{4} + \frac{\text{Var}[z(P_2)]}{16} + \frac{\text{Var}[z(P_1)] + \text{Var}[z(P_2)]}{16} \]

where \( \text{Var}(\Delta) \) is the sample variance for estimated \( \Delta \) value, \( \text{Var}[z(F_2)] \), \( \text{Var}[z(F_1)] \), \( \text{Var}[z(P_1)] \) and \( \text{Var}[z(P_2)] \) are the observed sampling variances for \( F_2 \), \( F_1 \), Black and Brown animals, respectively. The ratio of \( |\Delta|/\sqrt{\text{Var}(\Delta)} \) then provides a t test for evaluation of significance.
The joint scaling test (Cavalli, 1952; Gale, Mather, & Jinks, 1977; Mather & Jinks, 1971) was used to evaluate additivity. This test was designed to evaluate the increased variance observed in the $F_2$ generation. The test begins by fitting data to the simplest, additive model:

$$M = \begin{bmatrix} 1 & 1 \\ 1 & -1 \\ 1 & 0 \\ 1 & 0 \\ 1 & 0.5 \\ 1 & -0.5 \end{bmatrix}$$

Matrix $M$ represents the coefficients of effects for $\mu$ and $\sigma^2$ for Brown, Black, $F_1$, $F_2$, HB1 and LB1, respectively. A chi-squared test ultimately determines the adequacy of the model for the data.

There are several methods to estimate segregation variance; however, these methods can produce highly variable results (Lande, 1981; Lynch & Walsh, 1998). To avoid high variability, the least squares analysis method (Lynch & Walsh, 1998) was selected:

$$\text{Matrix } M \text{ represents the coefficients of effects for } \mu \text{ and } \sigma^2 \text{ for Brown, Black, } F_1, F_2, \text{ HB1 and HB2, respectively. A chi-squared test}}$$

Additive and dominance genotypic values were calculated in the population with means from Black, Brown and $F_1$ animals (Falconer & Mackay, 1996). Additive genotypic value ($\alpha$), was determined using the equation:

$$\alpha = \frac{\mu_{B} - \mu_{P}}{2}$$

In this equation, the parental line with the highest phenotypic value should be first in the numerator, or Black from Brown.

Dominance genotypic value ($d$) was determined using the equation:

$$d = \mu_i - \frac{\mu_P + \mu_{P_2}}{2}$$

In this equation, the mean of the parental strains is subtracted from the mean of the $F_1$ strain. To estimate the degree of dominance, the dominance value was divided by the additive value.

3 | RESULTS

A significant sex*strain interaction was observed in the dataset. This previously observed interaction (Haag et al., 2018; McGivern, Henschel, Hutcheson, & Pangburn, 1996; Reed, Bachmanov, & Tordoff, 2007), was accounted for by analysing each sex separately for all analyses in the study.

Brown animals consumed more water ($P < .0001$) than Black animals (Table 1). $F_1$ animals had higher water consumption but were much closer to that of the Black animals (Table 1). $F_2$ animals showed a range of phenotypes encompassing both parental phenotypes as well as higher variance than the $F_1$ animals. However, variance in the $F_2$ was not as high as that observed in the Brown animals. Backcross animals showed means and variances moving towards parental strain values each generation.

Prior to factor number estimation, the epistatic, additive and dominance effects were analysed to determine how well the data fit the assumptions for Castle-Wright. Both sexes showed a significant indication ($P < .0001$) of epistatic effects (Table 2), a violation of assumptions that would minimize the number of factors identified. Both sexes were, however, adequately fit by the additive model as evidenced by the significant chi-square value (Table 2). Degree of dominance estimations indicated that Black alleles are dominant over Brown alleles in this cross.

### TABLE 1 Number of animals per sex and strain, least squares analysis mean + SE (ml/g/wt$^{0.67}$) of water consumption for each sex and strain and the estimated variance for each sex and strain

| Line         | N   | $\mu$ ± SE | $\sigma^2$ |
|--------------|-----|------------|------------|
| Black Female | 40  | 0.619 ± 0.039 | 0.0072     |
| Black Male   | 28  | 0.582 ± 0.042 | 0.0086     |
| Brown Female | 53  | 1.459 ± 0.050 | 0.0805     |
| Brown Male   | 28  | 1.136 ± 0.044 | 0.0651     |
| $F_1$ Female | 56  | 1.034 ± 0.022 | 0.0193     |
| $F_1$ Male   | 61  | 0.874 ± 0.022 | 0.0103     |
| $F_2$ Female | 179 | 0.964 ± 0.012 | 0.0289     |
| $F_2$ Male   | 159 | 0.876 ± 0.013 | 0.0157     |
| HB1 Female   | 72  | 0.741 ± 0.032 | 0.0202     |
| HB1 Male     | 57  | 0.690 ± 0.032 | 0.0111     |
| LB1 Female   | 64  | 0.934 ± 0.031 | 0.0300     |
| LB1 Male     | 51  | 0.924 ± 0.033 | 0.0200     |

### TABLE 2 Significance test values for epistasis and additive by sex, estimated degree of dominance by sex

|                | Male | Female |
|----------------|------|--------|
| Epistasis test | 15.64| 127    |
| Additivity test| 0.44 | 4.00   |
| Degree of dominance | −0.593 | −0.010 |
Finally, \( n_e \) was used to estimate a more unbiased number of factors \( (n) \) (Table 3). Again, differences in effective factor number and \( n \) are likely related to estimation biases based on sex.

A similar analysis of weight data, however, yielded a negative value for the Castle–Wright estimator. This was likely due to the strains not being differentiated enough for weight. This lack of divergence was expected since lines were partially selected based on similar size. This was done to reduce the number of potential factors affecting water consumption differences.

### 4 | DISCUSSION

These results indicate many genes control the difference in water consumption between these two strains. In fact, this estimation is likely minimized due to violations of Castle–Wright estimator assumptions. To produce an unbiased prediction with the Castle–Wright estimator several assumptions must be met (Castle, 1921; Wright, 1968):

1. All alleles increasing the value of the phenotype are fixed in one line and all those that lower it are fixed in the other line.
2. Allelic effect differences are equal at all loci.
3. All loci are unlinked.
4. All alleles interact additively—no dominance or epistasis.

Expectedly, the data showed significant epistatic effects due to the quantitative nature of the trait (Cordell, 2002). Dominance effects were also observed which moved the \( F_1 \) and \( F_2 \) phenotypes nearer to the Black parent than the mid-parent value. However, joint scale testing indicated the data were adequately fit by the additive model. This signalled the data could be analysed using the Castle–Wright estimator. It should be noted though, research (Huang & Mackay, 2016) has indicated that data, regardless of genetic architecture, can typically be fitted to the additive model. Previous comparisons of Castle–Wright estimators and quantitative trait loci (QTL) analyses have yielded similar results. This indicates the robustness of the estimator and reduces concerns about the additivity of the data (Wu, Bradshaw, & Stettler, 1997).

The number of genes controlling the difference in consumption may be surprisingly high from two closely related strains (Beck et al., 2000). However, previous work has indicated higher than expected genetic variance and divergence in the strains, particularly in regard to copy number variation (Cutler, Marshall, Chin, Baribault & Kassner, 2007). Further, the Brown strain has been noted for its high degree of genetic distinctiveness potentially related to mutation rate (Taylor, 1972) as well as a higher level of haplotypic introgression than typically observed in inbred strains (Yang et al., 2011). Regarding water consumption specifically, previous research has indicated differential androgen regulation in males (Melinotou, Cohn, Bardin, & Janne, 1987); however, no differences in androgen receptor or affinity (Kemp & Drinkwater, 1989). Brown females have also been noted for lowered ovarian hormone production (Maronpot, 2009) which can increase water intake (McGivern et al., 1996; Tarttelin & Gorski, 1971). This level of phenotypic diversity in the strain could explain the high levels of variance observed in the Brown strain.

### 5 | CONCLUSION

In conclusion, the difference in water consumption between these two strains is controlled by many genes. This indicates a requirement for a very large number of animals to conduct a QTL analysis. It may be advisable to instead evaluate this trait using less genetically diverse strains such as C57BL/6J and C57BL/10J which still have a sufficient difference in water consumption (Tordoff et al., 2007).

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### CONFLICT OF INTEREST

The authors declare that they have no competing interests.

### ETHICAL STATEMENT

This study was designed and completed in accordance with University of Missouri ACUC Protocol #8656.

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**TABLE 3** Estimated segregation variance by sex, estimated number of factors using the Castle–Wright estimator by sex, estimated number of large effect factor by sex and estimated number of factors using the Zeng adjustment by sex

|          | \( \sigma^2_s \) | \( n_e \) | Large effect factors | \( n \) |
|----------|-----------------|----------|----------------------|------|
| Male     | 0.00372         | 10.20    | 8.10                 | 29.17|
| Female   | 0.00681         | 12.87    | 6.99                 | 43.21|

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