DOI: 10.1534/genetics.108.088070

Within-Generation Mutation Variance for Litter Size in Inbred Mice

Joaquim Casellas* and Juan F. Medrano†,1

**Genètica i Millora Animal, Institut de Recerca i Tecnologia Agroalimentàries-Lleida, 25198 Lleida, Spain and* †*Department of Animal Science, University of California, Davis, California 95616-8521*

Manuscript received February 14, 2008
Accepted for publication May 30, 2008

ABSTRACT

The mutational input of genetic variance per generation (σ^2_m) is the lower limit of the genetic variability in inbred strains of mice, although greater values could be expected due to the accumulation of new mutations in successive generations. A mixed-model analysis using Bayesian methods was applied to estimate σ^2_m and the across-generation accumulated genetic variability on litter size in 46 generations of a C57BL/6J inbred strain. This allowed for a separate inference on σ^2_m and on the additive genetic variance in the base population (σ^2_a). The additive genetic variance in the base generation was 0.151 and quickly decreased to almost null estimates in generation 10. On the other hand, σ^2_m was moderate (0.035) and the within-generation mutational variance increased up to generation 14, then oscillating between 0.102 and 0.234 in remaining generations. This pattern suggested the existence of a continuous uploading of genetic variability for litter size ($h^2 = 0.045$). Relevant genetic drift was not detected in this population. In conclusion, our approach allowed for separate estimation of σ^2_a and σ^2_m within the mixed-model framework, and the heritability obtained highlighted the significant and continuous influence of new genetic variability affecting the genetic stability of inbred strains.

THE importance of new mutations on polygenic variability has been suggested by several investigators in the last decades (Hill 1982a,b; Caballero *et al.* 1991; Keightley 1998). Direct evidence of new mutations with large effects in experimental selection lines was initially reported during the second half of the 20th century (Macarthur 1949; Yoo 1980; Bradford and Famula 1984). The mutational input of genetic variance per generation (σ^2_m) can be viewed as the ultimate source of polygenic variation and thus as the raw material for the maintenance of genetic variability in populations (Hill 1982a). Estimates of mutational heritabilities [$h^2_m = \sigma^2_m/(\sigma^2_m + \sigma^2_e)$, σ^2_e being the residual variance of the trait] in animals and cereal crops have ranged between 10^{-4} and 5×10^{-2} (Lynch 1988; Houle *et al.* 1996). Within this context, it is well known that spontaneous mutation continually contributes new alleles to the pool of genetic variation, allowing for response to long-term artificial selection experiments in both animals (Caballero *et al.* 1991; Keightley 1998) and plants (Hill 2007).

Most estimates of σ^2_m and h^2_m come from experiments focused on the rate of divergence between sublines from a highly inbred base population (Festing 1973; Mackay *et al.* 1994). These studies typically assumed a stringent scenario under mutation–drift equilibrium, which does not necessarily hold in experimental populations (Hill 1982a). Moreover, a long time is needed for generating the strains, and the analysis using the response to selection typically ignores information on covariances between relatives within lines, a proportion of which can be genetic (Keightley and Hill 1992). Alternatively, Wray (1990) developed a straightforward approach to account for mutation effects in mixed models, using the numerator relationship matrix, allowing for estimation of σ^2_m in unselected populations. This methodology has not been widely applied, although some σ^2_m estimates have been obtained in mice (Keightley and Hill 1992; Keightley 1998). A topic of interest in studies with laboratory mice is the genetic homogeneity of inbred strains across generations (Taft *et al.* 2006; Stevens *et al.* 2007) and Wray's (1990) approach seems to be the only available methodology for testing mutation effects, given that selection is avoided in these genetically controlled strains. Nevertheless, there are no published results on σ^2_m in highly inbred unselected mice, and the magnitude or impact of σ^2_m on the phenotypic variance remains unknown.

Taking the infinitesimal model (Fisher 1918) as the starting point, the additive genetic variance for a given phenotypic trait in a population characterizes the amount of genetic variability and the potential change due to natural or artificial selection, or genetic drift. Interestingly, analyses performed on the "new" genetic variance originated by mutation are commonly focused on the increment of genetic variation per generation (Caballero *et al.* 1991; Keightley 1998), but they do not estimate the accumulated genetic variability existing between

[1]*Corresponding author:* Department of Animal Science, University of California, One Shields Ave., Davis, CA 95616-8521.
E-mail: jfmedrano@ucdavis.edu

individuals. Even in highly inbred strains, the genetic variance in a given generation of interest could be viewed as the balance of an equilibrium between σ^2_m coming from current and previous generations and the loss of genetic variability due to selection, genetic drift, and/or inbreeding (Hill 1982a,b). Analyses of the mutation phenomenon in laboratory species have been mainly focused on σ^2_m (Festing 1973; Keightley and Hill 1992; Keightley 1998), whereas the magnitude of the overall within-generation genetic variability due to the accumulation of new mutations remains unclear.

In this study, we report estimates of mutation variance on litter size in C57BL/6J mice reared for 46 generations without selection. Wray's (1990) algorithm was modified to estimate the amount of genetic variance in the inbred base population and the increment of the per generation variance due to mutation. In addition, we applied the Bayesian approach proposed by Sorensen *et al.* (2001) to estimate the within-generation genetic variability, to examine if new mutation variance compensates for losses in genetic variability.

MATERIALS AND METHODS

Mice data source: *Mouse strain and breeding scheme:* A C57BL/6J inbred strain was kept in our vivarium at the University of California (Davis, CA) for 46 nonoverlapping generations (G_1–G_{46}), between October 1988 and May 2005. This strain was founded with two C57BL/6J males and six C57BL/6J females from The Jackson Laboratory (Bar Harbor, ME). Two to 5 generations per year were produced. Each generation involved between 2 and 28 males and between 6 and 49 females, producing an average of 21.6 litters (Table 1). In August 1995, a subline was derived from G_{21} and was maintained for five nonoverlapping generations (G_{22b}–G_{26b}), with a large number of litters per generation (Table 1). For each generation, males and females were selected at random from the offspring of a few litters of the previous generation, and full-sibs matings were favored. Only single (one male/one female) and group matings (one male/several females) were used to avoid multiple paternities. Each male and female produced an average of 3.5 and 1.8 litters, respectively, ranging from 1 to 16 litters for males and from 1 to 5 litters for females. Note that this strain was maintained to provide stock research mice in our colony and therefore a variable number of litters per generation were generated, depending on mice demand. All mice were fed with Purina 5008 diet (Ralston Purina, St. Louis; 23.5% protein, 6.5% fat, 3.3 kcal/g) and water was offered *ad libitum*. Mice were housed in polycarbonate cages under controlled conditions of temperature (21° ± 2°), humidity (40–70%), and lighting (14 hr light, 10 hr dark, lights on at 7 am) and managed according to the guidelines of the American Association for Accreditation of Laboratory Animal Care (AAALAC) (http://www.aaalac.org).

Data set and trait analyzed: Reproductive data were recorded accurately in all generations. Sire, dam, date of mating, date of birth, and number of pups at birth (alive and dead) were recorded for each litter, and pups were individually marked by ear notching at weaning (3 weeks after birth). Records were available on 1986 litters providing 15,044 pups. This study focused on litter size (LS), defined as the sum of live and dead pups at birth. Phenotypic records of LS ranged between 1 and 14 pups, with 7.58 pups per litter on average. The pedigree file included 572 males and 1116 females with a complete knowledge of all parental relationships.

TABLE 1

Number of males and females mated per generation (in parentheses, contributors to the next generation), number of litters, and average litter size per generation

Generation	Males	Females	Litters	Mean ± SE
G_1	2 (2)	6 (5)	19	9.42 ± 0.29
G_2	12 (4)	12 (4)	12	6.50 ± 0.48
G_3	5 (2)	5 (2)	5	8.40 ± 0.60
G_4	5 (1)	5 (1)	9	6.55 ± 0.60
G_5	4 (2)	5 (2)	7	8.42 ± 0.64
G_6	4 (4)	4 (4)	11	8.18 ± 0.81
G_7	7 (5)	7 (5)	8	7.12 ± 0.83
G_8	3 (3)	5 (4)	6	7.66 ± 0.55
G_9	5 (4)	7 (5)	9	7.88 ± 0.51
G_{10}	7 (6)	14 (8)	17	7.94 ± 0.34
G_{11}	7 (6)	9 (7)	9	7.55 ± 0.29
G_{12}	9 (6)	13 (7)	16	8.12 ± 0.44
G_{13}	10 (7)	14 (9)	17	6.64 ± 0.63
G_{14}	9 (6)	15 (9)	25	7.52 ± 0.44
G_{15}	9 (5)	19 (7)	19	7.73 ± 0.35
G_{16}	8 (3)	15 (6)	15	7.40 ± 0.48
G_{17}	9 (4)	18 (5)	24	7.29 ± 0.39
G_{18}	6 (3)	10 (3)	17	7.70 ± 0.45
G_{19}	4 (4)	8 (7)	18	7.16 ± 0.50
G_{20}	13 (11)	19 (15)	36	7.66 ± 0.31
G_{21}	28 (23)	49 (30)	79	7.86 ± 0.20
G_{22}	10 (5)	16 (5)	16	6.68 ± 0.45
G_{23}	7 (5)	9 (6)	14	6.78 ± 0.56
G_{24}	7 (2)	13 (2)	24	7.29 ± 0.48
G_{25}	4 (4)	5 (4)	5	6.40 ± 1.07
G_{26}	4 (4)	8 (6)	13	7.61 ± 0.52
G_{27}	10 (5)	18 (6)	18	7.72 ± 0.38
G_{28}	12 (3)	19 (4)	30	7.13 ± 0.40
G_{29}	11 (3)	19 (4)	24	7.33 ± 0.40
G_{30}	6 (3)	12 (4)	17	7.47 ± 0.52
G_{31}	8 (4)	11 (5)	14	5.78 ± 0.40
G_{32}	6 (4)	13 (5)	16	5.81 ± 0.48
G_{33}	6 (6)	10 (10)	16	6.75 ± 0.42
G_{34}	14 (6)	23 (6)	41	6.04 ± 0.44
G_{35}	7 (4)	12 (6)	27	5.92 ± 0.51
G_{36}	11 (6)	19 (8)	31	6.90 ± 0.49
G_{37}	6 (5)	14 (8)	32	6.53 ± 0.40
G_{38}	10 (5)	23 (9)	47	6.95 ± 0.34
G_{39}	12 (7)	27 (9)	35	6.82 ± 0.36
G_{40}	13 (8)	32 (9)	39	6.33 ± 0.36
G_{41}	10 (8)	20 (11)	20	7.10 ± 0.23
G_{42}	12 (4)	28 (6)	33	8.30 ± 0.33
G_{43}	7 (2)	18 (3)	43	8.55 ± 0.30
G_{44}	2 (1)	8 (2)	26	8.38 ± 0.45
G_{45}	4 (4)	11 (6)	18	8.61 ± 0.30
G_{46}	6	16	16	7.93 ± 0.29
G_{22b}	49 (16)	96 (31)	239	7.96 ± 0.13
G_{23b}	40 (24)	93 (40)	221	7.55 ± 0.14
G_{24b}	43 (22)	99 (37)	220	8.07 ± 0.14
G_{25b}	36 (17)	93 (27)	187	7.59 ± 0.17
G_{26b}	33	72	126	7.94 ± 0.20
Overall	572 (298)	1,116 (424)	1,986	7.58 ± 0.05

Bayesian analysis: *Model:* Litter size in the C57BL/J6 strain was analyzed with the following linear mixed model,

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_1\mathbf{p}_1 + \mathbf{Z}_2\mathbf{p}_2 + \mathbf{Z}_3\mathbf{a} + \mathbf{Z}_3\mathbf{m} + \mathbf{e},$$

where $\mathbf{y}$ was the vector of phenotypic data and $\mathbf{e}$ was the vector of residuals after accounting for systematic ($\boldsymbol{\beta}$), environmental ($\mathbf{p}_1$ and $\mathbf{p}_2$), and additive genetic effects ($\mathbf{a}$ and $\mathbf{m}$). Note that $\mathbf{X}$, $\mathbf{Z}_1$, $\mathbf{Z}_2$, and $\mathbf{Z}_3$ are appropriate incidence matrices. More specifically, $\boldsymbol{\beta}$ corrected for two systematic effects, parity number of the dam with the two levels proposed by Kirkpatrick *et al.* (1988; first parity and following parities), and generation number with 50 levels, accounting for environmental variability between generations (see Falconer 1960). Two environmental sources of variation common to all pups were fitted to the model, the effect of the sire ($\mathbf{p}_1$; Schilling *et al.* 1968) and the nongenetic effect of the dam ($\mathbf{p}_2$), with 572 and 1116 levels, respectively. Following in part Wray (1990), the infinitesimal genetic effect ($\mathbf{u}$; Fisher 1918) was partitioned into two terms, $\mathbf{u} = \mathbf{a} + \mathbf{m}$, the breeding value inherited from the genetic variability in the base generation ($\mathbf{a}$; Henderson 1973) and from the additional genetic variability originated by mutation ($\mathbf{m}$).

Prior distributions: Following a standard Bayesian development, the joint posterior distribution of the mixed model outlined above was constructed by multiplying the Bayesian likelihood with the prior distribution of all parameters in the model,

$$\begin{aligned} &p\left(\boldsymbol{\beta}, \mathbf{p}_1, \mathbf{p}_2, \mathbf{a}, \mathbf{m}, \sigma^2_{\mathrm{p}_1}, \sigma^2_{\mathrm{p}_2}, \sigma^2_{\mathrm{a}}, \sigma^2_{\mathrm{m}}, \sigma^2_{\mathrm{e}} \mid \mathbf{y}\right) \\ &\quad \propto p\left(\mathbf{y} \mid \boldsymbol{\beta}, \mathbf{p}_1, \mathbf{p}_2, \mathbf{a}, \mathbf{m}, \sigma^2_{\mathrm{e}}\right) p(\boldsymbol{\beta}) p\left(\mathbf{p}_1 \mid \sigma^2_{\mathrm{p}_1}\right) p(\sigma^2_{\mathrm{p}_1}) p\left(\mathbf{p}_2 \mid \sigma^2_{\mathrm{p}_2}\right) \\ &\quad \times\ p(\sigma^2_{\mathrm{p}_2}) p\left(\mathbf{a} \mid \mathbf{A}, \sigma^2_{\mathrm{a}}\right) p(\sigma^2_{\mathrm{a}}) p\left(\mathbf{m} \mid \mathbf{M}, \sigma^2_{\mathrm{m}}\right) p(\sigma^2_{\mathrm{m}}) p(\sigma^2_{\mathrm{e}}), \end{aligned}$$

where $\sigma^2_{\mathrm{p}_1}$, $\sigma^2_{\mathrm{p}_2}$, σ^2_{a}, σ^2_{m}, and σ^2_{e} were the appropriate variance components for $\mathbf{p}_1$, $\mathbf{p}_2$, $\mathbf{a}$, $\mathbf{m}$, and $\mathbf{e}$, respectively, $\mathbf{A}$ was the standard numerator relationship matrix (Wright 1922), and $\mathbf{M}$ was Wray's (1990) numerator relationship matrix adapted to accommodate the occurrence of mutations in the genome. Note that $\mathbf{M}$ was defined as $\sum_{k=0}^{t} \mathbf{A}_k$, where t is the number of generations, $\mathbf{A}_k$ is the numerator relationship matrix of additive genetic effects attributed to mutations arising in time unit k, and $\mathbf{A}_0 = \mathbf{A}$ (see the appendix). The elements of $\mathbf{A}_k$ are the additive genetic relationships if ancestors born in time unit $k - 1$ are ignored (Wray 1990).

Litter size data were assumed to be normally distributed as follows,

$$\begin{aligned} &p\left(\mathbf{y} \mid \boldsymbol{\beta}, \mathbf{p}_1, \mathbf{p}_2, \mathbf{a}, \mathbf{m}, \sigma^2_{\mathrm{e}}\right) \\ &\quad \sim\ N(\mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_1\mathbf{p}_1 + \mathbf{Z}_2\mathbf{p}_2 + \mathbf{Z}_3\mathbf{a} + \mathbf{Z}_3\mathbf{m}, \mathbf{I}_{\mathrm{e}}\sigma^2_{\mathrm{e}}), \end{aligned}$$

with $\mathbf{I}_{\mathrm{e}}$ being an identity matrix with dimensions equal to the number of records in $\mathbf{y}$. Model parameters $\boldsymbol{\beta}$, $\mathbf{p}_1$, $\mathbf{p}_2$, $\mathbf{a}$, $\mathbf{m}$, and σ^2_{e} were assumed mutually independent. *A priori* distributions for $\mathbf{p}_1$ and $\mathbf{p}_2$ were defined as multivariate normal,

$$\begin{aligned} p\left(\mathbf{p}_1 \mid \sigma^2_{\mathrm{p}_1}\right) &\sim N(0, \mathbf{I}_{\mathrm{p}_1}\sigma^2_{\mathrm{p}_1}) \\ p\left(\mathbf{p}_2 \mid \sigma^2_{\mathrm{p}_2}\right) &\sim N(0, \mathbf{I}_{\mathrm{p}_2}\sigma^2_{\mathrm{p}_2}), \end{aligned}$$

where $\mathbf{I}_{\mathrm{p}_1}$ and $\mathbf{I}_{\mathrm{p}_2}$ were identity matrices with dimensions equal to the number of elements in $\mathbf{p}_1$ and $\mathbf{p}_2$, respectively. Invoking the infinitesimal model (Fisher 1918), $\mathbf{a}$ and $\mathbf{m}$ were assumed to follow the multivariate normal distributions

$$\begin{aligned} p\left(\mathbf{a} \mid \sigma^2_{\mathrm{a}}\right) &\sim N(0, \mathbf{A}\sigma^2_{\mathrm{a}}) \\ p\left(\mathbf{m} \mid \sigma^2_{\mathrm{m}}\right) &\sim N(0, \mathbf{M}\sigma^2_{\mathrm{m}}). \end{aligned}$$

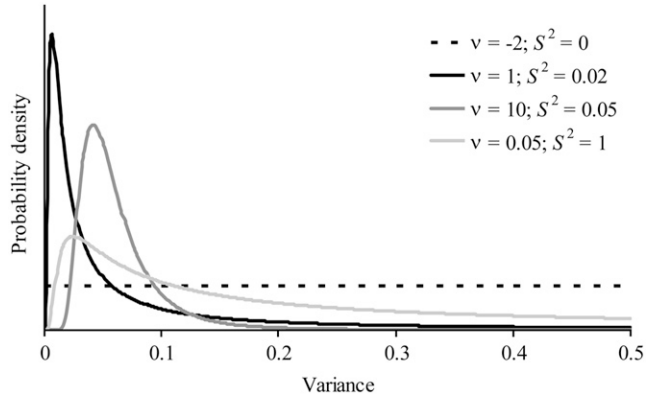

Figure 1.—*A priori* distributions for σ^2_{a} and σ^2_{m}.

Note that mutational effects are assumed *a priori* independent of $\mathbf{a}$ (Wray 1990) and therefore genetic correlation between $\mathbf{a}$ and $\mathbf{m}$ was arbitrarily fixed to 0. Improper uniform prior distributions were assumed for $\boldsymbol{\beta}$, $\sigma^2_{\mathrm{p}_1}$, $\sigma^2_{\mathrm{p}_2}$, and σ^2_{e} to approximate vague prior knowledge about systematic, environmental, and residual sources of variation.

According to Henderson (1973), Gianola *et al.* (1989), and Im *et al.* (1989), σ^2_{a} measures additive genetic variance at linkage equilibrium in the base population (G_1; see Table 1). Although σ^2_{a} should be null or very small in an inbred strain, if it exists, σ^2_{a} must originate from short-term mutations arising in previous generations and is highly related to σ^2_{m}. It seems reasonable to expect a similar behavior for σ^2_{a} and σ^2_{m} and therefore the same prior was assumed for both variance components. To evaluate the effects of *a priori* information on σ^2_{a} and σ^2_{m}, four different scaled inverted χ^2-prior distributions with hyperparameters ν and S^2 were assumed and tested independently on our data set. The first prior (PR1) generalized the scaled χ^{-2}-distribution to an improper uniform distribution by setting $\nu = -2$ and $S^2 = 0$ (Figure 1). This prior ignores previous knowledge on σ^2_{a} and σ^2_{m}, this being a typical assumption for variance components, where the variance is allowed to take any value between 0 and the phenotypic variance. Three more priors (Table 2) were defined on the basis of information from the literature and they varied on a trial and error basis until the desired shape of the distribution was obtained (Figure 1), following in part Blasco *et al.* (1998). Given the range of mutational heritabilities reviewed by Lynch (1988; 10^{-4}–5×10^{-2}) and the moderate phenotypic variance observed in our data set (4.1 pups^2), it seems reasonable to expect a σ^2_{m} between 4.1×10^{-4} and 2.1×10^{-1}, without disallowing for more extreme values. The second χ^{-2}-prior (PR2) illustrated a strong *a priori* opinion (sharp-contour distribution) about the probable distribution of the variance components, its mode being placed at the lower bound of Lynch's (1988) range. Prior 3 (PR3) was an attempt to cover the range of most plausible values, although a left-skewed prior (PR4) gave a vague *a priori* opinion of the distribution (Figure 1) of the variance components, its mode being 2.0×10^{-1}. Note that PR2 and PR4 were proper priors although they did not have a well-defined variance. See Table 2 for a detailed description of hyperparameters for all the scaled inverted χ^2-priors.

Additionally, a mixed model without $\mathbf{a}$ and $\mathbf{m}$ effects was analyzed (PR0), with its Bayesian likelihood reduced to $p(\mathbf{y} \mid \boldsymbol{\beta}, \mathbf{p}_1, \mathbf{p}_2, \sigma^2_{\mathrm{e}}) \sim N(\mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_1\mathbf{p}_1 + \mathbf{Z}_2\mathbf{p}_2, \mathbf{I}_{\mathrm{e}}\sigma^2_{\mathrm{e}})$ and the prior distributions for $\boldsymbol{\beta}$, $\mathbf{p}_1$, $\mathbf{p}_2$, $\sigma^2_{\mathrm{p}_1}$, $\sigma^2_{\mathrm{p}_2}$, and σ^2_{e} were the same as in the full model. It can be also viewed as the general model with $p(\sigma^2_{\mathrm{a}} = 0) = 1$ and $p(\sigma^2_{\mathrm{m}} = 0) = 1$, thereby allowing for testing

TABLE 2

χ^{-2} hyperparameter specifications and deviance information criterion (DIC) estimates

	Model (depending on priors for σ^2_a and σ^2_m)				
	PR0	PR1	PR2	PR3	PR4
χ^{-2} hyperparameters					
ν		−2	1	10	0.05
S^2		0	0.02	0.05	1
DIC					
Chain 1	3981.92	3976.65	3977.76	3975.51	3976.62
Chain 2	3981.98	3976.86	3977.74	3975.52	3976.72
Chain 3	3981.83	3976.86	3977.74	3975.47	3976.78
Mean	3981.91	3976.79	3977.75	3975.50	3976.71
SD	0.08	0.12	0.01	0.03	0.08

of the biological relevance of **a** and **m** effects in terms of model adequacy.

Markov chain Monte Carlo sampling: Within a Bayesian context, inferences are made on the joint posterior distribution or, for a given parameter of interest, on the relevant marginal posterior distribution. Given the multidimensional form of these posterior distributions, direct integration cannot be applied. Markov chain Monte Carlo (MCMC) techniques easily bypass this limitation and allow us to obtain draws from the appropriate marginal posterior distribution. For the mixed model described above, samples from the marginal posterior distribution of all unknowns in the model were obtained by Gibbs sampling (Gilks *et al.* 1996), following the procedures described by Sorensen *et al.* (1994).

For each prior distribution of σ^2_a and σ^2_m (PR1–PR4), as well as for PR0, three independent MCMC chains were launched, with 500,000 iterations after discarding the first 100,000 as burn-in. Convergence was confirmed on variance components by visual inspection and by Raftery and Lewis's (1992) approach. To arrive at the most preferable model, the deviance information criterion (DIC) (Spiegelhalter *et al.* 2002) was calculated.

Genetic drift and within-generation additive genetic variances: As mentioned above, breeding mice were randomly picked from the previous generation and no selection was applied along the 46 generations. Nevertheless, the small number of mice contributing to the next generation (see Table 1) could produce genetic drift on litter size if σ^2_a and/or σ^2_m were not null. Within this context, changes on the within-generation average breeding value (**a** and **m**) and environmental effects ($\mathbf{p}_1$ and $\mathbf{p}_2$) were evaluated with the Bayesian approach described by Sorensen *et al.* (1994).

Following Sorensen *et al.* (2001), both additive genetic and mutation variance components were estimated within generations, using data from all individuals in the population. By definition, the additive genetic value $a_{i(t)}$ of an individual randomly picked from generation t is a random variable with variance as defined by Sorensen *et al.* (2001),

$$\sigma^2_{a(t)} = E(a_t^2) - [E(a_t)]^2 = \frac{1}{n_t}\sum_{i=1}^{n_t} a^2_{i(t)} - (\bar{a}_{(t)})^2,$$

where $\bar{a}_{(t)}$ is the mathematical expectation of additive genetic values in generation t, $a_{i(t)}$ is the ith additive genetic value in generation t, and n_t is the number of individuals in generation t. Inferences on the within-generation additive genetic variance were made on their marginal posterior distribution, estimated via Markov chain Monte Carlo methods. A Gibbs sampler was applied following Sorensen *et al.* (2001). The same approach was applied for the within-generation mutation variance.

RESULTS

Litter size in this C57BL/6J strain averaged 7.58 ± 0.05 pups per litter with substantial variability between generations, from 9.42 ± 0.29 pups (G_1) to 5.78 ± 0.40 pups (G_{31}). Larger litter sizes were observed in earlier (G_1, G_3, and G_5) and later generations (G_{42}–G_{45}) with smaller values in the intermediate ones, although a relevant phenotypic trend was not observed (Table 1).

Table 2 shows the hyperparameters of the scaled χ^{-2}-priors (ν and S^2) tested for σ^2_a and σ^2_m. The values chosen for these hyperparameters generated a wide range of shapes for this distribution that reflected different *a priori* knowledge on the expected values of both variance components. Model fit and complexity were evaluated with the DIC (Spiegelhalter *et al.* 2002), and PR3 was favored (DIC = 3975.50) although the difference was slight with respect to PR1 and PR4 (DIC = 3976.79 and 3976.71, respectively; Table 2). PR2 was penalized with a 2-units greater DIC than PR3, and the model without genetic effects (PR0) was discarded (DIC = 3981.91). Note that differences in DIC > 3 are generally considered as statistically relevant (Burnham and Anderson 1998; Spiegelhalter *et al.* 2002), whereas lower discrepancies do not provide a strong evidence of a better fit and a lower degree of model complexity for a given comparison. It is important to highlight that three different MCMC chains were launched for each model and DIC showed a very small variance within models (Table 2).

As shown in Table 3, PR1, PR3, and PR4 models provided very similar estimates of variance components and their ratios, whereas PR2 had a lower value of σ^2_a. Taking the PR3 model as reference and after correcting for systematic effects (generation and parturition number of the female), the most important source of variation was that due to the uncontrolled factors that accounted for σ^2_e, its mode being 3.842 pups^2 (Table 3).

TABLE 3

Modal estimates (and highest posterior density region at 95%) for the variance components and heritabilities

	Model (depending on priors for σ^2_a and σ^2_m)				
	PR0	PR1	PR2	PR3	PR4
σ^2_a		0.155 (0.067–0.260)	0.020 (0.000–0.103)	0.151 (0.066–0.254)	0.158 (0.070–0.273)
σ^2_m		0.033 (0.015–0.048)	0.025 (0.009–0.043)	0.035 (0.017–0.049)	0.035 (0.019–0.050)
$\sigma^2_{p_1}$	0.112 (0.011–0.683)	0.105 (0.006–0.650)	0.099 (0.005–0.630)	0.092 (0.003–0.604)	0.099 (0.005–0.634)
$\sigma^2_{p_2}$	0.051 (0.009–0.612)	0.038 (0.006–0.555)	0.040 (0.008–0.602)	0.037 (0.002–0.596)	0.037 (0.003–0.606)
σ^2_e	3.901 (3.332–4.054)	3.887 (3.326–4.003)	3.965 (3.376–4.101)	3.842 (3.245–3.991)	3.880 (3.322–3.995)
h^2_m		0.008 (0.003–0.012)	0.006 (0.002–0.009)	0.008 (0.004–0.012)	0.008 (0.004–0.011)
$h^2_{G_1}$		0.045 (0.011–0.064)	0.011 (0.002–0.046)	0.045 (0.010–0.062)	0.046 (0.013–0.068)

$h^2_m = \sigma^2_m/(\sigma^2_a + \sigma^2_m + \sigma^2_{p_1} + \sigma^2_{p_2} + \sigma^2_e)$; $h^2_{G_1} = (\sigma^2_a + \sigma^2_m)/(\sigma^2_a + \sigma^2_m + \sigma^2_{p_1} + \sigma^2_{p_2} + \sigma^2_e)$.

Nevertheless, genetic variances σ^2_a and σ^2_m were high for an inbred strain, 0.151 and 0.035, respectively. It is important to note that the highest posterior density region at 95% (HPD95) for both variance components was away from zero, starting at 0.066 and 0.017, respectively (Table 3). Mutational heritability was 0.008, and the overall heritability at generation G_1 ($h^2_{G_1} = (\sigma^2_a + \sigma^2_m)/(\sigma^2_a + \sigma^2_m + \sigma^2_{p_1} + \sigma^2_{p_2} + \sigma^2_e)$) was 0.045 (HPD95 between 0.010 and 0.062), showing that enough additive genetic variance was available to develop a genetic trend under selection or drift. In a similar way, sire and (nongenetic) dam effects had moderate variance components (0.092 and 0.037, respectively), although with wide HPD95 (Table 3).

The C57BL/6J strain did not show notable genetic drift during 46 generations. The within-generation average breeding value ($\mathbf{a} + \mathbf{m}$) ranged between 0 (G_1, G_2, and G_{22}) and 0.02 pups (G_{44}), with all the HPD95 including the null value (Figure 2). Similarly, within-generation average values for environmental effect ($\mathbf{p}_1$ and $\mathbf{p}_2$) did not differ from zero. The between-generations phenotypic variability was mainly accounted for by the generation number effects (results not shown). Model fit was worse when this systematic effect was dropped out (DIC = 4018.89).

The within-generation $\sigma^2_{a(t)}$ quickly decreased (Figure 3) whereas the new genetic variance originated by mutation increased up to ~0.20 and oscillated around 0.15 thereafter (Figure 4). More specifically, $\sigma^2_{a(t)}$ started with a modal estimate of 0.159 in generation G_1 (HPD95 between 0.056 and 0.245) and fell to values <0.01 in <10 generations. Mutational variance accumulated during the first generations and reached its maximum at generation G_{14} (0.231), although it showed an oscillating pattern around 0.15 from generation G_7 (HPD95 reached values up to 0.400).

DISCUSSION

Prior distributions and Bayesian analysis: The mixed-model analysis of litter size in C57BL/6J mice was carried out using Bayesian methods. An important characteristic of the Bayesian analysis is that the final inference is based on the posterior distribution, resulting from combining two different sources of information. One of these sources is the experimental data, which are not influenced by arbitrary choices other than the model used for analysis. The other source of information is the assignment of prior distributions, which are arbitrarily chosen from previous knowledge of the parameters of

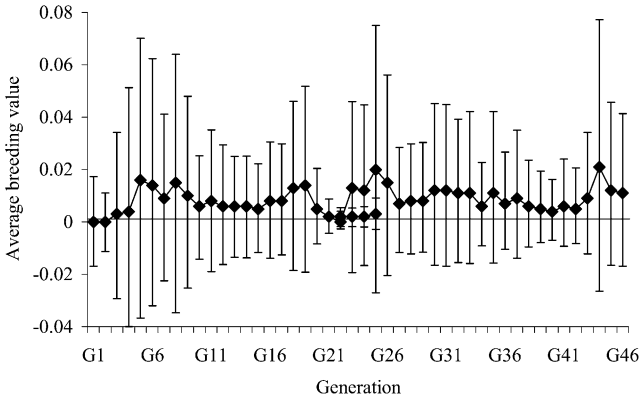

FIGURE 2.—Mode (solid diamonds) and highest posterior density region at 95% (whiskers) of the average breeding value per generation.

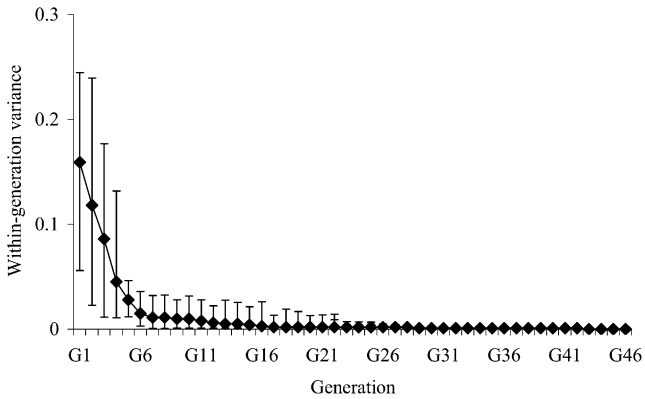

FIGURE 3.—Mode (solid diamonds) and highest posterior density region (whiskers) of the within-generation additive genetic variance ($\sigma^2_{a(t)}$).

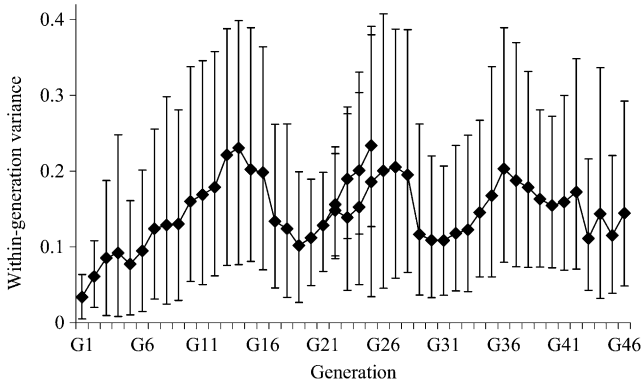

FIGURE 4.—Mode (solid diamonds) and highest posterior density region (whiskers) of the within-generation mutational variance ($\sigma^2_{m(t)}$).

interest. If previous information is not available, prior distributions become a blind choice and they could have a substantial impact on the posterior inference (GIANOLA and FERNANDO 1986; BLASCO 2001). Genetic components of litter size in highly inbred mice have not been previously analyzed and we lacked accurate information on the expected values of σ^2_a and σ^2_m. To assess influences of priors for both variance components, the analyses performed here made use of very different prior distributions for σ^2_a and σ^2_m, covering the range of mutational heritabilities reviewed by LYNCH (1988) and HOULE *et al.* (1996) in other traits and species. Model PR0, the one without genetic components, showed the poorest model fit and DIC substantially decreased when σ^2_a and σ^2_m were included in the model. This provided statistical evidence of the presence of additive genetic variance in this inbred strain. Models PR1–PR4 showed a similar fit, although the stringent prior for σ^2_a and σ^2_m in model PR2 was moderately penalized. It is important to note that posterior inferences from models PR1, PR3, and PR4 did not differ substantially (Table 3). This reassuring conclusion indicated that the experimental data had enough information content to override moderate influences of prior information, and the model performed better under a vague assumption for σ^2_a and σ^2_m over the parameter space.

Note that this analysis could also be performed under a frequentist approach by maximizing the likelihood function through iterative algorithms. These frequentist methods produce inferences based on the data and the previous knowledge of the distribution of estimators in the sampling space, without using prior information. As highlighted by BLASCO (2001), the distribution of the estimator is used for inferences instead of the distribution of the parameter, which leads to a rather unnatural form of expressing uncertainly about the results of an experiment. Within the Bayesian context, conceptual simplicity is gained because inferences are made from probabilities associated with values of the parameter of interest.

Genetic variability: Reported estimates of mutational heritability found in the literature commonly range between 10^{-4} and 5×10^{-2} (LYNCH 1988; HOULE *et al.* 1996), although this parameter has never been estimated for litter size in mice. Our estimate fell within this interval (0.008; Table 3) and was very close to the values reported by KEIGHTLEY and HILL (1992) and KEIGHTLEY (1998) for body size in mice. Although WRAY's (1990) approach assumes that mutations are small and additive, the inclusion of sire and dam environmental effects accounted for deviations from the infinitesimal model, allowing for a more accurate estimation of σ^2_m. Within this context, part of the variability that accounted for $\sigma^2_{p_1}$ and $\sigma^2_{p_2}$ could have originated from large mutations. Thus, σ^2_m likely underestimates all the genetic variation originated by mutation. Moreover, environmental variance could also account for nonadditive genetic mutations (ZHANG *et al.* 2004). Although $\sigma^2_{p_1}$ and $\sigma^2_{p_2}$ modal estimates suggested a greater impact of $\sigma^2_{p_1}$ on litter size, HPD95 were completely overlapping. It is important to note that **a** and **m** accounted for additive genetic effects whereas nonadditive genetic sources of variation such as inbreeding or heterosis could have a substantial impact on mouse litter size (BHUVANAKUMAR *et al.* 1985; HINRICHS *et al.* 2007). Given the difficulty in accommodating inbreeding and heterosis on the new mutational variability originated at each generation, as well as other nonadditive genetic effects (*i.e.*, epistasis or dominance), we restricted the model to pure additive genetic effects and assumed that the remaining nonadditive genetic influences were accounted for by $\mathbf{p}_1$ and $\mathbf{p}_2$ or were absorbed by the residual term.

Mutation variance in the mixed model was modeled as the dispersion term associated with random mutation effects arising in each offspring (WRAY 1990). This parameterization has been typically used in mutation experiments (LYNCH 1988; HOULE *et al.* 1996) and describes the potential effect of mutations in a very short time interval. Nevertheless, new mutations accumulate in successive generations, sometimes fixed or removed due to selection or genetic drift, and σ^2_m must be viewed in highly inbred strains as a lower limit of the accumulated (mutation) genetic variance. As shown in Figure 4, the within-generation mutation variance increased with generation, up to G_{14}. This is in agreement with a continuous input of new mutations with effects on litter size. The oscillating pattern around 0.15 after G_{14} agrees with $\sigma^2_{a(1)}$ at G_1 and suggests an equilibrium between new mutations and the fixation or loss of mutations due to genetic drift and inbred matings. In a similar way, σ^2_a lacks additional sources of new variation and its within-generation estimates have a rapid decrease after a few generations, which is related to the small population size and the mating system. Both within-generation σ^2_a and σ^2_m estimates support the conclusion that this C57BL/6J strain maintained a substantial degree of genetic variance across generations, accounting for an overall heritability around 0.05 (Table 3). This value is clearly lower than the

ones reported in outbred mouse populations (0.15–0.33; Falconer 1960; Joakimsen and Baker 1977; Long *et al.* 1991) although it indicates a substantial and generally unaccounted for degree of genetic variability in an inbred strain.

Absence of environmental and genetic trend across generations: Mice bred under inbred mating systems for prolonged periods should fix the vast majority (potentially all) of the genetic contribution to variation (Bailey 1982) and typically, individual mice within an inbred strain are considered genetically identical. Nevertheless, unexpected genetic variability has been observed in highly inbred mouse strains (Keightley and Hill 1992), even allowing for genetic drift (Bailey 1977) and genetic trend on phenotypic traits (Festing 1973; Keightley 1998). Indeed, incongruities between genetic homogeneity and phenotypic variability were first recognized >40 years ago (Wolff 1961) and this issue is an area of concern in laboratory species. The C57BL/6J strain was developed in the early 20th century from a very small founder population and it is considered a classical inbred strain with an almost homozygous genome (Wade and Daly 2005). As reported in other inbred mouse strains (Falconer 1960), our strain showed substantial phenotypic variation for average litter size across generations, average litter size being similar to the estimates reported by other authors (Kirkpatrick *et al.* 1998; Corva *et al.* 2004). Note that this population was not under selection for litter size.

Besides the continuous generation of additional genetic variability in our C57BL/6J population, genetic drift did not take place (Figure 2) and changes in the (within-generation) average environmental effect were also negligible. As mentioned above, our mixed-model analysis included generation number as a systematic effect and it accounted for the major differences between generations in litter size. Note that model fit was worse when this effect was dropped out (DIC = 4018.89). Theoretically, this effect must be viewed as the generation-specific contribution of multiple environmental sources of variation (*e.g.*, food, housing, and management among others) although some genetic contributions could be involved too. Additive genetic variability is accounted for by **a** and **m**, and residual effects can absorb (individual-specific) nonadditive genetic influences. Nevertheless, a small number of breeding individuals contribute to the next generation and commonly they are closely related (full sibs in the majority of cases). Nonadditive genetic effects from a given ancestor can be widely spread in the following generation, reducing between-individuals variability and therefore being partially accounted for by the within-generation overall mean (generation number effect). Within this context, genetic drift cannot be completely discarded although, if present, it would be due to nonadditive mutations.

In conclusion, we present a new approach to Wray's (1990) method for modeling σ^2_a and σ^2_m within a Bayesian framework, where all parameters in the mixed model can be inferred using the Gibbs sampling algorithm. The analysis of litter size in the C57BL/6J strain indicated a low mutational input of genetic variance per generation ($\sigma^2_m = 0.035$), although the accumulation of new mutations in successive generations led to a substantial amount of additive genetic variability ($h^2 = 0.045$). While genetic uniformity of highly inbred strains is a key point in several research areas (Stevens *et al.* 2007), our estimates do not support this assumption and confirm a continuous and unavoidable flow of new genetic variability. These results contribute to the understanding of mutation–drift equilibrium in experimental populations.

We are appreciative of the excellent efforts of Vince De Vera in mouse husbandry and phenotypic data collection. The useful comments of D. Gianola and an anonymous referee are greatly appreciated. This research was supported by the National Research Initiative grant no. 2005-35205-15453 from the U.S. Department of Agriculture Cooperative State Research, Education, and Extension Service and by the California Agricultural Experimental Station. The research contract of J. Casellas was partially financed by Spain's Ministerio de Educación y Ciencia (program Juan de la Cierva).

LITERATURE CITED

Bailey, D. W., 1977 Sources of subline divergence and their relative importance for sublines of six major inbred strains of mice, pp. 197–215 in *Origins of Inbred Mice*, edited by H. C. Morse. Academic Press, New York.

Bailey, D. W., 1982 How pure are inbred strains of mice? Immunol. Today **3:** 210–214.

Bhuvanakumar, C. K., R. C. Roberts and W. G. Hill, 1985 Heterosis among lines of mice selected for body weight. Theor. Appl. Genet. **71:** 52–56.

Blasco, A., 2001 The Bayesian controversy in animal breeding. J. Anim. Sci. **79:** 2023–2046.

Blasco, A., D. Sorensen and J. P. Bidanel, 1998 Bayesian inference of genetic parameters and selection response for litter size components in pigs. Genetics **149:** 301–306.

Bradford, G. E., and T. R. Famula, 1984 Evidence for a major gene for rapid postweaning growth in mice. Genet. Res. **44:** 293–298.

Burnham, K. P., and D. R. Anderson, 1998 *Model Selection and Inference. A Practical Information Theoretic Approach.* Springer-Verlag, New York.

Caballero, A., M. A. Toro and C. López-Fanjul, 1991 The response to artificial selection from new mutations in *Drosophila melanogaster.* Genetics **127:** 89–102.

Corva, P. M., N. C. Mucci, K. Evans and J. F. Medrano, 2004 Diet effects on female reproduction in high growth (*hg/hg*) mice that are deficient in the *Socs-2* gene. Reprod. Nutr. Dev. **44:** 303–312.

Falconer, D. S., 1960 The genetics of litter size in mice. J. Cell. Comp. Physiol. **56**(Suppl. 1): 153–167.

Festing, M., 1973 A multivariate analysis of subline divergence in the shape of the mandible in C57BL/Gr mice. Genet. Res. **21:** 121–132.

Fisher, R. A., 1918 The correlation between relatives under the supposition of Mendelian inheritance. Trans. R. Soc. Edinb. **52:** 399–433.

Gianola, D., and R. L. Fernando, 1986 Bayesian methods in animal breeding theory. J. Anim. Sci. **63:** 217–244.

Gianola, D., R. L. Fernando, S. Im and J. L. Foulley, 1989 Likelihood estimation of quantitative genetic parameters when selection occurs: models and problems. Genome **31:** 768–777.

Gilks, W. R., S. Richardson and D. J. Spiegelhalter, 1996 Introducing Markov chain Monte Carlo, pp. 1–19 in *Markov Chain Monte Carlo in Practice*, edited by W. R. Gilks, S. Richardson and D. J. Spiegelhalter. Chapman & Hall, London.

Henderson, C. R., 1973 Sire evaluations and genetic trends, pp. 10–41 in *Proceedings of the Animal Breeding and Genetics Symposium in Honor of Dr. Jay L. Lush*. American Society of Animal Science and American Dairy Science Association, Champaign, IL.

Hill, W. G., 1982a Rates of change in quantitative traits from fixation of new mutations. Proc. Natl. Acad. Sci. USA **79:** 142–145.

Hill, W. G., 1982b Predictions of response to artificial selection from new mutations. Genet. Res. **40:** 255–278.

Hill, W. G., 2007 A century of corn selection. Science **307:** 683–684.

Hinrichs, D., T. H. E. Meuwissen, J. Ødegard, M. Holt, O. Vangen *et al.*, 2007 Analysis of inbreeding depression in the first litter size of mice in a long-term selection experiment with respect to the age of the inbreeding. Heredity **99:** 81–88.

Houle, D., B. Morikawa and M. Lynch, 1996 Comparing mutational variabilities. Genetics **143:** 1467–1483.

Im, S., R. L. Fernando and D. Gianola, 1989 Likelihood inferences from animal breeding data subject to selection. Genet. Sel. Evol. **21:** 399–414.

Joakimsen, O., and R. L. Baker, 1977 Selection for litter size in mice. Acta Agric. Scand. **27:** 301–318.

Keightley, P. D., 1998 Genetic basis of response to 50 generations of selection on body weight in inbred mice. Genetics **148:** 1931–1939.

Keightley, P. D., and W. G. Hill, 1992 Quantitative genetic variation in body size of mice from new mutations. Genetics **131:** 693–700.

Kirkpatrick, B. W., J. A. Arias and J. J. Rutledge, 1988 Effects of prenatal and postnatal fraternity size on long-term reproduction in mice. J. Anim. Sci. **66:** 62–69.

Kirkpatrick, B. W., A. Mengelt, N. Schulman and I. C. A. Martin, 1998 Identification of quantitative trait loci for prolificacy and growth in mice. Mamm. Genome **9:** 97–102.

Long, C. R., W. R. Lamberson and R. O. Bates, 1991 Genetic correlations among reproductive traits and uterine dimensions in mice. J. Anim. Sci. **69:** 99–103.

Lynch, M., 1988 The rate of polygenic mutation. Genet. Res. **51:** 137–148.

Macarthur, J. W., 1949 Selection for small and large mice in the house mouse. Genetics **34:** 194–209.

Mackay, T. F. C., J. D. Fry, R. F. Lyman and S. V. Nuzhdin, 1994 Polygenic mutation in *Drosophila melanogaster*: estimates from response to selection of inbred strains. Genetics **136:** 937–951.

Quaas, R. L., 1976 Computing the diagonal elements and inverse of a large numerator relationship matrix. Biometrics **32:** 949–953.

Raftery, A. E., and S. M. Lewis, 1992 How many iterations in the Gibbs sampler?, pp. 763–773 in *Bayesian Statistics IV*, edited by J. M. Bernardo, J. O. Berger, A. P. Dawid and A. F. M. Smith. Oxford University Press, Oxford.

Schilling, P., W. North and R. Bogart, 1968 The effect of sire on litter size in mice. J. Hered. **59:** 351–352.

Sorensen, D., R. Fernando and D. Gianola, 2001 Inferring the trajectory of genetic variance in the course of artificial selection. Genet. Res. Camb. **77:** 83–94.

Sorensen, D. A., C. S. Wang, J. Jensen and D. Gianola, 1994 Bayesian analysis of genetic change due to selection using Gibbs sampling. Genet. Sel. Evol. **26:** 333–360.

Spiegelhalter, D. J., N. G. Best, B. P. Carlin and A. van der Linde, 2002 Bayesian measures of model complexity and fit. J. R. Stat. Soc. B **64:** 583–639.

Stevens, J. C., G. T. Banks, M. F. W. Festing and E. M. C. Fisher, 2007 Quiet mutations in inbred strains of mice. Trends Mol. Med. **13:** 512–519.

Taft, R. A., M. Davisson and M. V. Wiles, 2006 Know thy mouse. Trends Genet. **22:** 649–653.

Wade, C. M., and M. J. Daly, 2005 Genetic variation in laboratory mice. Nat. Genet. **37:** 1175–1180.

Wolff, G. L., 1961 Some genetic aspects of physiological variability. Cancer Res. **21:** 1119–1123.

Wray, N. R., 1990 Accounting for mutation effects in the additive genetic variance-covariance matrix and its inverse. Biometrics **46:** 177–186.

Wright, S, 1922 Coefficients of inbreeding and relationship. Am. Nat. **56:** 330–338.

Yoo, B. H., 1980 Long-term selection for a quantitative character in large replicate populations of *Drosophila melanogaster*. II. Lethal and visible mutants with large effects. Genet. Res. **35:** 19–31.

Zhang, X., J. Wang and W. G. Hill, 2004 Influence of dominance, leptokurtosis and pleitotropy of deleterious mutations on quantitative genetic variation at mutation-selection balance. Genetics **166:** 597–610.

Communicating editor: R. W. Doerge

APPENDIX

Following in part Wray (1990), the additive genetic (co)variance matrix including mutation effects can be partitioned as

$$\mathbf{A}_0\sigma^2_{\mathrm{a}} + \mathbf{M}\sigma^2_{\mathrm{m}} = \mathbf{A}_0\sigma^2_{\mathrm{a}} + \sum_{k=0}^{t}\mathbf{A}_k\sigma^2_{\mathrm{m}},$$

where t is the number of generations, $\mathbf{A}_k$ is the full relationship matrix of additive genetic effects attributed to mutations arising in time unit (or generation) k, and $\mathbf{A}_0$ is the full relationship matrix including all individuals in the pedigree. For the mixed-model equations, both $\mathbf{A}_0^{-1}$ and $\mathbf{M}^{-1}$ are required, computational efficiency becoming a key point. As developed by Quaas (1976), $\mathbf{A}_0^{-1}$ can be recursively computed from a list of individual sire and dam identifications ordered by age of individuals. Following in part Wray (1990), $\mathbf{M}^{-1}$ can also be computed from an age-ordered pedigree with n individuals and three vectors, $\mathbf{u}$, $\mathbf{v}$, and $\mathbf{h}$, all with dimension $n \times 1$. Computation efficiency is gained with n rounds, with the following calculations in the ith round,

$$v_i = \begin{cases} \sqrt{\frac{u_p + u_q}{4} - \frac{h_p + h_q}{2} + 1} & \text{both parents of } i \text{ are known } (p \text{ and } q) \\ \sqrt{\frac{u_q}{4} - \frac{h_q}{2} + \frac{1}{2}} & \text{only one parent of } i \text{ is known } (q) \\ 1 & \text{neither parent of } i \text{ is known,} \end{cases}$$

where v_l, u_l, and h_l are the lth elements in vectors $\mathbf{v}$, $\mathbf{u}$, and $\mathbf{h}$, respectively. For $j = i + 1, \ldots, n$,

$$v_j = \begin{cases} \frac{v_{p_i} + v_{q_i}}{2} & \text{if } i \le p_j < q_j \\ \frac{v_{p_i}}{2} & \text{if } p_j < i \le q_j \\ 0 & \text{if } p_j \le q_j < i, \end{cases}$$

where p_j and q_j are parents of the jth individual and $p_j < q_j$. For $j = i + 1, \ldots, n$,

$$h_j = \begin{cases} h_j + \frac{v_{p_j} v_{q_j}}{2} & \text{if } i \le p_j < q_j \\ h_j & \text{if } p_j < i \end{cases}$$

and for $j = i, \ldots, n$,

$$u_j = u_j + v_j^2.$$

To construct $\mathbf{M}^{-1}$, simply add the following:

a. If both parents of i are known (p and q), add

v_i^{-2} to m^{ii}
$-\ v_i^{-2}/2$ to m^{ip}, m^{pi}, m^{iq}, and m^{qi}
$v_i^{-2}/4$ to m^{pp}, m^{pq}, m^{qp}, and m^{qq},

where m^{kl} is the element in the kth row and lth column of $\mathbf{M}^{-1}$.

b. If only one parent of i is known (q), add

v_i^{-2} to m^{ii}
$-\ v_i^{-2}/2$ to m^{iq} and m^{qi}
$v_i^{-2}/4$ to m^{qq}.

c. If neither parent of i is known, add

v_i^{-2} to m^{ii}.

Note that this construction of $\mathbf{A}_0^{-1}$ and $\mathbf{M}^{-1}$ allows for a separate inference of additive genetic effects in the base population and new mutations arising in successive generations, $\mathbf{A}_0^{-1}$ and $\mathbf{M}^{-1}$ being independent from the remaining parameters in the model. If Bayesian mixed models are applied, this parameterization leads to well-known conditional posterior distributions for all parameters, allowing for standard Gibbs sampling. On the contrary, conditional posterior distributions for several parameters under the original Wray's (1990) approach do not lead to known densities, requiring generic sampling processes (*i.e.*, Metropolis–Hastings sampling) and the intensive reconstruction of the inverse of the relationship matrix within each sampling iteration (Wray 1990).