Heritability and Segregation Analysis of Deafness in U.S. Dalmatians

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ABSTRACT

Hereditary loss of hearing affects many breeds of the domestic dog, but the Dalmatian has the highest prevalence. Approximately 30% are affected in the United States (U.S.) population. It is widely accepted that a relationship exists between deafness and pigmentation in the dog and also in other animals. While the Dalmatian exemplifies this relationship, the genetic origin and mode of inheritance of deafness in this breed are unknown. The goals of this study were to: (1) estimate the heritability of deafness in an extended kindred of U.S. Dalmatians and (2) determine, through complex segregation analysis, whether there is a major segregating locus that has a large effect on the expression of deafness. A kindred of 266 Dalmatians was assembled, of which 199 had been diagnosed using the brainstem auditory evoked response to determine auditory status. Of these, 74.4% ($N = 148$) had normal hearing, 18.1% ($N = 36$) were unilaterally deaf, and 7.5% ($N = 15$) were bilaterally deaf. A heritability of 0.73 was estimated considering deafness a dichotomous trait and 0.75 considering it as a trichotomous trait. Although deafness in the Dalmatian is clearly heritable, the evidence for the presence of a single major gene affecting the disorder is not persuasive.

THE brainstem auditory evoked response (BAER; Kay *et al.* 1984; Marshall 1985) allows accurate detection of dogs that are either unilaterally or bilaterally deaf (Strain 2002). The BAER elicits an all-or-none response; a normal functioning ear will produce a specific waveform pattern while a nonfunctioning ear produces a flat line (Strain 2002). The prevalence of deafness has been determined in many breeds (Strain 2003). The Dalmatian is most affected with ~30% of the United States (U.S.) population exhibiting unilateral or bilateral deafness (Marshall 1986; Holliday *et al.* 1992; Famula *et al.* 2001; Strain 2003). Approximately 20% of Dalmatians are unilaterally deaf, with no significant preference for the left or right ear to be affected, and 10% are bilaterally deaf (Greibrokk 1994; Wood and Lakhani 1998; Famula *et al.* 2001; Muhle *et al.* 2002; Strain 2003).

Histological studies revealed that inner ear structures develop normally up to and after birth with atrophy of the stria vascularis occurring between 1 and 4 weeks of age in affected dogs (Anderson *et al.* 1968; Johnsson *et al.* 1973). These studies also showed an absence of melanocytes in stria of the affected dogs (Anderson *et al.* 1968; Johnsson *et al.* 1973), the first finding to support an association between deafness and pigmentation in the Dalmatian. The function of melanocytes in normal auditory function has been investigated in the mouse (Savin 1965; Steel *et al.* 1987). More specifically, these cells maintain the ionic composition of the cochlear endolymph, and their absence results in strial atrophy (Steel 1995).

A second finding supporting an association between deafness and pigmentation is that Dalmatians with at least one blue eye have a higher prevalence of deafness than brown-eyed Dalmatians (Greibrokk 1994; Wood and Lakhani 1998; Famula *et al.* 2000; Muhle *et al.* 2002; Juraschko *et al.* 2003; Strain 2003). A third finding to support a deafness-pigmentation association is that Dalmatians with a color patch have a lower prevalence of deafness than Dalmatians without a color patch (Greibrokk 1994; Famula *et al.* 2000; Muhle *et al.* 2002; Juraschko *et al.* 2003; Strain 2003). Dalmatians are born white and their spots appear during the first few weeks of life. Unlike a spot, a color patch is present at birth and is generally larger than any spot. While a color patch is negatively correlated with deafness, studies indicate that deafness and the color (*e.g.*, black or liver) of a Dalmatian's spots or patch are not associated (Greibrokk 1994; Wood and Lakhani 1998; Famula *et al.* 2000; Muhle *et al.* 2002; Strain 2003).

Similar associations of deafness with pigmentation have also been identified in the human and one example is that of Waardenburg syndrome (WS; Waardenburg 1951). WS has been proposed as a model for deafness in the Dalmatian (Hudson and Ruben 1962; Mair 1976; Brenig *et al.* 2003) and is the only known human condition in which unilateral and bilateral sensorineural deafness and pigmentation are associated.

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In addition to pigmentation, some groups have reported a significant association between deafness and gender (Holliday *et al.* 1992; Greibrokk 1994; Wood and Lakhani 1998) while others have not found such an association (Marshall 1986; Famula *et al.* 2001; Muhle *et al.* 2002; Strain 2003). Females were found to have a significantly higher prevalence of deafness than males in studies reporting a difference (Holliday *et al.* 1992; Wood and Lakhani 1998) with the exception of Greibrokk (1994) who reported a higher prevalence of deafness in males.

The mode of inheritance for deafness in the Dalmatian has not been determined, but various hypotheses have been proposed as researchers have tried to determine if a single major gene plays a role in the disorder. These hypotheses include transmission by an autosomal recessive, multifactorial gene with incomplete penetrance (Greibrokk 1994), a model of two interacting genes with incomplete penetrance (Strain 1999), a defect in a single major locus with an important role in auditory development but not solely responsible for deafness (Famula *et al.* 2000), and a recessive allele at a single biallelic major locus with incomplete penetrance in recessive homozygotes (Muhle *et al.* 2002).

Heritability estimates have been reported in Californian (Famula *et al.* 2000, 2001), Swiss (Muhle *et al.* 2002), and German (Juraschko *et al.* 2003) Dalmatians. Famula *et al.* (2000) and Muhle *et al.* (2002) also performed complex segregation analysis to examine evidence for the presence of a single major locus. Although Famula *et al.* (2000, 2001) reported heritability estimates and complex segregation analysis in Californian Dalmatians, no study utilizing a data set of U.S. Dalmatians collected from across the country has been performed.

The objectives of the present study were to (1) quantify the inheritance of deafness through the estimation of heritability in a threshold model and (2) use complex segregation analysis to determine if there is a major segregating locus that has a large effect on the expression of deafness in a newly assembled kindred representative of the U.S. Dalmatian population.

MATERIALS AND METHODS

Collection of data: BAER results, eye color, spot color, gender, birthdate, number of littermates, and registration pedigree were collected for each dog. Color patch data were not available for a significant portion of kindred members (>50%) and hence were not included. Data from a total of 266 Dalmatians were collected, 199 with auditory status determined by BAER and 67 with unknown auditory status. The phenotypes of the dogs with known auditory status are shown in Table 1.

Dalmatian kindred: A total of 74 matings between parents with known auditory status were present in the kindred; 60 matings occurred between unaffected parents, 13 matings occurred between an unaffected parent and a unilaterally deaf parent, and one mating occurred between two unilaterally deaf parents.

Nine complete litters (litters in which data concerning all offspring from a mating were collected, $N = 44$) are included in the kindred and contain at least one affected dog in each litter. Both parents and both sets of grandparents are included for each litter ($N = 54$) and all have known auditory status. Seven litters were the result of matings between two unaffected parents and two litters were the result of matings between two unaffected sires and unilaterally deaf dams. The remaining dogs ($N = 168$), including the 67 dogs with unknown auditory status, provided crucial information regarding relationships among the parents and grandparents of the complete litters, as multiple common ancestors create 72 inbreeding loops as identified by LOOPS (Xie and Ott 1992). There are four half-sib matings, three grandchild-by-grandparent matings, two niece/nephew-by-aunt/uncle matings, and one first cousin mating.

One breeder in Louisiana initially provided data for related Dalmatians ($N = 16$) that did not represent an entire family. Data from additional Dalmatians ($N = 31$) that are ancestors and offspring of the first dogs provided were collected from this breeder. Data from the remaining dogs ($N = 219$) were collected from each dog's respective breeder or owner and represent ancestors and offspring directly and indirectly related to the dogs collected from the breeder in Louisiana. Dogs were born in Alabama, California, Florida, Georgia, Kentucky, Louisiana, Massachusetts, Michigan, Mississippi, Missouri, New Hampshire, New Jersey, North Carolina, Tennessee, Texas, and Washington, representing the northern, southern, eastern, and western extents of the United States.

The data for Dalmatians collected from the breeder in Louisiana ($N = 47$) are also included in a data set assembled by Strain (2003). The remaining dogs in the kindred have not been included in any previous studies.

Comparison of kindred to U.S. population: Strain (1999) presented data for 5009 U.S. Dalmatians and has since added an additional 324 Dalmatians (Strain 2003). This is the most comprehensive data set of U.S. Dalmatians available, with the limitation that pedigree information was not recorded, precluding complex segregation analysis. However, the data set of Strain (2003) provides a standard for the U.S. Dalmatian population's phenotypic distribution with which to compare the Dalmatians that are part of this study.

The Dalmatians described here did not differ significantly from those of Strain (2003) when considering deafness as unaffected *vs.* affected (unilaterally and bilaterally deaf combined; $P > 0.19$) or unaffected *vs.* unilaterally deaf *vs.* bilaterally deaf ($P > 0.40$), nor did they differ in terms of eye color ($P > 0.13$). The Dalmatians in this study did differ significantly from Strain's data set in terms of spot color ($P < 0.0001$). Two factors can explain this result, the first being the smaller sample size of this kindred and the second being a preference by the breeders who contributed samples to this study for liver-spotted Dalmatians over black-spotted Dalmatians. This simply illustrates the phenotypic composition of the reported Dalmatians in terms of spot color, which has never been shown to correlate with deafness (Greibrokk 1994; Wood and Lakhani 1998; Famula *et al.* 2000; Muhle *et al.* 2002; Strain 2003). These results suggest that the kindred of Dalmatians reported here is representative of the U.S. Dalmatian population in terms of deafness and eye color.

Estimation of heritability: The estimation of heritability, as well as subsequent complex segregation analysis, is derived from analysis of a kindred of Dalmatians in which deafness segregates. The BAER is used to determine the auditory function of each ear, providing two possible deafness phenotypes in these dogs. One phenotype would be dichotomous, in which

unilaterally deaf and bilaterally deaf dogs would be classified as deaf (*i.e.*, affected *vs.* unaffected). A second phenotype would be trichotomous, with classes for normal hearing, unilateral deafness, and bilateral deafness.

Most data sets utilized in the study of hereditary diseases are constructed around probands, making correction for ascertainment bias necessary; this set of data is no exception. In estimation of heritability, mixed linear models are capable of accommodating nonrandomly sampled data (Henderson 1984). Accordingly, the estimation of the heritability of deafness should not be biased by family selection, provided that the animals at the top of the pedigree (those animals with no parents identified) can be considered a random sample of Dalmatians. This is more assumption than assertion because it is not feasible to create or discount a process of selection against deafness or for sampling such animals disproportionately among those animals at the top that have no known auditory status.

Estimation of heritability is conducted through use of threshold models (Falconer and Mackay 1996), an approach typical for study of binary and ordered categorical traits. The observation of deafness is considered as a binary trait, y_{ij} ($y_{ij} = 0$ when unaffected, 1 when affected) for the jth dog ($j = 1, 2, \ldots, 199$) of the ith gender ($i = 1$ for males, 2 for females). In threshold models, this categorical phenotype is assumed to be related to an underlying, unobservable, normally distributed continuous variable, θ, through a set of three fixed thresholds [$\gamma_0 = -\infty$; $\gamma_1 = 0$; $\gamma_2 = \infty$]; γ_1 is set to zero for computational convenience, with no loss in generality or impact on subsequent analysis of data. Specifically, we assume that the combination of continuous genetic and environmental terms thought to control the unobservable θ is translated into a categorical observation through comparison to the fixed thresholds (*i.e.*, observe an unaffected dog when $\gamma_0 \leq \theta < \gamma_1$ or an affected dog when $\gamma_1 \leq \theta < \gamma_2$).

In a later analysis we consider deafness to be a trichotomous trait, in which normal-hearing dogs are scored as a zero, unilaterally deaf dogs scored as a one, and bilaterally deaf dogs are scored as a two. Such a characterization of the auditory phenotype requires only minor modification of the threshold model. Specifically we need to add a fourth fixed threshold [$\gamma_0 = -\infty$; $\gamma_1 = 0$; γ_2; $\gamma_3 = \infty$], yet in this case γ_2 must be estimated from the available data. Furthermore, normal-hearing dogs would be observed when $\gamma_0 \leq \theta < \gamma_1$, unilaterally deaf dogs would be observed when $\gamma_1 \leq \theta < \gamma_2$, and bilaterally deaf dogs would be observed when $\gamma_2 \leq \theta < \gamma_3$.

The model for θ is similar to any that can be used for continuous phenotypes. The algebraic form of the model for this study is

$$\theta_{ijkl} = \mu + \text{gender}_i + \text{spot}_j + \text{eye}_k + a_l + e_{ijkl}, \qquad (1)$$

where θ_{ijkl} is an unobservable continuous variate for the lth ($l = 1, 2, \ldots, 199$) dog of the ith gender in the jth class of spot color ($j = 1$ for black, 2 for liver) and the kth eye color class ($k = 1$ for two pigmented eyes, 2 for one pigmented and one unpigmented eye). The component μ is an unknown constant while gender_i is the contribution of the ith gender to the expression of deafness. Spot_j and eye_k are similar contributions of these physical characteristics to the liability for deafness; a_l is the additive genetic contribution of the lth animal and e_{ijkl} is an unknown residual. Both a_l and e_{ijkl} are assumed to be random effects with zero means and variances of σ^2_a (the additive genetic variance) and σ^2_e (the residual variance), respectively. The additive genetic effect for each animal accounts for the covariance in phenotypes of relatives and is assumed to be multivariately normally distributed, with a covariance structure based upon the additive relationships among all 266 animals in the data set. Because the underlying scale is unobservable, the total variance is assumed to be $\sigma^2_P = \sigma^2_a + \sigma^2_e$, where $\sigma^2_e = 1.0$, with no loss of generality (Gianola and Foulley 1983; Harville and Mee 1984; Sorensen *et al.* 1995). The heritability of deafness, on the unobservable continuous scale, can be estimated as $h^2 = \sigma^2_a/(\sigma^2_a + \sigma^2_e)$.

A mixed-model Bayesian strategy outlined by Sorensen *et al.* (1995) was used to arrive at an estimate of σ^2_a. An advantage of Bayesian methods is the ability to arrive at not only a point estimate of the unknown parameters (*e.g.*, heritability), but also a distributional estimate. Although a more complete description of the statistical aspects of this analysis can be found in Sorensen *et al.* (1995), briefly, the assumed prior densities for the fixed effects (gender, spot, and eye effects) are the uniform density function, what Bayesian modelers refer to as a "flat" prior density. That is, we assume no prior knowledge of the behavior of the fixed effects. For the analysis of deafness as a binary observation there is no need to estimate the fixed thresholds. However, for the case of the trichotomous deafness, γ_2 must be estimated. The assumed prior distribution for this parameter is the uniform with bounds established by γ_1 and γ_3. As for the random contributions to θ, the additive genetic effects are assumed to be multivariately normally distributed with a null mean and variance-covariance structure consisting of the numerator relationship matrix times the unknown additive genetic variance, σ^2_a. Similarly the random residuals are assumed to be independently normally distributed with null mean with variance $\sigma^2_e = 1.0$ (with no loss of generality since θ is an unobservable variate). Finally, given our Bayesian approach to this problem, we also must establish a prior density for the unknown variance σ^2_a. Specifically, we look to the inverted Wishart distribution where the expected prior mean for the additive genetic variance was started at 1.0 and the shape parameter was 20. The shape parameter reflects the degree of certainty we have in the choice of prior mean for the additive genetic variance (the larger the value, the more certainty). A value of 20, speaking relatively, would be considered large and tend to keep the estimate of the posterior density of the additive genetic variance "close" to the prior density. Analyses were conducted with smaller shape parameters (as well as different starting mean values for the additive genetic variance), but all had the same general behavior of the estimate of the posterior density always returning with a heritability value much higher than the value where we began the search.

Estimation of the distribution of the unknown parameters employs a technique of numerical integration referred to as Gibbs sampling (Geman and Geman 1984). The algorithm is based on the iterative generation of a sequence of random variables from the known conditional distributions of the parameters, given the likelihood function of the data. Subsequent estimates of the parameters are found in the analysis of this sequence of random numbers, called the Gibbs sample. In this study, a total of 100,000 samples of possible heritability values were generated. The estimate of heritability was taken from the mean of every 25th iterate, after discarding the first 10,000 samples, for a total of 3600 sample observations (*i.e.*, $[100{,}000 - 10{,}000]/25 = 3600$). A more complete description of the Gibbs sampling process and its theoretical justification may be found in Sorensen *et al.* (1995) and in Van Tassell and Van Vleck (1995), published by the authors of the public domain software, MTGSAM (Van Tassell and Van Vleck 1995), with which this analysis was performed.

Complex segregation analysis: Regressive logistic models developed for complex segregation analysis (Bonney 1986) were used to evaluate the possible segregation of a single major locus with a large effect on deafness in the Dalmatian. A thorough discussion of complex segregation analysis is available (Lynch and Walsh 1998). The technique is intended to

integrate Mendelian transmission genetics, allelic frequency, and penetrance with the patterns of covariance expected in polygenic inheritance. Elston *et al.* (1975) outlined the criteria that must be satisfied before acceptance of the single major locus model. Adherence to these criteria reduces the number of false positives. Evaluation of the models necessary for complex segregation analysis was conducted with the statistical analysis for genetic epidemiology (SAGE) software (SAGE 1997).

SAGE requires a family structure without "loops" (*i.e.*, a pedigree free of inbreeding). This limitation is not genetic or statistical, but a computational requirement. Currently no software program is designed to analyze pedigrees with inbreeding loops to the extent observed in the kindred assembled for this study. Accordingly, the kindred was subdivided into 27 subfamilies to remove the loops created by inbreeding. Unfortunately, this may eliminate potentially important genetic information. Creation of the subfamilies began with the 199 dogs diagnosed by BAER and identification of their parents, grandparents, and great-grandparents (ignoring ancestors beyond three generations) to build all possible three-generation pedigrees from the kindred. Exclusion of ancestors beyond three generations for each subfamily represents a compromise between the added genetic information that could be gained by including more than three generations and the increase of inbreeding loops that more generations would introduce. Subfamilies still containing inbreeding loops as well as families in which the auditory status of all animals was identical were eliminated (*i.e.*, all normal hearing).

Most dogs were represented in more than 1 of the 27 families. The duplication was necessary to give the software the impression of two different dogs from what was actually one dog. Although not ideal, this was the only means to evaluate this potentially genetically informative kindred. The impact on the final complex segregation analysis was expected to make the detection of a major locus more difficult because ties that are known to exist were treated as being unrelated in the analysis. The magnitude of this effect could not be estimated but was assumed to be minor.

Methods for correcting for sampling bias begin with an assumption about the sampling process. Employing an inappropriate correction for ascertainment bias can be as damaging to the interpretation of results as ignoring ascertainment bias (Greenberg 1986). For this reason, analyses were done with and without correction for ascertainment bias, with founders as a conditioning subset (Elston and Bonney 1986), an option in the SAGE software. The results for both analyses were similar so only results from the analysis with correction for ascertainment bias are reported.

For the purpose of estimating heritability, the implication of biased sampling on the evaluation of inheritance must be considered at several levels. The bias should be minimal if the stated assumption of no selection in the animals in this set of data without identified parents is of little effect. Estimation of genetic variances with mixed-model methods for data that have been subjected to selection is unbiased when the base population can be considered a random sample (Henderson 1984). The impact of ascertainment bias on complex segregation analysis is less simply evaluated. Because the results are not from a randomly sampled cluster of Dalmatians, but rather a set constructed around several dogs with loss of hearing, this analysis must be corrected for such sampling bias.

RESULTS

Of the 199 dogs (87 males, 112 females) with known auditory phenotypes, 148 dogs (74.4%) had normal hearing, 36 (18.1%) were unilaterally deaf, and 15 (7.5%) were bilaterally deaf (Table 1). The relatedness of these dogs complicated generation of a graphical pedigree of all kindred members. A subset of 61 dogs with known auditory status, including six full litters with affected individuals, is shown in Figure 1. As an illustration of the relationships of the dogs, 125 of the total 266 were inbred, with an average inbreeding coefficient of 0.086 as calculated using the program MTGSAM (Van Tassell and Van Vleck 1995).

TABLE 1

Phenotypes of the 199 Dalmatians with known auditory status

Phenotype[a]	Male	Female	Total
Hearing/brown brown/black	47	50	97
Hearing/brown brown/liver	19	24	43
Hearing/brown blue/black	3	3	6
Hearing/brown blue/liver	1	1	2
Unilateral/brown brown/black	9	16	25
Unilateral/brown brown/liver	3	6	9
Unilateral/brown blue/black	0	1	1
Unilateral/blue blue/black	0	1	1
Deaf/brown brown/black	1	3	4
Deaf/brown brown/liver	2	3	5
Deaf/brown blue/black	1	4	5
Deaf/brown blue/liver	1	0	1
Total	87	112	199

[a] Auditory status/eye color/spot color.

Table 2 presents a summary of the analysis of the threshold model, including an estimate of the heritability of deafness on the underlying, unobservable scale for the two phenotypic classification schemes (*i.e.*, dichotomous and trichotomous). As shown, the mean heritability of the Gibbs sample is 0.73, with 95% of the values ranging from 0.55 to 0.89 for deafness when measured as a dichotomous trait and 0.75 (with 95% of the values ranging from 0.57 to 0.92) for deafness as a trichotomous trait.

Table 2 also contains evidence for equality in the incidence of deafness across genders. The mean difference in deafness between genders, on the underlying scale, was estimated as −0.49 with an empirical 95% confidence interval from −1.26 to 0.20. An interval that spans zero is evidence that no gender differences exist in the expression of deafness. The only descriptive character with a significant association with loss of hearing was eye color (Table 2), which did not have a confidence interval that spanned zero.

Table 3 presents results of the complex segregation analysis for dichotomous and trichotomous models of deafness with correction for ascertainment bias. The statistical models analyzed were: (1) a no major locus (NML) model, (2) a general major locus model with Mendelian transmission of the putative major allele [major locus Mendelian (MLM)], and (3) a general

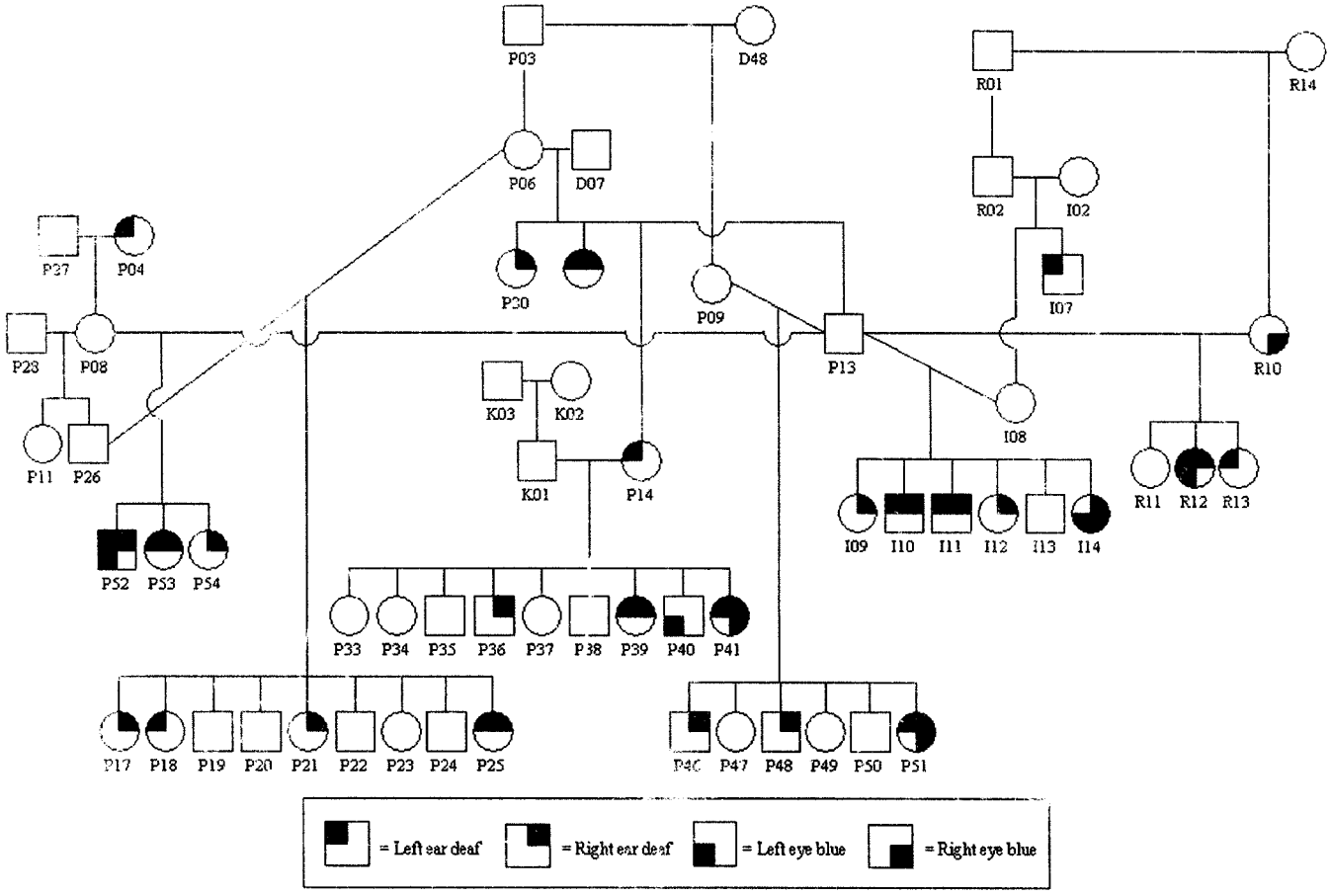

FIGURE 1.—Subset pedigree of 61 Dalmatians with known auditory status, drawn using the software package Progeny (Progeny Software, South Bend, IN).

major locus model in which the transmission probabilities are estimated from the pattern of inheritance revealed by the data [major locus arbitrary (MLA)].

First, considering deafness as a dichotomous trait the natural log of the likelihood ratio (Table 3) in comparing the NML and MLM models is calculated as $-2(-158.69 - (-148.30)) = 20.78$ (3 d.f., $P < 0.001$). This is a χ^2 statistic with degrees of freedom equal to the difference in number of parameters examined between models (in this case five parameters for the NML model and eight parameters for the MLM model) and the P values determined by the χ^2 distribution. This result shows that the MLM model provides a significantly better fit to the data than the NML model. However, the natural log of the likelihood ratio in comparing the MLM and MLA models equals 22.38 (3 d.f., $P < 0.0001$), showing the MLA model provides a significantly better fit to the data than the MLM model.

Second, considering deafness as a trichotomous trait the natural log of the likelihood ratio in comparing the NML and MLM models (Table 3) is 7.10 (3 d.f., $P < 0.07$), showing the NML model does not provide a significantly better fit to the data, at least when using the "standard" type I error at $P = 0.05$. This result differs from comparing the same models considering deafness as a dichotomous trait. The natural log of the likelihood ratio in comparing the MLM and MLA models equals 36.06 (3 d.f., $P < 0.0001$), showing the MLA model provides a significantly better fit to the data, the same result as comparing the same models considering deafness as a dichotomous trait.

DISCUSSION

Heritability and segregation analysis: It is clear from the results presented in Table 2 that deafness in the Dalmatian is hereditary and is influenced by genetic information passed from parent to offspring. Furthermore, the heritability of deafness is of sufficient magnitude that attempts to select against it are potentially successful. A heritability of this magnitude is suggestive, by itself, of the segregation of a single major locus exerting a large effect. MORTON and MACLEAN (1974) demonstrated that major loci tend to increase the heritability of a trait in a given population and a value >0.70 is comparatively large for many polygenic traits, indicating that deafness in the Dalmatian may not be polygenic.

TABLE 2

Estimate of additive genetic variance, heritability, eye color contrast, spot color contrast, and gender contrast in a threshold model for deafness measured in two and three categories

	Mean	Standard deviation	Empirical 95% confidence interval
Dichotomous trait			
Genetic variance	3.28	1.99	1.25, 8.49
Heritability	0.73	0.09	0.55, 0.89
Eye pigmentation[a]	−1.26	0.67	−2.76, −0.08
Black/liver spots	0.25	0.40	−0.48, 1.10
Male/female	−0.49	0.37	−1.26, 0.20
Trichotomous trait			
Genetic variance	3.87	3.09	1.30, 11.92
Heritability	0.75	0.09	0.57, 0.92
Eye pigmentation[a]	−1.97	0.77	−3.81, −0.68
Black/liver spots	0.14	0.40	−0.61, 0.97
Male/female	−0.49	0.38	−1.30, 0.21

Estimates are taken from a Gibbs sample of 3600 values.
[a] Dogs with two brown eyes contrasted with dogs of one brown eye and one blue eye.

This does not preclude other genes or loci exerting an effect on the major locus.

However, the results of Tables 2 and 3 raise important issues. First, the obvious question is, Which analysis is "correct"? The threshold model of heritability in Table 2 and the NML model of Table 3 are conceptually, though not identically, similar. That is, both seek to evaluate the inheritance of deafness with explanatory variables of sex, eye color, and spot color. Yet the approach is fundamentally quite different indeed. The threshold model is built around underlying normality in the distributions of genotypes and environmental contributions (Gianola and Foulley 1983). The complex segregation analysis is derived from logistic regression and the linearity of the log odds of deafness (Bonney 1986).

Conceptually, the threshold model provides a better approach for quantitative genetics analogous to the commonly used mixed models of polygenic continuous phenotypes. Moreover, the threshold model permits the inclusion and consideration of all known relationships, including the magnitude of inbreeding present in this kindred. This cannot be said of the logistic regression model for complex segregation analysis. The logistic regression model can accommodate only specific relationships, such as parent-progeny, and inbreeding loops cannot be present in families of the data set (SAGE 1997). Accordingly, owing to limitations of available software (specifically there being no complex segregation analysis packages for dichotomous and trichotomous traits in a threshold model), we have a two-step analysis of the kindred in this data set.

The comparison of the MLM and MLA models in Table 3, considering deafness as either a dichotomous or a trichotomous trait, is suggested by Elston *et al.* (1975) to reduce the probability of falsely declaring the presence of a major locus. Alleles at a genuine major locus should be transmitted from parent to offspring with probabilities that reflect Mendelian transmission. Table 3 demonstrates that a better fit to the data can be provided when the probabilities of transmission are significantly different from those expected under standard Mendelian transmission. Although from the results in Table 2 we can conclude that deafness is highly heritable, the exact genetic mechanism that leads to expression of this disease cannot be stated with certainty on the basis of the results in Table 3. Accordingly, we also conclude that a major locus with an impact on deafness cannot be established with the present data.

Nonetheless, we are encouraged to observe a rough equivalence in the threshold model results of Table 2 with those of the NML models of Table 3. Given the standard errors of Table 3, confidence intervals can simply be constructed (*i.e.*, 95% intervals computed from the parameter estimate ± 1.96 times the standard error) and evaluated for overlap with 0.0. As such, all the logistic regression coefficients are significantly different from zero, with the exception of differences in gender. Note, however, that the parent regression coefficient is negative, implying that normal-hearing parents are more likely than deaf parents to have deaf offspring. Figure 1 offers visual support of this result. That is, while it is only a snapshot of the kindred, only three unilaterally deaf dogs are parents (P04, P14, and R10); all other hearing-impaired dogs are without progeny in the figure. As previously stated, there were 74 matings between parents with known auditory status present in the kindred; 60 matings occurred between unaffected parents, 13 matings occurred between an unaffected parent and a unilaterally deaf parent, and 1 mating

TABLE 3

Parameter (PR) estimates [± standard error (SE)] from the logistic regression model in complex segregation analysis of dichotomous and trichotomous deafness in the Dalmatian with correction for ascertainment bias

			Major locus			
	No major locus		Mendelian		Arbitrary	
	PR	SE	PR	SE	PR	SE
Dichotomous trait						
$P(A)$[a]	NA		0.23	0.09	0.89	0.11
Pooled base	−1.08	0.47	NA		NA	
AA base	NA		−3.91	1.83	3.86	1.34
AB base	NA		1.18	2.70	−3.34	3.53
BB base	NA		−4.78	1.01	−1.61	0.91
τ_{AA}[b]	NA		1.00	Fixed	0.48	0.10
τ_{AB}	NA		0.50	Fixed	0.0	0.0
τ_{BB}	NA		0.00	Fixed	1.00	0.0
Sex[c]	−1.11	0.55	−1.55	0.44	−4.41	1.19
Eye color pattern[d]	−0.42	0.18	−0.07	0.31	−0.84	0.55
Spot color[e]	−1.03	0.43	−0.31	0.37	−0.62	0.58
Parent[f]	−0.32	0.15	−0.52	0.19	−0.35	0.28
Natural log of likelihood	−158.69		−148.30		−137.11	
Trichotomous trait						
$P(A)$[a]	NA		0.29	0.11	0.89	0.11
Pooled base	−1.71	0.47	NA		NA	
AA base	NA		−3.84	1.45	3.17	1.32
AB base	NA		−4.55	2.99	−4.03	3.42
BB base	NA		−0.33	0.78	−2.30	0.90
τ_{AA}[b]	NA		1.00	Fixed	0.48	0.10
τ_{AB}	NA		0.50	Fixed	0.0	0.0
τ_{BB}	NA		0.00	Fixed	1.0	0.0
Sex[c]	−1.01	0.61	−1.99	0.41	−4.41	1.18
Eye color pattern[d]	−0.56	0.21	−0.20	0.26	−0.84	0.55
Spot color[e]	−0.97	0.40	−0.61	0.33	−0.62	0.57
Parent[f]	−0.38	0.16	−0.48	0.18	−0.35	0.28
Natural log of likelihood	−210.68		−207.13		−189.10	

[a] Frequency of the putative major allele *A*.
[b] Major locus transmission probabilities.
[c] Regression effect for sex (0 for female, 1 for male).
[d] Regression effect for eye color (0 for two brown eyes; 1 for one brown eye, one blue eye).
[e] Regression effect for spot color (0 for black, 1 for liver).
[f] Regression effect for parent's deafness phenotype.

occurred between two unilaterally deaf parents. Interestingly, the heritability of hearing loss is still high for dichotomous deafness with a value of 0.73. It is not possible to directly relate the parent regression coefficient of the NML model to the well-recognized parameter of heritability. However, we can see how knowledge of all relationships, made possible in the threshold model, can provide a more thorough evaluation of inheritance than logistic regression can.

A manual review of the pattern of inheritance did not support a model of a simple autosomal Mendelian locus. For example, the majority of the affected progeny were the result of matings of two unaffected parents, eliminating models of a single dominant deafness allele. Discarding a model of a single recessive autosomal allele is not possible with the kindred, because there were not any matings of two bilaterally deaf dogs. However, there was a mating of two unilaterally deaf dogs (both deaf in the same ear, with two brown eyes, and with black spots) and the argument can be made that if the auditory phenotype is a dichotomous trait, this mating would support discarding the model of a single recessive autosomal allele because it produced normal-hearing offspring. Further support for discarding a single recessive allele is provided by several unrelated matings of bilaterally deaf parents not in this kindred (Strain 1999) that produced normal, unilaterally deaf, and bilaterally deaf offspring.

Famula *et al.* (2000) reported a heritability estimate of 0.32 in Californian Dalmatians, a value much lower than the estimates presented here. However, Famula *et al.* (2001) included more records of Californian Dalmatians in a larger data set and reported a heritability estimate of 0.76, a value comparable to the estimates presented here.

Although deafness in the Dalmatian is clearly inherited, the evidence for the presence of a single major gene affecting the disorder is not persuasive with the data from this kindred. Famula *et al.* (2000) and Muhle *et al.* (2002) provided evidence of a single major locus of large effect on deafness in Californian and Swiss Dalmatians, respectively. Jarvik (1998), in a review of complex segregation analysis, suggested prudence in the interpretation of complex segregation analysis until several sets of data had confirmed or rejected the presence of a Mendelian locus.

Future directions: While the absence of a clear mode of inheritance complicates genetic dissection of deafness in the Dalmatian, the assembling of this kindred provides a tool for eventually defining the genetic bases of this disorder. This set of Dalmatians provides a potentially informative group with which to perform a whole-genome scan and the analyses of the kindred described here will assist evaluation of linkage data generated by utilizing a set of multiplexed canine microsatellite markers (Cargill *et al.* 2002). Due to the uncertainty of the genetic mechanism of deafness, other experimental approaches such as examination of candidate genes may not be as effective as a genome scan. Brenig *et al.* (2003) reported that *PAX3*, a gene implicated in Waardenburg syndrome, is not responsible for deafness in the Dalmatian. Other candidate genes could be examined, but the possibility exists that the gene responsible for deafness in the Dalmatian has not been characterized in another species. The number of genes associated with deafness in the human and mouse (Steel and Bussoli 1999; Steel and Kros 2001) is quite large. Because of this, the time and cost required to analyze each gene in the Dalmatian is not justified when tools are available to localize chromosomal regions through a genome scan. Linkage analysis of observed microsatellites in this kindred of Dalmatians will hopefully reveal chromosomal regions harboring the gene(s) causative for deafness in the Dalmatian.

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