Genetic Structure of Susceptibility Traits for Hip Dysplasia and Microsatellite Informativeness of an Outcrossed Canine Pedigree


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Abstract

An outcrossed canine pedigree was developed for quantitative trait locus (QTL) mapping of hip dysplasia by breeding dysplastic Labrador retrievers to trait-free greyhounds. Measured susceptibility traits included age at onset of femoral capital chondroepiphyseal ossification (OSS), maximum hip distraction (laxity) index (DI), and the dorsolateral subluxation (DLS) score. The pedigrees consisted of 147 dogs representing four generations. For 59 dogs genotyped with 65 microsatellites, the median heterozygosity and polymorphic information content (PIC) values of the F1 generation were 0.82 and 0.68, respectively. Seventy-seven percent of microsatellites had a PIC greater than 0.59 in the F1s. Ninety-six percent of alleles showed Mendelian inheritance. Based on marker informativeness, approximately 350 randomly selected markers would be required for genome-wide screening to obtain an average interval between informative markers of 10 cM. Heritability was estimated as 0.43, 0.5, and 0.61 for OSS, DI, and the DLS score, respectively. Biometric estimates of the mean (± variance) effective number of segregating QTLs was 1.2 (± 0.05), 0.8 (± 0.02), and 1.0 (± 0.03) for OSS, DI, and the DLS score, respectively. The distributions of simulated backcross trait data suggested that the loci controlling these traits acted additively and that the DI may be controlled by a major locus. When combined with previous power and quantitative genetic analyses, these estimates indicate that this pedigree is informative for QTL mapping of hip dysplasia traits.

Canine hip dysplasia (CHD) is a common developmental trait that affects primarily large breed dogs and is characterized by poor hip joint congruity, functional subluxation, and development of debilitating secondary hip osteoarthritis (Lust 1997; Smith et al. 1990; Willis 1989). CHD is most commonly diagnosed by examination of ventrodorsal, hip-extended radiographs of the pelvis based on identification of subluxation and osteoarthritis (Henry 1992). The trait remains one of the most common affecting large dogs (Corley 1992; Leighton 1997; Swenson et al. 1997).

Heritability estimates for CHD range from 0.11 to 0.68 (Breur et al. 2001). Developmental dysplasia of the human hip has many of the same features as the canine condition (Todhunter and Lust 2002), and genetic and ethnic factors contribute to the expression of developmental dysplasia of the human hip (Weinstein 1996). In a Spanish population with a high incidence of developmental dysplasia of the hip, the most parsimonious hereditary transmission model was a two-locus, recessive gene model (Sollazzo et al. 2000). An interstitial duplication of HSA 15q24-26 associated with
CHD is a complex trait, the expression of which is influenced by genetic (Leighton 1997; Willis 1989), nutritional (Kealy et al. 1992), and possibly hormonal factors (Goldsmith et al. 1994). The molecular genetic control of the canine and human dysplastic phenotype is unknown. Theoretically several major and minor quantitative trait loci (QTLs) (Lander and Botstein 1989; Paterson 1995; Tanksley 1993) may contribute to trait expression, yet a biometric study of Labrador retrievers and German shepherds suggested a major locus may be controlling the dysplastic phenotype (Leighton 1997). In order to map QTLs underlying the dysplastic trait in dogs, we developed an outcrossed canine pedigree by breeding dysplastic Labrador retrievers and unaffected greyhounds (Todhunter et al. 1999). These founder individuals were selected because they expressed extremes of hip joint conformation. The emphasis in pedigree expansion has been to backcross the F1 generation to each founder population. The traditional hip radiograph has limited specificity for diagnosis of HD, especially in dogs of prebreeding age (Adams et al. 1998). Alternative methods for measurement of dysplastic features include the distraction index (DI) (Smith 1997; Smith et al. 1990), dorsolateral subluxation (DLS) test (Farese et al. 1999), and the age of detection of ossification in the capital femoral chondroepiphysis (OSS) (Madsen et al. 1991; Todhunter et al. 1997). Decomposition of the complex phenotype of CHD, characterized by functional hip subluxation and osteoarthritis, into potentially simpler traits of OSS, DI, and the DLS score should simplify genetic mapping, as these traits may be controlled oligogenically or even monogenically.

The current integrated canine radiation-hybrid linkage map places 1800 type I and II markers, easily sufficient for a 10 cM genome-wide scan (Breen et al. 2001). Prior to embarking on genome-wide screening for linkage to the QTLs associated with the expression of these specific traits, we determined that the power of this pedigree was adequate for mapping QTLs associated with susceptibility traits for hip dysplasia (Todhunter et al. 2000), that the mode of inheritance of OSS, DI, and the DLS score was additive, and that the DLS score also showed a dominant transmission pattern (Bliss et al. 2002). Because there were no paradigms to follow for canine complex trait analysis, it was important to further characterize the informativeness of this special pedigree and the nature of the underlying QTLs with the data in hand as a prelude to coarse genome-wide screening and linkage, and linkage disequilibrium mapping analysis. As a means to this end, we estimated the microsatellite informativeness in dogs from this outcrossed pedigree and the number of effective QTLs controlling these traits. We estimated each trait’s heritability and predicted the distribution of these traits by simulation of the current trait data in the backcrosses to the Labrador retriever and greyhound. These simulations predict the phenotype of those dogs bred in future family expansions and the trait segregation patterns.

### Materials and Methods

#### Animals

The development of the pedigree for studying CHD and osteoarthritis by breeding dysplastic Labrador retrievers and unaffected greyhounds has been described elsewhere (Bliss et al. 2002; Todhunter et al. 1999). The four generations include Labrador retriever (n = 8) and greyhound (n = 7) founders, F1s (n = 41; 11 of these dogs were used as breeders), 3/4 Labrador retriever backcrosses (n = 71), 3/4 greyhound backcrosses (n = 33), F1 intercrosses (F1×F1; n = 16), and one 3/4×3/4 Labrador retriever litter (n = 9) (Figure 1). The feeding regimen has been described elsewhere (Bliss et al. 2002; Todhunter et al. 1999). All procedures were approved by the university’s Institutional Animal Care and Use Committee.

#### Phenotyping of the Founders and F1 Breeders

Diagnostic ultrasound hip imaging was done every other day from 4 days of age until ossification onset in the proximal femoral capital epiphysis was confirmed. Mineralization was observed as an echoic region just proximal to the developing capital femoral growth plate (Figure 2A). The hip-extended, distraction, and dorsolateral subluxation radiographic projections were obtained at early maturity (8 months of age) and have been described elsewhere (Bliss et al. 2002; Todhunter et al. 1999). The hip-extended, traditional projection was scored for the presence or absence of osteoarthritis and CHD (Henry 1992) (Figure 2B). The maximum amount of passive hip laxity was measured on the distraction radiographic projection as the distraction index (DI) (PennHIP/Synbiotics; for a review see Smith [1997]) (Figure 2C). The DI ranges from about 0.1 to about 1. Large breed dogs with DIs less than 0.3–0.4 (tight hips) are non-susceptible to CHD, while those with DIs greater than 0.7 are highly susceptible (Lust et al. 1993; Smith 1997; Smith et al. 2001). The dogs were positioned in the dorsolateral subluxation (DLS) position and a dorsoventral radiograph was taken (Farese et al. 1998) (Figure 2D). The percentage of the femoral head beneath the respective craniodorsal acetabular rim (DLS score) is the most accurate measure in this pedigree of susceptibility to CHD (Lust et al. 2001a). Unaffected dogs had DLS scores greater than 55%, while dysplastic-susceptible dogs had DLS scores less than 45%, and all dogs with DLS scores less than 42% had CHD as marked by osteoarthritis (Lust et al. 2001b). Greyhounds had DLS scores between 65% and 80% and DIs less than 0.3.

#### Microsatellite Genotyping

Venous blood was collected from 59 dogs from the outcrossed pedigree into 10% by volume of sodium citrate. Founders, F1 breeders, and backcrosses to the Labrador retriever were sampled for genotyping because they represented the broadest range of phenotypes and may be the most informative for linkage analysis of CHD traits. DNA was isolated by standard protocols (Garner 2000). All DNA was diluted to a final concentration of 20 μg/ml in TE buffer.
Forward primer sequences for 62 canine tetranucleotide and 10 dinucleotide repeat markers were obtained from the Web site of the Fred Hutchinson Cancer Research Center (www.fhcrc.org/science/dog_genome/dog.html). Representative primer pairs from 28 of the 38 linkage groups of the canine radiation-hybrid linkage map were selected (Mellersh et al. 2000). Forward primers were synthesized with the 5' addition of one of three commercially available fluorochromes (Fam, Hex, Tet; Applied Biosystems, Foster City, CA) using a commercial oligonucleotide synthesizer (model 394 Oligo synthesizer; Applied Biosystems, Foster City, CA). Corresponding reverse primers were obtained commercially (Research Genetics, Huntsville, AL). Microsatellites were amplified in 12 μl reaction volumes containing final concentrations of 10.4 mM Tris-HCl, 41.6 mM KCl, 0.25 mM of each deoxynucleotide-triphosphate (dNTP), 1.5–4.0 mM MgCl₂, 0.3 U TaqGold DNA polymerase (AmpliTaqGold; Perkin-Elmer, Foster City, CA), 0.4–4.0 μM each of forward and reverse primers, and 25–50 ng of genomic DNA. Each sample was heated to 95°C for 15 min (predwell) as per the manufacturer’s instructions. Samples were amplified for 32–40 cycles at 95°C for 30 s, 52–66°C for 30 s, 72°C for 1 min, followed by a final extension at 72°C for 30–60 min.

Concentrations of forward and reverse primers, MgCl₂, and DNA were optimized for each primer pair. Separate optimization reactions were performed using paired DNA samples to determine the optimal reagent concentrations and cycling conditions for each primer pair. Appropriate annealing temperatures were determined empirically using a gradient thermal cycler (Mastercycler gradient; Perkin-Elmer, Foster City, CA) with a 10°C gradient across the block. Several markers were amplified using a “touchdown” protocol (Mellersh and Sampson 1993). Four microliters of polymerase chain reaction (PCR) product from each optimization reaction was mixed with an equal volume of formamide buffer, denatured at 95°C for 2 min, cooled on ice, and resolved on 10% polyacrylamide gels in 1× TAE buffer. Gels were stained with ethidium bromide (0.1 mg/ml) and visualized under ultraviolet (UV) light. Final PCR

Figure 1. Scheme illustrating outcrossed canine pedigree developed for linkage analysis of hip dysplasia. The first letter of a litter indicates the temporal sequence of litter birth for that year: A = the first litter born, B the second, etc. X = F₁, 2F = F₂, B = backcross, 2B = BC × BC. The first numeral is the order for each dog in that litter and the last numeral 60 is the year born: 5 = 1995, 6 = 1996, etc. So, for example, FX16 is the first F₁ dog in the sixth litter born in 1996.
products from optimized reactions were diluted 1:2–1:10 in distilled water. Amplified products from three markers, each with a different color label, were pooled at ratios of 1 part distilled water. Amplified products from optimized reactions were diluted 1:2–1:10 in the developing femoral capital chondroepiphysis. One microliter aliquots of products from three markers, each with a different color label, were pooled at ratios of 1 part

The percent Mendelian inheritance of alleles for each marker was calculated as the total number of alleles expressed in the F1, F2, and backcross generations for which a parental source could be identified divided by the total number of alleles in those generations. The median percent Mendelian inheritance was determined over all markers. The size of the canine genome is estimated to be 2700 cM (Neff et al. 1999). The minimum number of randomly selected markers required to obtain a mean interval between highly informative markers of 10 cM across the genome was estimated as 270 divided by the proportion of markers tested with a PIC value greater than 0.59.

**Trait Heritability**

To estimate the heritability for each trait, restricted maximum likelihood (REML) mixed models were used to compute the variances for the additive genetic component (SAS, version 8.1; SAS Institute, Cary, NC). These methods were an extension of the genetic modeling described by Bliss et al. (2002). Briefly, the model used to derive the variances was

\[ Y = \beta_0 + \beta_1 \text{ (gender)} + \beta_2 \text{ (litter)} + \beta_3 \text{ (x)} + \beta_4 \text{ (delta)} + \text{ residual}, \]

where \( Y \) is the mean value of an individual trait (OSS, DI, DLS score) and \( \beta_0 \) is the overall mean value of the trait within the entire study population. The coefficient of the additive effect \( \beta_3 \) is the difference between the expected proportion of GG (homozygous greyhound founder) and LL (homozygous Labrador retriever founder) loci, while the coefficient of the dominant effect \( \beta_4 \) is the expected proportion of GL (F1) loci (Todhunter et al. 1999). For convenience, the F1 genotype was coded as 0, the GG genotype as 1, and the LL genotype as –1. The model incorporated a “built-in” contrast between the GG and LL genotypes that allowed their relative contribution to an additive and/or dominant mode of transmission to be tested simultaneously (Bliss et al. 2002). By modeling the additive and dominant genetic effects as random variables, the covariance parameter estimates were obtained. The
heritability was estimated for each trait as the additive genetic variance divided by the total phenotypic variance (Falconer and Mackay 1996).

### Estimation of the Number of Effective QTLs

Two biometric methods were used to estimate the number of effective QTLs segregating in the backcross families in the pedigree. The first, the Castle–Wright (CW) method, related the means of the parental population traits to the variances of their hybrid offspring traits (Castle 1921; Wright 1968):

Number of effective QTLs \( (n_c) = \frac{(\mu_G - \mu_L)^2}{8\sigma^2_s} \)

where \( \mu_G \) is the mean of the greyhound parental population, \( \mu_L \) is the mean of the Labrador retriever population, and \( \sigma^2_s \) is the segregation variance of the backcross progeny. The additive genetic variances were used as the estimate of the segregation variance in the pedigree. The second estimation of the number of effective QTLs (the CWZ method) was derived from the method of Zeng, who modified the Castle–Wright estimator:

Number of effective QTLs

\[
= \left[ 2 \times \epsilon \times n_1 + (C - 1)(n_1 - 1) \right] / \left[ 1 - n_1(1 - 2\epsilon) \right],
\]

where \( \epsilon \) is the average recombination rate between loci, \( C \) is the coefficient of variation for the distribution describing the additive effects; and \( n_1 \) is the number of effective QTLs estimated by the CW method (above). The formula to estimate \( C \) is

\[
C = \frac{1}{\lambda_2} - 2L - M + \sum_{i=1}^{M} e^{-2\lambda_i},
\]

where \( M \) is the number of chromosomes, \( L \) is the chromosome length in Morgans, and \( \lambda_i \) is the length of the \( i \)th chromosome. There are 38 canine autosomes plus the X and Y. The total length of the canine genome has been estimated at 27 Morgans (Neff et al. 1999). The length of the individual chromosomes was estimated for each chromosome and linkage group (http://www.fhcrc.org/science/dog-genome/map/map.html). \( C \) can be assumed to be a particular value if the distribution of the gene effect is roughly known. Based on molecular mapping experiments, the additive effect of QTLs can be assumed to follow a normal distribution where \( \epsilon = 0.5 \), an exponential distribution where \( \epsilon = 1 \), or an L-shaped distribution where \( \epsilon = 2 \). For the calculations here we used \( \epsilon = 0.5 \), which minimized the variance.

The sampling variance (\( V' \)) of the CWZ method for the QTL estimation is

\[
V' = \frac{2[2\epsilon(1 + C^2)n_1^2]/(N + 2)[1 - n_1(1 - 2\epsilon)]^4}{},
\]

where \( N \) is the number of individuals in the backcross population.

### Predictive Density in the Backcross Generation

The predictive density shows the trait distribution expected in each backcross group by simulating the offspring traits resulting from \( F_1 \)’s crossed to either founder population. We assumed that the backcross population is a mixture of the founder and \( F_1 \) populations, with unknown subscript mixture weights. Using Markov chain Monte Carlo techniques (Robert and Casella 1999), specifically Gibbs sampling with latent variables, the backcross density was estimated for each trait and each backcross arm (i.e., the backcross of \( F_1 \) to the Labrador retriever and to the greyhound) based on simulation of 5,000 crosses.

### Results

#### Microsatellite Informativeness

Alleles were unable to be accurately scored for five dinucleotides due to stutter bands. Similarly, alleles could not be accurately sized for two tetranucleotides that generated PCR products in excess of 650 bp. Of the remaining 65 markers, alleles were scored for 99.2% of the final PCRs. Summary statistics, including the mean number of alleles per locus as well as the median heterozygosity and PIC values for dogs in the founder and \( F_1 \) generations for all markers, are listed in Table 1. Thirty microsatellites (29 tetranucleotides and 1 dinucleotide) are contained in the canine minimal screening set (Richman et al. 2001). The mean PIC value for this group of 30 microsatellites in the \( F_1 \) dogs in this pedigree was 0.69 (Table 2). Approximately 77% demonstrated high information content. Given this percentage and the size of the canine genome (2700 cM), the estimated minimum number of randomly selected markers necessary to achieve a mean interval between informative markers of \( \leq 10 \) cM across the genome was 270/0.769 \( \approx \) 351. Mendelian inheritance was less than 90% for 9 of 58

<table>
<thead>
<tr>
<th>Heterozygosity(^c)</th>
<th>PIC(^d)</th>
<th>No. of alleles/marker(^b)</th>
<th>Percent Mendelian inheritance(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labrador founders ((n = 7))</td>
<td>0.75 (0.0–1.0)</td>
<td>NA</td>
<td>4.51 (±0.295)</td>
</tr>
<tr>
<td>Greyhound founders ((n = 8))</td>
<td>0.71 (0.0–1.0)</td>
<td>NA</td>
<td>4.06 (±0.161)</td>
</tr>
<tr>
<td>F1 breeders ((n = 11))</td>
<td>0.82 (0.0–1.0)</td>
<td>0.68 (0.21–0.86)</td>
<td>4.14 (±0.243)</td>
</tr>
<tr>
<td>Entire group ((n = 59))</td>
<td>NA</td>
<td>NA</td>
<td>6.60 (±0.295)</td>
</tr>
</tbody>
</table>

\(^a\) Median (range).  
\(^b\) Mean (±SEM).  
\(^c\) Polymorphism information content; NA, not applicable.

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tetranucleotides. New alleles were not observed for any of the five dinucleotides.

**Trait Heritability**

The heritability estimates for OSS, DI, and the DLS score in this pedigree were 0.43, 0.5, and 0.61, respectively.

**Estimation of the Number of Effective QTLs**

The estimated number of effective QTLs for both the CW and CWZ methods were both small, with ranges from 0.5 to 1.4 (Table 3).

**Density Distribution Functions**

Density distribution functions for the backcross to the Labrador retriever are shown in Figure 3. OSS and the DLS score exhibited unimodality, suggesting additive or polygenic inheritance. For the DI, a bimodal density distribution was demonstrated, predicting separation of the backcross to the Labrador retrievers into two populations if sufficient breedings were carried out (Figure 3, middle). The predictive density distribution function for the DLS score in the backcrosses to the Labrador retrievers has long tails consistent with the broad distribution of phenotypes observed thus far in this particular backcross (Bliss et al. 2002) (Figure 3, bottom). We have observed both nondysplastic and dysplastic and osteoarthritic dogs in this backcross breed (Todhunter et al. 2000).

**Discussion**

Successful QTL mapping requires that the members of a pedigree be informative for both a sufficient number of mapped genetic markers and the QTLs associated with the phenotype of interest (Lynch and Walsh 1998). High microsatellite informativeness can be observed in outbred populations in which founder individuals are heterozygous at a high proportion of loci, but in which there is a low level of allele sharing between parental pairs. In pedigrees with high levels of ancestral heterozygosity at marker loci, marker informativeness is a function of both the mean number of alleles per locus as well as allele frequencies and is most accurately represented by the PIC (Botstein et al. 1980; Lynch and Walsh 1998). The high median PIC value of the microsatellites screened in this group shows that the pedigree is microsatellite informative for linkage analysis. QTL informativeness cannot be determined a priori and can only be demonstrated by the observation of linkage between a genetic marker and a QTL associated with a particular trait.

The Labrador retriever and greyhound founders in this pedigree were selected from inbred populations in which CHD occurs with high and low prevalence, respectively. Therefore it can be reasoned that these dogs are homozygous at those loci that contribute to (Labrador retrievers) or protect against (greyhounds) the development of the dysplastic phenotype. Moreover, clear phenotypic differences can be demonstrated between these dogs, as well as between the F1 and founder populations, with respect to OSS, DLS score, and DI (Bliss et al. 2002). The pedigree is thus predicted to be QTL informative.

Our biometric estimates of the effective (or minimum) number of QTLs (also called a “segregation index”) (Zeng 1992) segregating in this pedigree suggest that very few, or even a single, QTL(s) may be controlling the major differences in breed traits. These estimates seem small at first glance. However, it is becoming clearer that single QTLs can control large parts of phenotypic expression in complex traits (Frary et al. 2001). In addition, our goal in measuring susceptibility traits for CHD has been to decompose the complex phenotype into subphenotypes. Our hypothesis was that these simple traits may be controlled by fewer loci. A single dominant allele could explain the dominant effect of the protective greyhound alleles on the DLS score (Bliss et al. 2002). A single major QTL effect could also account for the bimodal DI distribution in the backcrosses we simulated from breeding of the F1s back to the Labrador retriever founders (Falconer and Mackay 1996).

**Table 3.** Estimated number of effective QTLs for age at detection of femoral capital ossification, distraction index, and dorsolateral subluxation score segregating in a dysplastic Labrador retriever–greyhound pedigree

<table>
<thead>
<tr>
<th>Estimation method</th>
<th>Trait</th>
<th>No. of QTL</th>
<th>Variance of estimate&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castle–Wright</td>
<td>OSS (days)</td>
<td>1.4</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>DI</td>
<td>0.9</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>DLS score</td>
<td>0.5</td>
<td>—</td>
</tr>
<tr>
<td>Castle–Wright–Zeng</td>
<td>OSS (days)</td>
<td>1.2</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>DI</td>
<td>1.0</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>DLS score</td>
<td>0.8</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<sup>a</sup> There is no variance estimate associated with the Castle–Wright estimates.
The estimation of the number of effective QTLs based on the CW method is error prone because of the underlying assumptions (Zeng 1992). One assumption of the CW estimator, which is clearly broken regarding the DLS score, is that the alleles interact purely additively—that is, there is no dominance or epistasis. However, even using the less biased modification of the CW estimates by Zeng (CWZ), the QTL estimate of the effective or minimum number of QTLs did not change appreciably. We have approximately satisfied the recommendations of Zeng (1992) to reduce the sampling variance problem. These include using populations strongly divergent in the trait, large sample sizes (>200), and better estimates of segregation variance. Our working hypothesis is that, although many loci will collectively influence trait expression, few major loci will explain a major proportion of the difference in hip conformation between these diverse breeds.

A formal, single-marker power analysis using methods previously described by Doerge and Churchill (1997) was completed for the pedigree based on phenotypic data collected from dogs in the founder and F1 generations (Todhunter et al. 2000). These two populations are involved in the backcross breedings and thus are the critical components of our strategy for linkage analysis. In anticipation of a genome-wide scan with a mean intermarker interval of 10 cM, recombination fractions (θ) of 0, 0.05, and 0.1 were used for this power analysis. Analyses performed based on a heterozygosity of 0.75 and θ = 0.05 indicated that sufficient numbers of 3/4 Labrador retriever and 3/4 greyhound individuals are already available to achieve a power of 0.8 for observation of linkage to these CHD susceptibility traits. The median heterozygosity estimated for the F1 generation based on the markers in the current report (heterozygosity = 0.82) supports these predictions.

Given an adequate sample size and high marker informativeness, a genome-wide scan based on an intermarker interval of 10 cM will disclose linkage between a marker and a QTL with a recombination fraction of 0.05 (since, on average, a QTL will reside no further than 5 cM from an adjacent marker). Little additional power for detection of linkage is gained through the use of markers spaced more closely than 10 cM (Darvasi 1998). Given a genome-wide screen based on a mean intermarker interval of 10 cM, a high interval variance can reduce the power for observation of linkage in regions of the genome with low marker density. This may necessitate utilization of additional markers to screen those regions with insufficient initial coverage. As indicated in Table 2, 77% of the markers screened in this study are considered highly informative in

![Figure 3. Predictive density (vertical axes) distribution functions for three susceptibility traits for canine hip dysplasia in the backcross offspring derived from 5000 simulations of crosses between the Labrador retriever founders and the F1 breeders. (A) The distribution of age at onset of femoral head ossification (OSS) for the F1s (a), Labrador retriever founders (b), and their crossbreed offspring (c). (B) The DI distribution for the F1s (a), Labrador retriever founders (b), and their crossbreed offspring (c). Similarly, (C) illustrates the respective distributions of the DLS score for the F1s (a), Labrador retriever founders (b), and their crossbreed offspring (c).]
this pedigree based on a PIC value greater than 0.59. Given the size of the canine genome (2700 cM), approximately 350 randomly selected markers would be necessary to achieve a mean interval of 10 cM between informative markers. This is well within the number of markers (1800 type I and II) currently mapped (Breen et al. 2001).

An alternative approach to initial genome-wide screening that is widely used in human mapping projects involves use of a minimal screening set (Reed et al. 1994; http://www.resgen.com/products/HuSCREENSET.php3). These sets are optimized groups of markers that demonstrate high PIC values in reference populations and are selected to provide a minimal density of coverage across the entire genome. A minimal screening set of informative microsatellites for canine mapping projects has been characterized (Richman et al. 2001). The set contains 172 markers with a mean PIC value of 0.74; 77% of the canine genome is estimated to fall within 10 cM of a marker in this set (Richman et al. 2001). The mean PIC value of the markers screened in this study that belong to the minimal screening set (0.69) is similar to the mean PIC reported for the set as a whole (0.74). This indicates that the minimal screening set may be a useful and cost-effective approach for initial genome-wide screening in this pedigree.

Overall, the inheritance of microsatellite alleles within the families of dogs in this study followed a Mendelian pattern. Tetranucleotides are known to exhibit higher mutation rates than those based on dinucleotide repeats (Koreth et al. 1996). Consistent with this, we observed non-Mendelian inheritance only for alleles of tetranucleotide-based markers. Various explanations for the origins of new microsatellite alleles have been proposed, including in vitro or in vivo mutation (Brinkmann et al. 1998; Di Rienzo et al. 1994), nonamplification of hidden alleles (Eggleston-Stott et al. 1997; Koorey et al. 1993), and nonspecific primer annealing during PCR (Koreth et al. 1996). Slippage of the copy DNA strand on the template during the synthesis cycle of the PCR is thought to be the mechanism for the origin of stutter bands that commonly occur following amplification of microsatellites, especially those based on dinucleotide repeats (Di Rienzo et al. 1994; Koreth et al. 1996). This may also represent the mechanism of in vivo mutation by which polymorphisms are generated within a population (Di Rienzo et al. 1994). New alleles may occur at a higher rate in vitro where strand slippage or other mutations occur in the absence of DNA proofreading and repair pathways (Strauss 1999). Since linkage analysis is based on the demonstrable inheritance of marker alleles, new alleles that arise through mutation or other sources are not informative.

Heritability tells us little about the trait differences between the greyhound and the Labrador retriever, but it does tell us how a breed would respond to selection. The higher the heritability, the more efficiently a breeder can elicit genetic improvement for that trait in a breeding program (Falconer and Mackay 1996; Leighton 1997; Smith 1997). Previous reported heritabilities for CHD have ranged from 0.11 to 0.68 (with most estimates ranging from 0.2 to 0.4) (see Breur et al. [2001] for a review). Heritability is a population-specific parameter and therefore is relevant only to the pedigree on which it was calculated. However, general comparisons can be made. The heritabilities we report here (albeit on a small number of dogs) for OSS, DI, and DLS score are high. We caution that they were estimated from an experimental pedigree specifically outbred and designed for linkage analysis of CHD and the dogs are bred and housed under the same environmental conditions (thus limiting environmental variance). Previous estimates of the heritability of DI in the Seeing Eye Labrador retriever and German shepherd populations were 0.5 and 0.6 (Leighton 2001) compared to our local estimate of 0.5. If the heritabilities we report here could be extended to pure breed populations, the application of selection pressure based both on the DI and the DLS score should influence the respective trait in the progeny more than reliance on the traditional hip-extended radiographic projection (Smith 1997). In addition, the higher the heritability, the more likely the probability of finding evidence of linkage to a genetic marker (Paterson 1998).

In conclusion, the single-marker power analysis (Todhunter et al. 2000), the clear inheritance patterns (Bliss et al. 2002), the high heritabilities of these traits, the informativeness of the microsatellites, the estimates of single effective QTLs controlling these individual traits, and the predicted broad backcross trait distributions in the backcross to the Labrador retriever lend support to the notion that this specially constructed pedigree is informative and powerful for initially mapping these traits that are strongly associated with CHD through a genome-wide screen. These factors are also important in designing statistical models for linkage analysis (Zeng 1992).

References


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