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Evaluating Evolutionary Divergence with Microsatellites

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Abstract. We report the use of microsatellites (MS) to track the recent evolution of swine. Allelic frequencies for nine MS loci linked on swine chromosome 6 (SSC6) representing four western and one Chinese swine breeds were used to estimate genetic distances and times of breed divergence. A phylogenetic tree was constructed which partitioned into western and Meishan breed branches. Yorkshire and Hampshire breeds exhibited the most recent divergence with a calculated distance of 391 years. The oldest divergence, of 2,227 years, was between Meishan and Hampshire swine. Estimates of breed divergence are consistent with historical records. Additional analysis suggests that polymorphic MS linked on a single chromosome are sufficient to determine evolutionary relationships within a single species.

Key words: Chromosome 6 microsatellites — Genetic variance — Genetic distance — Breed divergence — Swine breeds

Introduction

Genetic information obtained from highly polymorphic single-locus markers can improve estimates of genetic distances and assist in determining evolutionary relationships and preserving species diversity. Microsatellite (MS) abundance, polymorphism, ease of identification, and low mutation rates (in humans estimates are between 4.5×10^{-4} and 5×10^{-6} spontaneous mutation/locus/gamete) (Kwiatkowski et al. 1992; Ellegren 1995) in mammalian genomes make MS preferred genetic markers for estimating genetic variation (Milligan et al. 1994; Paetkau and Strobeck 1994). In cattle and sheep (Arranz et al. 1996; Buchanan et al. 1994; Manwell and Baker 1977) the increased heterozygosity of MS over protein polymorphisms allows clearer differentiation among closely related breeds and increases the accuracy of prediction for divergence with a smaller number of markers. MS have also proven superior to traditional blood-group and protein loci to estimate the date of origin for modern humans (Goldstein et al. 1995).

Current application of swine linkage maps (Ellegren et al. 1994; Rohrer et al. 1994, 1996; Schook et al. 1994; Archibald et al. 1995; Paszek et al. 1995) to genetic improvement while preserving genetic variation in existing swine populations requires a robust estimate of variation within and between swine breeds. Linkage maps also enhance the identification of quantitative trait loci (QTL) and the implementation of marker-assisted selection (MAS). A study based on phenotypic data for Chinese and several US pig breeds reported separate phylogenetic tree branches for Yorkshire and Duroc breeds from Landrace and Meishan breeds (Li and Enfield 1989). Initial information on relative heterozygosity between western swine breeds and the Chinese Meishan breed (Fredholm et al. 1993; Paszek et al. 1997) provides an opportunity to test whether markers linked to an economically important locus affecting meat quality in several breeds of western swine, but absent in Chinese breeds can be used to determine evolutionary relationships.

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Markers flanking the Calcium Release Channel locus (CRC1) (Paszek et al. 1995, 1997) located on chromosome 6 (SSC6) were sufficiently heterozygous to be useful in estimating the evolutionary divergence of western and Chinese swine breeds.

Materials and Methods

DNA Extraction and MS. Genomic DNA was extracted (Paszek et al. 1997) from the semen of unrelated (no shared grandparents or great grandparents) Yorkshire ($n = 73$), Hampshire ($n = 42$), Landrace ($n = 32$), and Duroc ($n = 29$) breeds and the spleen and liver of 27 male and female Meishan pigs. The mutant (n) allele at the CRC1 locus was absent in all DNA samples. Characteristics of the nine SSC6 MS markers used in this study (S0035, Sw1057, S0087, Sw1067, Sw1129, Sw316, S0031, Sw353, and Sw824) are described in Paszek et al. (1995). The Hampshire breed is known to carry a mutated CRC locus at fairly high frequency with Yorkshire, Landrace, and Duroc animals showing a lower frequency of the homozygous recessive locus (between 1.4 and 1.9%) (Houde et al. 1993).

Statistical Analyses. Hardy-Weinberg equilibrium (HWE) was tested for each breed population using the HWE procedure of LINKAGE (Terwilliger and Ott 1994). The maximum likelihood estimate of $\theta = 4N_e\mu$ was used as a measure of genetic variation at each MS locus (Ewens 1979) where N_e is the effective population size, and μ is the mutation rate per generation. Genetic variation for each locus is a function of θ based on the assumption of random mating and finite population size. Homogeneity of θ estimates across swine breeds was tested using the likelihood ratio test (Paetkau and Strobeck 1994). A Pascal program (courtesy of Dr. Strobeck, University of Alberta) was used to estimate and test the homogeneity of θ.

A recent study of genetic distances between human populations (Perez-Lezaun et al. 1997) based on tetranucleotide MS stressed the importance of genetic drift and to a lesser extent mutation in estimating genetic distances. Therefore, genetic distance(s) between breeds was estimated with Nei's (1972) formula contained in the GENDIST procedure of the Phylogenetic Inference Package (PHYLIP) version 3.5c (Felsenstein 1993). A similar rate of mutation at each locus and equilibrium between mutation and genetic drift were assumed. A phylogenetic tree was constructed using the KITSCH (Fitch and Margoliash 1967) and UPGMA (Unweighted Pair Group Arithmetic Average Clustering) methods of PHYLIP. The robustness of tree topology was tested with 1,000 replicates of bootstrap resampling (Felsenstein 1995) from the original data. Time of breed divergence (t) was calculated based on the equation $D = 2\alpha t$ (Nei 1976) where D is the estimated genetic distance between specific breeds and α is the estimated MS mutation rate (4.5×10^{-4} in humans [Kwiatkowski et al. 1992] and 8×10^{-5} in swine [Ellegren 1995]).

The MS set used in this study was from the genetic map of SSC6 (Paszek et al. 1997). Since closely linked MS loci may not be inherited independently and result in biased genetic distance estimates our analysis was also run using haplotype frequencies estimated with the HAPLO program (Hawley and Kidd 1995) for all MS with markers whose distances were less than 10 cM (S0087 and Sw1067—7 cM apart, and S0031–Sw353–Sw824 with genetic map distances of 2 and 5 cM, respectively). The robustness of tree topology was again tested using 100 replicates of bootstrap resampling and tree topologies compared based on unhaplotyped and haplotyped MS markers.

Results

MS Alleles

Figure 1 shows the fragment length and number of alleles for each SSC6 MS analyzed. MS alleles unique to at least one breed were found for all MS except S0035 and Sw824. Seven alleles specific to western breeds were found at the Sw1057 locus. Five such alleles were identified at the MS Sw316 and Sw353 loci, four at the Sw1129 and S0031 loci, three at the Sw824, and one at the Sw1067 locus. Conversely, three S0031 alleles were unique to the Meishan breed. The number of SSC6 MS alleles for the Meishan breed ranged from three (for MS Sw1057, Sw1129, and S0031) to six (for Sw1067). The largest distribution of MS allele fragment size and number was observed for MS Sw1057 (between 140 and 184 base pairs and seven alleles for Hampshire and Landrace breeds, respectively) (Fig. 1). A range of three to seven alleles for MS Sw1057, S0087, Sw1067, Sw1129, Sw316, Sw353, and Sw824 is comparable to the six to eight alleles reported for the genetically diverse United States Meat Animal Research Center (USMARC) map reference population that included crosses between a Chester White, Large White, Landrace, Yorkshire composite breed and either Meishan, Fengjing, or Minzhu breeds (Rohrer et al. 1994). We have also identified an additional allele (220 base pairs) for MS S0087 found in Hampshire, Duroc, and Meishan breeds (Fig. 1). Haplotyping of S0087 and Sw1067 has resulted in a total of 49 alleles. Haplotyping of S0031, Sw353, and Sw824 provided 392 alleles.

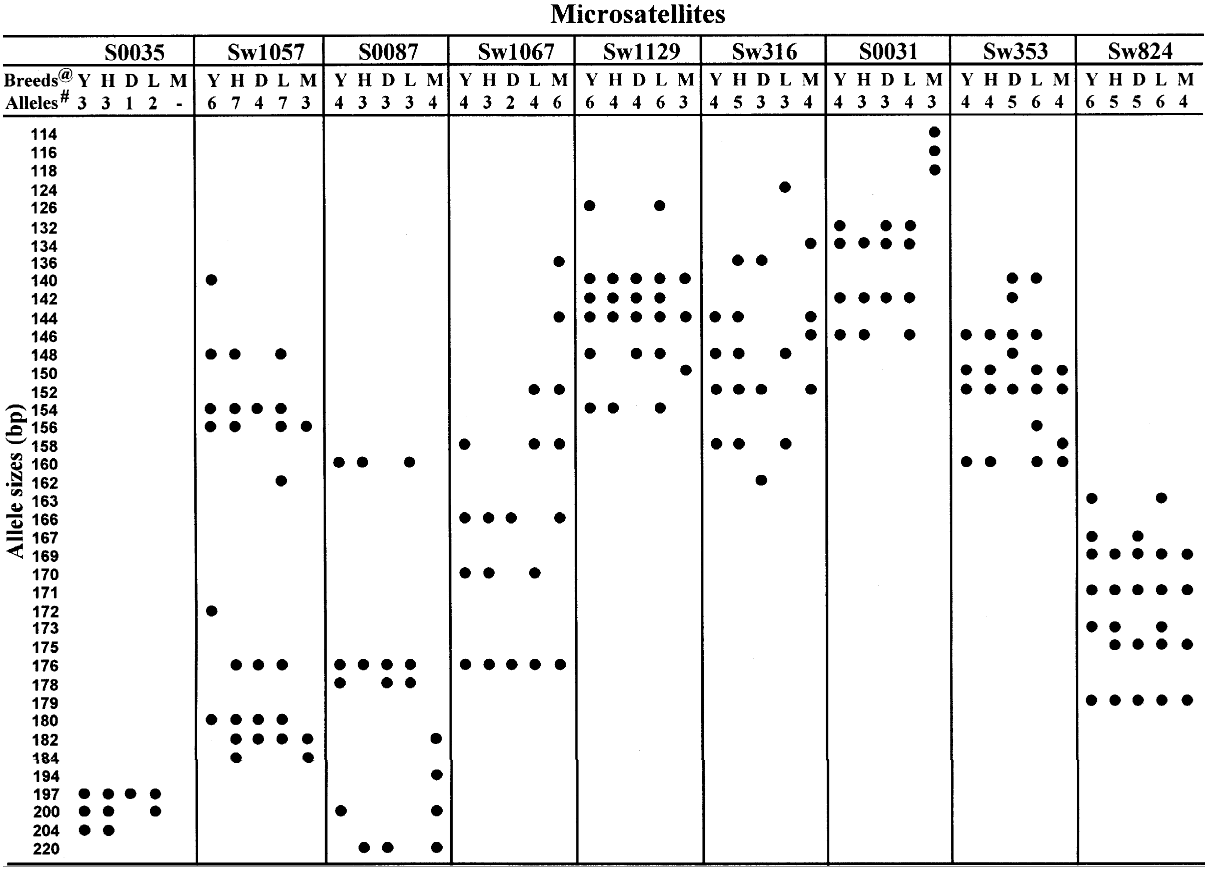
Genetic Variation

The within-breed χ^2 tests for Hardy-Weinberg equilibrium of genotypic frequencies resulted in significant evidence ($P < 0.001$) for a lack of equilibrium at the Sw824 locus in all breeds studied. No evidence for a lack of Hardy-Weinberg equilibrium was found for Sw1067 and Sw1129 loci. Disequilibrium of genotypic frequencies ($P < 0.05$) was found at five MS loci in Yorkshire and Hampshire breeds and at three MS loci in Meishan. The Duroc and Landrace breeds had only two MS loci that shared significant disequilibrium.

Estimates of genetic variation θ for each MS locus were compared using a χ^2 likelihood ratio test. Differences ($P < 0.1$) were found between breeds for MS Sw1067 (ranging from 0.282 to 2.086 for Duroc and Meishan, respectively), Sw1057 (0.636 to 2.465 for Meishan and Landrace, respectively), and S0035 (0.00 to 0.546 for Duroc and Hampshire, respectively). Similar estimates of θ across breeds were found for MS S0031 and Sw824. The largest average estimate of θ was found for Landrace, followed by Meishan, Hampshire, Yorkshire, and Duroc (1.289, 1.058, 0.957, 0.881, and 0.791, respectively). The presence of only one allele at the S0035 locus in Duroc (Fig. 1) resulted in a $\theta = 0$ (Table 1).

Genetic Distance

Table 2 presents genetic distances (Nei 1972) between swine breeds used in this study. The smallest genetic



[@] The following swine breeds (represented by first letter of the breed name) were included in this study: Y—Yorkshire (73 unrelated boars), H—Hampshire (42 unrelated boars), D—Duroc (29 unrelated boars), L—Landrace (32 unrelated boars) and M—Meishan (27 unrelated sows and boars).

[#] Number of observed alleles in studied breeds for specific chromosome 6 microsatellite. Animals of Meishan breed were not genotyped for microsatellite S0035.

Fig. 1. Number of alleles and molecular sizes for porcine chromosome 6 microsatellites observed in diverse breeds of swine[@].

Table 1. Homogeneity of maximum likelihood for estimates of θ^a in swine breeds

Microsatellite markers	Breeds						
	Yorkshire	Hampshire	Duroc	Landrace	Meishan[b]	Overall χ^2	*P* value
S0035	0.467	0.546	0.000	0.273	—	3.059	<0.08
Sw1057	1.363	2.145	1.016	2.465	0.636	3.406	<0.06
S0087	0.740	0.547	0.619	0.598	1.058	0.543	<0.46
Sw1067	0.740	0.547	0.282	0.976	2.086	4.402	<0.04
Sw1129	1.363	0.881	1.016	1.901	0.636	1.854	<0.17
Sw316	0.740	1.259	0.619	0.598	1.048	1.008	<0.32
S0031	0.740	0.547	0.619	0.976	0.636	0.410	<0.52
Sw353	0.740	0.881	1.477	1.906	1.048	1.789	<0.18
Sw824	1.039	1.259	1.477	1.906	1.313	0.834	<0.36
					Total	17.305	<0.05

[a] θ is a measure of variability at each locus in each population (Ewans 1979). A Pascal program provided by Dr. C. Strobeck (University of Alberta) was used for estimation of θ and χ^2 comparisons. Formulas for θ estimation and comparisons were reported by Paetkau and Strobeck (1994)

[b] Animals of Meishan breed were not genotyped for microsatellite S0035

distance (0.24) was between the Yorkshire and Hampshire breeds and the largest (1.34) between Hampshire and Meishan. Genetic distances between western breeds were quite similar (0.24 to 0.53), although the calculated distances between the Yorkshire and Duroc and the Landrace and Duroc breeds were twice as large as the distance between Yorkshire and Hampshire. Overall genetic distances between Meishan and western breeds

Table 2. Genetic distances and estimated time of breed diversion for swine breeds

Swine breeds	Yorkshire	Hampshire	Duroc	Landrace	Meishan
Yorkshire	—	0.2354[a]	0.5338[a]	0.3828[a]	1.2214[a]
Hampshire	391[b] 2,206[c]	—	0.4568[a]	0.4296[a]	1.3369[a]
Duroc	889[b] 5,004[c]	760[b] 4,283[c]	—	0.5337[a]	1.3056[a]
Landrace	637[b] 3,589[c]	715[b] 4,028[c]	889[b] 5,003[c]	—	1.1868[a]
Meishan	2,035[b] 11,451[c]	2,227[b] 12,533[c]	2,176[b] 12,240[c]	1,977[b] 11,126[c]	—

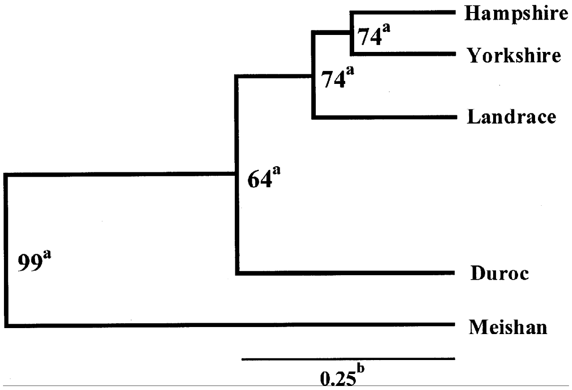
[a] Genetic distances
[b] Time of breed divergence (in years) based on the MS mutation rate of 4.5 × 10^{-4} (Kwiatkowski et al. 1992)
[c] Time of breed divergence (in years) based on the MS mutation rate of 8 × 10^{-4} (Ellegren 1995)

were large (1.18 to 1.34), indicating that breed divergence between these populations occurred much earlier than divergence among western breeds. This finding is not surprising based on the phenotypic differences between western breeds and Meishan, distant geographical location, and significant differences in the selection of performance traits (Li and Enfield 1989). All genetic distance estimates based on the haplotyped MS were similar.

Time of Breed Divergence and Evolutionary Tree

Estimates of the time of breed divergence were calculated for separation from a common ancestor (Nei 1976) and are presented in Table 2. A mutation rate of 4.5 × 10^{-4} per MS locus (Kwiatkowski et al. 1992) and an average generation interval of 1.5 years were used in these calculations. Estimates of the time of breed divergence ranged from 391 years for Yorkshire and Hampshire to 2,227 years between Yorkshire and Meishan. The time of breed divergence between western breeds ranged from 391 to 889 years. When a mutation rate of 8 × 10^{-5} for porcine MS (Ellegren 1995) was substituted, estimates for breed divergence ranged from 2,206 to 5,004 years for separation of western breeds and 11,126 and 12,533 years for the separation of western from Meishan breeds.

A phylogenetic tree is presented in Fig. 2. A closer tree location was observed between British breeds and the Landrace than between the British and the North American Duroc, based on average genetic distance (0.406 vs 0.495, respectively). As shown, the Meishan and western pig breeds were placed into separate tree branches. Tree topology was the same based on either the KITSCH or UPGMA method as well as after 1,000 bootstrap resamplings of the initial allele frequency data. A similar type of tree emerged based on resampled data and expressed as a percentage (Fig. 2). There was no change in the tree topology when either haplotyped or nonhaplotyped MS genotypes were used. Analysis of 100 bootstrap replicates of the haplotyped MS data confirmed this result.



[a] The percent occurrence in 1000 bootstrap replicates.
[b] The scale presents arbitrary genetic distances.

Fig. 2. Swine phylogenetic tree.

Discussion

Several studies have compared growth and carcass performance between western breeds of swine and the highly prolific Chinese breeds (Li and Enfield 1989; Bidanel et al. 1991; Yen et al. 1991; White et al. 1993). Western breeds are reported to be larger in body mass, to have increased growth rates, and to have slowed maturation in comparison to the Meishan breed. Allelic differences for three of the nine SSC6 loci presented in Fig. 1 identified breed differences ($P < 0.1$) for maximum likelihood estimates of genetic variance (θ) as a function of the number of observed alleles and the population size (Table 1). The largest ($P < 0.05$) breed differences for θ were found for the Sw1067 locus. The six Sw1067 alleles present in Meishan swine resulted in the largest measure of genetic variation for this breed, while the Duroc breed had the lowest measure of genetic variation at the Sw1067 locus, with only two observed alleles.

These results provide initial molecular evidence for breed differences at a region of SSC6 associated with an agriculturally important trait.

Estimates of genetic distance and time of breed divergence as well as the phylogenetic tree (Fig. 2) suggest that western swine breeds evolved from a common genetic ancestor. Breeds of European origin (Yorkshire, Hampshire, and Landrace) were genetically the closest. Consolidation of genetic distance estimates (Fig. 2) is in agreement with the cluster analysis of phenotypic data for western and Chinese pig breeds reported by Li and Enfield (1989). Similar results have been obtained for other domesticated species. Buchanan et al. (1994) constructed a phylogenetic tree for sheep and placed breeds of British origin into a common branch, while sheep breeds from the Middle East and Australia were placed into a separate branch of the phylogenetic tree. A recent study of human differentiation (Perez-Lezaun et al. 1997) presented four neighbor-joining (PHYLIP) evolution trees for 16 human populations based on four different genetic distance measures. Chinese and Northern European groups were placed on opposite sides of every tree. Additional analyses of data from this study using the neighbor-joining method on unhaplotyped and haplotyped data sets produced similar trees, with Chinese Meishan and European pig breeds located on opposite sides.

The magnitude of the estimates for breed divergence is directly related to assumed mutation rate. The larger the mutation rate the smaller the time estimate to breed divergence. Breed divergence estimates for western breeds based on the MS mutation rate of 8×10^{-5} reported by Ellegren (1995) were much larger than the literature estimates (approximately 400 vs at least 2,200 years). Estimates of divergence between western and Meishan breeds based on the lower mutation rate were larger than 11,000 years and do not appear compatible with historical records regarding swine breeding. The estimate of 391 years for the separation of the Yorkshire and Hampshire breeds is similar to written records from the late 1700s documenting the development of Hampshire and Yorkshire breeds (Porter 1993). Estimates of divergence obtained in this study compare favorably to the estimate of 1,094 years for divergence of British sheep breeds from Australian Merino reported by Buchanan et al. (1994).

In summary, we have identified SSC6 MS alleles specific to western and the Chinese Meishan pig breeds and provided evidence for larger genetic variation in the Meishan than in the highly selected Yorkshire, Hampshire, and Duroc breeds. A phylogenetic tree revealed separate branches for Meishan and breeds of European origin where breeds of British origin were included in a common sub-branch of the phylogenetic tree. Estimates of breed divergence were comparable to the historical documents and estimates obtained for other livestock species. Our observations suggest that MS loci (potentially) linked on a single chromosome provide sufficient resolution to provide a robust estimate of breed evolution within one animal specie.

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