Unexpectedly High Allelic Diversity at the *KIT* Locus Causing Dominant White Color in the Domestic Pig

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ABSTRACT

Mutations in *KIT* encoding the mast/stem cell growth factor receptor (MGF) are responsible for coat color variation in domestic pigs. The dominant white phenotype is caused by two mutations, a gene duplication and a splice mutation in one of the copies leading to skipping of exon 17. Here we applied minisequencing and pyrosequencing for quantitative analysis of the number of copies with the splice form. An unexpectedly high genetic diversity was revealed in white pigs. We found four different *KIT* alleles in a small sample of eight Large White females used as founder animals in a wild boar intercross. A similar number of *KIT* alleles was found in commercial populations of white Landrace and Large White pigs. We provide evidence for at least two new *KIT* alleles in pigs, both with a triplication of the gene. The results imply that *KIT* alleles with the duplication are genetically unstable and new alleles are most likely generated by unequal crossing over. This study provides an improved method for genotyping the complicated *Dominant white/KIT* locus in pigs. The results also suggest that some alleles may be associated with negative pleiotropic effects on other traits.

THERE has been selection for white-colored domestic pigs at least since medieval times (Wiseman 1986). White pigs with pigment spots are usually eliminated from breeding in white breeds like Landrace and Large White. Despite a strong selection for white color for at least 100 years breeders have not been able to completely fix the desired phenotype, white coat without pigment spots. Our studies of the inheritance of the dominant white coat color in an intercross between the European wild boar and Large White domestic pigs have revealed that the dominant white phenotype is caused by the combined effect of two mutations in *KIT*, one duplication of the entire coding sequence and one splice mutation (Johansson *et al.* 1992; Johansson Moller *et al.* 1996; Marklund *et al.* 1998).

KIT encodes the mast/stem cell growth factor receptor (MGF). Normal expression of *KIT* and its ligand MGF is essential for migration and survival of neural-crest-derived melanocyte precursors. Mutations in this gene cause pigmentation disorders in mice, called *Dominant white spotting/W* (Chabot *et al.* 1988; Geissler *et al.* 1988), and in humans, called piebald trait (Fleischman *et al.* 1991; Giebel and Spritz 1991). Structural *KIT* mutations in mice are often lethal or sublethal in the homozygous form, exhibit pleiotropic effects on the development of melanocytes, hematopoietic cells, primordial germ cells, and interstitial cells in the small intestine, and may affect hearing.

Four alleles have so far been identified at the porcine *Dominant white/KIT* locus: the recessive *i* allele for normal color, the semidominant I^P allele for the Patch phenotype, the fully dominant *I* allele for the Dominant white phenotype, and I^{Be} for the dominant Belt phenotype. The Patch phenotype has white and fully colored patches separated by sharp borders. It has been shown that the *I* and I^P alleles are both associated with a duplication of *KIT* (Johansson Moller *et al.* 1996). The size of the duplication is ~450 kb and includes the complete coding sequence (our unpublished results). The duplication most likely acts as a regulatory mutation. This could be a simple dosage effect due to the expression of two gene copies or could be because the duplicated copy lacks some regulatory elements and is dysregulated. The altered *KIT* expression may affect ligand availability, which in turn disturbs the migration of melanocyte precursors. The high sequence identity between the two *KIT* copies (>99%) is consistent with the duplication being a recent event, which is likely to have occurred after domestication (Marklund *et al.* 1998). In addition to the duplication, the *I* allele has a splice mutation—a G to A substitution—in the first nucleotide of intron 17 in one *KIT* copy (Marklund *et al.* 1998). This splice mutation disrupts the highly conserved GT dinucleotide at the 5′ splice site, leading to skipping of exon 17, and is therefore a structural mutation. Exon 17 encodes 41 amino acids of a highly conserved region of tyrosine kinases, comprising the catalytic loop and

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parts of the activation loop (Hubbard *et al.* 1994). There is clear evidence that the receptor form with splice mutation is expressed in a variety of cells in white pig embryos and we assume that this mutant receptor has normal ligand binding but absent tyrosine kinase activity (Marklund *et al.* 1998). A reduced number of white blood cells in *I/I* homozygous pigs was also observed, suggesting mild pleiotropic effects on hematopoiesis. The Belt phenotype constitutes a white belt across the shoulders and forelegs. The I^{Be} allele does not contain the duplication or the splice mutation, and no suggestive causative mutation was identified by sequencing the entire coding sequence (Giuffra *et al.* 1999). We assume that *Belt* is due to a regulatory *KIT* mutation.

It is difficult to genotype the *KIT* locus in pigs since the only known difference between some genotypes is quantitative rather than qualitative. The difference between the I/I^{P}, *I/i*, and *I/I* genotypes is that the ratio between the splice mutation and the normal form at the first nucleotide of intron 17 is 25, 33, and 50%, respectively. The objective of the present study was to apply pyrosequencing (Ronaghi *et al.* 1998) and minisequencing (Syvänen *et al.* 1993) for quantification of the ratio of the wild-type/mutant nucleotide at the splice site.

MATERIALS AND METHODS

Animals: An intercross pedigree comprising 2 European wild boar and 8 Large White founders, 23 F_1, and 178 F_2 animals was used. This pedigree has been extensively used for studies on coat color genetics (Johansson *et al.* 1992; Johansson Moller *et al.* 1996; Mariani *et al.* 1996; Kijas *et al.* 1998; Marklund *et al.* 1998). The distribution of *KIT* alleles in commercial populations was investigated using samples of 33 Swedish Large White and 48 Swedish Landrace pigs.

PCR amplification: Parts of exon 17 and intron 17 of *KIT* were amplified using the PCR primers KIT21 5′-GTATTCACA GAGACTTGGCGGC-3′ and KIT35 5′-AAACCTGCAAGGAA AATCCTTCACGG-3′ (Marklund *et al.* 1998). Primer KIT35 was 5′-biotinylated to allow capture of the PCR products onto avidin-coated solid supports. PCR reactions were carried out in a total volume of 50 μl containing 40 ng genomic DNA, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 μM dNTPs, 1.25 units AmpliTaq Gold DNA polymerase (PE Applied Biosystems, Foster City, CA), and 10 pmol of both forward and reverse primer.

Pyrosequencing: Biotinylated PCR product (25 μl) was immobilized onto streptavidin-coated paramagnetic beads (Dynal AS, Oslo) using binding-washing buffer (5 mM Tris-HCl, 1 M NaCl, 0.5 mM EDTA, 0.05% Tween 20, pH 7.6) in a total volume of 90 μl at 43° for 30 min. Single-stranded (ss) DNA was obtained by incubating the immobilized PCR product in 50 μl of 0.5 M NaOH for 1 min and washing the beads once in 100 μl of binding-washing buffer. A total of 15 pmol of detection primer KitSeq (5′-TAATTAC*X*TGGTCAAAGGAA AC-3′, *X* represents inosine), designed with its 3′ end immediately upstream of the splice mutation, was allowed to hybridize onto ssDNA in 40 μl of annealing buffer (20 mM Tris-Acetate, 5 mM $MgAc_2$, pH 7.6) at 80° for 2 min with subsequent cooling down to room temperature. Pyrosequencing was carried out using the PSQ96 instrument and the SNP Reagent kit containing dATPαS, dCTP, dGTP, dTTP, enzyme mixture (DNA polymerase, ATP sulfurylase, luciferase, and apyrase), and substrate mixture (APS and luciferin; Pyrosequencing AB, Uppsala, Sweden). The result of the pyrosequencing assay was expressed as the ratio between the signals from the incorporated dATPαS and dGTP.

Minisequencing: Four 10-μl aliquots of each PCR product were mixed with 40 μl of binding buffer (50 mM phosphate buffer, pH 7.5, containing 0.15 mM NaCl and 0.1% Tween 20) in streptavidin-coated microtiter plate wells (Combiplate 8, Labsystems, Helsinki, Finland) and incubated at 37° for 1.5 hr in a shaker (Thermomix 1415, Labsystems). The wells were washed with 40 mM Tris-HCl (pH 8.8), 1 mM EDTA, 50 mM NaCl, and 0.1% Tween 20 in an automatic plate washer (Wellwash, Labsystems). The nonbiotinylated strand of the PCR product was removed by denaturation with 60 μl of 0.1 M NaOH for 3 min. After washing as above, 50 μl of a minisequencing reaction mix, containing DNA polymerase buffer, 0.2 units of *Taq* polymerase (PE Applied Biosystems), 0.1 μCi of [^{3}H]dATP (TRK 633, 57–76 Ci/mmol) or [^{3}H]dGTP (TRK 627, 24–34 Ci/mmol; Amersham Pharmacia Biotech, Amersham, England), and 10 pmol of the detection primer KitSeq, was added to the wells. The plates were incubated at 50° for 10 min. The unincorporated label was removed by washing as above, and the sequencing primers were released with 100 μl of 0.1 M NaOH and measured in a liquid scintillation counter (1414, Wallac, Turku, Finland). The result of the minisequencing assay was expressed as the average ratio between the signals from the incorporated [^{3}H]dATP and [^{3}H]dGTP from duplicate assays.

Relative quantification of *KIT* copy number using real-time PCR: The copy number of *KIT* in different genotypes was estimated as previously described (Giuffra *et al.* 1999) using the comparative C_T method based on PCR amplification of the target *KIT* gene and the single copy control gene (*ESR*, estrogen receptor gene) in separate tubes. The PCR primers for *KIT* were forward 5′-CTACCTTTGCCATACCATGCA TTT-3′ and reverse 5′-TTGCATGCCCTCTAATTACACAATT-3′ and, for *ESR*, forward 5′-GCAGCTGCCAACCTATTCCA-3′ and reverse 5′-TGGGTTTAGGATGCAGCATTG-3′. The PCR reaction was performed using the ABI7700 instrument (PE Applied Biosystems) in 25-μl reaction volumes using the TaqMan universal PCR Master Mix (PE Applied Biosystems). The *KIT* specific TaqMan probe 5′-TGCAAAAGCACACTTCATC TGACGGCT-3′ was labeled with 6-carboxy fluorescein at its 5′ end and the *ESR* specific probe 5′-CATCTGCACCCTACAC CACAGCTCACA-3′ was labeled with VIC at its 5′ end. The time and temperatures in the thermal cycling were an initial 2-min hold at 50° and a 10-min hold at 95° for AmpErase and AmpliTaq Gold activation, respectively (PE Applied Biosystems), followed by 40 cycles of 15 sec at 95° and 1 min at 60°. Duplicate DNA samples were tested for each animal.

RESULTS

Segregation analysis of *Dominant white/KIT* alleles in the wild boar/Large White intercross reveals additional *KIT* alleles: Minisequencing and pyrosequencing were used to determine the ratio between the *KIT* sequence containing the splice mutation and the *KIT* sequence with the normal nucleotide at the first position in intron 17 in all animals in the wild boar/Large White intercross. The following four groups of ratios were expected: 0% splice variant (i/i, I^{P}/i), 25% (I/I^{P}), 33% (I/i), and 50% (I/I). By plotting the ratios obtained by the two methods, clusters consistent with our previous interpretation

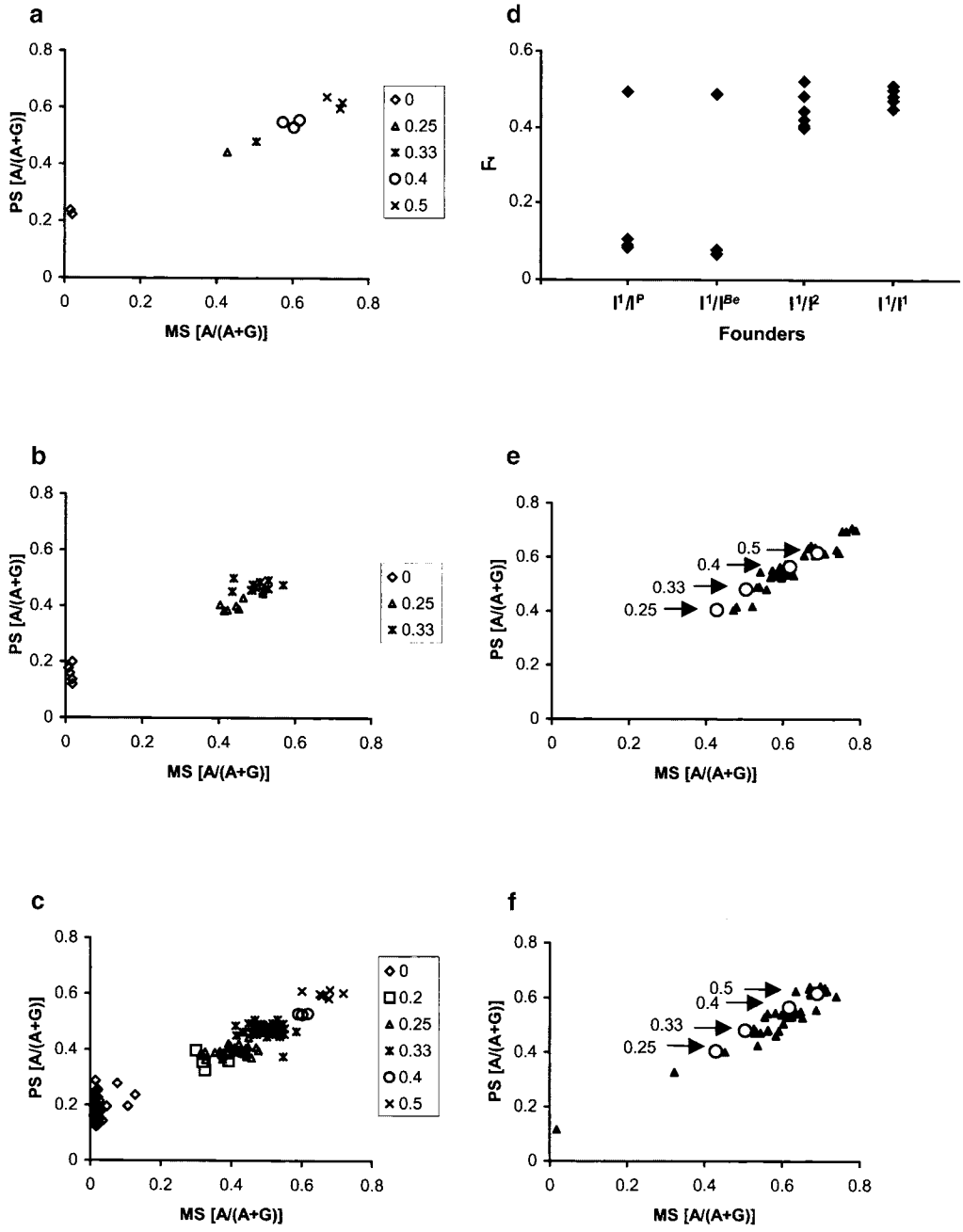

FIGURE 1.—Quantitative analysis estimating the ratio [A/(A + G)] of splice mutation (A) to normal (G) at the first nucleotide of intron 17 in the *KIT* gene using pyrosequencing (PS) and minisequencing (MS) in a wild boar/Large White intercross (a–d) and in commercial populations of Large White and Landrace pigs (e–f). The symbols represent the estimated ratio for the splice mutation (a–c). There is not a 1:1 relationship between the observed ratio and the true ratio since the efficiency of the incorporation of A and G nucleotides is not absolutely identical; the explanation for the animals with 0% splice mutation showing a ratio of ~20% using pyrosequencing is that the A nucleotide to some extent is used as a substrate in the enzymatic reaction. (a) The founder animals (W1–W10), $n = 10$. (b) F_1 animals, $n = 23$. (c) F_2 animals, $n = 178$. (d) The average splice ratio [(PS + MS)/2] for F_1 animals plotted according to the presumed KIT genotype of founder animals. (e) Large White ($n = 33$) and (f) Landrace ($n = 48$). The controls (indicated by circles) are from the European wild boar/Large White intercross and the ratio of the splice form has been deduced with great confidence for these selected animals.

of the composition of *KIT* alleles in this pedigree were observed (Figure 1). However, clear evidence for additional allelic heterogeneity was observed. The founder animals (Figure 1a) were assigned to five different clusters on the basis of the observed ratios and segregation data: 0% splice, the two wild boars being *i/i*; 25% splice, one Large White sow (W8) being heterozygous for the *Patch* allele I/I^P; 33%, a single Large White sow (W6) being heterozygous for an allele carrying a single *KIT* copy and no splice mutation; 40%, three females that were heterozygous for a new allele with three *KIT* copies; 50%, only three out of eight Large White sows appeared to be homozygous *I/I*.

The transmission of the I^P allele from the W8 female was evident from the analysis of F_1 animals (Figure 1d, W8 = I^1/I^P). The interpretation that the W6 female carried a novel allele with only one copy and no splice mutation was confirmed by our observation that two of its F_1 progeny and a proportion of its F_2 grand progeny did not carry the splice mutation at all. We can conclude that this allele is not identical to the wild-type allele (*i*) since none of the F_2 animals carrying this allele showed the wild-type color. Since we cannot formally exclude the possibility that this allele is identical to the *Belt* allele (I^{Be}), we suggest that it be given the same allele designation until molecular characterization or informative

pedigree material can reveal whether these are two distinct alleles. This allele was recessive to *Dominant white* since I/I^{Be} heterozygotes were white. The F_2 animals, being heterozygous I^{Be}/i, showed two different phenotypes due to interaction with the *Extension/MC1R* locus segregating in this cross (see Kijas *et al.* 1998; Giuffra *et al.* 1999). F_2 animals with the genotype I^{Be}/i, $E^+/-$ showed a distinct Roan phenotype characterized by white hairs intermingled with pigmented hair whereas F_2 animals with the genotype I^{Be}/i, E^p/E^p were predominantly white with some black spots.

Three of the Large White founder sows (W4, W5, and W10) showed a proportion of A *vs.* G at the splice site of ~40%, clearly distinct from the 50% expected for *I/I* homozygotes (Figure 1a). This observation, together with the observed segregation data described below, showed that these founders were heterozygous for a variant *Dominant white* allele designated I^2 with three copies of *KIT*, only one of which carries the splice mutation; the I^1 allele has two copies of *KIT*, one normal and one with the splice mutation. The F_1 progeny from the I^1/I^2 founders tended to form two groups with 25 and 33% of A, whereas the F_1 progeny from I^1/I^1 homozygotes were found in the 33% cluster only (Figure 1d). This interpretation was supported by a significantly higher average splice ratio among progeny from I^1/I^1 founders ($x = 0.48 \pm 0.006$, $n = 8$) than from I^1/I^2 founders ($x = 0.43 \pm 0.015$, $n = 8$; $P = 0.02$, Student's *t*-test). Further support for the presence of the I^2 allele was obtained by comparing the splice ratio of I^1/i ($x = 0.49 \pm 0.003$, $n = 48$) and I^2/i ($x = 0.40 \pm 0.007$, $n = 16$) F_2 animals, for which the genotype had been predicted using the assumed genotype of founders and F_1 animals in combination with the segregation data on linked genetic markers previously typed in this pedigree (Marklund *et al.* 1996). The difference in average splice ratio between the two groups was highly significant ($P \ll 0.001$, Student's *t*-test).There was no clear phenotypic difference between the two forms of the *Dominant white* allele.

In general, there was a good agreement between the predicted and observed ratio of the splice mutation among the F_2 animals (Figure 1c). The extensive variation in the splice ratio was interpreted to reflect the presence of six different classes of splice ratios: 0% (all possible genotype combinations of the *i*, I^{Be}, and I^P alleles), 20% (I^2/I^P), 25% (I^2/i, I^2/I^{Be}, and I^1/I^P), 33% (I^1/i, I^1/I^{Be}, and I^2/I^2), 40% (I^1/I^2), and 50% (I^1/I^1). The assignment of F_2 individuals into the various classes in Figure 1c was based on both the observed splice ratio and segregation data.

The constitution of the observed *KIT* alleles is compiled in Figure 2. The order along the chromosome of the different copies with or without the splice mutation has not been determined; this is difficult because the duplication is very large (~450 kb) and the different copies show a very high sequence identity (our unpublished results).

The variability in gene copy number among *KIT* alleles is confirmed by quantitative real-time PCR analysis: To exclude the possibility that part of the observed variability in the ratio of the splice mutation is due to a biased PCR amplification (*e.g.*, due to a polymorphism in a primer site), we tested our interpretation of the number of gene copies in different *KIT* alleles using real-time PCR analysis. The test was carried out by amplifying *KIT* and a single copy control sequence (*ESR*). The copy number of *KIT* and *ESR* sequences in samples of genomic DNA correlates with the Ct values, which are estimates of the number of cycles needed to reach a given fluorescence threshold. The difference in Ct(ESR) and Ct(KIT) was plotted against the predicted number of *KIT* copies in different genotypes according to our interpretation of all animals in the wild boar intercross (Figure 3). Although there was a large overlap between genotype classes, the Ct(ESR)-Ct(KIT) difference showed a highly significant positive correlation to the predicted copy number ($P < 0.0001$). The estimated means for the Ct difference for different genotype classes were as follows (means ± SE): two copies, -0.57 ± 0.17; three copies, -0.18 ± 0.13; four copies, 0.36 ± 0.17; five copies, 1.19 ± 0.40. This result is in good agreement with the theoretical expectation of a ΔCt value of −1.0 when the copy number of a DNA sequence is doubled. The results confirm our interpretation of variation in copy number among *KIT* alleles and the existence of a *KIT* triplication. The large overlap in ΔCt values between genotype classes makes this assay unsuitable for genotyping, at least with the experimental procedures used in this study.

Extensive allelic diversity in commercial white populations: Our observation of the presence of at least four different alleles (I^1, I^2, I^{Be}, and I^P) at the *KIT* locus among only eight Large White founder animals prompted us to investigate the allelic diversity in commercial white populations. Genomic DNA samples from 33 Swedish Large White pigs and 48 Swedish Landrace pigs were subjected to pyrosequencing and minisequencing analysis (Figure 1, e and f). The results revealed a considerable allelic diversity in both populations. It is not possible to deduce exactly which alleles are segregating in these two populations without any pedigree data, but it is obvious that alleles without the splice mutation (I^P, I^{Be}, and/or *i*) are segregating in both populations. Evidence for a sixth allele at the *Dominant white/KIT* locus was obtained since four Large White animals showed a significantly higher ratio of the splice mutation (~60%) than any of the genotype combinations formed by the alleles described above. Real-time PCR analysis using *KIT* and *ESR* indicated that these four animals carried five copies of *KIT*. We therefore postulate that they are heterozygous for a *KIT* allele with three gene copies of which two carry the splice mutation. We have

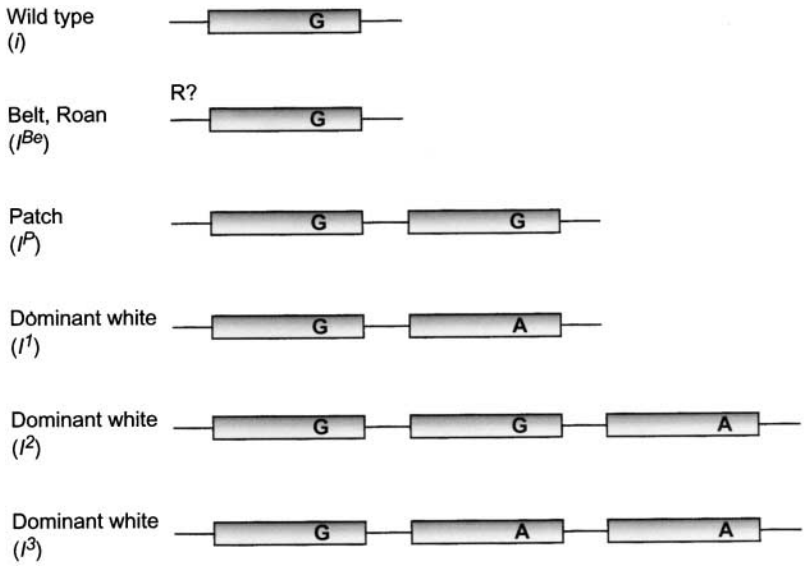

FIGURE 2.—Schematic description of *Dominant white/KIT* alleles in the pig. The duplication is ~450 kb. G and A reflect the normal and splice mutation, respectively, at nucleotide 1 in intron 17. R? indicates that we have postulated that the *Belt* allele is due to a regulatory mutation. It is possible that the Belt and Roan phenotypes are controlled by different alleles, both containing a single copy of *KIT* without the splice mutation (see text). We have not observed the phenotype associated with the I^3 allele but it is most likely Dominant white. The relative order of *KIT* copies (with and without the splice mutation) has not yet been established.

designated this allele I^3 (Figure 2). Two of the four animals carrying the I^3 allele were half-sibs and all four shared a common grandsire, suggesting that they had inherited I^3 from this common ancestor.

If we assume that the animals in the 50% cluster are homozygous I^1/I^1, a rough estimate of the frequency of this allele can be obtained as the square root of the frequency of this genotype class. This gives allele frequency estimates of 0.49 and 0.58 for I^1 in these Large White and Landrace populations, respectively. These are most likely slight overestimates since other possible genotype classes also give a 50% ratio.

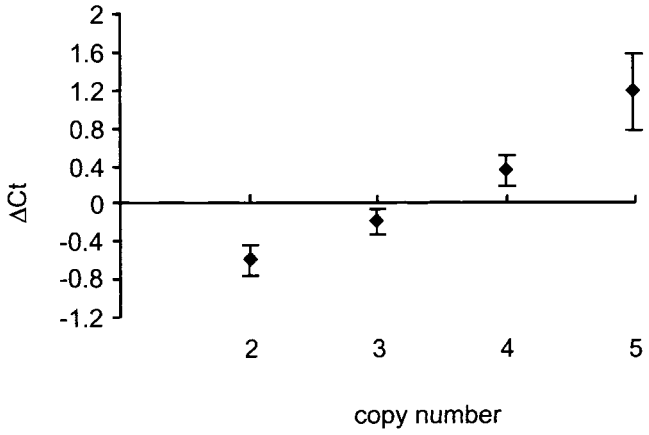

FIGURE 3.—Relative quantification of genomic copy numbers of *KIT* with real-time PCR using *ESR* as a single copy control. The material comprises a European wild boar/Large White intercross: founders, $n = 10$; F_1, $n = 23$; F_2, $n = 178$. The *x*-axis represents the predicted copy number using the quantification of the splice mutation and family segregation analysis. The *y*-axis represents the Ct(ESR)-Ct(KIT), reflecting the relative difference in copy number of *KIT* and *ESR* in genomic DNA samples. The data points are ΔCt ± SE.

DISCUSSION

This study revealed an extensive genetic diversity at the *Dominant white/KIT* locus in the Swedish Landrace and Large White breeds. This seems to be a general feature in populations carrying the *Dominant white* allele since limited screening of several other independent commercial white lines revealed a very similar degree of variation in the ratio of the splice mutation (our unpublished results). These findings were unexpected considering the strong selection for white color for more than 100 years. It was also unexpected from the literature on pig coat-color genetics in which it is assumed that white breeds are homozygous *I/I* (*e.g.*, LEGAULT 1998). However, the result is entirely consistent with the common observation of a variability in coat color among progenies from matings between white (*I/I*) and colored (*i/i*) commercial lines even though all progeny are expected to be heterozygous *I/i* and white. The present study clearly indicates that the *Dominant white/KIT* locus is genetically unstable. The reason for this is most likely that the duplication is large (~450 kb) and that the two copies show a very high sequence identity (>99%) facilitating the generation of new alleles by unequal crossing over and possibly by gene conversion. This is a very well-documented phenomenon for tandemly duplicated DNA fragments (OHTA 1990). For instance, unequal crossing over between the tandem copies of the genes for red/green eye pigment genes on the human X chromosome has generated haplotypes associated with color blindness (NEITZ and NEITZ 1995).

We have now documented at least six different *Dominant white/KIT* alleles. It is an open question whether the alleles associated with the Belt phenotype in Hampshire pigs and the Roan phenotype in our wild boar intercross are identical. Both alleles contain a single *KIT* copy

without the splice mutation. We have designated the allele associated with these two phenotypes I^{Be} to be conservative and will not introduce a new allele designation without compelling evidence for the allele being distinct from previously described alleles. The reason for our caution is that the phenotypic expressions of *KIT* alleles show interaction with other genes, in particular the *MC1R/Extension* locus (Marklund *et al.* 1998; Giuffra *et al.* 1999; this study). We have so far not observed the Belt-associated allele and the Roan-associated allele on the same genetic background.

It is possible that the presence of multiple *KIT* alleles in white pig breeds is explained simply by a high mutation rate. However, balancing selection may contribute to the maintenance of allelic diversity. It is well documented in the mouse that structural *KIT* mutations are associated with pleiotropic effects on hematopoiesis and fertility and that loss-of-function homozygotes are lethal (Jackson 1994). It is very likely that the splice mutation present in *I* alleles is a complete loss of function as regards *KIT* signaling since certain missense mutations in the corresponding exon in the mouse are nonfunctional and homozygous lethal. We have reported that *I/I* homozygotes had a lower number of white blood cells than *I/i* and *i/i* animals in our wild boar intercross, suggesting that the *I* allele is associated with mild negative effects on hematopoiesis (Marklund *et al.* 1998). It will therefore be of considerable interest to investigate hematopoietic parameters and possibly fertility traits among different *KIT* genotypes, in particular the phenotypic effect in I^3/I^3 homozygotes in which 66% of the expressed KIT protein is expected to possess the splice form lacking 41 amino acids of the tyrosine kinase domain. It is also possible that an allele containing a single *KIT* copy and the splice mutation occurs at a low frequency in some white populations and this allele is expected to be homozygous lethal.

One of the objectives of this study was to compare the power of minisequencing and pyrosequencing for resolving small differences in the splice ratio. The minisequencing assay has previously been applied to distinguish between one, two, and three copies of an allele on human chromosome 4 (Laan *et al.* 1995) and to accurately quantify alleles present in ratios ranging from 1 to 99% in pooled DNA samples (Olsson *et al.* 2000). This study shows that the pyrosequencing method also provides an excellent quantification of the incorporated nucleotides and there was a very good agreement between the two methods. In future applications it will be sufficient to use one of these methods but we recommend that duplicate samples from two independent PCR reactions be analyzed. The methods described in this study are major improvements with regard to *KIT* genotyping. Neither real-time PCR nor quantification of PCR-restriction fragment length polymorphism fragments that have previously been used for quantitative analysis of *KIT* alleles is able to resolve the allelic diversity to the same extent as reported here. The diagnostic test can be used to ensure that white boars are homozygous *I/I* and thus also breed true for white color in crosses with colored lines; in many markets there is a strong consumer preference for pig meat with white skin. However, the test described here is not able to resolve all possible genotype combinations. A further improvement in resolution would be obtained by adding an accurate quantification of the *KIT* copy number. Real-time PCR in our hands has not given sufficient resolution to reliably quantify the copy number of individual animals although highly significant differences are observed between groups of animals (Figure 3). We have recently cloned the *KIT* duplication breakpoint (our unpublished results), allowing us to establish a test for the presence/absence of the duplication breakpoint, and a test for quantification of the copy number is under development. However, even with this improvement, there will be some genotype combinations (*e.g.*, I^3/i *vs.* I^2/I^2; see Figure 2) that will require pedigree information to be resolved.

The present study implies that the genetic instability at the *KIT* locus causes a cost in pig breeding because part of the selection potential is devoted to maintaining the white color. The economic consequences are probably small in each generation but could be substantial when summed over many generations.

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