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# Genetic analysis of the diabetes-prone C57BLKS/J mouse strain reveals genetic contribution from multiple strains ☆

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**Abstract**

The C57BLKS/J (BKS) inbred mouse strain is a widely used animal model of type 2 diabetes. In the presence of the *diabetes* (*db*) mutation, obese BKS-*db* mice develop severe diabetes. Genetic studies of diabetes-susceptibility in this strain are facilitated by the fact that BKS is a genetic composite between the diabetes-resistant C57BL/6J (B6) and susceptible DBA/2J (DBA) strains. On this basis, it has been hypothesized that diabetes-susceptibility in BKS is conferred by DBA-derived alleles. However, recent studies revealed non-B6/non-DBA genetic material in BKS. To identify the origin of this genetic component, we generated a genomic map of BKS using 537 microsatellite markers. Our results demonstrate that, in addition to B6 and DBA, BKS contains alleles from at least three other strains, including 129, C57BL/10 and an unidentified mouse strain. We also analyzed two congenic strains, B6-*db* and BKS-*db*, which are widely used for the genetic mapping of diabetes-susceptibility loci. We identified several donor-derived genomic regions introduced during the generation of these congenic strains. In summary, our study reveals novel aspects of the genetic fine-structure of BKS and related strains and facilitates the identification of diabetes-susceptibility loci in this mouse model.


*Keywords:* Type 2 diabetes mellitus; Animal model; C57BLKS mouse strain; Leptin receptor mutation; Microsatellite marker

## 1. Introduction

Obesity is recognized as a major risk factor for the development of type 2 diabetes. However, only a subset of obese individuals is diabetic, whereas the rest remain disease free [1]. Despite the biomedical importance of these diseases, the factors determining diabetes-susceptibility in obese individuals are still unknown.

As inbred strains of mice show markedly different susceptibilities to obesity-induced diabetes, the mouse is an excellent model organism for genetic studies of this disease [2]. One of the best-characterized models involves the C57BL/6J (B6) and C57BLKS/J (BKS) strains with the homozygous *diabetes* (*db*) mutation, a genetic defect in the leptin receptor gene [3]. Obese B6-$Lepr^{db}$/$Lepr^{db}$ (B6-*db*) mice exhibit well-compensated diabetes characterized by mildly elevated glucose levels, hyperinsulinemia, and pancreatic islet hypertrophy. In contrast, similarly obese BKS-$Lepr^{db}$/$Lepr^{db}$ (BKS-*db*) mice develop severe diabetes associated with dramatically reduced insulin levels and islet atrophy. The two mouse strains display similar phenotypic differences in the presence of the *obese* (*ob*) mutation, a genetic defect of the leptin gene [4]. These observations indicate the presence of different genetic modifiers of diabetes in B6 and BKS mice. Early genetic studies suggested that diabetes-susceptibility in these strains is under multigenic control [5], and more recently, suggestive loci for plasma glucose were detected [6]. However, the genes underlying diabetes-susceptibility in this mouse model remain to be identified.

The BKS strain is the result of an inadvertent genetic contamination. In 1947, a wildfire destroyed the mouse colonies of The Jackson Laboratory and, in order to re-establish the B6 colony, a breeding pair was obtained from the Sloan-Kettering Institute in 1948. However, characterization of the re-derived

*Abbreviations:* B6, C57BL/6J; B10, C57BL/10J; BKS, C57BLKS/J; Chr, chromosome; *db*, *diabetes* mutation; QTL, quantitative trait locus; SNP, single nucleotide polymorphism

☆ The complete data set is available from the authors upon request at the address above or by e-mail (mpeterfy@ucla.edu).

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strain indicated that it was immunologically as well as genetically distinct from B6, and was named C57BLKS (BKS). Subsequent systematic genetic analysis of the BKS strain revealed that it was the result of a genetic contamination of the B6 strain with DBA/2J (DBA) [7]. A recent analysis of the BKS genome using SNP markers demonstrated that 71% of the BKS genome is derived from B6 and 29% is from the DBA strain [8].

Similarly to BKS-*db* mice, DBA-*db* mice are also susceptible to diabetes indicating that, in contrast to B6, the DBA strain background is diabetogenic [9]. On this basis, it has been widely assumed that the DBA-derived genetic component of BKS is responsible for diabetes-susceptibility in this strain. However, recent studies suggest that, in addition to B6- and DBA-derived DNA, BKS may carry additional genetic material [8]. Using high-resolution SNP mapping, we demonstrated that as much as 9% of the BKS genome originates from a non-B6/non-DBA donor [10]. A comparison of non-B6/non-DBA SNPs in BKS to a panel of inbred strains failed to identify the strain of origin, partly due to the bi-allelic nature and low information content of SNP markers. Thus, the source of non-B6/non-DBA genetic material in BKS remains to be determined. In the present study, we provide a high-resolution microsatellite map of the BKS genome, which allowed us to identify the location and strain origin of the non-B6/non-DBA genetic component.

Genetic mapping of diabetes-susceptibility loci in the BKS strain requires a cross between B6 and BKS mice segregating the *db* mutation. Two congenic strains with the *db* mutation are currently available for such crosses, B6-*db* and BKS-*db*. We assessed the genetic purity of these congenics by high-resolution microsatellite marker analysis. Our results indicate the presence of several donor-derived contaminating chromosomal regions in these congenic strains, which has implications for genetic mapping studies of diabetes-susceptibility in this mouse model.

## 2. Materials and methods

### 2.1. Mouse strains

DNA samples from C57BL/6J (stock #664), DBA/2J (671), C57BLKS/J (662), B6.Cg-*Lepr*$^{db}$/*Lepr*$^{db}$ (697), BKS.Cg-*Lepr*$^{db}$/*Lepr*$^{db}$ (642), C57BL/10J (665) and 129S1/SvImJ (2448) were obtained from the Mouse DNA Resource at The Jackson Laboratory.

### 2.2. Genotyping

Fluorophore-labeled primers for microsatellite amplification were obtained from Research Genetics-Invitrogen (Carlsbad, CA). PCRs were performed in 96-well format using 25 ng genomic DNA template and recombinant Taq DNA polymerase (Invitrogen) in 6 μl reaction volumes. A touch-down protocol consisting of 95 °C/5 min initial denaturation, 16 cycles of 95 °C/45 s, 58–50 °C (decreased by 0.5 °C/cycle increments)/30 s, and 72 °C/45 s, followed by a final 72 °C/6 min extension step. After PCR, 1 μl aliquots were diluted 10-fold with water and 2 μl of the diluted samples were mixed with 10 μl of HiDi formamide containing GeneScan 500 LIZ size standard in a 96-well optical plate (Applied Biosystems, Foster City, CA). Samples were denatured at 95 °C/5 min, cooled on ice, briefly spun and kept frozen until electrophoresis. PCR products were run on an ABI 3700 capillary sequencer at the UCLA Genotyping Core Facility. Data analysis was performed using the Genotyper 3.7 software (Applied Biosystems).

## 3. Results

### 3.1. High-resolution microsatellite map of the BKS genome

For initial genotyping of the BKS strain, we used a panel of 348 microsatellite markers polymorphic between B6 and DBA

Table 1
Marker distribution along the chromosomes

| Chr | Chromosome length (Mbp) | Number of markers | Minimal distance[a] (Mbp) | Maximal distance[a] (Mbp) | Average distance[a] (Mbp) |
|---|---|---|---|---|---|
| 1 | 200 | 36 (29) | 0.596 (0.595) | 16.97 (16.97) | 6.667 (8.356) |
| 2 | 181 | 37 (24) | 0.004 (0.057) | 18.92 (21.12) | 5.485 (7.542) |
| 3 | 160 | 29 (17) | 0.025 (0.025) | 17.90 (30.38) | 5.926 (8.889) |
| 4 | 153 | 38 (24) | 0.205 (0.497) | 18.62 (18.62) | 4.500 (6.652) |
| 5 | 150 | 35 (23) | 0.053 (0.053) | 17.12 (23.02) | 4.839 (7.895) |
| 6 | 150 | 20 (13) | 1.356 (1.791) | 16.37 (40.25) | 8.333 (12.50) |
| 7 | 140 | 31 (24) | 0.030 (0.130) | 13.07 (18.66) | 4.667 (5.833) |
| 8 | 130 | 29 (17) | 0.100 (1.484) | 16.04 (27.73) | 4.815 (8.125) |
| 9 | 130 | 28 (16) | 0.715 (0.715) | 23.01 (23.10) | 5.535 (8.667) |
| 10 | 130 | 24 (13) | 0.131 (0.382) | 37.83 (58.93) | 5.417 (10.00) |
| 11 | 120 | 46 (41) | 0.035 (0.035) | 12.80 (16.09) | 2.609 (5.000) |
| 12 | 110 | 22 (15) | 0.288 (0.288) | 16.89 (18.05) | 6.471 (8.462) |
| 13 | 120 | 21 (17) | 0.117 (0.117) | 19.86 (23.75) | 5.714 (8.000) |
| 14 | 120 | 23 (16) | 0.247 (0.723) | 16.27 (19.34) | 5.273 (7.500) |
| 15 | 100 | 26 (15) | 0.005 (0.122) | 20.73 (20.73) | 4.000 (6.933) |
| 16 | 99 | 17 (12) | 0.733 (1.842) | 19.46 (26.03) | 4.700 (8.250) |
| 17 | 94 | 21 (15) | 0.399 (0.766) | 15.23 (19.46) | 6.300 (5.875) |
| 18 | 91 | 19 (9) | 0.688 (2.942) | 21.43 (21.43) | 4.789 (10.11) |
| 19 | 61 | 20 (17) | 0.267 (0.267) | 15.93 (15.93) | 3.813 (4.067) |
| X | 160 | 15 (12) | 0.985 (1.063) | 35.30 (35.30) | 11.43 (13.33) |
| Total | 2599 | 537 (369) | | | |
| Average | | | 0.349 (0.695) | 19.49 (24.75) | 5.564 (8.100) |

[a] Distances refer to a composite map including data from Naggert et al. and Slingsby et al. Numbers in parentheses were calculated based on data from this study only.

[11]. Subsequently, 21 additional markers were typed in regions of interest, as discussed below. The chromosomal distribution and inter-marker distances are shown in Table 1. The allele distribution of the randomly distributed initial marker set indicates that 71% of the BKS genome is derived from B6, 25% from DBA and 4% of markers exhibit alleles different from both, in agreement with recent estimates based on SNP genotyping [8,10].

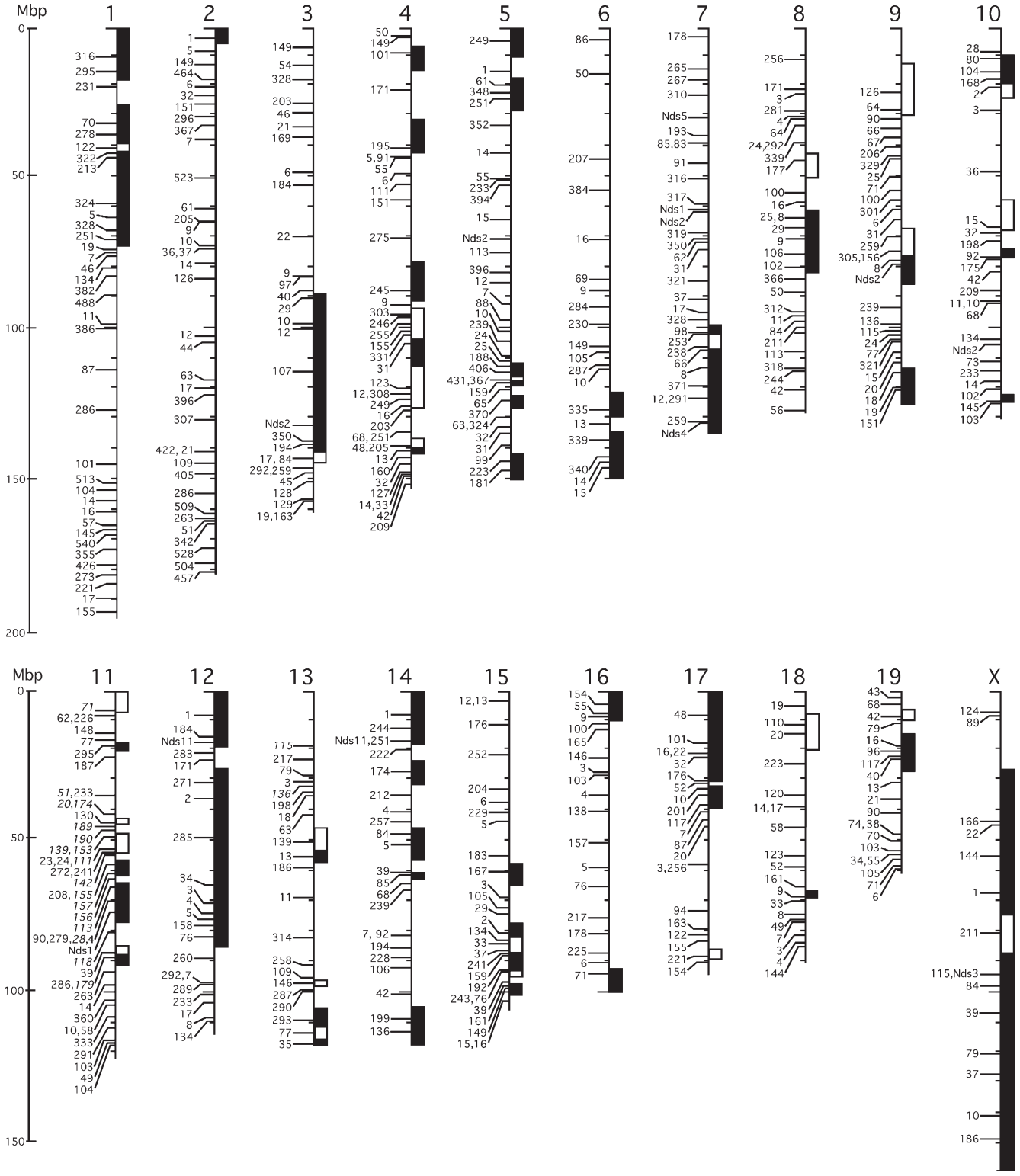


Fig. 1. Composite microsatellite map of the BKS genome. Black boxes represent DBA-derived chromosomal regions and empty boxes indicate genomic intervals where markers exhibit non-B6/non-DBA alleles. The symbols for the Mit microsatellite markers have been abbreviated. Tick marks on chromosomes are spaced at 10 Mbp.

In order to generate a high-resolution composite map of the BKS genome, we combined our data set (369 markers) with those generated previously by Naggert et al. (161 markers) [7] and Slingsby et al. (19 markers) [12], as shown in Fig. 1. As a result of significantly increased marker density, the new map defines the boundaries of genomic segments at considerably higher precision than the previous microsatellite map [7]. The locations of DBA-derived regions obtained in our study are generally in good agreement with those in SNP-based maps [8,10]. However, some of the DBA-derived segments reported here were undetected by the SNP studies (e.g., those on proximal Chr 2 and distal Chr 13), probably due to the limited degree of polymorphism of SNPs compared to microsatellite markers.

### 3.2. The origin of non-B6/non-DBA regions in BKS

Although several previous studies recognized the presence of non-B6/non-DBA alleles in the BKS genome, the origin of these alleles is currently unclear. Naggert et al. hypothesized that non-B6/non-DBA alleles represent mutations, which accumulated since the origin of the BKS strain [7]. In contrast, results by Slingsby et al. indicate that some of these alleles cluster in the genome and may be of C57BL/10 (B10) origin [12]. In a recent large-scale SNP genotyping study, the non-B6/non-DBA SNP pattern in BKS best matched that of BTBR *T*$^{+}$*tf*/J (BTBR) among 102 strains tested [8].

As the BTBR strain was established several years after the genetic contamination of B6 resulting in BKS [13], BTBR itself cannot be the contaminating strain. However, BTBR is genetically closely related to the 129 strain [13], which was derived in the late 1920s, many years before BKS originated [14]. To test the hypothesis that BKS contains genetic contribution from 129, we compared microsatellite allele sizes between the BKS and the 129S1/SvImJ (129) strains. Since previous work indicated the presence of B10 alleles in BKS [12], we also included the B10 strain in our analysis. We genotyped 15 microsatellite markers exhibiting non-B6/non-DBA alleles in our original screen of the BKS genome and subsequently tested additional markers in the vicinity of the 15 core markers.

As listed in Table 2, a total of 42 markers showed non-B6/non-DBA alleles. The majority (69%) of these markers occur in chromosomal regions recognized as non-B6/non-DBA-derived in previous SNP studies [8, 10]. This observation indicates that these alleles are not the result of de novo mutations accumulated since the separation of the BKS strain, but they are derived from a strain(s) other than B6 and DBA. Frequent clustering of non-B6/non-DBA alleles (e.g., Chr 4, 9 and 11) also indicates that

Table 2
Location of non-B6/non-DBA markers in BKS

| Chr | Marker | Position (Mbp) | Allele[a] | Probability of de novo mutations in cluster (%)[b] | Chr region detected in SNP studies | |
|---|---|---|---|---|---|---|
| | | | | | Petkov et al. [8] | Davis et al. [10] |
| 1 | D1Mit122 | 40.8 | other | | no | yes |
| 3 | D3Mit17[c] | 144.2 | | 7.8 | no | yes |
| | D3Mit84 | 144.3 | 129 | | | |
| 4 | D4Mit246 | 97.0 | 129 | $4.7\times10^{-2}$ | yes | yes |
| | D4Mit155 | 100.9 | 129 | | | |
| | D4Mit308 | 122.5 | 129 | | | |
| | D4Mit16 | 126.5 | 129 | | | |
| | D4Mit13 | 141.0 | B10 | | no | no |
| 5 | D5Mit431 | 117.3 | other | 7.8 | no | yes |
| | D5Mit367 | 117.3 | other | | | |
| 7 | D7Mit253 | 102.9 | other | 7.8 | no | no |
| | D7Mit238 | 106.4 | 129 | | | |
| 8 | D8Mit177 | (31.7 cM)[d] | B10 | | | |
| 9 | D9Mit126 | 23.1 | other | 7.8 | yes | yes |
| | D9Mit64 | 28.5 | other | | | |
| | D9Mit259 | 70.1 | other | 0.61 | no | yes |
| | D9Mit156 | 75.0 | other | | | |
| | D9Mit305 | 75.7 | other | | | |
| 10 | D10Mit2 | 21.3 | other | | no | no |
| | D10Mit15 | 66.8 | B10 | | no | no |
| 11 | D11Mit71[e] | 6.8 | B10 | | no | yes |
| | D11Mit130 | 44.5 | other | | no | no |
| | D11Mit139[e] | 48.8 | B10 | $2.9\times10^{-4}$ | no | yes |
| | D11Mit153[e] | 49.5 | B10 | | | |
| | D11Mit24 | 53.1 | B10 | | | |
| | D11Mit111[e] | 53.3 | B10 | | | |
| | D11Mit241 | 54.2 | B10 | | | |
| | D11Mit272 | 55.5 | B10 | | | |
| | D11Mit118[e] | 80.2 | | | yes | yes |
| 13 | D13Mit139 | 50.8 | 129 | | no | no |
| | D13Mit146 | 96.8 | 129 | | no | no |
| | D13Mit77 | 113.9 | other | | no | yes |
| 15 | D15Mit134 | 83.4 | other | 0.61 | no | yes |
| | D15Mit33 | 84.1 | 129 | | | |
| | D15Mit37 | 87.0 | 129 | | | |
| | D15Mit243 | 93.4 | other | 7.8 | yes | yes |
| | D15Mit76 | 95.4 | other | | | |
| 17 | D17Mit221 | 88.7 | B10 | | no | no |
| 18 | D18Mit110 | 12.1 | other | 7.8 | no | no |
| | D18Mit20 | 15.1 | other | | | |
| 19 | D19Mit42 | 9.2 | other | | no | no |
| X | DXMit211 | 80.7 | other | | no | no |

Notes to Table 2:
Markers representing contiguous chromosomal segments are grouped together.
[a] Abbreviations: 129S1/SvImJ, 129; C57BL/10, B10; other, different from B6, DBA, 129 and B10.
[b] Probability of de novo-mutated microsatellites occurring in cluster calculated as $0.078^{n-1}$x100%, where 0.078 is the frequency of non-B6/non-DBA alleles (42 out of 537) and 'n' is the number of markers in the cluster.
[c] Different from B6 and DBA according to Naggert et al. (1995); not analyzed in this study.
[d] As physical position for this marker is not available in UniSTS, genetic location is provided.
[e] Data from Slingsby et al. (1995); not analyzed in this study.

these alleles were introgressed into the BKS genome in concert. This conclusion is supported by the low probability of clustering of mutant microsatellites by chance (Table 2). In some regions (e.g., 7 and 18), allele clustering suggests the presence of genuine non-B6/non-DBA genomic intervals even in the absence of SNP support. Finally, nine markers (21%) occur isolated in chromosomal regions unsupported by SNP studies and probably represent de novo mutation events.

About 25% of the non-B6/non-DBA markers were identical to 129 and different from all other strains tested (B6, B10, DBA) indicating genetic contribution from a 129 strain. Again, this conclusion is strongly supported by the observation that 129-derived alleles occur in clusters on Chr 4 and 15. Consistent with previous results [12], several microsatellite markers exhibited B10-like alleles. Four of these markers cluster together with additional B10-like markers reported by Slingsby et al. on Chr 11, whereas the rest occur isolated on other chromosomes. Finally, 50% of the microsatellite markers (labeled as 'other' in Table 2) show alleles that are different from all strains analyzed in this study. Importantly, these markers also occur in clusters on several chromosomes, including Chr 5, 9, 15 and 18. These results strongly suggest genetic contamination, as opposed to mutation, as the source of at least some of the 'other' alleles in the BKS genome. In an attempt to identify the unknown contaminating strain, we compared allele sizes in BKS to those in other strains catalogued in the Mouse Genome Informatics (www.informatics.jax.org) and Center for Inherited Disease Research (www.cidr.jhmi.edu) databases. Although some markers exhibited allele identities between BKS and other strains, other markers within the same cluster never matched the same strains (Supplementary Table 1). Thus, the observed allele identities are likely random occurrences and do not reflect genuine lineage relationships between the matching strains and BKS. In summary, our analysis of non-B6/non-DBA alleles in the BKS genome revealed multiple sources of genetic contamination in this strain. In addition to the previously reported B10-derived genetic component, we demonstrate for the first time contributions from 129 as well as an additional, unidentified strain in BKS.

### 3.3. Genetic characterization of the BKS-db and B6-db congenic strains

The *db* mutation arose on the BKS genetic background and was subsequently introduced into B6 [15]. Since the original BKS-*db* strain has been lost, BKS-*db* mice currently available from The Jackson Laboratory are congenic, in which the chromosomal region harboring the *db* mutation is derived from B6-*db*. In addition, to facilitate the identification of *db*/+ heterozygotes for breeding, the closely linked coat color mutation *misty* (*m*) has also been introgressed into current stocks of BKS-*db* [16]. The *m* mutation originally arose on the DBA background, but was introduced to BKS-*db* via a B6-*m* congenic (E. Leiter, personal communication). Similarly to BKS-*db*, current stocks of B6-*db* mice also represent second generation congenics harboring the *m* mutation. The complex history of BKS-*db* and B6-*db* suggests that, apart from the *db* congenic region, these strains may carry additional chromosomal regions that are different from the BKS and B6 background strains, respectively. Knowledge of the chromosomal locations of these intervals would facilitate the genetic analysis of phenotypic differences between BKS-*db* and B6-*db* mice.

To identify and map donor-derived genetic material in the BKS-*db* and B6-*db* congenic strains, we genotyped 369 microsatellite markers. As shown in Table 3, six markers in BKS-*db* exhibit alleles different from BKS and 14 markers are different between B6-*db* and B6. Some of these alleles derive from neither B6 nor BKS (labeled as 'other' in Table 3) and occur isolated. These alleles likely represent de novo mutations that arose since the separation of the congenic from the parental strains. In contrast, a total of 9 markers in the congenics exhibit DBA or B6 alleles, which are different in the recipient strains. Most of these markers likely correspond to contaminating genomic regions fixed during the generation of *db* congenics. A clear example for such a genomic

Table 3
Donor-derived alleles in the BKS-*db* and B6-*db* congenic strains

| Strain | Chr | Marker | Position (Mbp) | Allele in congenics [a] | Allele in background strain |
|---|---|---|---|---|---|
| BKS-*db* | 1 | D1Mit122 | 40.8 | DBA | other |
| | 5 | D5Mit431 | 117.3 | other | other [b] |
| | 17 | D17Mit52 | 41.7 | DBA | B6 |
| | 19 | D19Mit68 | 3.4 | other | B6 |
| | | D19Mit42 | 9.2 | B6 | other |
| | | D19Mit40 | 24.6 | other | DBA |
| B6-*db* | 4 | D4Mit31 | 105.5 | DBA | B6 |
| | | D4Mit308 | 122.5 | het [c] | B6 |
| | | D4Mit249 | 124.1 | het [d] | B6 |
| | 5 | D5Mit55 | 51.1 | het | B6 |
| | | D5Mit233 | 51.3 | het | B6 |
| | | D5Mit394 | 53.0 | het | B6 |
| | 6 | D6Mit14 | (63.4 cM) [e] | DBA | B6 |
| | 7 | D7Mit62 | 71.8 | other | B6 |
| | 10 | D10Mit92 | 77.4 | DBA | B6 |
| | | D10Mit175 | 78.2 | DBA | B6 |
| | | D10Mit42 | 82.3 | DBA | B6 |
| | 11 | D11Mit49 | 117.8 | DBA | B6 |
| | 17 | D17Mit221 | 88.7 | het [f] | B10 |
| | 18 | D18Mit161 | 66.2 | het | B6 |

Markers representing contiguous chromosomal segments are group together.
[a] Abbreviations: DBA, DBA/2J; B6, C57BL/6J; B10, C57BL/10J; other, different from B6, DBA, and 129; het, heterozygous between B6 and DBA, unless noted otherwise.
[b] BKS-*db* and BKS alleles are different.
[c] Heterozygous between B6 and 129/DBA (129 and DBA alleles are identical).
[d] Heterozygous between B6 and B10.
[e] As physical position for this marker is not available in UniSTS, genetic location is provided.
[f] Heterozygous between B6 and B10.

contamination is the large DBA-derived region in B6-*db* on Chr 10 between 77.4 and 82.3 Mbp (Table 3). We also detected heterozygosity at several chromosomal loci in B6-*db*, which may reflect limited back-crossing during the development of this congenic strain (E. Leiter, personal communication). In summary, our analysis of the BKS-*db* congenic strain revealed non-BKS genetic material on three chromosomes (Chr 1, 17 and 19), whereas B6-*db* showed four non-B6 genomic intervals (Chr 4, 6, 10 and 11) as well as several regions of heterozygosity (Chr 4, 5, 17 and 18).

## 4. Discussion

In this report, we present a high-resolution microsatellite map of the BKS genome. Consistent with other studies of this mouse strain, a relatively high fraction (4%) of the markers we tested exhibited non-B6/non-DBA alleles. The frequent chromosomal clustering of these markers suggests that genomic contribution from a strain(s) other than B6 and DBA is responsible for the presence of these alleles. In contrast to recent studies using SNPs [8,10], the use of multiallelic markers enabled us to identify the source of non-B6/non-DBA genetic material with high confidence. We provide evidence for the first time that, in addition to B6 and DBA, BKS contains genetic material from at least three other mouse strains, including 129, B10 and an unidentified strain. We cannot exclude the possibility that the source of non-B6/non-DBA alleles is a single unidentified strain with 129- and B10-like alleles. However, the observed chromosomal clustering of 129- and B10-like markers strongly argues against this possibility, and supports the hypothesis that contamination occurred with these strains or a hybrid between the two. As the 129 strain originated in the late 1920s [14] and B10 was derived in the 1930s [17], both of these strains existed at the time when the genetic contamination of B6 occurred [7].

One of the attractive features of the BKS strain for genetic studies is the mosaic nature of its genome. The majority of genetic material in BKS is derived from B6, a strain that is resistant to diabetes induced by the *db* mutation. Thus, diabetes-susceptibility genes are expected to reside in non-B6 chromosomal regions, which are present in blocks and comprise less than 30% of the BKS genome. The fact that genetic variation is restricted to defined blocks offers a powerful way to reduce the size of critical intervals underlying QTL peaks in mapping studies. The largest (20-25%) component of non-B6 genetic material in BKS is derived from DBA, which is a diabetes-susceptible strain. Therefore, DBA-derived genomic blocks represent the most likely locations of diabetes-susceptibility loci. The 129 genetic component is unlikely to contribute to diabetes-susceptibility in BKS, as 129/J-$Lepr^{db\text{-}3J}$/$Lepr^{db\text{-}3J}$ mice are resistant to hyperglycemia [18]. The B10 strain is genetically closely related to B6, therefore it is also unlikely to contribute diabetes alleles in BKS. However, since genetically obese B10 mice have not been evaluated for diabetes sensitivity, this possibility cannot be fully excluded. Indeed, a B10 allele on distal Chr 4 was reported to be associated with diabetes [19]. Finally, we uncovered a genetic component in BKS, the origin of which is currently unknown. It is conceivable that genetic interactions between B6 and non-B6/non-DBA alleles contribute to diabetes sensitivity of the BKS strain. Therefore, non-B6/non-DBA chromosomal regions should be included in the analysis of candidate intervals.

Genetic mapping of diabetes loci in BKS requires a cross segregating the *db* mutation. To initiate such a cross, two congenic strains, B6-*db* and BKS-*db*, are used as parental strains. As congenics often carry unwanted donor-derived chromosomal segments, the genetic composition of the congenics may be different from the B6 and BKS background strains. Indeed, our analysis revealed the presence of several donor-derived genomic regions in these congenic strains. We identified several chromosomal regions containing DBA-derived alleles in B6-*db* (Chr 4, 6, 10 and 11) and an interval in BKS-*db*, where a marker of unknown origin was replaced by a B6 allele (Chr 19). Therefore, these genomic regions are unlikely to harbor diabetes-susceptibility loci and may be used to limit candidate intervals in QTL mapping studies.

The B6-*db* and BKS-*db* strains have previously been used to identify loci affecting diabetes-susceptibility. Using an F2 cross, Coleman [20] mapped a major diabetes QTL on proximal Chr 12 to a genomic interval containing a B6-derived segment flanked by DBA-derived regions. More recently, Mu et al. [6] identified suggestive QTLs for plasma glucose levels on Chr 8 and 17. Both peak QTL markers, D8Mit195 (81.8 Mbp) and D17Mit24 (35.2 Mbp), are located within DBA-derived genomic regions in BKS. For each of these QTLs, knowledge of the precise boundaries of DBA blocks results in significant reductions of the candidate QTL intervals.

In conclusion, our studies revealed novel aspects of the genetic composition of the diabetes-prone BKS strain, as well as the BKS-*db* and B6-*db* congenic strains. The results presented here will facilitate the genetic dissection of diabetes-susceptibility in this mouse model and the identification of underlying genes.

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## Appendix A. Supplementary data

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