Complex Quantitative Traits Cracked by the Mouse Inter-Subspecific Consomic Strains

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Abstract: Mammalian quantitative traits that are observed at the whole-body level, such as body weight and length and blood biochemical parameters, are determined by the cooperative effects of multiple genetic and epigenetic factors as well as environmental factors. This complexity has hampered the genetic analysis of quantitative traits. To overcome this difficulty, we have established a full set of consomic mouse strains, also known as chromosome substitution strains, by replacing every chromosome of the classical inbred strain C57BL/6J with its counterpart from the Japanese wild-mouse-derived inbred strain MSM/Ms. The core components of the genomes of these two strains originated from different mouse subspecies. The inter-subspecific large-genome divergence and phenotypic differences between the two strains allowed the identification of genetic determinants for many quantitative traits by comprehensive phenotype screening. For some quantitative traits, the genetic determinants could be dissected into multiple chromosomes, thereby reflecting strain differences between C57BL/6J and MSM/Ms and their simple additive effects on the background of the consomic host strain. For other quantitative traits, the measured values of some consomic strains often far exceeded the range of the two parental strains, which suggests that nonadditive genetic interactions occur among multiple genes located on the substituted MSM/Ms chromosomes and the consomic host chromosomes. Thus, the inter-subspecific consomic strains are unique tools that can be used to identify both additive and nonadditive genetic effects on quantitative complex traits.

Key words: C57BL/6J, consomic strain, Japanese wild-mouse-derived inbred strain, mammalian quantitative traits, MSM/Ms

Introduction

More than 400 inbred strains of laboratory mouse [2] have long served as important animal models for identifying the genetic components of quantitative complex traits and for determining the pathogenesis of human diseases [37, 38]. Since the inception of mouse genetics, many mutations with simple Mendelian traits on pure genetic backgrounds of these inbred strains have been successfully used to identify causative genes in diseases [11, 14]. Moreover, the phenotypic variations of morphological and physiological quantitative traits observed among inbred strains have attracted the attention of mouse geneticists. Genetic crosses of inbred mouse strains have been performed to map the quantitative trait loci (QTLs) underlying the phenotypic variations between the strains [22, 58]. To date, more than 4,000 QTLs have been assigned to the mouse genome [3]. However, the cloning of genes for QTLs remains an arduous task because most quantitative traits have a complex genetic architecture and are also affected by epigenetic factors, age, sex and environmental factors.
Therefore, most quantitative trait genes or genetic polymorphisms have not been identified, regardless of the efforts involved [11, 14]. Identifying every gene associated with these QTLs and thereby elucidating the genetic network that controls quantitative complex traits remains a challenging task.

Commonly used inbred mouse strains, also known as the classical inbred strains, have a compound genome architecture that consists of a limited number of ancestries that originate from different mouse subspecies [17, 27, 61]. Their genomes are thought to be a mixture of those of different mouse subspecies, consisting predominantly of a west European subspecies, *Mus musculus domesticus*, and, to a lesser degree, several Asian subspecies. The genomes of the Asian subspecies have highly diverged from those of the west European subspecies. The incorporation of this inter-subspecific genome difference into genetic studies expands the spectrum of target complex traits and enhances the ability to genetically dissect the complex traits because the extent of phenotypic differences and the density of genetic markers are significantly larger in the inter-subspecific crosses than in the intra-subspecific crosses between the classical inbred strains. In this review, we present a new trend in the genetic approach to dissecting complex traits that includes the development and application of newly established inter-subspecific consomic strains.

**Japanese Wild-Mouse-Derived Inbred Strain for Studies of Quantitative Complex Traits**

In 2002, the whole genome sequence of a standard classical inbred mouse strain, C57BL/6J (B6), was reported. Since then, SNP information for inbred mouse strains has accumulated [63]. Recently, oligonucleotide array-based resequencing was performed for 11 classical inbred strains and four wild mouse-derived inbred strains. A comparative analysis of these sequences detected a total of 8.27 million SNPs across 16 strains [13, 64]. More recently, deeper genome resequencing of 14 classical inbred strains and three wild mouse-derived inbred strains revealed additional novel SNPs [19]. These studies confirmed that the genomes of the classical inbred mouse strains have a mosaic structure with...
the haplotype blocks derived from different subspecies (Fig. 1). They also clearly showed that the genomes of the classical inbred strains are predominantly derived from *M. m domesticus*, with a small portion overwhelmingly derived from the Japanese subspecies, *M. m. molossinus* [4, 10, 27, 66, 67].

The inbred strain MSM/Ms (MSM) was established from the Japanese wild mouse, *M. m. molossinus* [27, 28]. Ancestors of this strain were captured in Mishima City in the central region of the Honshu Island of Japan in 1978. Since then, the mouse colony has been maintained at the National Institute of Genetics (NIG) by full sibmating, and an inbred strain was later established. In 2010, this strain reached its 100th inbreeding generation. *M. m. molossinus* is known to be a hybrid of *M. m. musculus*, which ranges in a wide area from eastern Europe to Far East Asia, and *M. m. castaneus*, which ranges throughout southeastern Asia [66]. Many studies have shown that *M. m. molossinus* is genetically distant from *M. m. domesticus* [1, 27, 44, 45, 50]. It was traditionally thought that the classical inbred mouse strains, particularly “Castle’s stocks,” originated from Japanese fancy mice [20]. From recent SNP data, it is instead estimated that, on average, *M. m. molossinus* contributes to only 6–10% of the genomes of the classical inbred strains [13, 64]. Interestingly, most commonly used classical strains have a Y-chromosome with *M. m. molossinus*-specific polymorphisms, which suggests that male Japanese fancy mice were crossed with *M. m. domesticus* during generation of the classical inbred mouse strains, likely in Europe [31, 60]. The time of divergence between the two subspecies is estimated at approximately 0.5 to 1.0 million years ago [27, 66], which has resulted in a great genomic difference (~1% divergence) between B6 and MSM, as we previously reported [1]. More than ten million SNPs have been identified between B6 and MSM, and the SNP data are now available to the public (NIG Mouse Genome Database: http://molossinus.lab.nig.ac.jp/msmdb/index.jsp).

As a consequence of its genome divergence from B6 and other classical inbred strains, MSM has unique complex traits [27, 28, 55, 68]. For example, MSM has an extremely low susceptibility to the development of a variety of tumor types [29]. Significant resistance to age-dependent hearing loss has been reported [26, 32]. Unique wild-derived behavioral traits including very high locomotor activity are also characteristic of MSM [21, 33, 56, 57]. Additionally, this strain was selected as one of the target strains in the Mouse Phenome Project of the Jackson Laboratory, and the accumulated phenotypic data are now available online [16, 39]. The MSM strain likely bears additional intriguing phenotypes that are specific to *M. m. molossinus* and not found in other mouse subspecies.

**Mouse Consomic Strains as New Tools for the Study of Quantitative Complex Traits**

Consomic strains, also known as chromosome substitution strains, are powerful tools for assigning the polygenes that control quantitative complex traits to specific chromosomes. The concept of chromosome-substituted strains is not new. In plants and Drosophila genetics, early studies with chromosome-substituted strains [12, 41, 46] were performed as early as 50 years ago. By definition, each consomic strain has a single chromosome that was transferred from a donor strain into the genetic background of a host strain. Currently, consomic strains are constructed in many model organisms, such as Caenorhabditis elegans, Arabidopsis thaliana, and Zea mays, for the genetic dissection of various biological traits [8, 54, 59]. Mouse consomic strains are used for the dissection of genetic factors responsible for quantitative polygenic traits [17, 30]. The first mouse consomic strain was used to study the susceptibility to testicular germ cell tumors in the 129/sv strain with a Ter mutation. A consomic strain with chromosome 19 from MOLF/Ei (*M. m. molossinus* derived inbred strain) in the 129/Sv-Ter/+ background was successfully used to map the locus that modifies the incidence of testicular tumors [24]. The first full set of consomic strains was established using A/J as the donor and B6 as the host. For these strains, each chromosome of the A/J strain was individually transferred into the B6 background [51]. This consomic strain panel is now widely applied to the genetic dissection of complex traits, including anxiety [52], pubertal timing [22], cis- and trans-regulation of transcripts in the kidney and liver [49], obesity [5, 25, 47, 65] and epistatic effects on many complex traits [47].

We have reported a consomic strain in which the X chromosome of the donor strain MSM was transferred into the B6 background. This strain showed reproductive failure characterized by male sterility, which was caused by an incompatibility between the MSM-derived genes on the X chromosome and other genes in the B6 background [34–36]. A gene controlling late-onset hearing...
loss was studied using a consomic strain in which chromosome 17 of MSM was transferred into the B6 background [26, 32]. These studies have clearly shown that the use of consomic strains facilitates the evaluation of QTLs assigned to the substituted chromosome without the influence of genetic noise from other chromosomes.

Establishment of a Full Set of Inter-Subspecific Consomic Strains, B6-ChrN^{MSM}

A full set of consomic strains, referred to as a consomic panel, provides a tool for identifying all QTLs for a complex trait of interest, which are detected by genetic crosses between donor and host strains. The major advantages of consomic panels are as follows: 1) Linkage analysis is not required. At least one QTL is immediately mapped to a single chromosome if the phenotype of interest is detected in a particular strain with the relevant donor chromosome. 2) When a QTL is identified on a particular chromosome, the consomic strain with the corresponding donor chromosome can be used for backcrosses to the host strain, which accelerates fine mapping of the QTL and improves the resolution of QTL linkage. 3) Because of the increased detection power, QTLs with a weak effect on a complex trait can be identified by phenotyping a relatively low number of animals. 4) Epistatic effects can be validated reproducibly by analyzing the results of a cross between consomic strains with donor chromosomes relevant to the epistatic effect.

In the mid-1990s, we began development of new mouse consomic strains as a collaborative project with Dr. Hiromichi Yonekawa of the Tokyo Metropolitan Institute of Medical Science. To identify the polygenes that control quantitative complex traits, the choice of parental mouse strains with distinct phenotypes and genotypes is important [1]. When we initiated the development of a full set of consomic strains, we selected the inbred strain MSM as the donor and B6 as the host. Because the commonly used inbred strain genomes were derived from a relatively small gene pool [13, 61, 64], the genetic variation among common laboratory strains is thought to be limited [17]. Because MSM and B6 are two inbred strains whose core genomes originate from two different subspecies, the new strains are correctly referred to as inter-subspecific consomic strains. After repeated backcrossing over 10 generations to introduce each chromosome of MSM into the B6 background, we have established a full set of consomic strains (Fig. 2A).

The complete inter-subspecific consomic panel that we established consists of 29 strains with MSM autosomes and an X chromosome as well as two other strains, each of which harbors the MSM Y chromosome or mitochondrial DNA (Fig. 2B). According to the consomic nomenclature, each strain was named B6-ChrN^{MSM}, where N is the number of the chromosome transferred from MSM. The conplastic strain carrying MSM mitochondrial DNA was named B6-mt^{MSM}(conplasmic). When it was difficult to transfer the entire MSM chromosome into the host background, mostly because of reduced reproductive performance, we also established sub-consomic strains that carry the proximal (centromeric) or distal half of the MSM chromosome. In these cases, the strains were named B6-ChrNC^{MSM} and B6-ChrNT^{MSM}, with C (centromere) or T (telomere) following the chromosome number N of the consomic strain. The break points of the MSM sequence in the corresponding chromosome of these subconsomic strains are shown in Fig. 2C. [The B6-ChrN^{MSM} strains are available from the Genetic Strains Research Center of the National Institute of Genetics (http://www.shigen.nig.ac.jp/mouse/strain/) and RIKEN Bioresource Center (http://www.brc.riken.jp/lab/animal/en/) for the academic and scientific community.]

Genetic Dissection of Morphological and Physiological Quantitative Complex Traits by Systematic Phenotype Screening of the Panel of Consomic Strains, B6-ChrN^{MSM}

General outlook for physiological phenotyping

The systematic phenotyping of the inter-subspecific consomic strains focused on morphological traits and physiological complex traits related to reproduction, growth and energy metabolism and successfully detected statistically significant QTLs that affected over 40 traits. Statistical significance was determined in a t-test between each consomic strain and the chromosome host strain B6. Statistical results were subjected to a Bonferroni correction. A measured value was considered significant if the corrected significance level was 0.05 and highly significant if the level was 0.001, which implies that the expected rate of false positives was only 0.05 to 0.001, respectively, per trait (or trait/sex combination) across the entire consomic panel. The minimum number of QTLs that we estimated with the consomic strains is summarized in Table 1. The number of highly...
Fig. 2. Development of the B6-ChrN\textsuperscript{MSM} consomic panel. A) Strategy for developing B6-ChrN\textsuperscript{MSM} consomic strains. The first step was the generation of F\textsubscript{1} crosses between the B6 and MSM strains. The F\textsubscript{1} progeny were then backcrossed to the host B6 strain. Progeny with a nonrecombinant target chromosome derived from the donor MSM strain were backcrossed to the host strain B6 at each generation. After the proper backcross generation (>N10), males and females with nonrecombinant target chromosomes derived from the donor strain, MSM, were intercrossed. Progeny from this intercross that were homozygous for the target chromosome were used to propagate the consomic strain. The Y chromosome consomic strain was generated by selecting males for backcrossing to the host strain at each generation. The plasmatic (mitochondrial DNA consomic) strain was generated by selecting females for backcrossing to the host strain at each generation. B) Genomes of the consomic mouse strains. The centromeres are shown at the top. To name the subconsomic strains, we included C (Centromere) or T (Telomere) after the chromosome number to indicate that the proximal or distal half of the B6 chromosome was substituted by the corresponding region of the MSM chromosome. Black, MSM-derived chromosome; White, B6-derived chromosome. The details of the genetic markers used for genotyping at each backcrossing generation for each chromosome are listed in C).

\begin{table}
\centering
\begin{tabular}{|l|l|}
\hline
Strain Name & MSM genome region* \\
\hline
B6-Chr\textsuperscript{MSM} & D1Mit16 - D1Mit54 \\
B6-Chr2\textsuperscript{MSM} & D2Mit1 - D2Mit15 \\
B6-Chr27\textsuperscript{MSM} & D2Mit15 - D2Mit145 \\
B6-Chr3\textsuperscript{MSM} & D3Mit46 - D3Mit45 \\
B6-Chr8\textsuperscript{MSM} & D4Mit106 - D4Mit51 \\
B6-Chr11\textsuperscript{MSM} & D5Mit146 - D5Mit102 \\
B6-Chr6\textsuperscript{MSM} & D6Mit166 - D6Mit12 \\
B6-Chr7\textsuperscript{MSM} & D6Mit274 - D6Mit15 \\
B6-Chr9\textsuperscript{MSM} & D7Mit305 - D7Mit141 \\
B6-Chr10\textsuperscript{MSM} & D7Mit315 - D7Mit222 \\
B6-Chr17\textsuperscript{MSM} & D7Mit200 - D7Mit141 \\
B6-Chr19\textsuperscript{MSM} & D8Mit149 - D8Mit13 \\
B6-Chr18\textsuperscript{MSM} & D9Mit43 - D9Mit151 \\
B6-Chr11\textsuperscript{MSM} & D11Mit154 - D11Mit369 \\
B6-Chr12\textsuperscript{MSM} & D11Mit71 - D11Mit659 \\
B6-Chr12\textsuperscript{MSM} & D12Mit103 - D12Mit34 \\
B6-Chr17\textsuperscript{MSM} & D12Mit34 - D12Mit8 \\
B6-Chr13\textsuperscript{MSM} & D13Mit236 - D13Mit21 \\
B6-Chr15\textsuperscript{MSM} & D13Mit43 - D13Mit196 \\
B6-Chr16\textsuperscript{MSM} & D14Mit132 - D14Mit267 \\
B6-Chr10\textsuperscript{MSM} & D15Mit174 - D15Mit40 \\
B6-Chr18\textsuperscript{MSM} & D16Mit79 - D16Mit51 \\
B6-Chr17\textsuperscript{MSM} & D17Mit246 - D17Mit123 \\
B6-Chr18\textsuperscript{MSM} & D18Mit66 - D18Mit145 \\
B6-Chr19\textsuperscript{MSM} & D19Mit68 - D19Mit33 \\
B6-Chr20\textsuperscript{MSM} & D20Mit89 - D20Mit65 \\
B6-ChrY\textsuperscript{MSM} & D20Mit95 - D20Mit160 \\
B6-\textsuperscript{MSM} & D2Mit15 - D2Mit145 (76.65 - 91.84) \\
B6-Chr2\textsuperscript{MSM} & D2Mit15 - D2Mit100 (91.84 - 106.34) \\
B6-Chr2\textsuperscript{MSM} & D2Mit93 - D2Mit15 (76.65 - 91.84) \\
B6-Chr11\textsuperscript{MSM} & D6Mit126 - D6Mit259 (124.32 - 142.7) \\
B6-Chr11\textsuperscript{MSM} & D6Mit167 - D6Mit269 (19.77 - 34.66) \\
B6-Chr11\textsuperscript{MSM} & D7Mit331 - D7Mit304 (122.53 - 133.65) \\
B6-Chr11\textsuperscript{MSM} & D7Mit233 - D7Mit200 (68.35 - 80.04) \\
\hline
\end{tabular}
\footnote{Marker location based on the NCBI m36 mouse assembly (April 2006, strain B6).}
\end{table}
Fig. 3. Representative traits of the B6-ChnM<sup>MSM</sup>-consomic panel. The upper and lower halves of each box indicate the result obtained for males and females, respectively. Plus signs (+ and ++) indicate values higher than those of the host B6 strain. In this figure, highly significant values ($P<0.001$) are indicated by 2 plus signs (++), and all other statistically significant values ($P<0.05$) are indicated by 1 plus sign (+). Minus signs (− and −−) indicate values lower than those of B6. Highly significant values ($P<0.001$) are indicated by 2 minus signs (−−), and all other statistically significant values ($P<0.05$) are indicated by 1 minus sign (−). All of the data were adapted from ref. 55.
significant QTLs detected in this study was 74 for males and 53 for females. Moreover, 208 significant QTLs that affect 26 traits were detected. Many QTLs were detected only for males or females, and less than half (34.1%) of the significant QTLs were commonly observed in both males and females. Furthermore, some simple additive effects were found for the traits of body weight, body length, fat pad weight, relative ratios of kidney and heart weight to lean body weight, and blood constituents such as triglycerides, amylase, and BUN (blood urea nitrogen) (Fig. 3).

**Body size regulation**

The MSM strain has a significantly lower body weight and length than B6, which is characteristic of the Japanese wild mouse, *M. m. molossinus*. This strain also has a higher relative ratio of heart and kidney weight to lean body weight compared with B6 and other commonly used classical inbred strains. Our phenotyping of B6-ChrNMSM showed that many MSM chromosomes significantly reduce the body weight and length, whereas many chromosomes increase the relative ratios of the heart and kidney weight to lean body weight. These results indicate that polygenes of the MSM strain that control body and organ size have additive effects in the B6 genomic background (Figs. 3 and 4).

The genetic control of body and organ size is complex and regulated at different layers of biological processes, such as cell proliferation and growth, cell localization, and cell death [6]. The mechanism underlying the effect of cell proliferation on the control of body and organ size is still not well understood, but various growth factors, such as growth hormone and insulin-like growth factor (Igf1), are known to trigger cell proliferation. Moreover, the phosphoinositide 3-kinase (PI3K) pathway that is activated by growth factors such as Igf1 is known to be a key signal for cell growth [6]. A recent study showed that a polymorphism of the Igf1 gene is a major determinant of small size in dogs [53]. The cardiac-specific expression of constitutively regulated PI3K was reported to augment heart size in mice [48]. Mouse Igf1 has been mapped to chromosome 10 [18], and multiple QTLs affecting the serum levels of Igf1 have been mapped to chromosomes 6, 10, and 15 [43]. The Igf1 receptor gene (*Igf1r*) is on chromosome 11 [43]. In our study, highly significant QTLs affecting body length were detected on mouse chromosomes 1, 2, 3, 4, 5, 8, 9, 13, and 18. Notably, the body size of the consomic strain B6-Chr10MSM is comparable with that of the B6 strain (data not shown). Therefore, as yet unidentified genes, other than those involved in the Igf1 pathway, likely also regulate mouse body size and are responsible for the inter-subspecific difference in body size between MSM and B6.

**Energy metabolism**

When we initiated the genetic study of complex traits with wild mouse-derived strains, we anticipated that MSM has a frugal metabolic system that prevents the waste of energy inputs because wild mice have survived

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**Table 1. Minimum number of QTLs (modified from ref. 55)**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Male P&lt;0.001*</th>
<th>Female P&lt;0.001*</th>
<th>Total P&lt;0.001*</th>
<th>Male P&lt;0.05*</th>
<th>Female P&lt;0.05*</th>
<th>Total P&lt;0.05*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body growth</td>
<td>12</td>
<td>18</td>
<td>74</td>
<td>15</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Body growth between 10–20wk</td>
<td>7</td>
<td>10</td>
<td>15</td>
<td>0</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Body length</td>
<td>11</td>
<td>17</td>
<td>19</td>
<td>9</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Organ weight</td>
<td>21</td>
<td>45</td>
<td>50</td>
<td>9</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Visceral fat</td>
<td>2</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous fat</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Dorsal brown fat</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Adiposity index</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>7</td>
<td>18</td>
<td>11</td>
<td>8</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Other blood composition</td>
<td>11</td>
<td>43</td>
<td>15</td>
<td>14</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>168</td>
<td>208 (71*** )</td>
<td>53</td>
<td>119</td>
<td></td>
</tr>
</tbody>
</table>

*Significance levels were subjected to a Bonferroni correction. **At least one QTL in males or females was included. ***The number of QTLs in common between male and female animals.
over a long evolutionary time period under conditions of starvation or near starvation. By contrast, laboratory mice, which have been maintained in animal facilities and fed by humans over several hundred generations, may have lost such a system. To capitalize on the unique features of the wild-mouse-derived MSM strain, extensive phenotyping of the panel of consomic strains for energy-metabolism-related complex traits was performed with the goal of identifying the genetic components of age-dependent metabolic control. The accumulation of fat pads is important for overall body weight gain and serves as a good indicator of an energy-conserving phenotype. To date, many QTLs affecting obese or lean phenotypes have been detected by genetic linkage analysis using various combinations of laboratory mouse strains [7, 40]. Our extensive measurements of fat pads of the consomic panel revealed that many chromosomes of MSM affect fat pad deposition (Fig. 3). Notably, several chromosomes tend to increase the deposition of subcutaneous and brown fat. These chromosomes also increase the deposition of visceral fat, whereas other chromosomes decrease the deposition of visceral fat. Because visceral but not subcutaneous fat is a known risk factor for cardiovascular disease in humans, MSM likely has a genetic system that promotes the safe storage of excessive energy as subcutaneous fat. Among the many MSM chromosomes that affect fat pad deposition, the distal half of the X chromosome is particularly intriguing. Males of the consomic strain B6-ChrXT MSM showed a dramatic increase in body weight compared with males of the B6 host strain after 18 weeks of age (Fig. 3). Previous studies based on results from two different inter-specific genetic crosses showed that at least two QTLs on the X chromosome affect the body weight of adult males from puberty onward. Of particular interest are other consomic strains that have shown age- and sex-dependent obese phenotypes after sexual maturation.

The increase in body weight of the B6-Chr13T MSM strain between 10 and 20 weeks of age (36.02% increase) is greater than that of any other consomic strain (Fig. 3). Previous studies based on results from two different inter-specific genetic crosses showed that at least two QTLs on the X chromosome affect the body weight of adult males at 40 weeks of age [9]. These two independent studies indicated that the X chromosome has genes that control the body weight of males from puberty onward. Of particular interest are other consomic strains that have shown age- and sex-dependent obese phenotypes after sexual maturation.

The concentrations of plasma HDL cholesterol and triglycerides (TG) are important indicators of metabolic syndrome and correlate with the pathogenesis of cardiovascular disease in humans. In particular, low HDL cholesterol and high TG levels are the most prominent predictors of cardiovascular disease [15]. Plasma TG levels also positively correlate with the plasma HDL cholesterol level [23]. To date, many QTLs affecting plasma lipid levels have been reported on all autosomes based on the results of 23 different genetic crosses including the B6 strain and the wild mouse-derived strains CASA, CAST, PERA, and SPRET [42]. Many of the detected QTLs are likely located on the same chromosomal segments, which suggests that the different crosses pinpointed identical QTLs.

None of the previous genetic crosses using various mouse strains revealed QTLs on the X chromosome that affect plasma HDL cholesterol levels. In this study, the MSM X chromosome was shown to reduce plasma HDL cholesterol levels. By 10 weeks of age, B6-Chr4 MSM females showed lower HDL cholesterol levels and higher TG levels than B6 females. This observation suggests that the consomic strain B6-Chr4 MSM would be a useful model to study the genetic system that regulates the levels of plasma HDL cholesterol and TG.

Reproductive performance

We also performed phenotype screening of the consomic panel for reproductive performance-related traits [55]. Average litter size and viability at 4 weeks of age shows large variation among the consomic strains (Table 2). As described above, the consomic strain, B6-ChrX MSM, which carries the X chromosome of MSM in the B6 background, shows reduced testis weight and abnormal morphology of the spermatozoa, resulting in male sterility [34]. To circumvent the difficulty in transferring the whole X chromosome of MSM into the B6 background, we established two subconsomic strains, B6-ChrXC MSM and B6-ChrXT MSM, which harbor the...
centromeric and distal half of the MSM X chromosome, respectively. Males of both strains are fertile, but B6-ChrX\textsuperscript{MSM} showed reduced testis weight. A similarly reduced testis weight was observed in the consomic strains for chromosome 5, 7C, 11, and 18 (Fig. 3), which suggests that these chromosomes are likely involved in male fecundity.

### Table 2. Reproductive performance-related traits of the inter-subspecific consomic strain panel (modified from ref. 55)

<table>
<thead>
<tr>
<th>Strain</th>
<th>LS</th>
<th>SE</th>
<th>Min</th>
<th>Max</th>
<th>M/F ratio (%)</th>
<th>VI (%)</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>8.0</td>
<td>0.4</td>
<td>4</td>
<td>12</td>
<td>49.5</td>
<td>96.0</td>
<td>27</td>
</tr>
<tr>
<td>MSM</td>
<td>5.7</td>
<td>0.2</td>
<td>2</td>
<td>7</td>
<td>47.2</td>
<td>88.8</td>
<td>41</td>
</tr>
<tr>
<td>1</td>
<td>6.2</td>
<td>0.3</td>
<td>3</td>
<td>9</td>
<td>52.2</td>
<td>90.7</td>
<td>35</td>
</tr>
<tr>
<td>2C</td>
<td>7.1</td>
<td>0.4</td>
<td>2</td>
<td>10</td>
<td>50.5</td>
<td>92.3</td>
<td>30</td>
</tr>
<tr>
<td>2T</td>
<td>4.9</td>
<td>0.3</td>
<td>1</td>
<td>9</td>
<td>48.6</td>
<td>55.4</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>5.2</td>
<td>0.6</td>
<td>2</td>
<td>10</td>
<td>40.3</td>
<td>77.3</td>
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LS, average litter size; SE, standard error; Min, minimum litter size; Max, maximum litter size; M/F, ratio of males to females of total offspring; VI, viability at 4 wk of age; L, number of litters observed in this study.

Comprehensive phenotypic screening of the B6-ChrN\textsuperscript{MSM} consomic strains revealed that some phenotypic effects of the chromosome donor strain MSM are mediated by multiple chromosomes (Table 1). For example, several consomic strains have significantly smaller body sizes than that of the host strain B6, and their measured values are within the range between MSM and B6 (Fig. 4A). This result may indicate that the MSM alleles have additive effects on the phenotype of body size. In contrast, with respect to fat pad deposition in adipose tissues and some serum components, many consomic strains showed measured values that far exceeded the range between the two parental strains (Fig. 4B). In extreme cases, some consomic strains exhibited measured values beyond those of B6. This effect may have been caused by the segregation of MSM alleles, with phenotypic effects opposing the net effect of MSM. Alternatively, chromosome substitution may give rise to nonadditive interactions or disrupt the proper genetic interaction between multiple genes located in the substituted chromosome and other host chromosomes. One prominent example of such epistasis was observed in the case of B6-ChrX\textsuperscript{MSM}. Notably, this chromosome substitution causes male sterility, although the two parental strains show full fertility. A genome-wide linkage analysis successfully detected significant QTLs on chromosomes 1 and 11 that interact with the X chromosome; the
disruption of this interaction causes male sterility [36].

At present, it is not clear whether the segregation of alleles or epistasis gives rise to the measured values in the consomic strains for some quantitative complex traits that exceed the ranges of the B6 and MSM parental strains. It is clear, however, that the use of inter-subspecific consomic strains provides a unique opportunity to determine latent genetic components and/or genetic interactions that are otherwise difficult to elucidate.

Database for Phenotype Data of Consomic Strains

As phenotype screening of the inter-subspecific consomic strains proceeds, additional useful information on phenotypes will accumulate. To increase the usability of this information, we are developing the NIG Mouse Functional Genomics Database (NIGMFGD: http://molossinus.lab.nig.ac.jp/) and integrating two types of information (Fig. 5). First, NIGMFGD provides the genome sequences of the MSM and B6 strains and supports the searching of SNPs between MSM and the classical inbred strains, including B6. It also supports in silico screening of the BAC library of MSM genome DNA (1). Second, NIGMFGD provides the phenotype data and QTLs detected by comprehensive phenotype screening of the panel of inter-subspecific consomic strains, B6-ChrNMSM [55, 56]. In this database, users can explore the candidate SNPs for the QTL by giving map positions of physical borders of the interval on the query page if users confine the interval of the genome region that contains the QTL of interest (Fig 5C). The ultimate goal of this database is to provide a platform to identify genes and genome functions that underlie complex quantitative traits by linking genotype and phenotype data.

Acknowledgments

We thank H. Yonekawa and his colleagues, Y. Kikawa, H. Shitara, T. Sakai, and S. Takahama for the grand design and production of the inter-subspecific consomic strains B6-ChrNMSM, and K. Moriwaki, founder of the MSM/Ms strain, for valuable advice throughout this study. We also thank A. Mita for careful breeding and

Fig. 4. Distribution of the measured values for 10-week body weight and perirenal fat pad deposits of male animals from the consomic strains compared with the range observed for the parental strains. Error bars, standard error of the mean. Gray shading, the range of values between the MSM and B6 strains. A, body weight; B, inguinal white adipose tissue. The measured value of the inguinal white adipose tissue was used to determine the fat pad weight with respect to the total fasted body weight. All the data were adapted from ref. 55.
Fig. 5. The NIG Mouse Functional Genomics Database (NMFGD). This database consists of two sub-data sets. A) Sub-data set of genomic polymorphisms between B6 and MSM/Ms. B) Sub-data set of phenotypic variation of B6-ChrN MSM and the conplasmic panel. C) Procedures for searching candidate SNPs. This panel shows the navigation windows to find the SNPs for a QTL of interest by three different procedures, (1) giving names of genes or MSM BAC clones, (2) giving physical map positions of the borders of a chromosomal interval and (3) pinpointing a chromosomal position.
maintenance of all the consomic strains. We thank the members of the NIG, especially A. Matsusue, Y. Yamabe, H. Watanabe, N. Harima, K. Nishimoto, K. Masuyama, T. Aoki, F. Murofushi, A. Yakamaghe, M. Sekine, F. Kobayashi, K. Aida, A. Fujii, M. Nagai, F. Iwase, K. Takada, K. Fukunaga, A. Okagaki, H. Nakazawa, and Y. Mizushina for maintaining the mouse strains and for technical assistance. We also thank Y. Yamazaki for management of the server for the NIGMFG database. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas “Comparative Genomics” from the Ministry of Education, Culture, Sports, Science and Technology of Japan and supported in part by the Biodiversity Research Project of the Transdisciplinary Research Integration Center (TRIC), Research Organization of Information and Systems. The pheno-type database is supported by a Grant-in-aid for Scientific Research on Priority Areas “Comparative Genomics” from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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