Genetic dissection of testis weight in a mouse strain having an extremely large testis: major testis weight determinants are autosomal rather than Y-linked on the basis of comprehensive analyses in Y-chromosome consomic strains

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Abstract: I investigated the potential contribution of Y-linked genes by analyzing 16 Y-consomic strains that had been established on a DH-strain background. The results provided evidence that only the Y chromosome from the C3H/HeJ strain was different from most other inbred strains. The CBA strain has the lightest testis and the DDD strain has the heaviest testis among mouse strains; however, Y-consomic analysis revealed that there were no significant differences in testis weight among DH, DH-Chr Y^{DDD}, and DH-Chr Y^{CBA} strains, suggesting that Y^{DDD} and Y^{CBA} themselves do not influence testis weight. QTL analysis in DDD × DH F_2 mice identified significant testis weight QTLs on chromosomes 9, 14, and 17, and the DDD allele at all these loci was associated with an increase in testis weight. Contribution of Y chromosome itself to testis weight was thus rather modest, and therefore, major testis weight determinants are autosomal. However, it was uncertain whether there would be any effects by interactions between Y chromosomal and autosomal genes.

Keywords: mouse, QTL, testis weight, Y-consomic strain

Introduction

Normal development of the testis is crucially important for ensuring reproductive success. Testis weight has a direct connection with male fertility; that is, spermatogenic ability. The rate of sperm production largely depends on the total length and/or diameter of the seminiferous tubes, which, in turn, are the primary determinants of testis weight.[1)] In fact, testis weight depends on the rate of sperm production; for example, the testis weight in polygamous males is heavier than that in monogamous males in primates.[2)] A seasonal change in testis weight is reported in wild animals (seasonal breeders); they tend to have a greater testis weight in their breeding season.[3)] On the other hand, there is an apparent genetic aspect to the control of testis weight. Indeed, in laboratory mice, strain differences in testis weight have been apparent to many biologists and geneticists who work with divergent strains.[4),5)]

Testis weight is probably determined by the action of multiple genes under the influence of non-heritable environmental effects. In addition to genes on autosomes and the X chromosome, the relevance of Y-chromosome-linked (hereafter called Y-linked) genes has been suggested, because the testis develops only in males, and the testis-determining *Sry* gene is Y-linked. The effect of Y-linked genes on testis weight has been debated, but the results are conflicting.[1),5)–8)] For a definitive evaluation of the effect of the Y chromosome, it is crucial to synchronize the genetic background other than the Y chromosome. Analyzing Y-chromosome consomic strains (hereafter called Y-consomic strains) is the best way of accomplishing this. Accordingly, I established a series of Y-consomic strains, and I

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Non-standard abbreviation: QTL, quantitative trait locus; LRS, likelihood ratio statistics; LOD, logarithm of odds.

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addressed whether there was any contribution of Y-linked genes to testis weight. In addition, involvement of genes on autosomes and/or on the X chromosome has been suggested.[7),8)] Another line of evidence also supports the probability of an autosomal contribution; that is, in mouse lines selected for testis weight in males, the ovulation rate in females increased.[9),10)] Because marked variations in testis weight are observed among mouse inbred strains in terms of either absolute weight or weight relative to body weight, the genetic basis for this variability can be investigated genetically with the aid of QTL analysis. To date, several QTL analyses have addressed the issue of the genetics of testis weight.[5),11)–14)] Although they demonstrated the presence of testis weight genes on several chromosomes, it is expected that novel genes or loci underlying testis weight will be identified in different genetic crosses. In this study, I performed QTL analysis on testis weight in the inbred DDD/Sgn mouse strain. The DDD/Sgn is one of the mouse strains that have an extremely large testis. The testis weight in DDD/Sgn is about one and a half times greater than that in common inbred mouse strains, such as C57BL/6J.

Materials and methods

Mice and genetic cross. Inbred mouse strains DDD/Sgn (hereafter called DDD for convenience), DH/Sgn (DH), CF1/Sgn (CF1), RR/Sgn (RR), and SS/Sgn (SS) were maintained in the National Institute of Agrobiological Sciences (Tsukuba, Japan). A/J (A), CAST/EiJ (CAST), AKR/J (AKR), RF/J (RF), SJL/J (SJL), and SWR/J (SWR) strains were purchased from the Jackson Laboratory (Bar Harbor, ME). BALB/cA (BALB), C3H/HeJ (C3H), C57BL/6J (B6), and KK/Ta (KK) were purchased from CLEA Japan (Tokyo). CBA/N (CBA) was purchased from Japan SLC (Hamamatsu, Japan). I would like to mention the origin and characteristics of the DDD strain briefly on the basis of information from Mouse Genome Informatics (MGI, `http://www.jax.org`). In 1928, the original colony of dd mice was introduced from Germany into the Kitasato institute, Tokyo. Their descendants were shipped to the Health Institute of Manchuria Railway, Tailen, China in 1934. Two males and eight females from the Tailen colony were shipped back to the Institute for Infectious Diseases (Denken), Tokyo. Inbreeding of dd mice maintained at Denken was commenced in 1957, and the resulting inbred strain was named DDD after dd at Denken. As described below, F_1-*Dh*/+ male mice resulting from a cross between DDD females and DH-*Dh*/+ are essentially lethal during neonatal period; however, this does not occur in the reciprocal cross.[15)]

For analysis of the effect of Y-linked genes by use of a series of Y-consomic strains, a Y-consomic strain, which has a Y chromosome from DDD (hereafter called Y^{DDD}), onto a DH background (hereafter called DH-Chr Y^{DDD}) has been produced by successive backcrossing (backcross generation: N_{30}, sample size: $n = 41$). In a similar way, Y-consomic strains DH-Chr Y^{A} (N_{21}, $n = 27$), DH-Chr Y^{B6} (N_{29}, $n = 32$), DH-Chr Y^{BALB} (N_{29}, $n = 24$), DH-Chr Y^{CAST} (N_{30}, $n = 26$), DH-Chr Y^{CBA} (N_{11}, $n = 11$), DH-Chr Y^{CF1} (N_{19}, $n = 21$), DH-Chr Y^{C3H} (N_{28}, $n = 40$), DH-Chr Y^{KK} (N_{12}, $n = 5$), DH-Chr Y^{RR} (N_{11}, $n = 18$), DH-Chr Y^{SS} (N_{11}, $n = 10$), DH-Chr Y^{AKR} (N_{30}, $n = 37$), DH-Chr Y^{RF} (N_{30}, $n = 32$), DH-Chr Y^{SJL} (N_{31}, $n = 29$), and DH-Chr Y^{SWR} (N_{34}, $n = 27$), were established and used in this study.

Structurally, two kinds of *Mus musculus* Y chromosomes are known to coexist among the inbred mouse strains, that is, *M. m. musculus* Y (Y^{Mus}) and *M. m. domesticus* Y (Y^{Dom}), on the basis of nucleotide sequences of the *Sry* gene.[16),17)] Y chromosomes, Y^{A}, Y^{B6}, Y^{BALB}, Y^{CAST}, Y^{CF1}, Y^{C3H}, Y^{KK}, and Y^{RR} belong to Y^{Mus}, Y^{AKR}, Y^{RF}, Y^{SJL}, and Y^{SWR} belong to Y^{Dom}, and Y^{CBA} and Y^{SS} are unknown.

For QTL analysis, reciprocal F_1 males were produced between DDD and DH. For F_2 analysis, DDD females were crossed with DH males to produce F_1, and F_1 males and females were intercrossed to produce F_2 males. Hereafter, I defined the DDD as having D alleles, and the DH as having H alleles, throughout the genome.

All mice were maintained in a specific-pathogen-free facility with a regular light cycle of 12 hr light: 12 hr dark, with controlled temperature and humidity. They had free access to food (CE-2, CLEA Japan) and water. Experiments were approved by the Institutional Animal Care and Use Committee of the National Institute of Agrobiological Sciences.

Phenotype measurements. At the age of 80 ± 1 days after birth, mice were weighed with an

electric balance to the nearest 0.01 g. Then the mice were killed, and the testis on both sides was removed and placed in physiologic saline. After they were rinsed, I wiped excessive moisture with wet chromatography paper, and the paired testes weight was determined with an electric balance to the nearest 1 mg. The spleen weight was determined in the same way. The weight of the spleen was analyzed as a reference for a parenchymatous organ. Trait names have been abbreviated as follows: Bw for body weight (g), Tw for absolute paired testis weight (mg), rTw for relative testis weight [Tw (mg)/Bw (g)], Sw for absolute spleen weight (mg), and rSw for relative spleen weight [Sw (mg)/Bw (g)]. Although rTw (and rSw) has been used to minimize the inevitable effects by Bw traditionally, Tw and rTw were analyzed separately in the study.

Genotyping. Genomic DNA was isolated from the tails of mice with a commercial DNA extraction kit (Wizard Genomic DNA Purification Kit, Promega, Madison, WI). Microsatellite sequence length polymorphism was detected by electrophoresis subsequent to PCR. Most microsatellite primers were purchased as MapPairs (Research Genetics, Huntsville, AL), whereas others were synthesized on the basis of information from MGI. PCR amplification was carried out by use of a Takara PCR thermal cycler MP (TaKaRa Biomedicals, Tokyo) under the following conditions: 1 cycle at 94 °C for 5 min; 35 cycles at 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 45 s; 1 cycle at 72 °C for 7 min. All PCR products were electrophoresed on 10% polyacrylamide gels for 70 min and visualized by ethidium bromide staining.

QTL analysis. For identifying putative testis weight QTLs, a total of 72 F_2 mice (which were selected out of 143 F_2 mice), including 24 mice showing the highest Tw and 24 mice showing the lowest Tw. These 72 F_2 mice also included 20 mice showing the highest rTw and 22 mice showing the lowest rTw. Furthermore, these 72 F_2 mice were shown to include 19 out of 24 mice having the highest Bw and 18 out of 24 mice having the lowest Bw. The 72 F_2 mice were genotyped for a total of 92 microsatellite marker loci distributed on all autosomes and the X chromosome. Initially, the QTL analysis was performed in 143 F_2 mice with the Mapmaker/EXP version 3.0b and the Mapmaker/QTL 1.1b computer program.[18] Of these 143 F_2, 72 mice were genotyped completely, and the genotypes of most of the remaining mice were labeled as missing data. In general, once a log logarithm of odds (LOD) score of more than 2.8 (threshold LOD score for suggestive linkage as recommended by Lander and Kruglyak[19]) was obtained, the remaining 71 F_2 mice, and newly produced 30 F_2 were genotyped for all microsatellite markers located on relevant chromosomes. At this stage, QTL analysis was again performed in all 173 F_2 mice with the Map Manager QTX b20 software.[20] The interval mapping was performed with this program. Because the interval mapping function of Map Manager QTX is most accurate when the phenotypic data are normally distributed, the normality was assessed by Kolmogorov-Smirnov test. As a result, because distribution of Bw in F_2 mice did not follow a normal distribution, log-transformed Bw data was analyzed. Significant threshold values at genome-wide 5% level were calculated by performing 1,000 permutations of the phenotypic data. Although it depends on the traits, approximate threshold LOD scores (because QTL calculates a LRS, it is converted to a LOD score by dividing by 4.605) for five phenotypic traits are as follows: 2.1 for suggestive linkage, 3.6–3.7 for significant linkage, and 5.1–5.7 for highly significant linkage. Once a significant QTL was identified, the 95% confidence interval (CI) for the QTL was defined as a 1.5-LOD score support interval. Potential interaction between marker loci was evaluated pairwise. For this analysis, the threshold LOD score for significance at genome-wide 5% level was obtained for all traits by performing 1,000 permutations on the interaction model of Map Manager QTX b20, and then the significance of the total effect of the two loci was tested. Significant threshold LOD scores for total effects are as follows: 8.3 for Bw, 8.6 for Tw, and 8.4 for rTw. For a pair of loci showing the significant total effect, interaction testing was performed according to the User Manual for QTX (by Chmielewicz KM and Manly KF, `http://www.mapmanager.org/mmQTX.html`).

Statistics. A statistical analysis between two groups was performed by use of Student's or Welch's t-test, and the statistical analysis among mouse groups with three possible genotypes in QTL analysis was performed by one-way ANOVA. Statistical analysis on Y-consomic strains was performed by use of Tukey's multiple comparison tests

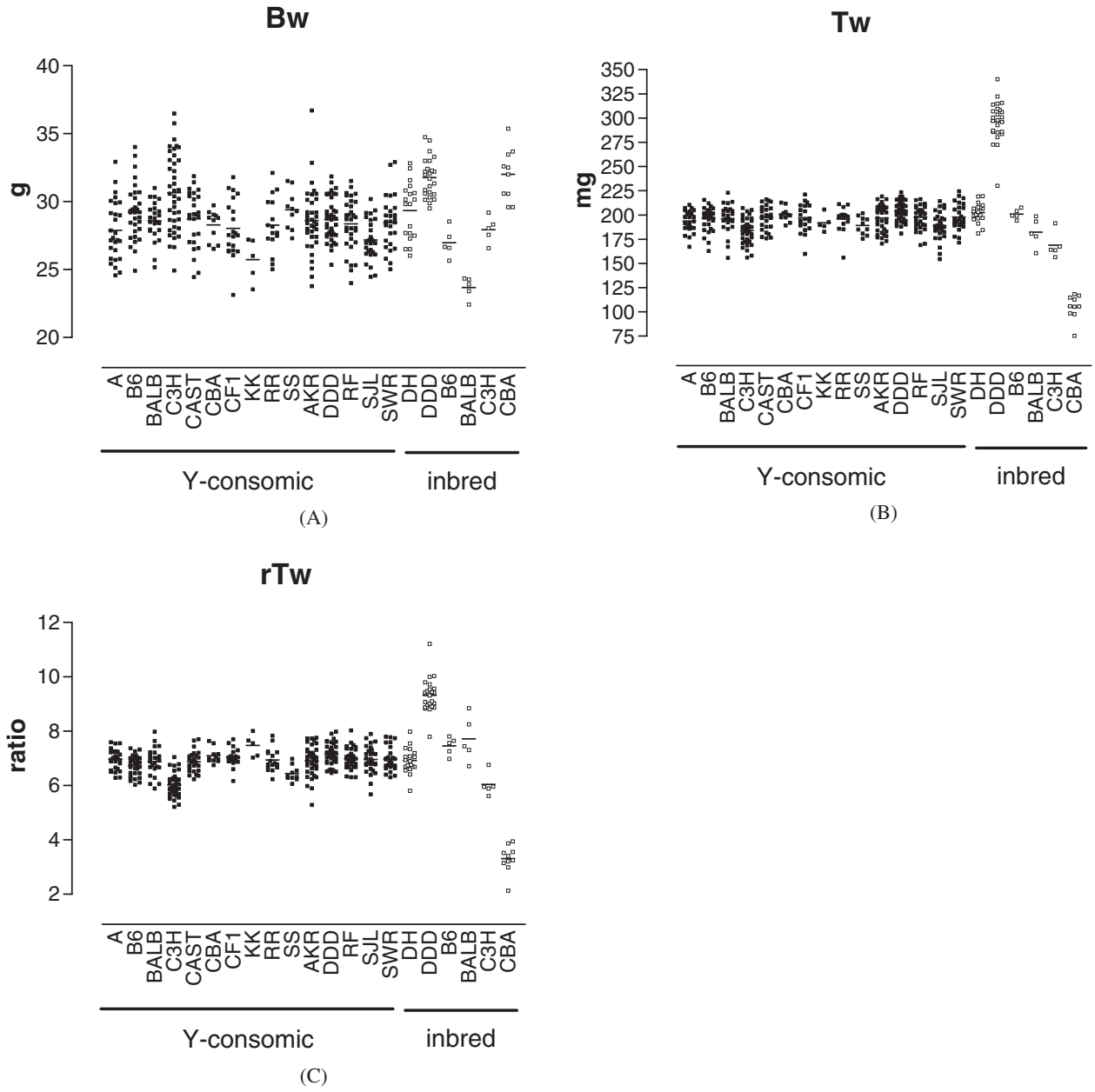

Fig. 1. Scatter plots of trait values in 15 Y-consomic strains (■) and 6 inbred strains (□). Each point represents the trait value of an individual mouse. As to the Y-consomic strains, only the donor strain symbols are presented at X-axis. Each horizontal bar indicates the mean of the trait value. **A**: Bw (body weight, g), **B**: Tw (absolute paired testis weight, mg), **C**: rTw [relative testis weight is expressed as Tw (mg)/Bw (g)]. All measurements were done at the age of 80 ± 1 days after birth.

with SPSS software (SPS for Windows Release 7.5.1J, SPSS Inc., Chicago, IL). $P < 0.05$ was considered to indicate significant difference.

Results

Analyses of Bw, Tw, and rTw in Y-consomic strains.

Scatter plots of trait values (Bw, Tw, and rTw) in 16 Y-consomic strains (including DH) and 6 inbred strains are shown in Fig. 1(A–C), and the results of Tukey's multiple comparison tests are listed in Tables 1–6. DH was listed in plots as one of the inbred strains, but was also included in the

Table 1. Result of Tukey's multiple comparison tests for Bw in Y consomic strains

	B6	BALB	C3H	CAST	CBA	CF1	KK	RR	SS	AKR	DDD	RF	SJL	SWR	DH
A	—	—	***	—	—	—	—	—	—	—	—	—	—	—	—
B6		—	—	—	—	—	*	—	—	—	—	—	**	—	—
BALB			**	—	—	—	—	—	—	—	—	—	—	—	—
C3H				*	*	***	***	**	—	***	***	***	***	***	—
CAST					—	—	—	—	—	—	—	—	—	—	—
CBA						—	—	—	—	—	—	—	—	—	—
CF1							—	—	—	—	—	—	—	—	—
KK								—	—	—	—	—	—	—	*
RR									—	—	—	—	—	—	—
SS										—	—	—	—	—	—
AKR											—	—	—	—	—
DDD												—	—	—	—
RF													—	—	—
SJL														—	*
SWR															—

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, —: Not Significant

Table 2. Result of Tukey's multiple comparison tests for Bw in six inbred strains

	DDD	B6	BALB	C3H	CBA
DH	***	—	***	—	**
DDD		***	***	***	—
B6			*	—	***
BALB				**	***
C3H					***

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, —: Not Significant

statistical analysis in Y-consomic strains as DH-Chr Y^{DH}. B6, BALB, and C3H are progenitor strains for the DH. CBA is known as having the smallest testis among mouse strains on the basis of some publications[1),5),7),21)] and information from the commercial breeder (Japan SLC Inc., `http://www.jslc.co.jp`).

The Bw of a DH-Chr Y^{C3H} was greater than that of other Y-consomic strains except for DH-Chr Y^{B6}, DH-Chr Y^{SS}, and DH-Chr Y^{DH} (Fig. 1A and Table 1), suggesting that Y^{C3H} was different from the Y chromosome of most inbred strains in the ability to control Bw. In inbred strains, the Bw of the BALB was significantly smaller than that of all other strains (Fig. 1A and Table 2). Although there was no significant difference in Bw between DDD and CBA, these were significantly heavier than other strains. Because there was no significant difference in Bw among DH-Chr Y^{BALB}, DH-Chr Y^{DDD}, and DH-Chr Y^{CBA} (Table 1), this suggested that Y^{BALB}, Y^{DDD}, and Y^{CBA} themselves had no significant effects on Bw.

The DH-Chr Y^{C3H} had a significantly lower Tw than did other Y-consomic strains except for DH-Chr Y^{A}, DH-Chr Y^{KK}, DH-Chr Y^{RR}, DH-Chr Y^{SS}, and DH-Chr Y^{SJL} (Fig. 1B and Table 3), suggesting that Y^{C3H} was different from the Y chromosomes of most inbred strains in the ability to control Tw. Among inbred strains, DDD had a significantly higher Tw than did all other strains, whereas CBA had a significantly lower Tw than did all other strains (Table 4). Because there was no significant difference in Tw among DH-Chr Y^{DDD}, DH-Chr Y^{CBA}, and DH-Chr Y^{DH} (Table 3), this suggested that Y^{DDD} and Y^{CBA} themselves had no significant effects on Tw. In contrast, C3H had a significantly lower Tw than did other inbred strains except for BALB (Table 4); thus, it was possible that the lower Tw in C3H is partly due to the effect of Y^{C3H}.

Regarding rTw, DH-Chr Y^{C3H} had a significantly lower rTw than did other Y-consomic strains except for DH-Chr Y^{SS} (Fig. 1C and Table 5), suggesting that Y^{C3H} was different from the Y chromosomes of most inbred strains in the ability to control rTw. Among inbred strains, DDD had a

Table 3. Result of Tukey's multiple comparison tests for Tw in Y consomic strains

	B6	BALB	C3H	CAST	CBA	CF1	KK	RR	SS	AKR	DDD	RF	SJL	SWR	DH
A	—	—	—	—	—	—	—	—	—	—	*	—	—	—	—
B6		—	**	—	—	—	—	—	—	—	—	—	—	—	—
BALB			**	—	—	—	—	—	—	—	—	—	—	—	—
C3H				**	**	*	—	—	—	***	***	***	—	***	***
CAST					—	—	—	—	—	—	—	—	—	—	—
CBA						—	—	—	—	—	—	—	—	—	—
CF1							—	—	—	—	—	—	—	—	—
KK								—	—	—	—	—	—	—	—
RR									—	—	—	—	—	—	—
SS										—	*	—	—	—	—
AKR											—	—	—	—	—
DDD												—	***	—	—
RF													—	—	—
SJL														—	*
SWR															—

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, —: Not Significant

Table 4. Result of Tukey's multiple comparison tests for Tw in six inbred strains

	DDD	B6	BALB	C3H	CBA
DH	***	—	—	**	***
DDD		***	***	***	***
B6			—	*	***
BALB				—	***
C3H					***

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, —: Not Significant

significantly higher rTw than did all other strains, whereas CBA had a significantly lower rTw than did all other strains (Table 6). The finding that there was no significant difference in rTw among DH-Chr Y^{DDD}, DH-Chr Y^{CBA}, and DH-Chr Y^{DH} (Table 5) suggested that Y^{DDD} and Y^{CBA} themselves had no significant effects on rTw. Again, C3H had a significantly lower rTw than did the other inbred strains (Table 6); thus, it was possible that the lower rTw in C3H is partly due to the effect of Y^{C3H}. In all traits examined, there were also significant differences among several Y-consomic strains (Tables 1, 3, and 5), but, as can be seen, DH-Y^{C3H} was stood out among Y-consomic strains. The singularity of the Y^{C3H} was expressed most prominently in rTw, probably because the Bw-increasing effect and the Tw-decreasing effect of Y^{C3H} were combined.

These results suggest that some Y chromosomes have effects on Bw, Tw, and rTw, but the Y chromosomes themselves of most inbred mouse strains had only modest effects on these traits. Instead, it is suggested that these traits are controlled mainly by autosomal and/or X-linked genes. Therefore, to search for such genes, I performed a subsequent QTL analysis.

QTL analysis. The 72 F_2 mice were genotyped for a total of 92 microsatellite marker loci distributed on all autosomes and on the X chromosome; the average distance between markers was approximately 17.4 cM (1,600 cM/92). A list of microsatellite markers used in the present study with their chromosomal location from the information retrieved from the MGI (February 5, 2008) is available upon request.

In the initial screening of 72 selected from 143 F_2 mice, a significant linkage (LOD score ≥ 4.3 was applied to an initial analysis) was identified on chromosome 17 (*D17Mit164* for rTw), and suggestive linkages (LOD score ≥ 2.8 was applied to an initial analysis) were identified on chromosomes 4 (near *D4Mit214* for Tw), 9 (*D9Mit229* for Tw), 11 (*D11Mit236* for Bw and Sw), and 14 (*D14Mit165* for rTw). The remaining 71 F_2 mice and 30 newly

Table 5. Result of Tukey's multiple comparison tests for rTw in Y consomic strains

	B6	BALB	C3H	CAST	CBA	CF1	KK	RR	SS	AKR	DDD	RF	SJL	SWR	DH
A	—	—	***	—	—	—	—	—	—	—	—	—	—	—	—
B6		—	***	—	—	—	*	—	—	—	**	—	—	—	—
BALB			***	—	—	—	—	—	—	—	—	—	—	—	—
C3H				***	***	***	***	***	—	***	***	***	***	***	***
CAST					—	—	—	—	—	—	—	—	—	—	—
CBA						—	—	—	*	—	—	—	—	—	—
CF1							—	—	*	—	—	—	—	—	—
KK								—	***	—	—	—	—	—	—
RR									—	—	—	—	—	—	—
SS										—	***	*	—	—	—
AKR											—	—	—	—	—
DDD												—	—	—	—
RF													—	—	—
SJL														—	—
SWR															—

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, —: Not Significant

Table 6. Result of tukey's multiple comparison tests for rTw in six inbred strains

	DDD	B6	BALB	C3H	CBA
DH	***	—	—	*	***
DDD		***	***	***	***
B6			—	**	***
BALB				***	***
C3H					***

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, —: Not Significant

produced F_2 mice were genotyped for all of the loci on chromosomes 9, 11, 14, and 17, and for *D4Mit214*. In addition, *D1Mit293*, *D5Mit240*, *D7Mit250*, *D10Mit188*, *D12Mit141*, *D13Mit139*, *D18Mit60*, and *D18Mit123* were genotyped, because these loci showed LOD scores with a near-suggestive threshold for some traits.

Pearson's correlation coefficient between Bw and Tw was 0.46 ($P = 9.68 \times 10^{-11}$), that between Bw and Sw was 0.42 ($P = 6.76 \times 10^{-9}$), and that between Tw and Sw was 0.06 ($P = 0.42$). It was thus suggested that both of Tw and Sw are influenced by Bw to a certain extent, but distinct genetic bases may underlie in the control of Tw and Sw.

Bw QTLs. Scatter plots of Bw for DDD, DH-Chr Y^{DDD}, DH, DDD × DH F_1, DH × DDD F_1, and F_2 are shown in Fig. 2A. In the comparison of Bw among DDD, DH-Chr Y^{DDD}, and DH, DDD mice were significantly heavier than DH and DH-Chr Y^{DDD}, but no significant difference was identified between DH and DH-Chr Y^{DDD}. The results suggested that Y^{DDD} itself had no significant effect on Bw in the DH background, and the larger Bw is attributed to autosomal, X-linked, and/or mitochondrial genes. Therefore, it was anticipated that some of these gene loci would be revealed by QTL analysis.

Interestingly, DH F_1 were significantly heavier than HD F_1 mice ($P < 0.02$). Because Y^{DDD} did not differ from Y^{DH} in its effect on Bw, this reciprocal cross effect should again be attributed to the effect of X-linked genes, mitochondrial genes, or imprinted genes.

One significant Bw QTL was identified on proximal chromosome 11, near *D11Mit236*, with a peak LOD score of 5.3 (Table 7, Fig. 3A). I named this locus *Bwdq1* (body weight in DDD male QTL 1). The D allele at *Bwdq1* increased the Bw in an additive manner (Tables 7, 8). In addition, two separate suggestive Bw QTLs were identified on proximal- and distal-part of chromosome 17 (Table 7, Fig. 3B). At the proximal locus (near *D17Mit164*), the D allele was associated with a

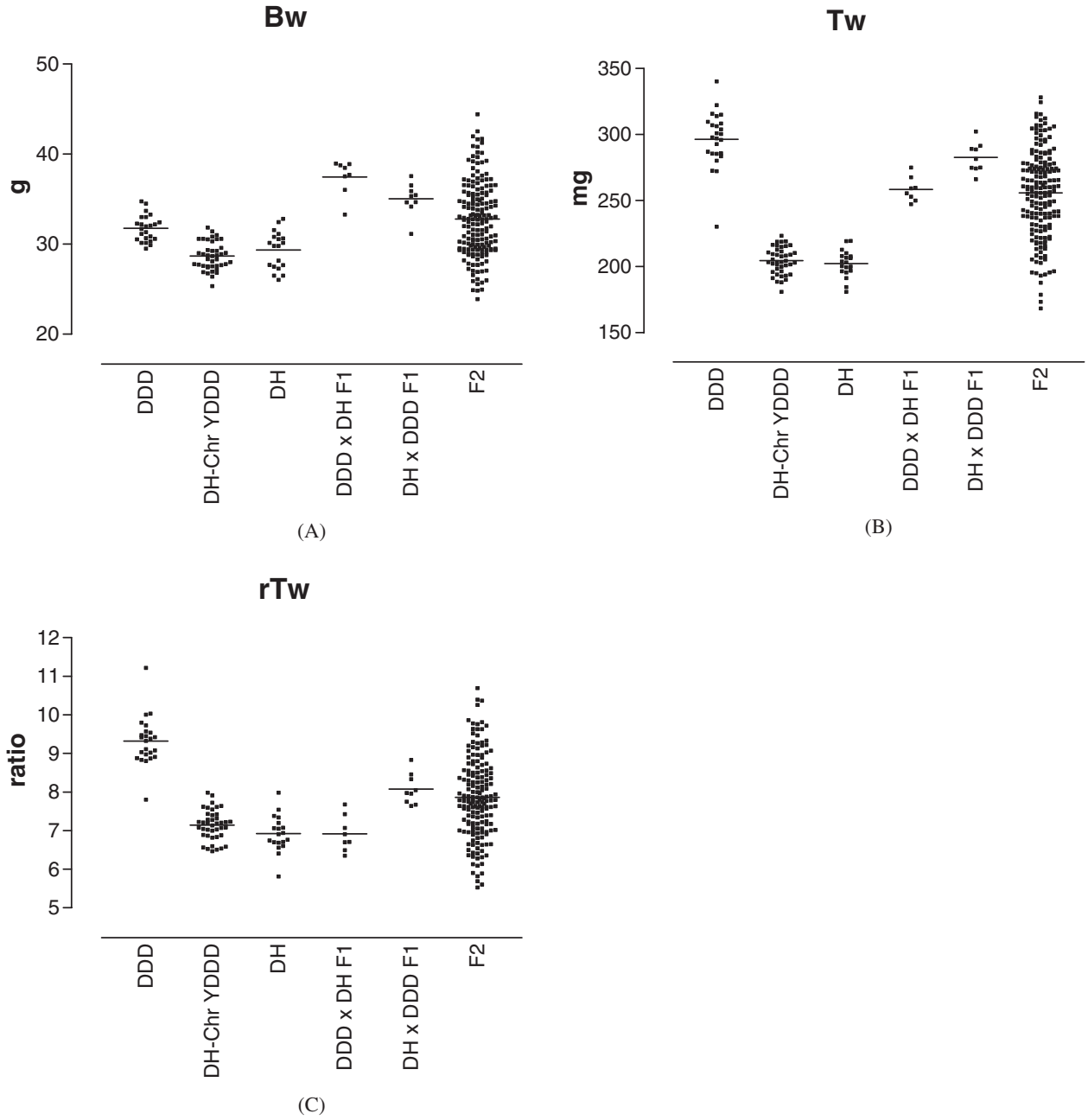

Fig. 2. Scatter plots of trait values in DDD (n = 24–25), DH-Chr Y^{DDD} (n = 41), DH (n = 19), ♀DDD × ♂DH F_1 (n = 8), ♀DH × ♂DDD F_1 (n = 9), and ♀DDD × ♂DH F_2 (n = 171–173). Each point represents the trait value of an individual mouse. Each horizontal bar indicates mean of the trait value. **A**: Bw (body weight, g), **B**: Tw (absolute paired testis weight, mg), **C**: rTw [relative testis weight is expressed as Tw (mg)/Bw (g)]. All measurements were done at the age of 80 ± 1 days after birth.

decrease in Bw, while the D allele was associated with an increase in Bw at the distal locus (near *D17Mit123*).

Tw QTLs. In a similar way as for the Bw, scatter plots of the Tw for DDD, DH-Chr Y^{DDD}, DH, DDD × DH F_1, DH × DDD F_1, and F_2 are shown in Fig. 1B. In the comparison of Tw among DDD, DH-Chr Y^{DDD}, and DH, DDD mice had a

Table 7. QTLs identified in this study

Traits[a]	Chr (Closest marker)	Peak position[b] (CI[c])	LOD (% Variance[d])	Add[e]	Dom[f]	QTL symbol[g]
Bw	11 (*D11Mit236*)	23 (17–32)	5.3 (13)**	0.03	−0.01	*Bwdq1*
	17 (*D!7Mit164*)	4	2.3 (6)*	−0.02	−0.00	
	17 (*D17Mit123*)	57	2.7 (7)*	0.02	−0.00	
Tw	4 (*D4Mit214*)	18	2.9*			
	5 (*D5Mit240*)	59	2.6*			
	9 (*D9Mit229*)	24 ([h]–50)	4.3 (11)**	15.96	−6.30	*Twdq1*
	14 (*D14Mit165*)	51	2.7 (7)*	12.50	−5.64	
	17 (*D17Mit139*)	33	2.6 (7)*	13.15	−0.21	
rTw	9 (*D9Mit229*)	22	3.1 (8)*	0.45	−0.08	
	11 (*D11Mit236*)	28	2.2 (6)*	−0.39	0.21	
	14 (*D14Mit165*)	52 (46–57)	5.2 (13)***	0.45	−0.46	*Rtwdq1*
	17 (*D17Mit164*)	7 ([h]–13)	6.8 (17)***	0.64	−0.28	*Rtwdq2*
Sw	9 (*D9Mit229*)	25	2.1 (6)*	−4.60	−4.11	
	11 (*D11Mit236*)	18 (14–26)	6.8 (17)***	9.76	3.83	*Swdq1*
rSw	9 (*D9Mit229*)	22	2.4 (6)*	−0.17	−0.08	

a: Bw, log-transformed Bw (g); Tw, paired testis weight in mg; rTw, paired testis weight relative to Bw [Tw (mg)/Bw (g)]; Sw, spleen weight in mg; rSw, spleen weight relative to Bw [Sw (mg)/Bw (g)].
b: Peak position of the LOD score plot curve is expressed as distance from the centromere in cM.
c: 95% confidence interval (CI) is defined by 1.5-LOD support interval. CI is given only to significant QTLs.
d: Total variance explained by QTL at this locus is expressed as percent. When only one or two markers are fully genotyped in all 173 F_2 as to the relevant chromosome, this value is not given. *: suggestive, **: significant, and ***: highly significant.
e: The additive component of the QTL D allele effect. Positive value indicates that the D allele is associated with increased trait values, and negative value indicates that the D allele is associated with decreased trait values. When only one or two markers are fully genotyped in all 173 F_2 as to the relevant chromosome, this value is not given.
f: The dominance component of the QTL D allele effect. When only single marker is fully genotyped in all 173 F_2 as to the relevant chromosome, this value is not given.
g: Assignment of the QTL symbol is limited to significant and highly significant QTLs.
h: Proximal end of CI cannot be defined because it extends proximally.

significantly larger Tw than did DH and DH-Chr Y^{DDD}, but no significant difference was identified between DH and DH-Chr Y^{DDD}. The results suggest that Y^{DDD} itself had no significant effect on Tw in the DH background.

A reciprocal cross effect was observed; that is, DH × DDD F_1 had a significantly larger Tw than did DDD × DH F_1 ($P < 0.0003$).

One significant Tw QTL was identified on chromosome 9, near *D9Mit229*, with a LOD score of 4.3 (Table 7, Fig. 3C). I named this locus *Twdq1* (testis weight in DDD male QTL 1). The D allele at *Twdq1* increased the Tw in an additive manner (Tables 7, 8). In addition, four suggestive Tw QTLs were identified on chromosomes 4, 5, 14, and 17 (Table 7). At all these loci, the D allele was associated with an increase in Tw.

rTw QTLs. Scatter plots of rTw for DDD, DH-Chr Y^{DDD}, DH, DDD × DH F_1, DH × DDD F_1, and F_2 are shown in Fig. 1C. In the comparison of rTw among DDD, DH-Chr Y^{DDD}, and DH, DDD mice had significantly larger rTw than did DH and DH-Chr Y^{DDD}, but no significant difference was identified between DH and DH-Chr Y^{DDD}. The results suggest that Y^{DDD} itself had no significant effect on rTw in the DH background.

A reciprocal cross effect was again observed; that is, DH × DDD F_1 had a significantly larger rTw than did DDD × DH F_1 ($P < 0.00006$).

Two highly significant rTw QTLs were identified on chromosomes 14 (near *D14Mit165*) and 17 (near *D17Mit164*) (Table 7, Figs. 3B and D). I assigned the locus symbols *Rtwdq1* (relative testis weight in DDD male QTL 1) and *Rtwdq2* (relative testis weight in DDD male QTL 2) to these QTLs, respectively. The peak LOD score for *Rtwdq1* was 5.2, and this locus explained 13% of the F_2 variance. The D allele at *Rtwdq1* was recessive to the H allele, and it increased the rTw (Tables 7, 8). On the other hand, the peak LOD score for *Rtwdq2* was 6.8, and

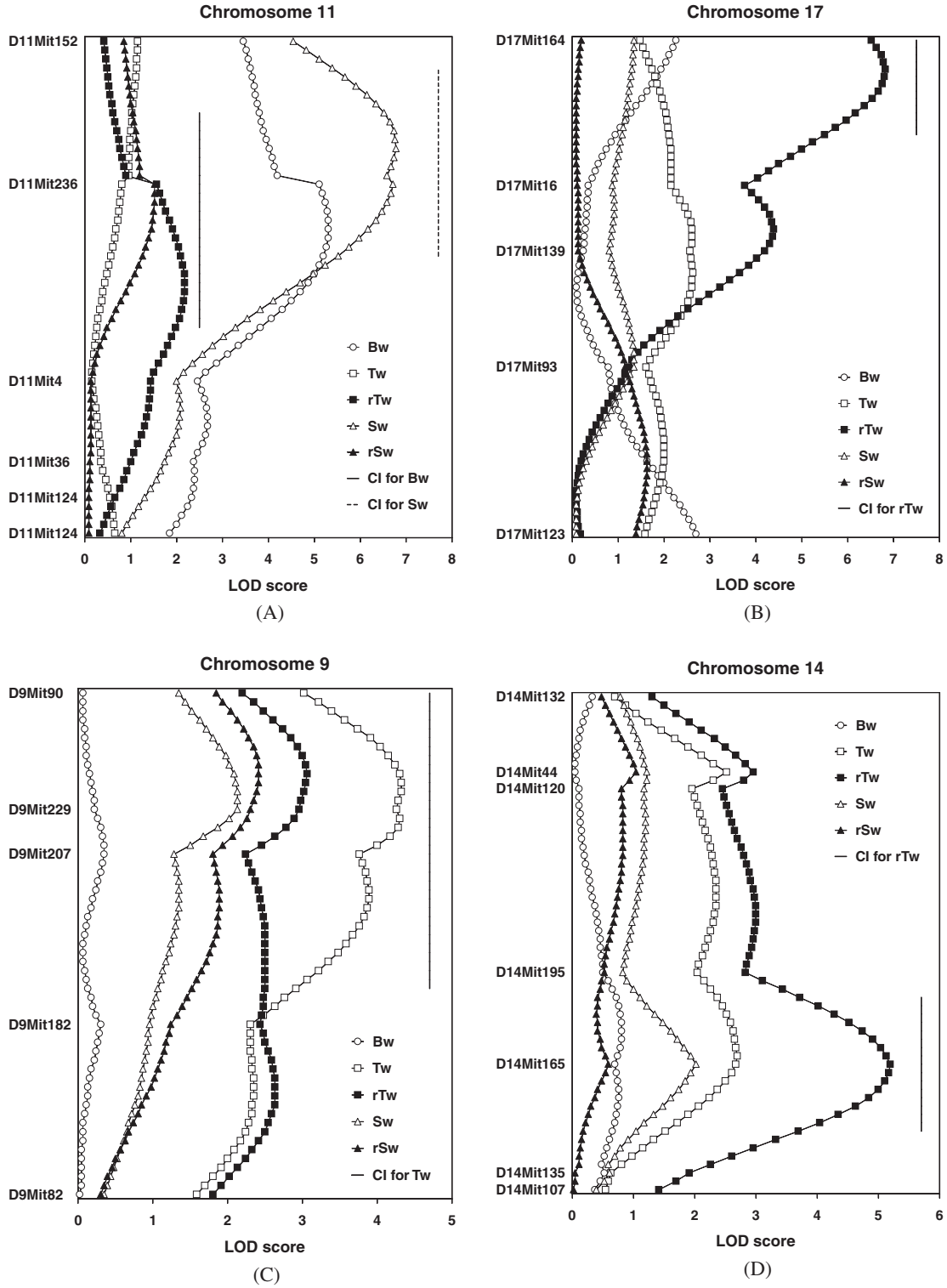

Fig. 3. LOD score plots of Bw (body weight), Tw (absolute paired testis weight), rTw (relative testis weight), Sw (absolute spleen weight), and rSw (relative spleen weight) on chromosomes 11 (**A**), 17 (**B**), 9 (**C**), and 14 (**D**). Each vertical line indicates 95% confidence interval (CI) for a defined trait. X-axis represents LOD score. Y-axis represents microsatellite localization; the upper part of the graph is in the direction of the centromere, and the lower part is in the direction of the telomere.

Table 8. Effect of significant QTLs on the basis of single point statistics

QTL (Closest marker)	Trait	Mean ± S.E.M. trait values by marker genotype			Nominal P value
		H/H	H/D	D/D	
Bwdq1 (*D11Mit236*)	Bw (g)	30.93 ± 0.58	32.64 ± 0.41	35.34 ± 0.68	0.0000087
	Tw (mg)	249.83 ± 5.32	255.27 ± 3.36	263.95 ± 5.23	0.16
	rTw	8.14 ± 0.18	7.87 ± 0.11	7.51 ± 0.14	0.029
Twdq1 (*D9Mit229*)	Bw (g)	32.86 ± 0.61	32.48 ± 0.43	33.37 ± 0.74	0.539
	Tw (mg)	244.61 ± 4.78	253.12 ± 3.44	274.28 ± 4.42	0.000067
	rTw	7.49 ± 0.14	7.84 ± 0.12	8.31 ± 0.16	0.0013
Rtwdq1 (*D14Mit165*)	Bw (g)	32.36 ± 0.70	33.29 ± 0.44	31.96 ± 0.59	0.19
	Tw (mg)	246.93 ± 4.94	253.02 ± 3.36	271.13 ± 4.90	0.0023
	rTw	7.67 ± 0.15	7.65 ± 0.10	8.56 ± 0.18	0.0000081
Rtwdq2 (*D17Mit164*)	Bw (g)	34.02 ± 0.55	32.46 ± 0.45	31.25 ± 0.67	0.0056
	Tw (mg)	252.40 ± 3.87	253.01 ± 3.87	269.26 ± 5.46	0.036
	rTw	7.49 ± 0.13	7.81 ± 0.10	8.69 ± 0.19	0.00000040

this locus explained 17% of the F_2 variance. The D allele at *Rtwdq2* increased rTw in an additive manner (Tables 7, 8). In addition, two suggestive QTLs were identified on chromosomes 9 and 11. Of these, a locus on chromosome 9 was mapped to near *D9Mit229*, with a peak LOD score of 3.1 (Fig. 3C). At this locus, the D allele was associated with an increase in rTw. This locus was suggested to be allelic with *Twdq1*.

It was thus revealed that Y^{DDD} itself had no significant effects on Bw, Tw, and rTw, and significant QTLs for these traits were confirmed on DDD autosomes; however, it was uncertain whether there would be any effects by interactions between Y chromosomal and autosomal genes.

Sw and rSw QTLs. Although a data plot is not shown, the DDD strain had very large spleen among the inbred strains, and F_2 mice showed a spectrum of Sw and rSw.

One highly significant Sw QTL was identified on chromosome 11 (near *D11Mit236*) (Table 7, Fig. 3A). I assigned the locus symbol *Swdq1* (spleen weight in DDD male QTL 1) to this QTL. The peak LOD score for *Swdq1* was 6.8, and this locus explained 17% of the F_2 variance. The D allele at *Swdq1* increased the Sw in an additive manner. *Swdq1* was suggested to be allelic with *Bwdq1*, and probably for this reason, only one suggestive rSw locus was identified on chromosome 9. This locus was also suggestive QTL for Sw.

Next, potential interaction between marker loci was evaluated pairwise for Bw, Tw, and rTw. One significant interaction was detected for rTw. A marker pair of *D1Mit293* and *D12Mit141* showed LOD score of 8.9 as a total LOD score for association, and LOD score of 4.1 as an interaction LOD score. Interaction LOD score was higher than that for significant threshold LOD score obtained for interval mapping, and the P value for interaction effect was 0.002 (recommended P value was less than 0.01); these results satisfied a criterion for declaring significant pairwise interaction. Because both loci had not been genotyped completely till then, I genotyped both loci in 173 F_2 mice. As a result, the interaction of this marker pair was revealed to be not statistically significant.

Finally, the effect of a significant QTL on other traits was examined. Because Bw and Tw are mutually interrelated traits, it is appropriate to assess whether the QTL has any effect on other related traits by using a point-wise, rather than a genome-wide, significance threshold of $P = 0.05$.[22] Table 8 summarizes the results. It can be seen that *Bwdq1* had a significant effect on the rTw, but the D allele was associated with a decreased rTw. *Twdq1* also had a significant effect on the rTw, and the D allele was associated with an increased rTw. *Rtwdq1* had no significant effect on Bw, but had significant effect on Tw. In contrast to *Rtwdq1*, *Rtwdq2* had significant effect on the Bw and Tw. The D allele at the *Rtwdq2* was associated with a decreased Bw, but was associated with an increased Tw. These results suggested the presence of a complex interrelation between Bw and Tw, and therefore rTw.

Discussion

Effect of Y-linked genes on testis weight is rather modest. The effect of the Y-linked genes on testis weight has been argued about for many years, and the results are still conflicting.[1),5)–8)] Hayward and Shire,[1)] Le Roy *et al.*,[5)] and Hunt and Mittwoch,[7)] and reported results that support the presence of a Y chromosomal effect. In contrast, Herrick and Wolfe[6)] and Chubb[8)] claimed that it is unlikely that the Y chromosome has a significant effect. A problem is that the former three studies were done on some CBA substrains, whereas the latter two did not use the CBA strain. This is why I analyzed Y-consomic strains by incorporating the CBA strain in this study. Nevertheless, there was still a major discrepancy among the results of the preceding three studies[1),5),7)] and those of the present one with regard to the effect of Y^{CBA}. Hayward and Shire[1)] produced several genetic crosses including F_1, F_2, F_3, F_4, and N_2 backcross progeny between CBA/FaCam and SF/Cam strains, and they showed that rTw is clearly segregated with the type of Y chromosome; that is, Y^{CBA} is associated with a lower rTw. Although they admitted that there was an autosomal contribution, they estimated that 41% of the difference in rTw was due to the Y chromosome. Hunt and Mittwoch[7)] produced F_1, F_2, and N_2 backcross progeny between CBA/Gr and BALB/c, and they showed that the Tw is affected by factors on the Y chromosome as well as those on autosomes and the X chromosome. They estimated that approximately 46% of the difference in Tw was attributable to the effect of the Y chromosome. These two studies are similar in presenting the argument that a relatively large portion of the difference in testis weight is due to the Y chromosome. Le Roy *et al.*[5)] produced F_1 and F_2 progeny between CBA/H and NZB/BINJ, performed a QTL analysis on Tw, and identified testis weight determinants on several autosomes and the X chromosome. They also showed evidence that a Y-consomic strain carrying Y^{CBA} (to be exact, the non-recombining part of the Y^{CBA}) on an NZB background had a significantly lower Tw than did the NZB strain. The most plausible explanation for the inconsistency between Le Roy's study[5)] and this study is the difference in the background strain. Y^{CBA} reduced the Tw on the NZB background, but not on the DH background. A CBA substrain difference was unlikely to be suspected, because all CBA substrains, including the present strain CBA/N, had very small Tw and rTw. The combination of the Y chromosome and the background genome may be crucially important for controlling testis weight, and native Y^{NZB} may be essential for sustaining a relatively larger Tw in the NZB strain, because the Tw was not changed in the CBA strain when its Y chromosome was replaced by that from the NZB strain.[5)] In this regard, it is interesting to replace the Y chromosome in the DDD strain by the Y chromosome of a different strain (i.e. DH-Chr Y^{DH}, see below), but this cannot be tested immediately.

In addition, although I did not measure trait values in the inbred AKR strain, several studies reported that the AKR strain has a smaller testis than do the other inbred strains.[4),23)] However, Y^{AKR} itself had no significant effects on any of the traits examined in the present study (Tables 1, 3, and 5). This result also suggested the absence of a testis weight determinant on the Y chromosome in the AKR strain. Overall, the effect of the Y chromosome itself on testis weight was surely present, but it was generally rather modest. However, I cannot rule out a possibility that there would be any effects by interactions between Y chromosomal and autosomal genes. It seems to be crucially important to establish and analyze DDD-Chr Y^{DH} for verifying the possibility.

Concerning the singularity of Y^{C3H}, colleagues and I have previously reported that Y^{C3H} was different from Y^{B6}, Y^{BAL}, and Y^{DH}[24)] in the ability to cause neonatal lethality in (♀DDD × ♂DH-*Dh*/+) F_1-*Dh*/+ males.[15),24)] Colleagues and I previously reported that F_1-*Dh*/+ male mice resulting from a cross between DDD females and DH-*Dh*/+ males were essentially lethal during neonatal period; however, this did not occur in the reciprocal cross.[15)] Subsequent genetic mapping analysis revealed that the lethality was caused by a combination of three independent gene loci; that is the *Dh* locus on chromosome 1, *Grdhq1* locus on the X chromosome, and the Y-linked gene locus from some inbred strains.[24)] As to the Y-linked gene, Y^{B6}, Y^{BAL}, and Y^{DH} caused lethality, but Y^{C3H} did not. The singularity of Y^{C3H} among these four strains was supported by the nucleotide polymorphisms of the *Sry* gene.[24)] On the basis of the results of statistical comparison presented in Tables 2, 4, and

6, Y^{DH} was suggested to be the same as Y^{B6} rather than as Y^{BALB}.

Major testis weight determinants are autosomal. Several coincidental QTLs or candidate genes can be postulated for the present QTLs. In particular, there are numerous potential genes or loci within a 95% CI for *Bwdq1*. Among nearly ten candidate genes picked up in an MGI search, colony stimulating factor 2 (granulocyte-macrophage) (*Csf2*, 29.5 cM)[25),26)] and glycine receptor, alpha 1 subunit (*Glra1*, 30.0 cM),[27)] are known to have effects on spleen weight; therefore, taking the coincidental occurrence of *Bwdq1* and *Swdq1* into consideration, these two genes are the best candidates for these QTLs. Several overlapping QTLs are also known. Among them, weight gain in high growth mice 7 (*Wg7*)[28)] and body weight, 10 weeks, QTL 3 (*Wt10q3*),[29)] can be regarded as coincidental QTLs.

Although I do not enumerate them in detail, many candidate genes can be postulated for *Twdq1*; this is partly because of a very large CI for this locus (Table 7). According to the mutant phenotypes provided by MGI, many of the candidate genes may have crucial roles in spermatogenesis. It is impossible to point out which is the best candidate gene for *Twdq1* at the moment.

In contrast to *Twdq1* on chromosome 9, only a few candidate genes can be postulated for *Rtwdq1* on chromosome 14 and *Rtwdq2* on chromosome 17. Indeed, the fibronectin type III domain containing 3a (*Fndc3a*)[30)] is the only plausible candidate gene for *Rtwdq1*. For *Rtwdq2*, there are three possible candidate genes; of these, one is a gene and the remaining two are QTLs. The first candidate is a high mobility group AT-hook 1 (*Hmga1*).[31)] The second candidate is a male hybrid sterility QTL 1 (*Mhstq1*).[12)] *Mhstq1* causes male infertility as well as a small testis. Because proximal chromosome 17 holds five hybrid sterility gene loci (*Hst1*, *Hst4-7*), an apparent association of *Rtwdq2* with male fertility is suggested. Third, an association between the *H2* haplotype and testis weight has been reported.[22),32)] Iványi *et al.*[32)] assigned the gene symbol *Hom1* (hormone metabolism 1) to this locus. Shukri and Shire[33)] also reported that the segregation pattern for Tw was identical to that for the *H2* locus. However, *H2* is located at 23 cM, which is outside the CI for *Rtwdq2*; therefore, *H2* is clearly excluded from being a candidate. However, assuming that the gene causative of *Hom1* is not *H2*, and that *Hom1* is allelic to *Rtwdq2*, there are no discrepancies between the present result and previously reported results, because there was a pointwise significant linkage for rTw at 23 cM position in the present study (Fig. 3B). Interestingly, Gregorová and Iványi[23)] produced and analyzed C57BL/10ScSnPh × AKR/J F_2 mice and found that the AKR allele at the *H2* locus was associated with an increased Tw (not significant) and rTw (significant), but was associated with a decreased Bw (significant). With regard to the effect of a locus on proximal chromosome 17, the inverse correlation between Bw and rTw (Tw) was also observed in this study. Finally, a suggestive QTL on chromosome 11 for rTw has an overlapping QTL, low testis weight 1 (*Lstw1*), which was identified in an interspecific recombinant congenic mice between B6 and *Mus spretus*.[14)]

Because of the limitation of the experimental cross design, I could not investigate the effects of mitochondrial and imprinted genes as well as the effects by interactions between Y chromosomal and autosomal genes, although the presence of these effects was suggested in this study. Nevertheless, I identified one suggestive and one significant QTLs for Tw, and two highly significant QTLs for rTW. The identification of the genes underlying these QTLs will provide insight into the genetic control of testis weight.

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