

Marker-assisted introgression of Trypanotolerance QTL in mice

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Abstract

A marker-assisted introgression (MAI) experiment was conducted to use genetic markers to transfer each of the three trypanotolerance QTL from a donor mouse strain, C57BL/6, into a recipient mouse strain, A/J. We used a backcross strategy that consisted of selecting two lines, each carrying two of the donor QTL alleles through the backcross (BC) phase. At the fourth BC generation, single-carrier animals were selected for the production of homozygous animal in the intercross phase. The QTL regions (QTLR) were located on chromosomes MMU1, MMU5, and MMU17. Groups of mice with different genotypes and the parental lines were subjected to a challenge with *Trypanosoma congolense*. The results show that trypanotolerance QTL was successfully moved into the recipient background genotype, yielding a longer survival time. The mean estimated survival time was 57.9, 49.5, and 46.8 days for groups of mice carrying the donor QTL on MMU1, MMU5, and MMU17 on A/J background. The mean estimated survival time was 29.7 days for the susceptible A/J line and 68.8 days for the resistant C57BL/6 line. The estimated QTLR effects are close to 30% smaller than those in the original mapping population which was likely caused by the difference in the background on which the effects of QTLR are tested. This is the first report of successful marker-assisted introgression of QTL in animals. It is experimental proof of the use of genetic markers for marker-assisted introgression in animal breeding.

Trypanosomiasis is the most important constraint to livestock development in the subhumid and non-forested portions of the humid zone of Africa. The disease costs approximately US \$1340 million per year for livestock producers in Africa (Kristjanson et al. 1999). This cost excludes losses due to reduction of manure availability and the inability to use draught power.

N'Dama and West African Shorthorn cattle (Baoulé, Muturu, Lagune) are recognized for their ability to withstand the effect of trypanosome infection and to remain productive in areas where trypanosomiasis prevents the presence of other cattle types, or significantly reduces their productivity (Murray and Trail 1984; Trail et al. 1989). This ability to withstand trypanosome infection, called trypanotolerance, is an innate feature of the Longhorn N'Dama and other Shorthorn cattle from West Africa (Roberts and Gray 1973; Roelants 1986; Doko et al. 1991). At the International Livestock Research Institute (ILRI) in Nairobi, Kenya, a linkage study was initiated on an F₂ generation of a cross between N'Dama and Boran cattle to identify genes or QTL involved in trypanotolerance (Teale 1993). Hanotte et al. (2003) analyzed data of this experiment and detected several QTL controlling resistance to trypanosomiasis in cattle. Further, they found that some of the trypanotolerant QTL alleles originated from the susceptible Boran cattle. The detection and the identification of genes related to trypanotolerance offer opportunity for marker-assisted introgression (MAI) of these genes in more susceptible cattle.

Various laboratory inbred mouse strains show variation in resistance to *Trypanosoma congolense* infection. Among these laboratory mouse strains, the C57BL/6 strain appears to be one of the most resistant, with mean survival time of 110.2 days, whereas the A/J strain, with mean survival of 15.8 days, appears to be the least resistant (Morrison et al. 1978). Thus, although the survival times following

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challenge vary considerably among strains, infection with *T. consolense* usually results in death of all strains of mice (Teale et al. 1999). This stands in contrast to the situation in cattle, where resistant breeds are able to clear the infection.

Based on two F₂ populations, A/J × C57BL/6 and BALB/c × C57BL/6, Kemp et al. (1996, 1997) showed that three chromosomal regions were associated with trypanotolerance in mice. The marker density now available on the mouse genome and the identification of markers linked to these QTL allow a MAI experiment in mice. This provides a unique opportunity to experimentally verify the efficacy of MAI for this trait, as mice are more convenient in terms of costs and generation interval than cattle and can serve as a model.

Marker-assisted introgression is a crossbreeding program that aims at incorporating genes from a donor into a recipient line through a backcross design. It consists of creation of an F₁ generation from founder individuals, followed by a number of backcross generations and completed with an intercross phase to fix the introgressed genes (Soller and Plotkin-Hazan 1977; Koudandé et al. 2000). Koudandé et al. (1999) revealed that simultaneous introgression of three QTL is practically impossible. Based on these results an alternative MAI experiment involving two backcross lines was designed that aimed at the production of three different lines homozygous for each of the QTL.

This article aims at investigating the effectiveness of a MAI experiment aimed at incorporating each of the three different trypanotolerance QTL alleles from the mouse strain C57BL/6 (donor) into the mouse strain A/J (recipient). Whereas reports on MAI in animals are scarcely available (e.g., Yancovich et al. 1996; Markel et al. 1997), they flourish in plant breeding (e.g., De Vries et al. 1992; Oertel and Matzk 1999; Khurstaleva and Kik 2000; Lim et al. 2000). Our report, therefore, fulfills this gap between experimental animal and plant breeding.

Materials and methods

The introgression strategy is based on the model calculations of Koudandé et al. (2000). The strategy consisted of selecting two lines of mice, each carrying two out of the three donor QTL alleles through the backcross phase followed by the intercross phase. During the intercross phase, selection aimed at achieving three different lines.

Breeding program. Inbred mouse strains A/J (OlaHsdnd) and C57BL/6 (OlaHsd) (purchased from Harlan UK Ltd., Bicester, UK), are maintained at

ILRI as pure strains for research purposes. In the present experiment, C57BL/6 being resistant was the donor and A/J being sensitive was the recipient. A set of markers was chosen to define each QTL-containing region (QTLR) and donor and recipient marker allele haplotypes defined for each region. For the breeding program, a reciprocal cross was performed between founder parents (10 males C57BL/6 × 10 females A/J and 10 males A/J × 10 females C57BL/6) to produce F₁ animals. F₁ males were crossed to A/J females and F₁ females were crossed to A/J males to produce the first backcross generation (BC₁). Male mice from BC₁ were genotyped and two lines of mice were selected, each carrying two of the three donor QTLR: Line L_{1,5}, mice carrying donor marker allele haplotypes for the QTLR on MMU1 and MMU5 in the heterozygous state, and Line L_{15,17}, mice carrying donor marker allele haplotypes for the QTLR on MMU5 and MMU17 in the heterozygous state. In both lines, no selection was applied on the markers of the third QTLR. These two lines were maintained separately throughout the backcross phase as suggested by Koudandé et al. (2000). Selected BC₁ males were backcrossed to A/J females to produce BC₂ mice within each of the two lines. From BC₂ onward males and females were genotyped and individuals heterozygous for donor marker allele haplotypes were selected as parents for the next BC generation as noted. This continued through production of the BC₄ generation.

In the BC₄ generation, the selection procedure for L_{1,5} and L_{5,17} was slightly modified with the aim of producing different genotypes during the intercrossing phase. In addition to selection for donor marker allele haplotypes at the QTLR defining the line, there was also selection for recipient haplotypes at the remaining QTLR (that is, for the recipient MMU17 QTLR in L_{1,5}, and for the recipient haplotype for the MMU1 QTLR in L_{5,17}). Also, in this generation, in addition to continuation of lines L_{1,5} and L_{5,17} three new "single-carrier" lines were developed from them: L₁, consisting of mice carrying donor haplotype at the QTLR on MMU1 and recipient haplotypes at the QTLR on MMU5 and MMU17; L₅, consisting of mice carrying donor haplotype at the QTLR on MMU5 and recipient haplotypes at the QTLR on MMU1 and MMU17; and L₁₇, consisting of mice carrying, donor haplotype at the QTLR on MMU17 and recipient haplotypes at the QTLR on MMU1 and MMU5.

After BC₄ an intercrossing was performed within each of the new lines L₁, L₅, and L₁₇, aimed at producing homozygous individuals for the donor haplotypes at the target QTLR. Because of the limited number of selected females in L₁₇, males from L₁₇

were also crossed with females from L_{5,17} to generate additional mice homozygous for the donor haplotype on MMU17. A second generation of intercrossing was performed to increase the number of homozygous mice in each line by primarily crossing selected homozygous mice either with each other or with heterozygous mice of the same line, as well as crossing heterozygous mice inter se. Meanwhile, mice that were homozygous for recipient (sensitive) haplotypes at all three QTLR were selected from all three intercrosses as internal controls (group 10 in Table 2). For the challenge phase of the experiment, 12 groups of mice were produced (Table 2). Nine of the 12 groups were derived from the homozygous mice obtained in lines L₁, L₅, and L₁₇, namely, all females from the three lines were crossed with corresponding males to produce homozygous mice for groups 1, 4, and 7; other males from these three lines were used for backcrossing to both parental lines to generate more combinations and groups to be challenged (groups 2, 5, and 8 when backcrossed to A/J, and groups 3, 6, and 9 when backcrossed to C57BL/6). The remaining three groups consisted of the two parental lines (A/J and C57BL/6) and of the internal control selected from the intercrossed L₁, L₅, and L₁₇ (group 10 noted above).

Genotyping. Total genomic deoxyribonucleic acid (DNA) was extracted from tail tissue of three-week-old mice using the conventional method described by Sambrook et al. (1989). The extracted DNA was diluted and quantified and each DNA solution was adjusted to 0.05 mg/ml for polymerase chain reaction (PCR).

Three fluorescent-labeled primers for microsatellite marker amplification (Research Genetic Inc., Huntsville, AL, USA) were used for each QTL region to assess the alleles inherited by each mouse from BC₁ through BC₄. These microsatellite markers as located on chromosomes from the centromere were *D1Mit60*, *D1Mit87*, and *D1Mit217* for QTL region on MMU1; *D5Mit200*, *D5Mit113*, and *D5Mit10* for QTL region on MMU5; and *D17Mit29*, *D17Mit16*, and *D17Mit11* for QTL region on MMU17 (MGD 1997). Because of ambiguous amplification results for marker *D5Mit113* on MMU5, it was decided to change from the first intercross onward to markers *D5Mit58*, *D5Mit201*, and *D5Mit157*, resulting in a total of five markers investigated in the QTL region on MMU5. All microsatellite markers were fixed at alternative alleles for the two mouse strains used to start the experiment.

The amplification of markers was performed according to the supplier recommendations using a thermocycler PTC100 (MJ Research, Inc., USA). PCR

products were analyzed on a 4.25% polyacrylamide gel using an automated DNA sequencer ABI 377 (Applied Biosystems). Subsequently, the tracking of the gel was checked and adjusted manually and analyses were performed using Genescan version 2.1 and Genotyper version 2.0 software (Applied Biosystems).

In the backcross generations, all animals in each of the two lines (L_{1,5} and L_{5,17}) were genotyped for all relevant markers, i.e., animals in L_{1,5} were genotyped for markers defining the QTLR on MMU1 and MMU5, and animals in L_{5,17} were genotyped for markers defining the QTLR on MMU5 and MMU17. Only animals heterozygous at all relevant markers were chosen to continue the line. Genotyping was performed first on the QTLR of MMU5 because this QTLR was common to both L_{1,5} and L_{5,17} lines. Heterozygous animals for all donor markers on this chromosome were selected for subsequent genotyping for markers on MMU1 or MMU17 depending on the line.

Challenge experiment with *T. congolense*. Animals of different experimental groups, together with control parental strain mice, were challenged with *T. congolense* clone IL1180 (Masake et al. 1983) according to procedures described by Kemp et al. (1996). The number of days of survival, i.e., the number of days postinoculation before death, was recorded and analyzed. The experiment was terminated at day 150 after inoculation. The age of mice at challenge varied from 9 through 18 weeks. Mice were grouped in cages according to their sex and age. Most cages contained five mice but the number varied from two to six mice per cage given the availability of age and sex classes. The cages were placed on a mobile stall in the small animal unit at the International Livestock Research Institute in Nairobi, Kenya. Three challenge experiments were conducted according to the availability of desired mice in April, May, and July 2000.

Statistical analysis. A preliminary analysis using the GLM procedure of SAS (1990) was used to find survival times of all challenged mice to determine the influence of sex, batch, and age at test. None of these variables were found to have a significant effect on survival times. These variables were, therefore, ignored in subsequent analysis.

Survival analysis was performed using the LIFE-TEST procedure of SAS (1990). This analysis utilizes the information from the animals that died during the experiment as well as the animals that were alive at the end of it. The procedure computes the estimates of the survival function by the product-

limit method, also known as the Kaplan–Meier method. It consists of calculating the survival probabilities for each group of mice from the numbers of mice alive on a daily basis after parasite infection. The log-rank test was used to compare survival curves across different groups of mice (Kalbfleisch and Prentice 1980).

Results

Breeding. The first 53 F₁ animals (30 males and 23 females) resulting from the reciprocal cross between the founder mouse strains were backcrossed to the recipient line A/J. The number of progeny born in each generation and the observed proportion of desired genotypes are summarized in Table 1. There were only three females within the 17 selected heterozygous mice in L₁₇ at BC₄. For MMU1, MMU5, and MMU17, the width of the respective QTLR was 0.05 M, 0.18 M, and 0.07 M so that the probability that a backcross offspring is both heterozygous and nonrecombinant for the QTLR was 0.4756, 0.4176, and 0.4661, respectively. The expected number of offspring of the desired type can be calculated using this probability (Koudandé 1999). The first intercross within lines L₁, L₅, and L₁₇ followed by genotyping resulted in 16 selected homozygous mice (9 males and 7 females) in L₁, two homozygous males in L₅, and four homozygous mice (2 males and 2 females) in L₁₇. The four homozygous mice in L₁₇ resulted from the cross L₁₇ × L_{5,17} and not from the intercross of L₁₇. There were no homozygous females in L₅ and few homozygous mice were selected in L₁₇. To overcome this problem, heterozygous mice (males and females) were selected for a second generation of intercrossing. The second intercrossing generation resulted after genotyping in 53 additional homozygous animals in L₁, 46 in L₅, and 26 in L₁₇.

From these homozygous mice, all females and part of the males were crossed to produce mice for groups 1, and 4, and 7 of the challenge experiment. The remaining males were backcrossed to A/J females to produce mice for groups 2, 5, and 8 and to C57BL/6 females to produce mice for groups 3, 6, and 9 (Table 2). Respective numbers of challenged, infected, and censored mice are summarized in Table 3. None of offspring produced from L₁₇ homozygous mice survived after weaning. Animals in group 7, therefore, were produced from the second intercross generation, i.e., from the mating of parents heterozygous at the QTL region on MMU17.

Challenge experiment. One mouse died three days after challenge, even before infection control had started, and was eliminated from the analysis.

Table 1. Observed frequencies of the desired genotype through the introgression phases (number of selected mice)

Steps ^a	Lines	Number of genotyped animals	Observed frequencies of desired genotype
BC ₁	L _{1,5}	164	0.0609 (10)
	L _{5,17}	-	0.0548 (9)
BC ₂	L _{1,5}	68	0.1912 (13)
	L _{5,17}	90	0.1555 (14)
BC ₃	L _{1,5}	104	0.1250 (13)
	L _{5,17}	206	0.0919 (16)
BC ₄	L ₁	299	0.1037 (31)
	L ₅	457	0.1138 (52)
	L ₁₇	158	0.1076 (17)
	L _{1,5}	299	0.1170 (35)
	L _{5,17}	158	0.1582 (25)
IC ₁	L ₁	93	0.1720 (16)
	L ₅	134	0.0149 (2)
	L ₁₇	16	0.0000 (0)
	L ₁₇ × L _{5,17}	25	0.1600 (4)
IC ₂	L ₁	53	1.0000 (53)
	L ₅	368	0.1250 (46)
	L ₁₇	97	0.2680 (26)

L = line of mice with subscripts indicating the chromosomes which carry the donor allele.

^aBC = backcross, IC = intercross; subscript represents generation.

At day 14 postinoculation, 36 animals were still not parasitemic and were discarded from subsequent analyses. The infection rate was 95% for 731 inoculated mice. The noninfected mice originated from group 9 (8 mice), group 6 (6 mice), and groups 2 and 5 (4 mice for each group), and they showed a strong tendency to be associated with crossbred groups.

The survival data of all groups are summarized in Table 3. In total 53 animals were censored (i.e., still alive at day 150) while 642 had died. For the original inbred lines, the mean survival time was 29.7 days for A/J and 68.8 days for C57BL/6. The mean survival time of the internal control group (group 10) and the number of censored mice were found to be very close to those of the recipient line (Table 3). During the four backcrossing generations, the expected proportion of donor line genetic material was reduced to 3.1% at the unmarked chromosomes and 100% at the three QTLR. The good correspondence between the internal control group (group 10) and the recipient line A/J demonstrates the success of the backcrossing process to eliminate the donor line genetic material.

The mean estimated survival time of synthetic mice groups homozygous for the individual donor QTLR on MMU1, MMU5, and MMU17 (groups 1, 4, and 7) has increased significantly compared with that of the recipient line. Mean estimated survival times of the introgressed A/J mice with donor QTLR on MMU1, MMU5, and MMU17 were 57.9, 49.5,

Table 2. Different groups of mice that have been challenged, their genotype profile [*q* = A/J allele (recipient), *Q* = C57BL/6 allele (donor)], and their origin

Groups	MMU1	MMU5	MMU17	Origin
0	<i>qq</i>	<i>qq</i>	<i>qq</i>	Control recipient line A/J from stock
1	QQ	<i>qq</i>	<i>qq</i>	Homozygous on MMU1 for donor alleles (synthetic)
2	Qq	<i>qq</i>	<i>qq</i>	Cross of group 1 with the recipient A/J
3	QQ	Qq	Qq	Cross of group 1 with the donor C57BL/6
4	<i>qq</i>	QQ	<i>qq</i>	Homozygous on MMU5 for donor alleles (synthetic)
5	<i>qq</i>	Qq	<i>qq</i>	Cross of group 4 with the recipient A/J
6	Qq	QQ	Qq	Cross of group 4 with the donor C57BL/6
7	<i>qq</i>	<i>qq</i>	QQ	Homozygous on MMU17 for donor alleles (synthetic)
8	<i>qq</i>	<i>qq</i>	Qq	Cross of group 7 with the recipient A/J
9	Qq	Qq	QQ	Cross of group 7 with the donor C57BL/6
10	<i>qq</i>	<i>qq</i>	<i>qq</i>	Internal control (synthetic)
11	QQ	QQ	QQ	Control donor line C57BL/6 from stock

and 46.8 days, respectively. The number of mice in all three groups that survived the challenge experiment was very small.

The crosses of mice homozygous at one of the QTLR with the donor line C57BL/6 (groups 3, 6, and 9) resulted in higher mean estimated survival times than of the resistant pure donor line C57BL/6. The mean estimated survival time of these groups ranged from 120.4 to 128.0 days (Table 3). The better ability of these groups of mice to resist the challenge is also reflected by the larger number of animals alive at the end of the experiment.

Crosses of mice homozygous at the QTLR with the recipient line A/J (groups 2, 5, and 8) resulted in mean survival times that were on average similar to those of animals homozygous at the respective QTLR. The difference in mean survival time between the groups homozygous and heterozygous at the QTLR was equal to 9.8, 6.7, and 3.3 days for QTLR on MMU1, MMU5, and MMU17, respec-

tively. None of these differences was statistically significant ($p = 0.10$).

The effects of the different genotypes on the survival functions are presented in Fig. 1. The survival curves of groups 3, 6, and 9 were strikingly different from all other groups. In these groups, all animals survived the period of 70 days after infection while in the other groups, except for the donor line, deaths started to occur between days 5 and 10 after infection. Within 20 days postinoculation mortality was only 1% in the C57BL/6 donor (group 11), whereas mortality was 50% in the internal control (group 10) and 56% in the A/J recipient line (group 0). The mortality of all other synthetic mice and their backcross to the recipient line varied between 14% and 43%.

Discussion

The present experiment addresses introgression of chromosomal regions (or QTLR) involved in a

Table 3. The number of mice challenged, infected, and censored for each group, the estimated proportion of C57BL/6 genotype (Background),^a and the mean and standard error (JE) of the survival time estimated using a nonparametric analysis for different groups of mice (*q* = A/J recipient allele, *Q* = C57BL/6 donor allele)

Group	Genotype			Background ^a	Challenged mice (No.)	Infected mice (No.)	Censored mice (No.)	Mean survival (days)	SE mean survival
	MMU1	MMU5	MMU17						
0	<i>qq</i>	<i>qq</i>	<i>qq</i>	0.000	80	78	0	29.7	2.6
1	QQ	<i>qq</i>	<i>qq</i>	0.031	60	60	1	57.9	3.3
2	Qq	<i>qq</i>	<i>qq</i>	0.015	84	80	4	48.1	2.9
3	QQ	Qq	Qq	0.515	54	52	11	120.4	3.6
4	<i>qq</i>	QQ	<i>qq</i>	0.031	66	63	2	49.5	3.8
5	<i>qq</i>	Qq	<i>qq</i>	0.015	74	70	3	56.2	3.3
6	Qq	QQ	Qq	0.515	62	56	17	128.0	2.8
7	<i>qq</i>	<i>qq</i>	QQ	0.031	21	21	2	46.8	5.1
8	<i>qq</i>	<i>qq</i>	Qq	0.015	54	51	2	43.5	4.3
9	Qq	Qq	QQ	0.515	37	29	6	126.3	3.9
10	<i>qq</i>	<i>qq</i>	<i>qq</i>	0.031	59	58	0	34.2	3.4
11	QQ	QQ	QQ	1.000	80	77	5	68.8	2.1

^aEstimated donor's background genotype according to Stam and Zeven (1981) and Young and Tanksley (1989).

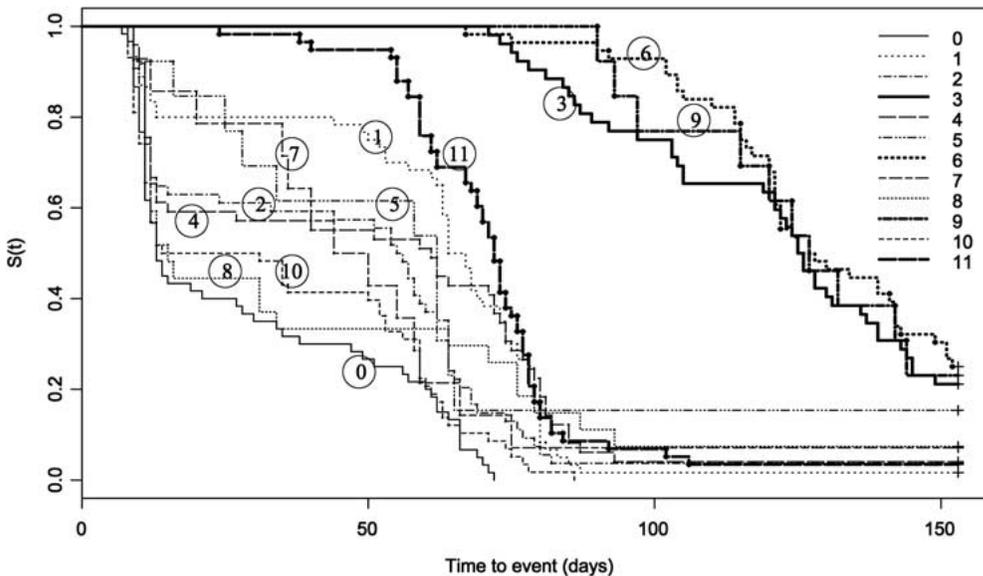


Fig. 1. Kaplan-Meier estimates of the survivor function across groups for survival postchallenge with *Trypanosoma congolense*. A/J = group 0, C57BL/6 = group 11, 1–10 correspond to groups 1–10 in Table 2.

quantitative trait, trypanotolerance. This is also the first report of successful marker-assisted introgression of QTL in animals. It is experimental proof of the use of genetic markers for marker-assisted introgression in animal breeding.

Experimental design. In the initial stages of planning this experiment we aimed at simultaneous introgression of the three QTLR. Model calculations of Koudané et al. (1999) demonstrated very clearly that a strategy that used only one single backcrossing line is intractable in mice given the relatively small number of offspring carrying the desired donor alleles at three QTLR. An alternative introgression strategy was designed by Koudané et al. (2000) which used two lines during the backcrossing phase. The modeling work has increased our understanding of bottlenecks to be encountered in an introgression experiment and has also resulted in some of the changes in the experiment described in this article.

During the introgression experiment a few bottlenecks were encountered: (1) small number of animals with the desired genotype, e.g., IC₁ generation (Table 1); and (2) availability of animals of a single sex only during the intercross phase, e.g., L₅ within IC₁ generation where both selected animals were males. These bottlenecks delayed the introgression program and increased the cost. Markel et al. (1997) experienced similar problems when introgressing the *ApoE null* allele to multiple inbred strains of different genetic backgrounds using selection against the donor's genome to speed up the

recovery of the recipient background. This reinforces that risk needs to be considered when designing experiments as pointed out by Koudané et al. (1999).

From the breeding program of our current experiment, offspring produced from homozygous individuals for donor's QTL allele on Chromosome 17 failed to survive after weaning. Observations of the offspring when still alive showed that animals grew slowly, became weaker, and died before the age of 9 weeks, i.e., the minimal age required to undergo trypanosome challenge. Postmortem investigation did not provide any explanation. The cause of this mortality is not known but it might have resulted from a negative interaction between genes linked to the QTLR on MMU17 and the A/J background. It could be due to the presence or the absence of a genetic modifier (Dietrich et al. 1993; Ikeda et al. 1999; Moore and Nagle 2000).

Marker-assisted introgression. The mice that were homozygous for one of the introgressed QTLR clearly showed longer survival times than the recipient strain of mice (Table 3). The mice homozygous for the C57 allele for the QTLR on MMU1, MMU5, and MMU17 on average lived 20.2, 19.8, and 17.1 days longer than the A/J mice. The QTLR were first localized by Kemp et al. (1997). They used two types of F₂ populations to estimate the expected differences in survival time between mice homozygous for the C57 allele and those homozygous for the susceptible allele. The cross between the susceptible BALB/C strain and C57BL/6 resulted in a difference of 32, 22, and 36 days for MMU1, MMU5, and

MMU17, respectively. The F_2 population originating from the susceptible A/J strain and C57BL/6 resulted in a difference of 22 and 31 days for MMU5 and MMU17, whereas no significant effects due to MMU1 were found. The results of our introgression experiment are in line with those in the original mapping population but the estimated effects are 30% smaller. The smaller effects might be caused by the difference in the background on which the effects of QTLR are tested. In our experiment, the gene effects were estimated in mice which contained only 3.1% of the donor genome at unmarked chromosomes. In the F_2 population used by Kemp et al. (1997), the proportion of donor genome was 50%. The comparison of the different groups in our experiment demonstrated a marked influence of the proportion of donor genome on the survival times (Table 3).

Groups 3, 6, and 9 resulted from the backcross of synthetic single QTLR mice from groups 1, 4, and 7 with the donor line C57BL/6 (Table 2). The survival curves showed that groups 3, 6, and 9 were more resistant than the purebred donor mice (Fig. 1), demonstrating their higher ability to survive trypanosome challenge, as also shown by the mean survival time in Table 3. Except for these three groups, the remaining crossbred groups as well as the synthetic groups lie between the two parental lines as would be expected. All three groups showed a much higher resistance during the initial period postinoculation. The higher resistance was not observed in the crosses with the recipient parental line and therefore cannot be explained by the dominance effects of QTLR. The explanation may be found in the interaction between the QTLR or in the expression of heterosis on other parts of the genome. Apart from the introgressed QTLR regions, the background genotype is expected to carry 3.1% of the donor's genomic DNA at the end of the backcross phase (Stam and Zeven 1981; Young and Tanksley 1989). Crosses with the donor line, therefore, are expected to reveal high levels of heterozygosity, which might explain the observed heterosis.

A number of animals survived the experiment, especially in groups 3, 6, and 9 (Table 3). Kemp et al. (1997) observed that 2% of their F_2 animals survived the experiment. They demonstrated that these animals became aparasitemic and recovered. In our experiment, all animals included in the analyses were shown to be parasitemic by examination of blood sampled from the tail tip. At the end of our experiment the animals were not examined but most likely some did become aparasitemic. More detailed analysis of the data (Koudandé et al. unpublished) revealed a biphasic pattern of time to death, with

highly distinct early and late mortality phases. They found clear evidence for QTL having different effects on different mortality phases.

Typing animals in group 1 for additional markers flanking those used to trace the QTLR on MMU1 showed that less than 10 cM of DNA is dragged with the introgressed portion (results not shown). In addition, this additional analysis revealed that the introgressed chromosomal region on MMU1 included *Tir3c*, one of the three distinct trypanoresistant QTL on MMU1 resulting from the fine mapping performed by Iraqi et al. (2000).

The results of this study strongly revealed that all three donor QTLR alleles have an effect on survival after parasite infection. In a recent analysis, Hanotte et al. (2003) revealed that several QTLR control resistance to trypanosomosis in an F_2 cross between N'Dama and Boran cattle. They suggested that selection for trypanotolerance within an F_2 cross between N'Dama and Boran cattle could produce a synthetic breed with higher trypanotolerance levels than currently exist in the parental breeds. This hypothesis is supported by the high survival rates that we observed in the cross between the homozygous lines and the resistant C57BL/63 lines (Table 3, Fig. 1).

In summary, a successful introgression experiment based solely on genetic markers has been conducted. The merit of this experiment is that it gives support to theories advocating the use of genetic markers linked to a QTL to trace that QTL in a selection program. The marker-assisted introgression was confronted with some bottlenecks but procedures were found to circumvent those. This experiment provided valuable insights which will have important implications for the development of novel breeding strategies for control of trypanosomiasis in livestock.

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References

- De Vries JN, Wietsma WA, De Vries T (1992) Introgression of leaf blight resistance from *Allium roylei* Stearn into onion (*A. cepa* L.). *Euphytica* 62, 127–133
- Dietrich WF, Lander ES, Smith JS, Moser AR, Gould KA, et al. (1993) Genetic identification of Mom-1, a major modifier locus affecting Min-induced intestinal neoplasia in the mouse. *Cell* 75, 631–639
- Doko A, Guedegbe B, Baelmans R, Demey F, N'Diaye A, et al. (1991) Trypanosomiasis in different breeds of cattle from Bénin. *Vet Parasitol* 40, 1–7
- Hanotte O, Ronin Y, Agaba M, Nilsson P, Gelhaus A, et al. (2003) Mapping of quantitative trait loci controlling trypanotolerance in a cross of tolerant West African N'Dama and susceptible East African Boran cattle. *Proc Natl Acad Sci USA* 100, 7443–7448
- Ikeda A, Zheng QY, Rosenstiel P, Madatu T, Zuberi AR, et al. (1999) Genetic modification of hearing in tubby mice: evidence for the existence of a major gene (*moth1*) which protects tubby mice from hearing loss. *Hum Mol Genet* 8, 1761–1767
- Iraqi F, Clapcott SJ, Kumari P, Haley CS, Kemp SJ, et al. (2000) Fine mapping of trypanosomiasis resistance loci in murine advanced intercross lines. *Mamm Genome* 11, 645–648
- Kalbfleisch JD, Prentice RL (1980) *The statistical analysis of failure time data* (New York: John Wiley & Sons)
- Kemp SJ, Darvasi A, Soller M, Teale AJ (1996) Genetic control of resistance to trypanosomiasis. *Vet Immunol Immunopathol* 54, 239–243
- Kemp SJ, Iraqi F, Darvasi A, Soller M, Teale AJ (1997) Localization of genes controlling resistance to trypanosomiasis in mice. *Nat Genet* 16, 194–196
- Khrustaleva LI, Kik C (2000) Introgression of *Allium fistulosum* into *A. cepa* mediated by *A. roylei*. *Theor Appl Genet* 100, 17–26
- Koudandé OD, Thomson PC, Van Arendonk JAM (1999) A model for population growth of laboratory animals subjected to marker-assisted introgression: how many animals do we need? *Heredity* 82, 16–24
- Koudandé OD, Iraqi F, Thomson PC, Teale AJ, Van Arendonk JAM (2000) Strategies to optimize marker-assisted introgression of multiple QTL. *Mamm Genome* 11, 145–150
- Kristjanson PM, Swallow BM, Rowlands GJ, Kraska RL, de Leeuw PN (1999) Measuring the costs of African animal trypanosomiasis, the potential benefits of control and returns to research. *Agric Syst* 59, 79–98
- Lim K-B, Chung J-D, Van Kronenburg CE, Ramana MS, De Jong JH, et al. (2000) Introgression of *Lilium rubellum* Baker chromosomes into *L. longiflorum* Thunb.: a genome painting study of the F1 hybrid, BC1 and BC2 progenies. *Chromosome Res* 8, 119–125
- Markel P, Shu P, Ebeling C, Carlson GA, Nagle DI, et al. (1997) Theoretical and empirical issues for marker-assisted breeding of congenic mouse strains. *Nat Genet* 17, 280–283
- Masake RA, Musoke AJ, Nantulya VM (1983) Specific antibody responses to the variable surface glycoproteins of *Trypanosoma congolense* in infected cattle. *Parasite Immunol* 5, 345–355
- Moore KJ, Nagle DL (2000) Complex trait analysis in the mouse: the strengths, the limitations and the promise yet to come. *Annu Rev Genet* 34, 653–686
- Morrison WI, Roelants GE, Mayor-Withey KS, Murray M (1978) Susceptibility of inbred strains of mice to *Trypanosoma congolense*: correlation with changes in spleen lymphocyte populations. *Clin Exp Immunol* 32, 25–40
- Mouse Genome Database (MGD) (1997) Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME (URL: <http://www.informatics.jax.org/ccr/>)
- Murray M, Trail JCM (1984) Genetic resistance to animal trypanosomiasis in Africa. *Prev Vet Med* 2, 541–551
- Oertel C, Matzk F (1999) Introgression of crown rust resistance for *Festuca* spp. into *Lolium multiflorum*. *Plant Breed* 118, 491–496
- Roberts CJ, Gray AJ (1973) Studies on trypanosome-resistant cattle II. The effects of trypanosomiasis on N'Dama, Muturu and Zebu cattle. *Trop Anim Health Prod* 5, 220–233
- Roelants GE (1986) Natural resistance to African trypanosomiasis. *Parasite Immunol* 8, 1–10
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press)
- SAS(1990) User's Guide, Statistics, Version 6.0, 4th ed. (Cary, NC: SAS Inst., Inc)
- Soller M, Plotkin-Hazan J (1977) The use of marker alleles for the introgression of linked quantitative alleles. *Theor Appl Genet* 51, 133–137
- Stam P, Zeven AC (1981) The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. *Euphytica* 30, 227–238
- Teale AJ (1993) Improving control of livestock disease. Animal biotechnology in the Consultative Group for International Agricultural Research. *Bioscience* 43, 475–483
- Teale A, Agaba M, Clapcott S, Gelhaus A, Haley C, et al. (1999) Resistance to trypanosomiasis: of markers, genes and mechanisms. *Arch Tierz Dummerstorf* 42, 36–41
- Trail JCM, d'Ieteren GDM, Teale AJ (1989) Trypanotolerance and the value of conserving livestock genetic resources. *Genome* 31, 805–812
- Yancovich A, Levin I, Cahanar A, Hillel J (1996) Introgression of the avian naked neck gene assisted by DNA fingerprints. *Anim Genet* 27, 149–155
- Young ND, Tanksley SD (1989) RFLP analysis and size of chromosomal segments retained around the Tm-2 locus in tomato during backcross breeding. *Theor Appl Genet* 77, 353–359