subspecies groups according to their genetic profiles. Furthermore, genetic admixture between these major subspecies groups have been observed. Japanese mice, *M. m. molossinus*, show that genetic admixture has occurred between the musculus and castaneus groups (Yonekawa et al., 1988). Some research groups including us have been involved in establishing a variety of inbred strains from wild mice captured in different areas of the world (Bonhomme and Guénet, 1996; Gregorová and Forejt, 2000; Moriwaki et al., 1994). The descendants of these original mice were established as wild-derived inbred strains (wild strains) after at least 20 generations of brother-sister matings. These genetically defined wild strains have proven to be useful for a variety of genetic and behavioral studies due to the high frequency of genetic polymorphism among the strains (Koide et al., 2000). A panel of wild-derived strains including a reference laboratory strain, B6, is now known as the Mishima battery (Table 1) (Furuse et al., 2002). One of these wild derived inbred strains, MSM, was originally captured in Mishima city, Japan, and classified as *M.m. molossinus* (Abe et al., 2004; Ogasawara et al., 2005; Moriwaki et al., 1994). In the last part of this chapter, we focus on a comparison of MSM with B6.

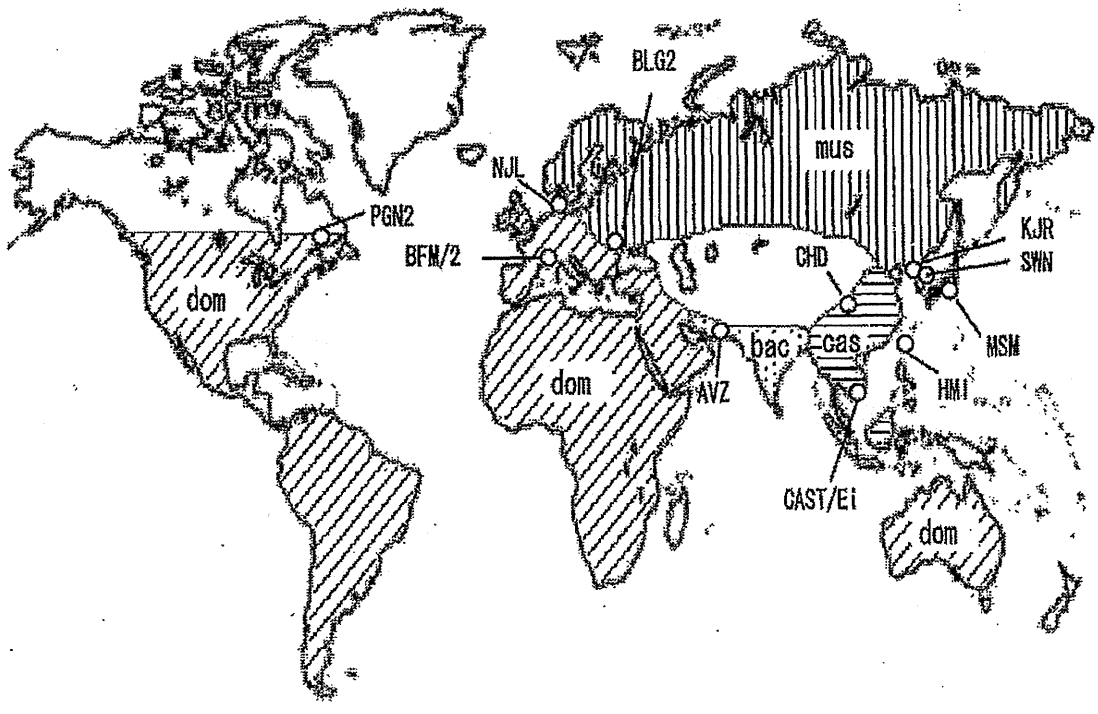

Figure1. Geographical distribution of subspecies groups for wild mice and origin of wild-derived strains. dom, domesticus subspecies group; mus, musculus subspecies groups; cas, castaneus subspecies group. Each subspecies group is categorized based on the genetic profile of wild mice which were systemized further in a taxonomic way. Wild-derived strains and their origin are indicated.

Table 1. A list of the Mishima battery of mouse strains

Origin	Strain	Subspecies group	Subspecies	Place of collection
Laboratory	C57BL/6J	domesticus		
Laboratory	DBA/1J	domesticus		
Wild mice	PGN2/Ms	domesticus	*M.m.domesticus*	Canada
Wild mice	BFM/2Ms	domesticus	*M.m.brevirostris*	France
Wild mice	HMI/Ms	castaneus	*M.m.castaneus*	Taiwan
Wild mice	CAST/Ei	castaneus	*M.m.castaneus*	Thailand
Wild mice	NJL/Ms	musculus	*M.m.musculus*	Denmark
Wild mice	BLG2/Ms	musculus	*M.m.musculus*	Bulgaria
Wild mice	SWN/Ms	musculus	*M.m.yamasinai*	Korea
Wild mice	KJR/Ms	musculus	*M.m.yamasinai*	Korea
Wild mice	MSM/Ms	musculus	*M.m.molossinus*	Japan
Fancy mice	JF1/Ms*	musculus	*M.m.molossinus*	Denmark*

JF1 was found in Denmark, but characterized as a Japanese fancy mouse by a genetic study (Koide et al., 1998).

Genetic Profiles of Wild and Laboratory Strains

Several studies characterizing genomic sequence polymorphisms have been carried out in order to analyze the genetic profiles of these wild strains clearly. Koide and colleagues (Koide et al., 2000) analyzed polymorphism frequency in microsatellite markers among wild strains by SSLP analysis (Figure 2). These results showed that polymorphism frequencies among wild strains are much higher than among laboratory strains. The frequency of simple sequence length polymorphism (SSLP) for two laboratory strains, B6 and DBA/2, was 49% but increased to 83.7% when B6 was compared with MSM. This result indicates that higher diversity can be expected by adding these wild strains to the mouse resource pool of laboratory strains.

Triplet-repeat sequences are frequently observed in the coding regions. Two trinucleotide repeats, CAG and CAA, coding for poly-glutamines have been studied by many research groups since repeat length contributes to differences in protein function or diseases. We systematically searched for genes carrying a CAG repeats in the public databases and found 62 loci carrying CAG/CAA trinucleotide repeat. We then analyzed variations of the repeat length in the 62 loci among 16 inbred mouse strains by PCR amplification following sequence analysis. We found that the maximum repeat number was 37 and 51.6% of the loci maintained their length among strains. Higher polymorphism frequency was observed when the repeat number was over 10. Phylogenetic relationships among the 16 inbred mouse strains were analyzed using the CAG/CAA repeat numbers in the coding regions.

	MSM	JF1	KJR	SWN	HMI	CAST	BLG2	NJL	BFM/2	B6	DBA/1
MSM		45.2	69.2	73.1	87.5	88.5	81.7	72.1	92.3	86.7	86.5
JF1			64.4	60.6	85.6	88.5	78.8	73.1	92.3	82.7	80.8
KJR				67.3	82.7	92.3	83.7	77.9	91.3	89.4	89.4
SWN					84.6	87.5	75	76	91.3	87.5	91.3
HMI						69.2	64.6	87.5	89.4	87.5	86.5
CAST							87.5	88.5	86.5	88.5	86.5
BLG2								77.9	85.6	86.5	93.3
NJL									87.5	84.6	91.3
BFM/2										86.7	80.8
B6											49
DBA/1											

Figure 2. A matrix diagram showing degrees of polymorphism based on SSLP typing for 104 different microsatellite markers distributed in the entire genome. The value indicates the frequency of polymorphism between two strains. Two laboratory strains are indicated by grey shadowing. This figure is modified from Koide et al. (2000).

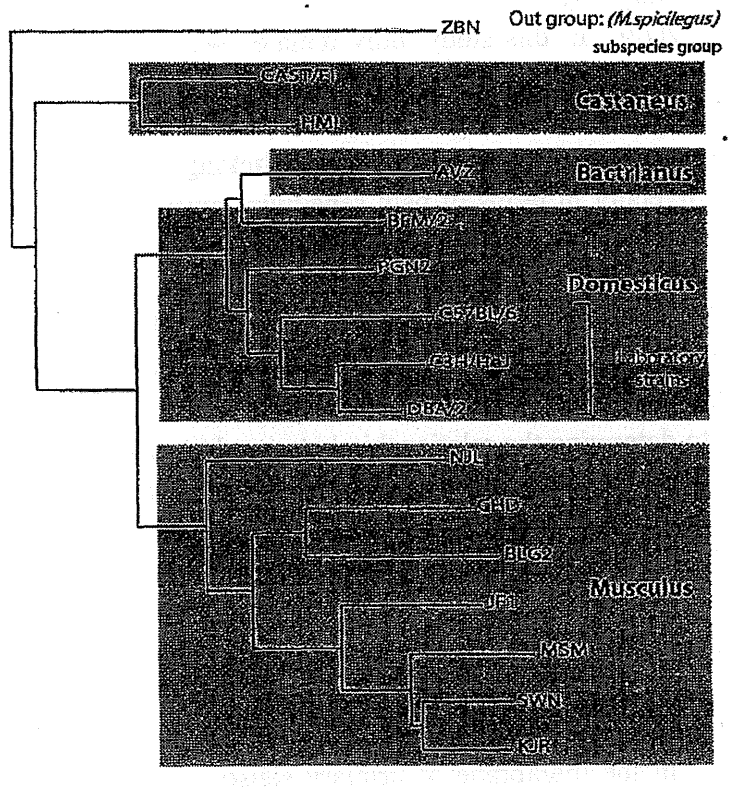

Figure3. Phylogenetic tree for wild-derived strains. A Neighbor-joining tree was constructed based on CAG/CAA repeat polymorphism for 31 repeat loci of 16 inbred mouse strains. Three subspecies groups, domesticus, castaneus, and musculus, were clearly different in terms of their genetic profile. The AVZ strain is categorized as a bactrianus subspecies group, but this group is not well differentiated. This figure is modified from Ogasawara et al. (2005).

The branching patterns from reconstructed Neighbor-joining (NJ) trees showed that 14 inbred strains, excluding ZBN and AVZ, were clustered into 3 groups: domesticus, castaneus and musculus (Figure 3). Laboratory strains B6 and DBA/2 were also shown to co-cluster into the domesticus subspecies group, as previously mentioned.

In addition to these repetitive sequences, 21 nuclear genes were characterized in the polymorphism of these wild-derived strains (Liu et al., 2008). A neighbor-joining tree constructed from the sequence polymorphism data showed clustering of wild-derived strains into three subspecies groups, and coclustering of a laboratory strain, C57BL/10, into the domesticus subspecies group.

These data indicate that three subspecies groups, domesticus, castaneus, and musculus, are genetically different from each other. Wild-derived strains classified into different subspecies are genetically different and very useful for locating genetic polymorphisms in the desired gene/genes.

Pain and Capsaicin Sensitivity in Wild Strains

In order to study the strain differences in pain sensitivity, Koide and colleagues conducted tail-flick (Figure 4B) and hot-plate tests at 52°C (Figure 4A) on a Mishima battery (Koide et al., 2000). In this study, only females were used. It is thought that the hot plate response at 52°C mediates the central response to moderate heat stimuli, but the tail flick response mediates the spinal reflex to high temperatures. As a result of these tests, significant strain-effects were observed in both hot-plate (licking) and tail-flick tests. The results clearly showed that diversity in terms of pain sensitivity exists among strains. In the hot-plate test, three strains, JF1, KJR and SWN, proved to be insensitive to heat, and MSM was moderately insensitive, while the laboratory strains, B6 and DBA/1, and castaneus strains, CAST/Ei and HMI, were highly sensitive. In the tail-flick test, the KJR strain proved to be insensitive, MSM, JF1, SWN, and BLG2 were moderately insensitive, but CAST/Ei, HMI, NJL, BFM/2, B6 and DBA/1 were highly sensitive.

In order to determine the relationship between hot-plate and tail-flick tests, we conducted Pearson's correlation analysis of data from the two pain sensitivity tests (Figure 4D). A comparison between both tests showed a high correlation (R^2=0.646), suggesting the existence of a partially overlapping underlying mechanism of strain difference for pain sensitivity.

These results also raised the possibility that there could be strain differences for capsaicin perception among these strains, since perception for both hot taste and a moderate level of heat is mediated by the same sensory receptor, *Trpv1* (*vanilloid receptor 1*, a nonselective cation channel in the membrane of primary sensory nerve endings) (Caterina et al., 1999; Caterina et al., 2000; Caterina et al., 1997). Capsaicin is the chemical component of hot chili peppers which causes its hot taste and stimulates the physiological pain system. Since response to both moderate heat (> 43°C) and capsaicin are mediated by *Trpv1*, a similar sensitivity to the hot-plate test at moderate temperature (52°C) and capsaicin intake test was expected.

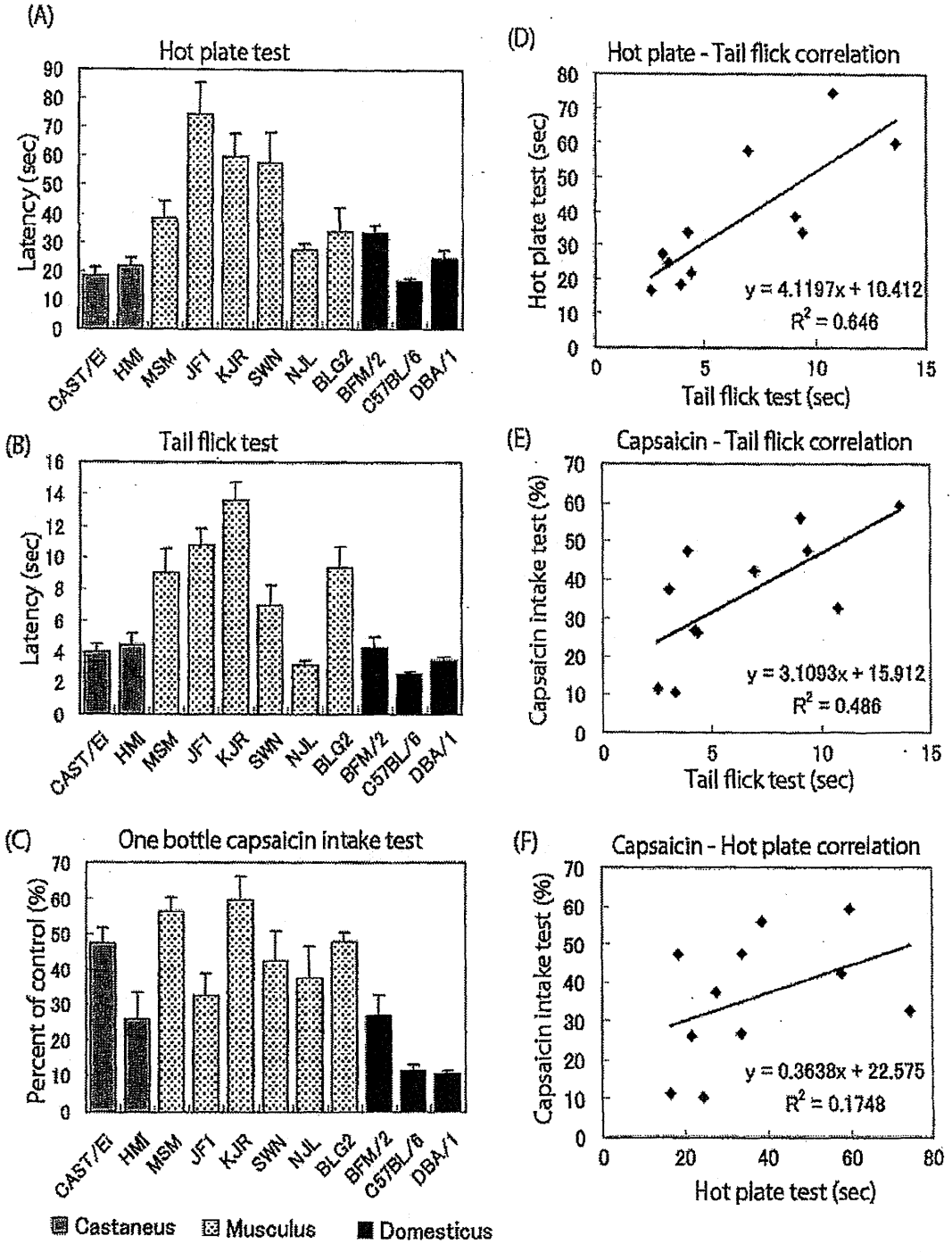

Figure 4. Strain difference in pain sensitivities and correlation between different pain tests. Strain comparison of sensitivities in (A) hot-plate, (B) tail-flick, and (C) capsaicin intake tests. Correlation analyses of different pain sensitivities. (D) Correlation between hot-plate and tail-flick sensitivities, (E) correlation between capsaicin sensitivity and tail-flick sensitivity, (F) correlation between capsaicin sensitivity and hot-plate sensitivity. Data for hot-plate and tail-flick tests were taken from a previous paper (Koide et al. 2000). Data from capsaicin sensitivity were taken from our previous paper (Furuse et al., 2002).

Thus, we carried out 1-bottle capsaicin intake tests (Figure 5) on a Mishima battery to measure hot-taste sensitivity (capsaicin intake is expressed as a percentage of baseline water intake). Results at 15 μM of capsaicin are shown in Figure 4C.

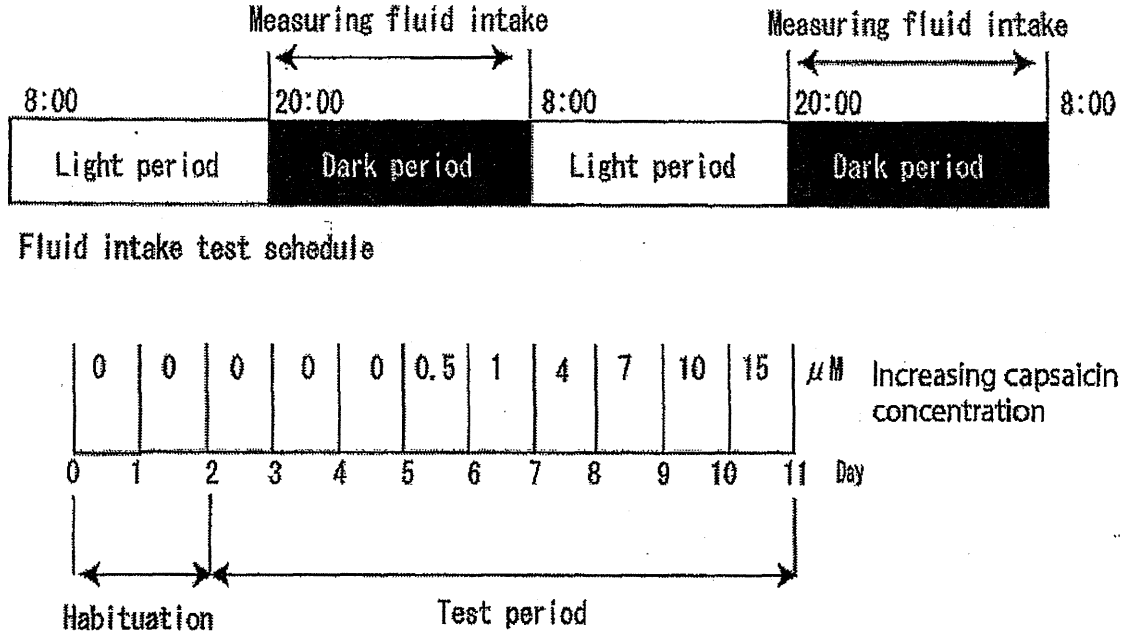

Figure5. Method for the 12-h 1-bottle test. Test schedule for measuring capsaicin sensitivity.

When fluid intake was compared among strains, the KJR and MSM strains consumed 15 μM of capsaicin solution at 60% of the control water intake, which was significantly higher than for all other strains (a post hoc analysis, $P < 0.03$). BFM/2, BLG2, CAST/Ei, HMI, JF1, NJL and SWN strains consumed 15 μM capsaicin at 26 – 47% of the controls. Two strains, B6 and DBA/1, consumed 15 μM capsaicin solutions at 7 - 11% of the controls, which was significantly lower than for all other strains (a post hoc analysis, $P < 0.01$). The KJR and MSM strains were less sensitive to capsaicin, but two laboratory strains, B6 and DBA/1, were highly sensitive. The pattern of strain differences in sensitivity was similar to that displayed by these strains for the hot-plate test.

To determine the relationship between capsaicin sensitivity and other pain sensitivity tests, we conducted Pearson's correlation analysis on data from the combination with the capsaicin sensitivity test and either the hot-plate test or tail-flick test. Neither combinations of capsaicin - hot plate test nor capsaicin - tail flick test showed a high correlation (R^2=0.486 and 0.175, respectively) suggesting an independent basis for the strain difference in sensitivity to capsaicin and heat sensation (Figure 4E,F).

Concerning the functional basis for pain sensitivity, it is possible that further variations in the central pathways may be associated with the strain differences in pain sensitivity. Pain perception can be modified by two types of endogenous analgesic systems: opioid-dependent and opioid-independent (Mogil, 1999). Therefore, investigation of the relationship between pain sensitivities and opioid-dependent pathways is important to understand the basic mechanisms for different pain sensitivities in these mouse strains.

Differences in Opioid Pathways

Opioid drugs are among the most used analgesics in human history, since some of them are available as natural compounds extracted from the juice of *Papaver somniferum*, such as morphine and codeine. Both natural and synthetic (including fentanyl and methadone) opioids have been widely used in clinical practice to alleviate many types of pain from general surgery to terminal cancer. However, it is known that there is a large individual diversity in opioid sensitivities in human populations. The existence of large inter-individual differences in the response to opioid analgesics complicates appropriate pain treatment in clinical practice. This individual diversity in opioid sensitivity is thought to be caused by complex interactions of psychological, environmental, and genetic factors. For this reason, understanding the genetic basis of an individual difference in morphine sensitivity would be useful information for developing better analgesic drugs and an appropriate administration method for morphine-related drugs.

Ikeda and colleagues have investigated morphine effects in the Mishima battery (Shigeta et al., 2008). The morphine effect on spontaneous activity was examined in these strains with the open-field test (Figure 6A). Half of the strains, JF1, KJR, MSM, SWN, and NJL, showed significantly increased open-field ambulation when morphine was administered.

The antinociceptive effects of morphine were examined using hot-plate and tail-flick tests (Figure 6B and 6C, respectively; Shigeta et al., 2008). In the hot-plate test, response latency was significantly longer when mice were treated with morphine than with saline in all of the strains tested. For the tail-flick test, the response latency was significantly longer in the morphine-treated group than in the saline-treated group for all of the strains tested. Next, we tried to measure the antinociceptive effect of morphine by scoring it as a percentage of the maximal possible effect (%MPE), which was calculated as follows: %MPE = {[(latency at morphine injection) – (latency at saline injection)] / [(cut-off time) – (latency at saline injection)]} x 100%.

When we calculated the %MPE of morphine antinociception, diversity of morphine sensitivity was observed among strains (Figure7). Particularly, we found that the CHD, KJR, JF1 and MSM strains showed a pronounced response to morphine for multiple tests in each strain. These data indicated that morphine effects were highly variable among mouse strains.

We have shown that there is a wide diversity of sensitivities in pain-related tests and the antinociceptive effect of morphine. In order to investigate the functional relationship between pain sensitivity and morphine sensitivity, we conducted Pearson's correlation analysis. The results showed a moderate correlation of nociceptive response and the morphine effect in the hot-plate test (Figure 8A), and a high correlation between nociceptive response and the morphine effect in the tail-flick test (Figure 8B).

These results suggested that there is a functional association of the opioid-dependent analgesic pathway to pain perception in these mouse strains. Therefore, investigating the genetic basis for different pain sensitivities will lead to greater understanding of the underlying mechanisms for different pain sensitivities and opioid-dependent analgesic pathways. This in turn may lead to the development of new antinociceptive drugs.

Genetic Mapping of Loci Related to Pain Sensitivity

As we explained above, a variety of wild derived strains are a powerful resource for research into the genetic basis of diversity in pain sensitivity-related phenomena. By adding wild derived strains to the list of mouse strains to investigate, we were able to detect differences in pain-related phenotypes. In particular, two strains, B6 and MSM, have proven to be a highly useful resource to study these phenotypes as the two strains exhibit differences.

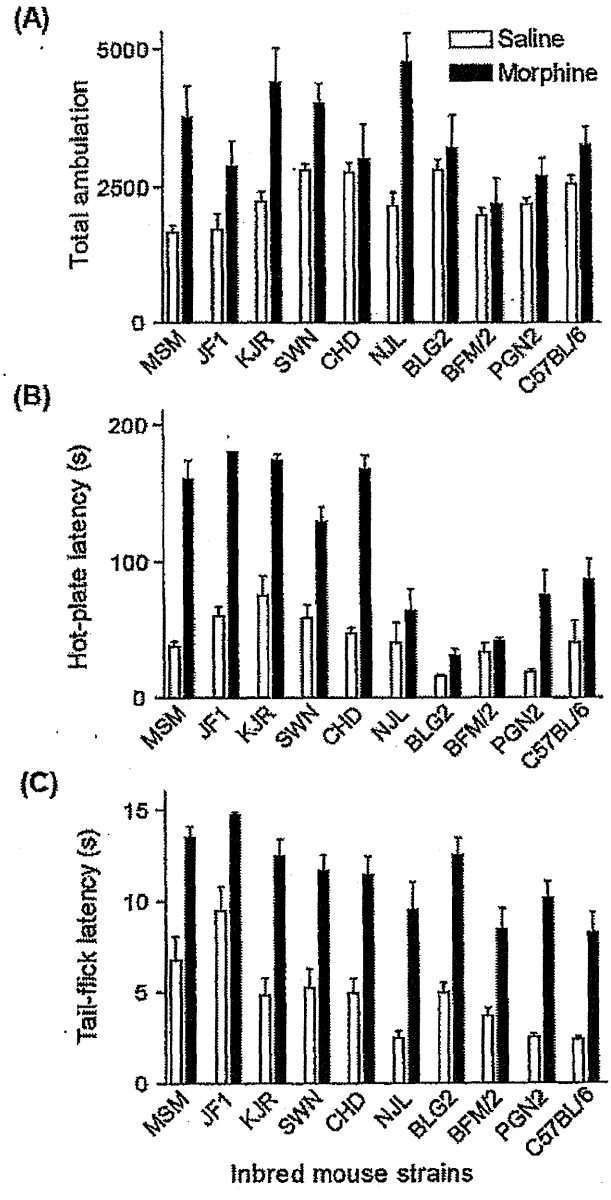

Figure 6. Comparison of strain differences in morphine sensitivity in 10 inbred mouse strains. Effects of morphine were compared after administration of saline or morphine (10 mg/kg) intraperitoneally. Effect on spontaneous activity in open-field test (A), hot-plate sensitivity (B), and tail-flick sensitivity (C) were examined. Each bar represents the mean±SEM. Data on tests for open-field, hot-plate, and tail-flick were taken from a previous paper (Shigeta et al., 2008).

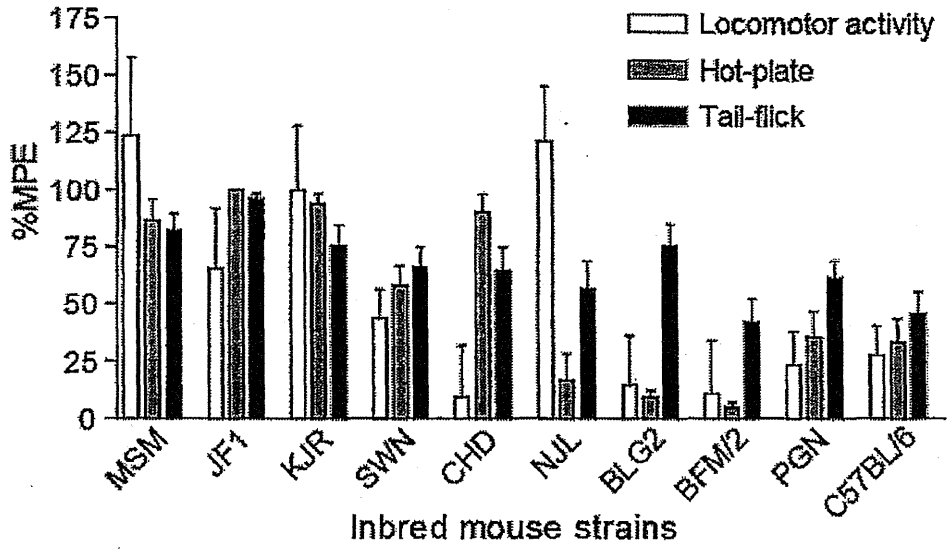

Figure7. Comparison of morphine effects in 10 inbred mouse strains including wild-derived strains. Data on tests for open-field, hot-plate, and tail-flick were taken from a previous paper (Shigeta et al., 2008).

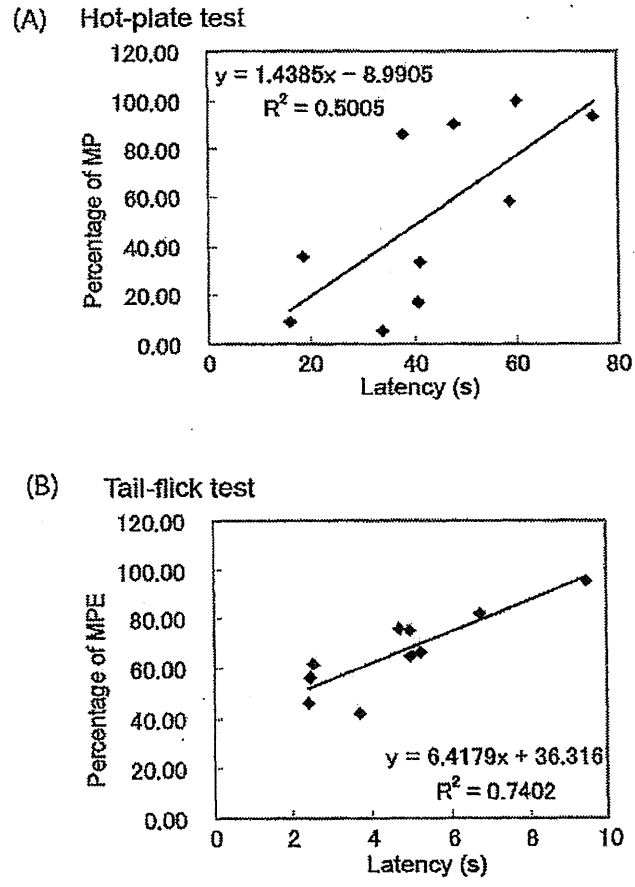

Figure 8. Correlation between pain perception and morphine effect. Strain comparison of sensitivities in (A) hot-plate test, and (B) tail-flick test. Correlation analyses of different pain sensitivities. Data on morphine effects in the hot-plate and tail-flick tests were taken from a previous paper (Shigeta et al., 2008).

In order to investigate the genetic basis of the phenotypic difference, QTL studies have been conducted on mice and rats by using F2 intercross, N2 backcross, recombinant inbred strains, and heterozygote stocks (Flint, 2002; Flint, 2003; Valdar et al., 2006). Flint and colleagues surveyed previously reported QTL studies and found that most QTLs have just a small effect, contributing approximately 6% on average of the total phenotypic variance for behavioral and physiological phenotypes (Flint et al., 2005). In addition, they also conducted genome-wide high-resolution mapping using heterogeneous stock mice and revealed 843 QTLs for a variety of phenotypes, including behaviors. Among these QTLs, only 10 had effect sizes of higher than 5% and 109 QTLs of less than 2% (Valdar et al., 2006). Because of this small effect of each QTL, it is proposed that an enormous amount of effort will be required to identify the quantitative trait gene (QTG) for behavior.

In order to study QTLs more systematically, new mouse resources have been developed. Consomic strains, also known as chromosome substitution strains, are a favored resources for approaching the QTL because genotyping to map the chromosome is unnecessary, results are reproducible, QTL detection is statistically significant, and creating subconsomic strains is efficient (Belknap, 2003; Nadeau et al., 2000). Shiroishi and colleagues have established a new panel of consomic strains, dubbed B6-ChrN^{MSM}, in which each chromosome of B6 was substituted with a corresponding set of chromosomes from MSM (Figure 9) (Takada et al., 2008). Recently, systematic QTL mapping was conducted for a series of behavioral tests related to emotionality and incidence of hydrocephalus; multiple loci were successfully mapped for these phenotypes on many chromosomes in the genome (Takahashi et al., 2008a; Takahashi, 2008b). Thus, we also attempted to map genetic loci related to pain sensitivity using hot-plate and tail-flick tests (unpublished data). In this chapter, we report on the results of these tests using a panel of consomic strains and subsequent further mapping using subconsomic strains established from one of the consomic strains.

Mapping the Chromosomes Related to Pain Sensitivity

We first identified those chromosomes related to pain sensitivity by comparing each consomic strain with B6 in hot-plate (Figure 10A) and tail-flick (Figure 10B) tests.

In the hot-plate test, two-way ANOVA revealed a significant strain effect [$F(22, 726)=24.345$, $P<0.001$] and sex-strain interaction [$F(21,726)=1.737$, $P<0.05$]. Females of B6-Chr2T^{MSM}, B6-Chr8^{MSM}, B6-Chr14^{MSM}, and B6-Chr17^{MSM}, and males of B6-Chr2T^{MSM}, B6-Chr8^{MSM} and B6-Chr9^{MSM} showed reduced pain sensitivity in the hot-plate test. Although only females of B6-Chr17^{MSM} showed significantly reduced sensitivity in the hot-plate test, the levels of sensitivity were similar between males and females. In contrast, females of B6-Chr14^{MSM} clearly had reduced sensitivity compared to males. Thus, Chr14 reduces hot-plate sensitivity only in females. In the tail-flick test, two-way ANOVA showed a significant effect of strain [$F(22, 739)=25.48$, $P<0.001$], but no significant sex-strain interaction. Thus, data from both sexes were combined for the tail-flick test and are shown in Figure 10. Fifteen consomic strains for Chrs 1, 2C, 2T, 3, 4, 7T, 8, 11, 12C, 12T, 14, 15, 16, 17 and Y showed reduced sensitivity compared to B6.

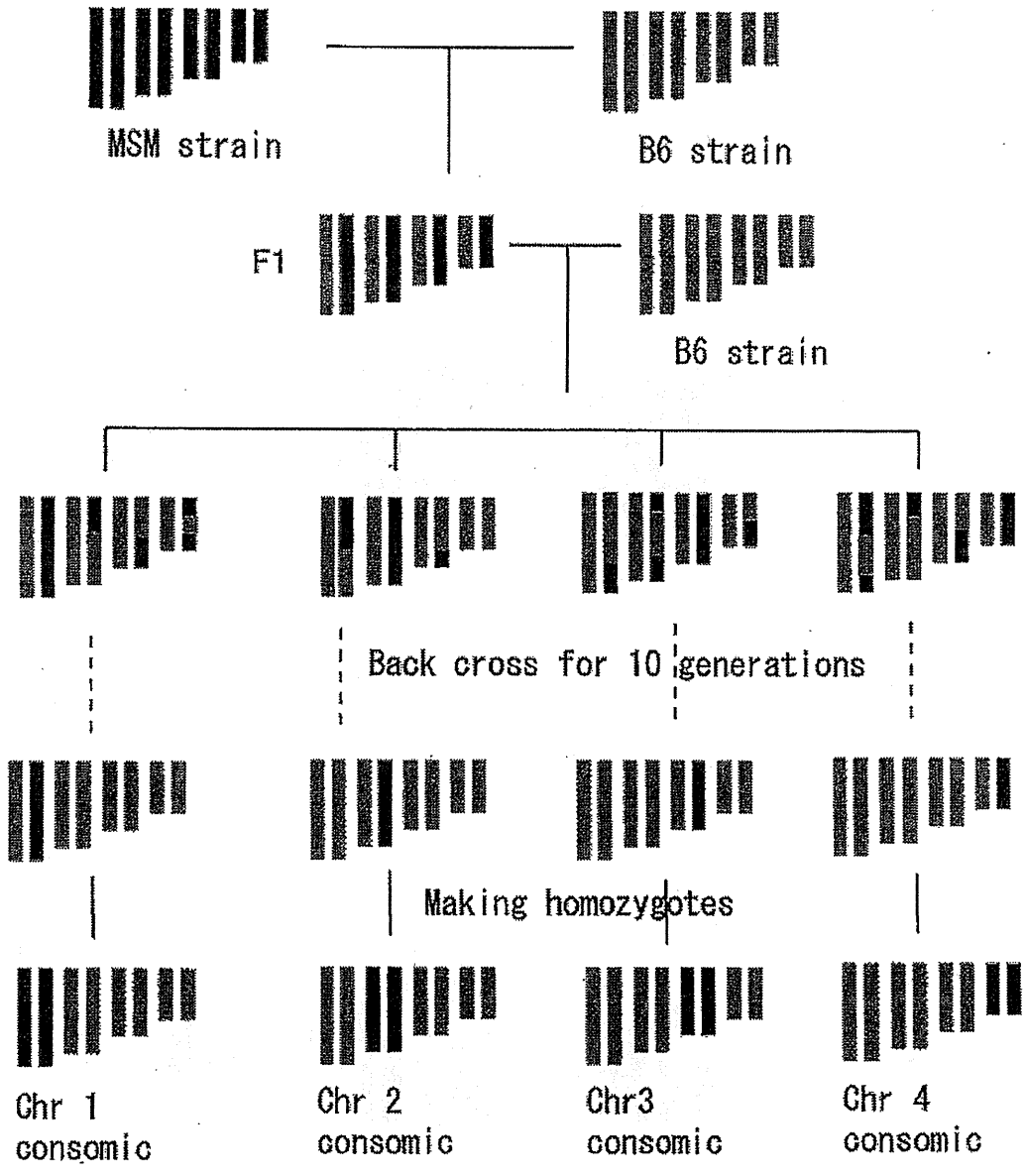

Figure 9. Breeding scheme for establishing a panel of consomic strains.

Our results suggested a larger number of chromosomes related to different pain sensitivities to the tail-flick test than the hot-plate test. We found consomic strains for Chrs 1, 2C, 3, 4, 7T, 11, 12C, 12T, 15, 16 and Y related to reduced sensitivity to only the tail-flick test, but not the hot-plate test. Chrs 2T and 9 related to increased sensitivity in the hot-plate test. It is interesting that Chr2T related to increased sensitivity in the hot-plate test, but reduced sensitivity in the tail-flick test. It is known that these two pain-sensitivity tests reflect distinct biological pathways; the tail-flick test for spinal reflex pain and the hot-plate test for centrally mediated acute pain reflexes (Crawley, 2007). Our results indicated that there are some common genetic loci for pain perception, and also many test-specific QTLs related to the spinal reflex reaction.

A male-specific QTL on Chr 4 has been reported for the hot-plate test in F2 intercross mice between DBA and B6 (Mogil et al., 1997).

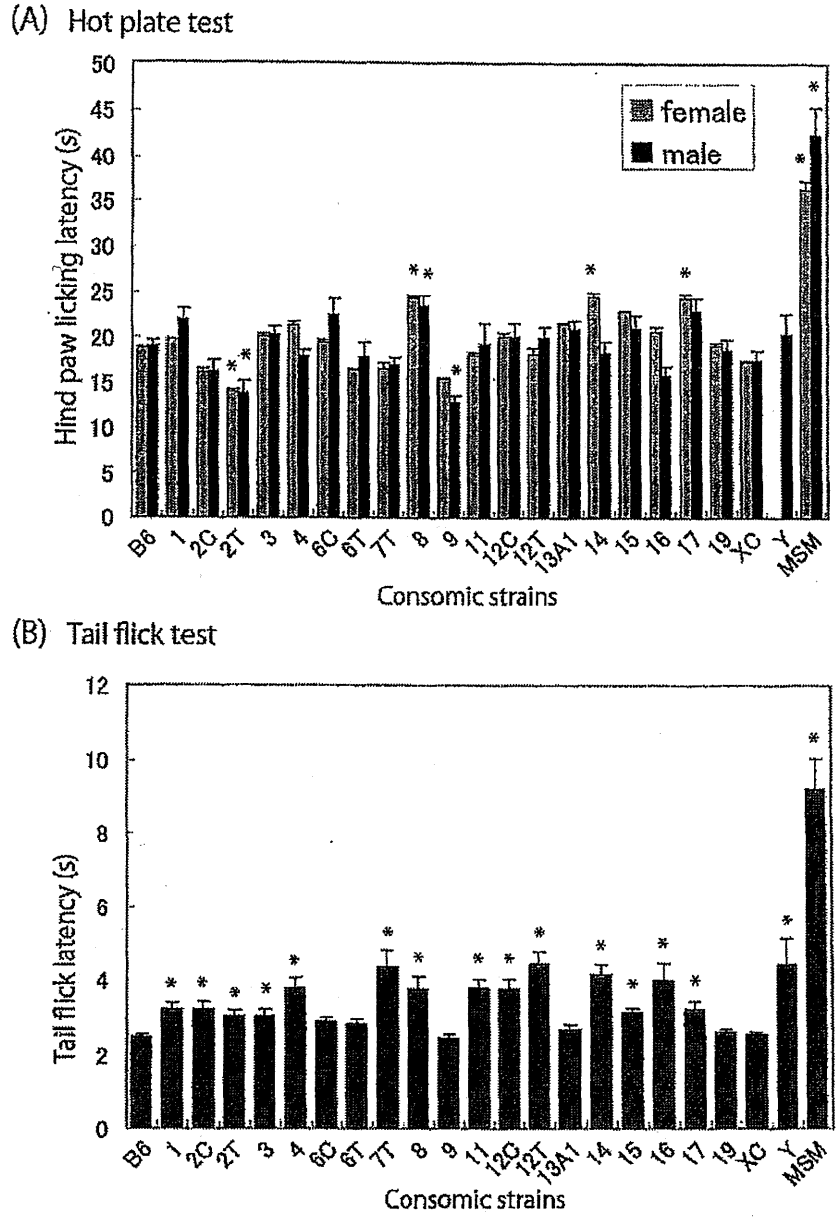

Figure10. Results of chromosomal mapping for pain sensitivities.

Astarisks indicate significant difference between each consomic strain and B6.

Methods: Mouse strains, C57BL/6J (B6), MSM/Ms (MSM), and consomic strains were maintained in the animal facility of the National Institute of Genetics (NIG), Mishima, Japan. All strains were maintained at NIG in a controlled room at 23°C (±2°C) on a 12/12 light/dark cycle, light period starting at 8.00 h with food and water available ad libitum. Throughout this study, 9- to 13-week-old females were used for testing. Before the two pain sensitivity tests, hot-plate and tail-flick, all the animals were tested for home-cage activity and emotionality-related behavior (Takahashi et al., 2008a) in the same test order. All procedures were carried out with approval by our institutional animal care and use committee. The hot-plate test and tail flick test were conducted as follows:

Hot-plate test (HP)

The hot plate apparatus consisted of an acrylic resin cage (20 × 25 × 25 cm) and thermo-controlled aluminum plate (model MK-350B, Muromachi Kikai Co., Tokyo, Japan). The plate temperature was adjusted to 52 ± 0.2°C. Latency to the first licking of the hind limb was measured. When the mouse jumped on the plate before licking a hind limb, the test was stopped and excluded from the data.

Tail flick test (TF)

The tail flick apparatus consisted of a radiant heat source and a photo sensor to detect the tail flick (model MK-330B, Muromachi Kikai Co., Tokyo, Japan). Latency, being the median of three independent tests from the start of irradiation to the tail flick reaction, was measured. The cut-off time was 15 s in order to prevent tail damage.

Data analysis was performed using the SPSS version 14.0J software package. The significance for each consomic strain was determined by a t-test with a Bonferroni correction compared to B6 ($p = \alpha/m$: where $\alpha=0.05$ and $m=20$ for males and $m=19$ for females). In order to avoid an interactive influence from sex chromosomes on the effect of substituting chromosomes, we first performed t-tests for males and females independently. Sex-genotype interaction in consomic strains was analyzed by performing 2-way ANOVA in all consomic strains and B6. Because the tail flick test did not show strain-sex interaction, data for both females and males were combined and presented in Figure 10(B).

In some consomic pairs (B6-Chr2C^{MSM}/B6-Chr2T^{MSM}, B6-Chr6C^{MSM}/B6-Chr6T^{MSM} and B6-Chr12C^{MSM}/B6-Chr12T^{MSM}), different parts of a chromosome were substituted (Chr 2, 6 and 12), and the MSM region overlapped in the middle of the chromosome. Thus, to avoid overestimation, we considered it as one consomic strain even if both of them (C and T) showed significant behavioral differences from B6, as well as if one of those (C or T) showed a significant difference. A phenotypic effect was also calculated in one of a pair of the consomic strains which showed larger differences from B6.

However, our B6-Chr4^{MSM} did not show significant differences in the hot-plate test, but exhibited differences in the tail-flick test. This discrepancy may result from the strain differences between DBA and MSM.

Pain sensitivity can be affected by stress-induced analgesia (Imbe et al., 2006), which may reflect consomic mouse sensitivity toward stress (e.g. holding by experimenter). We previously investigated emotionality-related behavior using this panel of consomic strains (Takahashi et al., 2008a). The same animals used in the emotionality investigation were also used for these pain sensitivity analyses at the end of a series of behavioral tests. By using these data, genetic correlation analysis was conducted for emotionality-related indices and pain sensitivity. The results showed that there were moderate correlations between pain sensitivity measurements and some emotionality-related behaviors (Table 2). Particularly, locomotion, leaning and rearing were negatively correlated, but stretching was positively correlated with sensitivity to the hot-plate test. Therefore, this indicates that pain sensitivity in the hot-plate test and an emotionality-related phenotype have genetically related bases. Further analysis is required to interpret these correlations between pain related indices and an emotionality-related phenotype.

Table 2. Correlation of pain sensitivity with behavioral parameters related to emotionality

		HP	TF
OF	Ambulation	-0.295	-0.100
	Center area	-0.136	0.069
	Center area %	0.236	0.217
	Center time	0.243	0.189
	Defecation	0.139	-0.262
	Locomotion	-.463(*)	-0.135
	Stretching	.595(**)	0.143
	Leaning	-.555(**)	-.480(*)
	Rearing	-.438(*)	0.316
	Grooming	0.276	0.127
	Face-wash	0.007	0.015
	Jumping	-0.191	-0.422
	Pausing	0.294	-0.130
L/D	Transition number	-0.385	-0.142
	Dark box duration	0.327	-0.070
	Latency to first transition	0.213	-0.097
EPM	Total distance (cm)	-0.380	-0.168
	Total number of arm entry	-0.415	-0.188
	Closed arm entry	-0.400	-0.037
	Open arm entry	-0.379	-0.345
	Open arm %	0.090	-.464(*)
	Open arm time	-0.075	-0.373

Genetic correlation was caluculated by using strain mean values of all consomic strains and B6 in all consomic strains and B6 using SPSS version 14.0J software packages. OF, Open-field test; L/D, Light-dark box test; EPM, Elevated plus maze test; HP, hot-plate test; TF, tail-flick test. Asterisks indicate a significant correlation (**, P<0.01; *, P<0.05) between emotionality related parameter and either hot-plate or tail-flick sensitivity.

Further Mapping Using Subconsomic Strains

In order to identify loci responsible for reduced pain sensitivity in the hot-plate test for B6-Chr17^{MSM}, we analyzed subconsomic strains encompassing the entire Chr 17 (Figure 11). In the hot-plate test, C2 and C3 strains showed significantly prolonged latency for hind paw licking comparing to B6 (Dunnet's t-test: B6-C2, $p<0.05$; B6-C3, $p<0.01$) (Figure 12). As a result, we found that a region between a centromere to *D17Mit34* (10cM, 34.3Mb), had an effect of reducing the thermo-sensitivity at 52°C. Mogil and colleagues have previously reported that a congenic strain carrying a Chr17-proximal region of a *M.m.molossinus*-derived strain, MOLF/Ei, had an effect on reducing pain sensitivity (Mogil et al., 2006). This region, a spans of 24.5cM from the centromere, was named *Tpnr3* (*thermal pain response 3*) and partially overlapped with the region which we mapped in our present study. Thus, it is

possible that these two studies have clarified the same locus related to pain sensitivity as both studies used *M.m.molossinus*-derived mice.

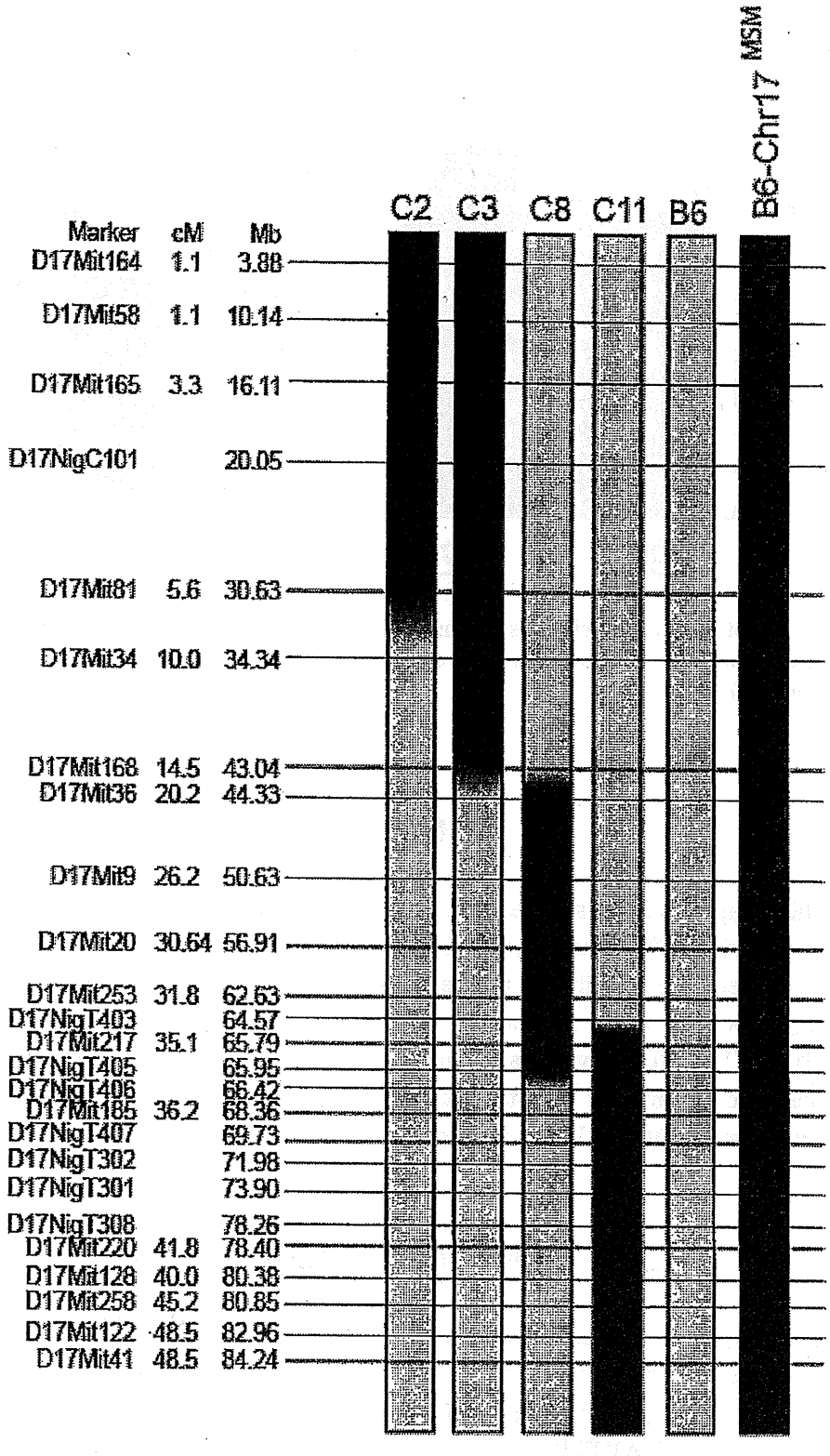

Figure 11. Genetic mapping of reduced hot-plate sensitivity in MSM-derived Chr17. Diagram of subconsomic strains used for analysis. Substituted chromosomal regions from MSM are black.

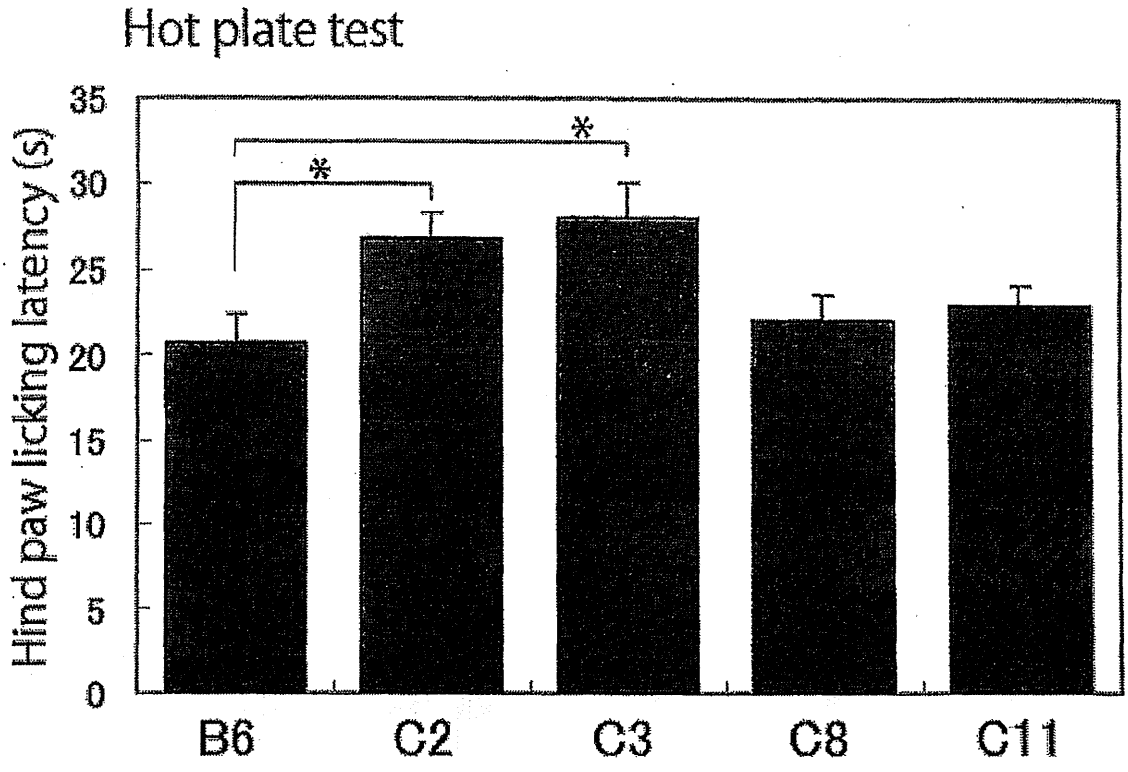

Figure 12. Hot-plate sensitivity in subconsomic strains. Mean latency for hind-paw licking in each subconsomic strain is shown. Error bars represent SEM. Asterisks show $P<0.05$ with Dunnet's t-test compared with B6.

Conclusion

This chapter clearly showed that consomic strains are a powerful resource for addressing pain sensitivity differences among mouse strains. We successfully identified one of the loci related to reduced sensitivity in the hot-plate test. The screening of a panel of consomic strains showed a large number of chromosomes are related to different levels of heat sensitivity. Therefore, systematic analysis of consomic strains and subsequent analysis of subconsomic strains will give further information about the complex genetic basis regulating different heat sensitivity levels in mice.

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Chapter V

Association between Analgesic Requirements after Major Abdominal Surgery and Polymorphisms of the Opioid Metabolism-Related Gene *ABCB1*

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Abstract

Analyses of opioid-related molecules using genetically modified animals have clarified the mechanisms of the analgesic action of morphine, its related side-effects, and individual variabilities in sensitivity. In the present study, we examined the relationship between the dosage of analgesics, including opioids, required after major abdominal

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