Population Differentiation and Gene Flow Revealed by Microsatellite DNA Markers in the House Mouse (Mus musculus castaneus) in Taiwan

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Source: Zoological Science, 19(4) : 475-483

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.19.475
Population Differentiation and Gene Flow Revealed by Microsatellite DNA Markers in the House Mouse (Mus musculus castaneus) in Taiwan

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ABSTRACT—We analyzed population subdivision and gene flow of the Southeast Asian house mouse (Mus musculus castaneus) in Taiwan by using six microsatellite DNA markers. Seven populations of the house mouse (187 individuals), including one from Fukien Province in southeastern China, which is separated from Taiwan by the Taiwan Strait, were analyzed in this study. The overall polymorphic level at the six loci was high (He=0.76) although individual populations varied in their levels of heterozygosity (He=0.35–0.83). For the populations within Taiwan, there was no evidence of isolation by distance and the level of gene flow was not (inversely) correlated to geographic distances. Gene flow was estimated to be higher across the Taiwan Strait than within the island of Taiwan. These observations of gene flow cannot be understood unless in the context of the historical human settlements and agricultural expansion, and the commensal habits of the species. We also discussed the causes of population subdivision and genetic variation among populations in terms of ecological characteristics of the house mouse in Taiwan.

Key words: house mice, Mus musculus castaneus, gene flow, microsatellite, population genetics, human migration

INTRODUCTION

Population subdivision and gene flow in the house mouse (Mus musculus) are of particular interest because of its commensal habit associated with human activities and modern cosmopolitan distribution resulting from this commensalism (Sage et al. 1993; Silver 1995). Previous studies have been focused primarily on one of the four genetically differentiated subspecies, Mus musculus domesticus (Boursot et al. 1993; Bonhomme et al. 1994; Yonekawa et al. 1994; Boursot et al. 1996, Din et al. 1996). These studies were based on two types of approaches, either primarily a genetic analysis (e.g., Petras 1967; Selander 1970; Berry et al. 1987; Britton-Davidian 1990; Ryan et al. 1993; Dallas et al. 1995) or an eco-behavioral study (e.g., Berry and Jakobson 1974; Myers 1974; Lidicker 1976; Baker 1981; Singleton 1983; Singleton and Hay 1983). These studies were based on two types of approaches, either primarily a genetic analysis (e.g., Petras 1967; Selander 1970; Berry et al. 1987; Britton-Davidian 1990; Ryan et al. 1993; Dallas et al. 1995) or an eco-behavioral study (e.g., Berry and Jakobson 1974; Myers 1974; Lidicker 1976; Baker 1981; Singleton 1983; Singleton and Hay 1983). Genetic analyses indicate that population subdivision in M. m. domesticus is present, in general, at three levels. At the continental level, the subdivision was presumed to be driven by genetic drift after human influences (Britton-Davidian 1990). One level below, subdivision has been demonstrated among different villages and farmlands (Petras 1967; Selander 1970; Britton-Davidian 1990; Dallas et al. 1995), with gene flow restricted to neighboring subpopulations. This level of gene flow is weakly correlated with geographical distance (Dallas et al. 1995). At the lowest level, substantial genetic heterogeneity and limited gene flow existed among breeding groups or demes defined by social territorial interactions (Selander 1970; Singleton 1983; Singleton and Hay 1983). Eco-behavioral studies revealed that gene flow could occur both at short distances among buildings within a farm or among farmlands, and at long distances reaching out an area of about 100 hectares (Berry and Jakobson 1974; Myers 1974; Lidicker 1976; Baker 1981; Berry et al. 1991; Dallas et al. 1995). Nonetheless, the population structures in the house mouse are far from stable, usually subjected to high turnover rates, and influenced by repeated local extinction and re-colonizing events (Myers 1974; Stickel 1979; Baker and Petras 1986; Singleton 1989; Carlsen 1993; Ardill and Silver 1998; Chou et al. 1998). Overall, the genetic and ecological characteristics of house mouse populations are vicissitudinous and amenable to many circumstances, since it is an extremely adaptable species (Berry 1981; Bronson 1984).
In this paper we apply polymorphic microsatellite DNA markers to study the genetic population subdivision and gene flow of the South East Asian house mouse (*Mus musculus castaneus*) in Taiwan. The house mouse in Taiwan has been confirmed to be the subspecies of *M. musculus castaneus* based on its D-loop sequences (Prager et al. 1996; Yang 1998) and the result was corroborated by the evidence of Zfy-2 gene on Y chromosomes (Boissinot and Bourriot 1997; Wu 1999). This is the first report on the population genetics of this subspecies. In addition, we discuss the patterns of population subdivision and gene flow in connection with historical human settlements and agricultural expansion.

**MATERIALS AND METHODS**

**Field samples**

In Taiwan, most house mice are found in or near human dwellings, outbuildings, and rice granaries (Chou et al. 1998). Rice is the major staple food crop in Taiwan and, therefore, almost every township in the rice-producing lowlands has its own centralized rice granaries. From July 1995 through February 1997, we collected a large number of house mice from rice granaries in various townships to embark on a study of the population biology of the species in Taiwan (Chou et al. 1998; Peng 1998). Samples from 11 townships were used for this study (Fig. 1) and sample sizes from each township varied from 2 to 76 (see Peng 1998). Although feral house mice were rare, some were caught in the field from Jia-li (n = 2), Shih-gang (n = 3), and Jin-men (n = 17) townships (Fig. 1). These mice are also included in this study. Detailed trapping protocols were given in Chou et al. (1998).

For population analyses, we treat samples from the rice warehouse of each township (Fig. 1) as a single population except those with small sample sizes. We pooled the small samples according to their geographical locations. Samples from Jia-li, Ma-dou and Shi-gang (map nos. 4, 5, and 6 in Fig. 1) were combined to represent a population for Tainan area; those from Mei-nung, Pin-dung City and Wan-dan (map nos. 7, 8 and 9 in Fig. 1) were combined for Gau-ping area; finally, mice from several trapping sites in Jin-men were treated as one population. As a result, seven populations (n = 187) were used for these analyses: Guan-shi (GS), Shin-pu (SP), Lin-nei (LN), Tainan (TN), Gau-ping (GP), Shou-feng (SF), and Jin-men (JM) (see Table 1).

**Tissue samples and DNA extraction**

Mice caught were kept alive in cages until the end of the trapping session and were brought back to the lab for further processing. After autopsy, tissue samples of heart, liver, spleen, kidney, and muscle were placed in cryogenic tubes for storage in liquid nitrogen tanks (Chou et al. 1998). In addition, mice of three inbred strains (BALB/CJ, B6 and CBA/J) and F1 hybrids between B6 and CBA/J were bought from Laboratory Animal Center, College of Medicine, National Taiwan University. The inbred mice were used as controls to recognize allele bands for homozygous (two inbred strains) and heterozygous (F1 hybrids) individuals.

Total DNA was extracted from tissue samples by standard phenol-chloroform procedures (Ausubel et al. 1992). The chromosomal location and core sequences of the six loci are as follows: 34, 9 / (CAAG)n; 105, 7 / (ATT)n; 150, 11 / (ATT)n; D6Mit138, 6 / (GA),(GAA)nm; D10Mit20, 10 / (TAA)n; D15Mit16, 15 / (TAA)n.

**Genotyping by native polyacrylamide gels**

We first genotyped each mouse by native polyacrylamide gels. PCR reactions (10 μl) were carried out, each containing the following: 200 ng of genomic DNA, 0.1 μM of each primer, 0.25 mM dNTPs, 0.25 U of DNA Tag polymerase (Promega), and 1–4 mM MgCl₂. Amplifications were done in a Biotronics AG-9600 Thermostycer. Thermal profiles started with an initial denaturation at 94°C for 4 min, followed by 6 “touch down” cycles, and 34 cycles consisting of 1 min denaturation at 94°C, 15 s annealing at the proper annealing temperature, 20 s extension at 72°C. Finally, a 10 min extension step was added to complete the thermal profile. The “touch down” cycles were the same as the main cycles except that the annealing temperature of “touch down” cycles contains 3 steps, each having two cycles. The annealing temperature of the first step was at 6°C higher above the main annealing temperature and decreases by 2°C each in the next two steps until the main cycles began. Such procedures were used to reduce nonspecific bands. Amplified products were resolved by 7% (19:1) nondenaturing polyacrylamide gel electrophoresis (13.5X14.5X0.075 cm), and visualized by ethidium-bromide staining. Allele sizes were estimated by running a pBR322/MspI size marker (NEB) along the PCR products. This system provided satisfactory results in most cases. However, some heteroduplex bands appeared to cause confusions in reading the correct bands from gels. Whenever confusions occurred, we used denaturing sequencing gel electrophoresis to re-score the results (see below).

**Genotyping by denaturing sequencing gels**

For denaturing sequencing gel electrophoresis, radioactive PCR amplifications were performed in similar conditions except that 0.02 μ Ci/μl α-35S-dATP was added to each reaction. Amplified products were mixed with 4 μl stop solution in an AmpliCycle™ Sequencing kit (Perkin-Elmer). For electrophoresis, 4 μl of the final mixture were denatured and run on 6% denaturing polyacrylamide sequencing gels. Gels were dried and exposed to a Biomax X-ray film (Kodak) for up to 6 days. Products were sized by reference to sequence of control DNA supplied in an AmpliCycle™ Sequencing kit (Perkin-Elmer), or a known sequence fragment of D-loop from the house mouse (Yang 1998). Furthermore, we ran a few representatives of every known allele revealed by native gels to compare and confirm the estimated allele sizes.

**Data analysis**

Both observed heterozygosity (H₀) and unbiased expected heterozygosity (Hₑ; Nei 1978) were calculated to estimate the genetic variability for the 7 mouse populations. The calculations were performed with the BOTTLENECK package (Estoup et al. 1995; Cornuet and Luikart 1997).

The genotype frequencies within samples were tested for agreement with Hardy-Weinberg expectations by Fisher’s exact test (Gou and Thompson 1992; Raymond and Rousset 1995a), using Markov chain procedures. When a population deviated from the Hardy-Weinberg expectation, we used a score test (Rousset and Raymond 1995) to examine if the deviation was caused by excess or deficiency of heterozygous individuals. GENEPOP was used to evaluate the level of population subdivision. Values (f, θ, and F as defined by Weir and Coakesham 1984) were estimated for each locus and averaged over loci. The calculations were done by...
Fig. 1. Map of Taiwan showing 10 townships where house mouse populations were sampled. 1. Guan-shi; 2. Shin-pu; 3. Lin-nei; 4. Ma-dou; 5. Jia-li; 6. Shi-gang; 7. Mei-nung; 8. Pin-dung; 9. Wan-dan; 10. Shou-feng. Inset map shows the location of Jin-men (11), an offshore island of China’s province of Fu-kien. The gray areas indicate elevation above 1000 m and dark areas above 2000 m.
Table 1. Allele frequencies at six microsatellite loci in Taiwanese house mouse populations. GS: Guan-shi; SP: Shin-pu; LN: Lin-nei; TN: Tainan; GP: Gau-ping; SF: Shou-feng; JM: Jin-men

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele (bp)</th>
<th>GS (n=22)</th>
<th>SP (n=10)</th>
<th>LN (n=76)</th>
<th>TN (n=27)</th>
<th>GP (n=9)</th>
<th>SF (n=24)</th>
<th>JM (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6Mit138</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
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<td>0.007</td>
<td>0.056</td>
<td>0.019</td>
<td>0.148</td>
<td>0.222</td>
<td>0.079</td>
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<td></td>
<td>0.111</td>
<td>0.079</td>
<td>0.053</td>
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<td></td>
<td></td>
<td>0.227</td>
<td>0.074</td>
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<td>0.011</td>
<td>0.167</td>
<td>0.105</td>
<td>0.053</td>
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<tr>
<td>D15Mit16</td>
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<td></td>
<td></td>
<td>0.278</td>
<td>0.188</td>
<td>0.039</td>
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<td></td>
<td></td>
<td>0.111</td>
<td>0.079</td>
<td>0.053</td>
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<td>D15Mit17</td>
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<td></td>
<td>0.026</td>
<td>0.079</td>
<td>0.053</td>
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<td>0.063</td>
<td>0.278</td>
<td>0.111</td>
<td>0.063</td>
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<td>0.056</td>
<td>0.121</td>
<td>0.079</td>
<td>0.111</td>
<td>0.188</td>
<td>0.053</td>
<td>0.026</td>
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<td>0.130</td>
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<td>0.130</td>
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<td>0.130</td>
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<td>0.130</td>
<td>0.079</td>
<td>0.053</td>
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<td></td>
<td></td>
<td>0.130</td>
<td>0.079</td>
<td>0.053</td>
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<tr>
<td>Average</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.021</td>
<td>0.079</td>
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<tr>
<td>allele no. (N)</td>
<td>3.17</td>
<td>4.17</td>
<td>7.17</td>
<td>8.50</td>
<td>5.00</td>
<td>4.67</td>
<td>8.50</td>
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</table>

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GENEPOP (Raymond and Rousset 1995b). Significant departures from zero of the values were tested using permutations (see Dallas et al. 1995) and were calculated by FSTAT (Goudet 1995). In addition, we also calculated Slatkin’s (1995) $F_{ST}$, which is similar to Wright’s $F_{IS}$ except that its calculation is based on variance of allele size.

Gene flow was estimated by three measures: $M_n$, $M_S$ and “private allele” methods. $M_n$ was estimated by the formula $F_{ST} = 1/(1+4N_m)$M_n$. In this formula, $S_{ST}$ was estimated as $\theta$ (Weir and Cockeryam 1984) and $\alpha = (n-1)^2$, where $n$ is the number of subpopulations (see Nagylaki 1998). The relationship between $F_{ST}$ and $N_m$ is derived for neutral alleles adopting an island model of a subdivided population at migration-drift equilibrium (Wright 1978). Similarly, gene flow was estimated by $M_S = (dS-1)(1-R_S)/(4dS-R_S^2)$ that is derived using Slatkin’s $R_{ST}$. $d$ is the number of subpopulations or number of demes. Finally, we used Slatkin’s “private alleles” method to estimate the gene flow (Slatkin 1985). In this calculation, when sample size $(n)$ is smaller than 25, we multiply $(25/n)$ to the estimate to yield a corrected value (Slatkin 1985).

To test the hypothesis of isolation by distance, correlations between matrices of gene flow (using $F_{ST}$ / $(1-F_{ST})$ as indicator) and geographic distances (in logarithm) among populations were analyzed based on Mantel’s test (Manly 1985; Rousset 1997) and were performed by the program GENEPOP. The geographic distances were estimated from a map by the connecting straight lines between two localities on one side of the Central Mountain Range. When two localities are separated by the Central Mountain Range, the distances were estimated by lines that go through plains. The rationale behind this treatment is that the Central Mountain Range is not suitable for human settlement and no rice has ever been cultivated there, i.e., the mountain range poses a insurmountable barrier to dispersal for house mice.

### RESULTS

#### Microsatellite variability and heterozygosity

Complete lists of allele frequencies for each population are given in Table 1. Levels of genetic variation and related parameters at each locus for all populations combined are summarized in Table 2. The following provides a summary of the results.

Each locus was revealed to be highly polymorphic. The observed heterozygosity ($H_o$) ranged from 0.37 to 0.71, with an average of 0.57; the expected heterozygosity ($H_e$) ranged from 0.59 to 0.84, with an average of 0.76 (Table 2). The numbers of alleles at each locus ranged from 7 to 12.

In terms of individual populations (Table 1), the population of Guan-shi was the least polymorphic, with an average of 3.17 alleles per locus and expected heterozygosity ($H_e$) 0.35. The populations of Tainan and Jin-men were the most polymorphic: both with an average of 8.50 alleles per locus, and the expected heterozygosity was slightly lower in Tainan (0.76) than in Jin-men (0.83).

For 7 populations at 6 loci, 17 out of 42 cases contained a single dominant allele with frequency exceeding 0.5 (Table 1): 3 populations (Guan-shi, Shin-pu, and Shou-feng) at $D6Mit138$, 2 populations (Guan-shi and Shou-feng) at $D15Mit16$, 2 populations (Guan-shi and Shin-pu) at 34, 6 populations (Shin-pu, Lin-nei, Tainan, Gau-ping, Shou-feng and Jin-men) at 105, and 4 populations (Guan-shi, Lin-nei, Gau-ping, and Shou-feng) at 150. Otherwise, most populations contained more than 4 alleles at each locus and the frequencies were largely spread out among all alleles.

#### Hardy-Weinberg expectation

We compared genotype frequencies at 6 loci with Hardy-Weinberg expectations using Fisher’s exact test. The overall genotype frequencies deviated significantly from the expectations $(P<0.001)$. Considering 42 combinations of 7 populations over 6 loci, significant departures $(P<0.05)$ were found in 18 cases, all showing deficiencies in heterozygotes $(P<0.05)$. The distribution of the 18 cases were somewhat clustered by 2 loci and 3 populations. $D6Mit138$ and 105 showed significant deviations in more than half of the 7 populations: 5 out of 7 and 4 out of 7 (for $D6Mit138$ and 105), respectively. In terms of individual populations, Guan-shi, Lin-nei and Gau-ping had 3 or more loci that showed heterozygote deficiencies. While the departure from Hardy-Weinberg expectation in Gau-ping may be due to the combination of 3 separated samples, the causes for the other populations are not clear.

#### Genetic population differentiation

Wrights $F$-statistics ($F_{ST}$, $F_{IS}$, and $F_{IT}$) in the notation of Weir and Cockerham (1984) (i.e., $f$, $\theta$, and $F$, respectively) were calculated to reveal population genetic subdivision

<table>
<thead>
<tr>
<th>Locus</th>
<th>Motif</th>
<th>N</th>
<th>Allele size range</th>
<th>Allele size interval</th>
<th>$H_o$</th>
<th>$H_e$</th>
<th>$\theta$</th>
<th>$F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6Mit138</td>
<td>2+4</td>
<td>12</td>
<td>133–169</td>
<td>2 or 4</td>
<td>0.57</td>
<td>0.77</td>
<td>0.179***</td>
<td>0.136***</td>
</tr>
<tr>
<td>D10Mit20</td>
<td>4</td>
<td>169–201</td>
<td>4</td>
<td>0.71</td>
<td>0.84</td>
<td>0.060</td>
<td>0.133***</td>
<td>0.185***</td>
</tr>
<tr>
<td>D15Mit16</td>
<td>4</td>
<td>121–161</td>
<td>4</td>
<td>0.71</td>
<td>0.84</td>
<td>0.047</td>
<td>0.142***</td>
<td>0.183***</td>
</tr>
<tr>
<td>34</td>
<td>4</td>
<td>198–230</td>
<td>4</td>
<td>0.60</td>
<td>0.81</td>
<td>0.102*</td>
<td>0.216***</td>
<td>0.296***</td>
</tr>
<tr>
<td>105</td>
<td>5</td>
<td>125–155</td>
<td>5</td>
<td>0.37</td>
<td>0.59</td>
<td>0.240***</td>
<td>0.200***</td>
<td>0.392***</td>
</tr>
<tr>
<td>150</td>
<td>3</td>
<td>115–148</td>
<td>3</td>
<td>0.45</td>
<td>0.69</td>
<td>0.158***</td>
<td>0.281***</td>
<td>0.395***</td>
</tr>
<tr>
<td>All loci</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.57</td>
<td>0.76</td>
<td>0.122***</td>
<td>0.182***</td>
</tr>
</tbody>
</table>
within Taiwanese house mice (Table 2). The population sub-
division was inferred when either single-locus or multi-locus 
θ values were high and significant. The multi-locus θ was sig-
nificant, but the trend was not consistent across all loci. Sin-
gle-locus θ was significant in 4 out of 6 loci. Both multi-locus 
and single-locus F were significant, implying overall het-
erozygote deficiencies in the whole population.

Table 3 gives measures (both θ and RST) of population 
differentiation in a pairwise fashion. All the θ values are sig-
nificant (P<0.001) and thus indicate substantial population 
substructure. Two aspects are noteworthy. First, the levels 
of differentiation between Jin-men and the 6 Taiwanese 
populations (mean θ=0.113) were less than those among 
the 6 Taiwanese populations (mean θ=0.219). The popula-
tion of Jin-men was particularly similar to those of western 
Taiwan (Shin-pu, Lin-nei, Tainan, and Gau-ping; Fig. 1), 
baring that of Guan-shi. Second, population of Guan-shi 
(mean θ=0.346) was quite different from the rest of Taiwan-
iese populations (mean θ=0.155). The same pattern was 
observed for RST estimates of subdivision.

### Gene flow

The levels of gene flow were estimated by three meth-
ods: Nm, “private allele” estimate and M (Table 4). These 
estimates are consistent with the data of Table 3, in showing 
that gene flow was higher across the Taiwan Strait (between 
Jin-men and Taiwan; Fig. 1) than that within Taiwan. In one 
estimate (Nm), the gene flow across the Taiwan Strait (1.12) 
was almost twice that of within Taiwan (0.60). This high 
over-water gene flow across the Taiwan Strait is extraordi-
nary and requires further interpretation in the context of his-
torical human settlements in Taiwan.

The result of the Mantel test did not support the isola-
tion by distance hypothesis (P>0.05), therefore, no evidence 
for contiguous short-distance gene flow in Taiwanese house 
mouse populations.

### DISCUSSION

#### Human mediated gene flow and genetic drift

The gene flow estimated here for *M. musculus casta-
neus* cannot be understood without considering the com-
mensal habits of the species in relation to human settle-
mements in Taiwan’s history. Observed gene flow was higher 
between populations of Taiwan and Jin-men (i.e. across the 
Taiwan Strait) than among the populations within Taiwan 
(Table 4). The waters separating the two landmasses 
should have been an effective barrier to inhibit the gene 
exchange, presumably more effective than whatever barri-
ers have been existing among the populations living in dif-
ferent rice warehouses. Nonetheless, we found the opposite 
trends.

Before the 17th century, the island of Taiwan was 
sparsely inhabited by aboriginal peoples who had colonized 
Taiwan since in pre-historical times (Diamond 1997; Chen 
1993). However, the Han Chinese began in late 17th century 
to immigrate to Taiwan to cultivate rice and sugar cane (Fig. 
2; Chen 1993). This wave of human settlement had contin-
ued for more than 2 centuries until the end of 19th century. 
There were two provinces in southeastern China where 
these immigrants came from, roughly 85% from Fukien 
Province and 15% from Canton Province (Chen 1993). 
Many of them landed in Tainan, which was one of the oldest 
and major historical ports to receive these settlers. From 
there they began to move into the western floodplain, either 
to the north or the south. By the mid-19th century, the land 
in the western floodplain had largely been developed for 
agriculture. Two other floodplains that remained suitable for 
agriculture are situated in the eastern part of the island (Fig. 
2), where the settlers had been previously kept out due to 
the massive Central Mountain Range (Fig. 1). Agriculture 
was expanded to the northeastern I-Lan plain in late 19th 
century and to the eastern Hua-Dong plain as late as the 
early 20th century (Fig. 2).

Among the six mouse populations in Taiwan, Shou-feng 
was the most distinct (Table 3) as revealed by microsatellite 
markers. Moreover, over 50% of the mice from Shou-feng 
carried a mitochondrial D-loop haplotype that is separated 
by a deep branch from the other haplotypes and is largely 
confined to the Hua-Dong plain (Yang 1998). It, therefore, 
appears that the Shou-feng population may still maintain 
some descendants of the "indigenous" mice.

We consider the mice from Jin-men as representatives 
of the mouse genomes from southeastern China because of 
its proximity to the mainland Fukien province, merely 1.2 km 
apart (Fig. 1). This is corroborated by the genetic data. The 
genetic diversity of Jin-Men’s mouse population is the great-

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**Table 3. Measures of population differentiation for all pairwise combinations of house mouse populations in Taiwan. Both θ (=FST; above diagonal) and RST (below diagonal) are given**

<table>
<thead>
<tr>
<th>GS</th>
<th>SP</th>
<th>LN</th>
<th>TN</th>
<th>GP</th>
<th>SF</th>
<th>JM</th>
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<tbody>
<tr>
<td>GS</td>
<td>0.393</td>
<td>0.243</td>
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<td>LN</td>
<td>0.241</td>
<td>0.130</td>
<td>0.082</td>
<td>0.123</td>
<td>0.241</td>
<td>0.105</td>
</tr>
<tr>
<td>TN</td>
<td>0.322</td>
<td>0.060</td>
<td>0.118</td>
<td>0.095</td>
<td>0.197</td>
<td>0.025</td>
</tr>
<tr>
<td>GP</td>
<td>0.282</td>
<td>0.202</td>
<td>0.068</td>
<td>0.222</td>
<td>0.267</td>
<td>0.075</td>
</tr>
<tr>
<td>SF</td>
<td>0.437</td>
<td>0.260</td>
<td>0.356</td>
<td>0.289</td>
<td>0.361</td>
<td>0.132</td>
</tr>
<tr>
<td>JM</td>
<td>0.350</td>
<td>0.054</td>
<td>0.222</td>
<td>0.026</td>
<td>0.285</td>
<td>0.195</td>
</tr>
</tbody>
</table>

**Table 4. Estimates of gene flow in house mouse populations within Taiwan and across the Taiwan Strait**

<table>
<thead>
<tr>
<th>Estimate</th>
<th>Within Taiwan</th>
<th>Between Taiwan and Jin-men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nm</td>
<td>0.60</td>
<td>1.12</td>
</tr>
<tr>
<td>“Private allele”</td>
<td>2.43</td>
<td>3.45</td>
</tr>
<tr>
<td>M</td>
<td>0.45</td>
<td>0.58</td>
</tr>
</tbody>
</table>
est for all the populations studied, even though the island of Jin-men has an area of just 132 km². This suggests the general assumption that the source population should contain the greatest diversity. Furthermore, the population of Tainan, where the settlement started and flow of immigration had continued, is the most similar to the population of Jin-men (see pairwise $\theta$ in Table 3).

All in all, the high gene flow across the Taiwan Strait and close genetic relatedness between Jin-men’s and western Taiwan’s populations indicate that there had been human-mediated gene flow in *Mus musculus castaneus* populations due to historical agriculture expansions. This interpretation is also supported by mtDNA (Yang 1998) and Y chromosome data (Wu 1999). The connection between human movement and rapid gene flow has been demonstrated in *Mus musculus domesticus* in southern Europe (Britton-Davidian 1990), where human seafaring activities were frequent.

Moreover, within the island of Taiwan there was not isolation by distance in the mouse populations. One population (Guan-shi; map #1 in Fig. 1) is genetically distinct among the six Taiwanese populations (Table 3) and another (Shou-feng; map #10 in Fig. 1) is geographically isolated from the others (Table 3). However, when the two populations were excluded, the result of Mantel test was still not significant ($P > 0.05$). It is, therefore, evident that the prevailing gene flow cannot result from constant migration among stable neighboring populations as assumed in the island or stepping-
stone model (Wright 1978; Dallas et al. 1995). It is likely that human activities facilitated some form of long-distance gene flow in *M. musculus castaneus*.

Ecological observation shows that these mouse populations in rice granaries are not stable (Chou et al. 1998). These populations are typically ephemeral and unstable due to the regular turnover of grain, which is stored and processed within a period of 2–3 years. Although mice in the granaries are not in short supply of food, they are subjected to regular depletion of the habitat (when all the grain is emptied), as well as to applications of poison. These populations must go through regular episodes of bottleneck, extinction, and re-colonization which can result in genetic subdivision. Under these circumstances, some populations could lose their polymorphism if the founding populations were small. There is evidence to show this may be the case for the population of Guan-shi. First, this population is distinct from the others, and particularly it is quite different from its neighbouring population (10.5 km apart) of Shin-pu ($\theta=0.393$ second greatest in pairwise comparisons; Table 3). Second, Guan-shi is the least polymorphic population (Table 1) and the mean relatedness values (Queller and Goodnight 1989) among its members (0.75; unpublished data) is above the average for siblings (0.5), implying a small founding population and inbreeding. Furthermore, populations of Guan-shi and Shin-pu were each fixed for two separate mtDNA haplotypes (Yang 1998), also indicating some genetic drift due to bottleneck/founder effect.

**Inbreeding and deme structure**

The inbreeding effect is indicated by significant $f$ values (Table 2) and deviation from Hardy-Weinberg equilibrium due to heterozygote deficiency in, at least, three populations (Guan-shi, Shin-pu and Lin-nei). The three populations comprised mice from a single warehouse, reducing the likelihood of the Wahlund effect (Hartl and Clark 1989). However, the potential presence of null alleles that can also cause the deficiency in heterozygotes could not be completely ruled out. Yet, such single populations could still be subdivided into social demes (Selander 1970; Lidicker 1976; Singleton 1983; Singleton and Hay 1983), giving rise to the observed heterozygote deficiency. We are currently focusing on a mark-recapture study in rice granaries in two townships to resolve the issue of population substructure caused by social interactions.

**Population structure and genetic diversity**

At the level of granaries from different townships in Taiwan, the population subdivision is apparent. Not only the overall $\theta$ (Table 2) but also all the $\theta$ between pairs of populations (Table 3) were significantly different from 0. Each population except Guan-shi appeared to maintain a high level of polymorphism (all $H_o > 0.5$; Table 1). Although the gene flow estimated was low among Taiwanese populations, gene flow may still occur occasionally through some pockets of feral populations (Chou et al. 1998) or by long-distance dispersal associated with human activities. Nevertheless, each population seems to be drifting away from each other by the fluctuating population sizes in individual townships that tend to promote genetic drift in the process (Whitlock 1992). On the other hand, successful colonizers, if derived from genetically differentiated populations, will tend to re-introduce new polymorphisms in single populations (Wade and McCauley 1988). It is, therefore, of great interest to investigate, in various townships, the size and genetic relatedness of founding populations, to further elucidate the nature of gene flow in this human commensal species.

**ACKNOWLEDGMENTS**

Many keepers of the rice warehouses kindly offered help and permission to work in the areas under their supervision. C. M. Kuo lent us his field vehicle for many collecting trips. Kristin G. Ardlie offered her expertise in microsatellite screening. Kristin G. Ardlie, Jim Patton, Hwei-Yu Chang, Jim O’Connor, Hung-I Wang, Kateryna Makova and Anton Nekrutenko read an earlier draft of the manuscript. Two anonymous reviewers offered valuable comments on the manuscript. We thank them all. This research was supported by the National Science Council of the Republic of China (85-2311-B-002-023-B17, 86-2311-B-002-030-B17, 87-2311-B-002-016-B17).

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