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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 (H_e=0.76) although individual populations varied in their levels of heterozygosity (H_e=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; Gerlach 1990; Berry *et al.* 1991).

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; Ardlie 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).

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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 Boursot 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), Shigang (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 Shigang (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.* 1995) and the extracts were stored at –20°C for future use.

Microsatellite loci

Six unlinked microsatellite loci were employed; three (*34*, *105*, *150*) were taken from Hearne *et al.* (1991), two (*D6Mit138*, *D10Mit20*) from database of the Whitehead Institute for Biomedical Research (http://www.genome.wi.mit.edu), and one (*D15Mit16*) from Dietrich *et al.* (1992). The chromosomal location and core sequences of the six loci are as follows: *34*, 9 / $(CAAG)_n$; *105*, 7 / $(ATTTT)_n$; *150*, 11 / $(ATT)_n$; *D6Mit138*, 6 / $(GA)_n(GAAA)_m$; *D10Mit20*, 10 / $(TAGA)_n$; *D15Mit16*, 15 / $(TAGA)_n$.

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 $MgCl_2$. Amplifications were done in a Biotronics AG-9600 Thermocycler. 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 rescore 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-^{35}S-dATP was added to each reaction. Amplified products were mixed with 4 μl stop solution in a 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 (H_o) and unbiased expected heterozygosity (H_e; 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 Raymont 1995) to examine if the deviation was caused by excess or deficiency of heterozygous individuals. GENEPOP was used to perform the tests (Raymond and Rousset 1995b; http://wbiomed.curtin.edu.au/genepop).

Wright's *F*-statistics (Wright 1965; 1978) were calculated according to Weir and Cockerham (Weir and Cockerham 1984) to evaluate the level of population subdivision. Values (f, θ, and F as defined by Weir and Cockerham 1984) were estimated for each locus and averaged over loci. The calculations were done by

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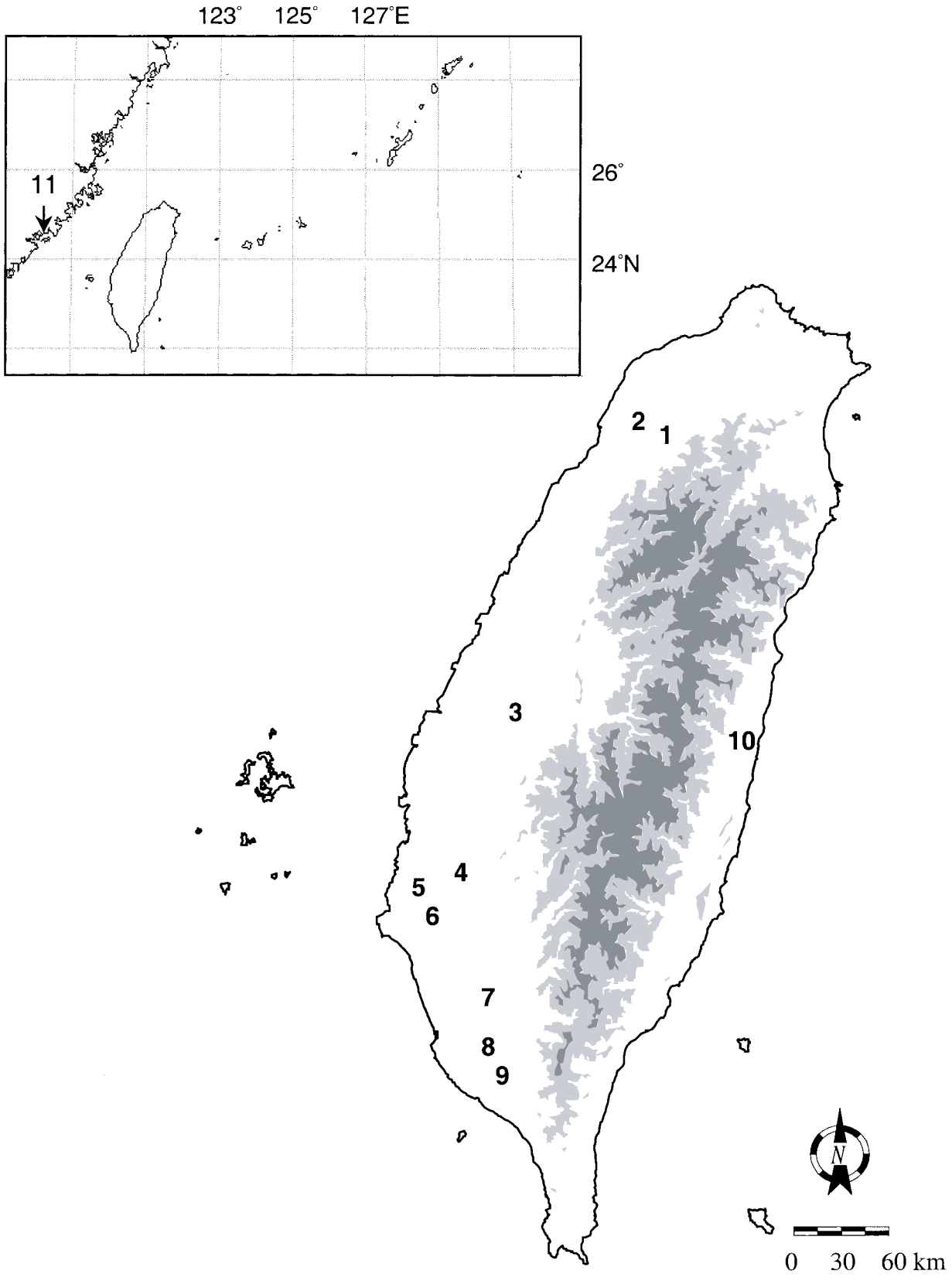


Fig. 1. Map of Taiwan showing 10 townships where house mouse populations were sampled. 1. Guan-shi; 2. Shin-pu; 3. Lin-nei; 4. Ma-dou; 5. Jia-li; 6. Shi-gang; 7. Mei-nung; 8. Pin-dung; 9. Wan-dan; 10. Shou-feng. Inset map shows the location of Jin-men (11), an offshore island of China's province of Fu-kien. The gray areas indicate elevation above 1000 m and dark areas above 2000 m.

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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

Locus	Allele (bp)	Sample locality and sample size (n)						
		GS (n=22)	SP (n=10)	LN (n=76)	TN (n=27)	GP (n=9)	SF (n=24)	JM (n=19)
D6Mit138	133			0.007	0.056			
	137			0.007	0.019			0.026
	141		0.150	0.033	0.148	0.222		0.079
	143				0.019			
	145	0.045		0.020	0.148	0.011	0.167	0.105
	147			0.033	0.019			
	149	0.864	0.100	0.355	0.241	0.278		0.316
	153		0.550	0.349	0.130	0.389	0.563	0.211
	157		0.200	0.118	0.130		0.208	0.211
	161	0.068			0.056		0.063	0.053
	165	0.023		0.007	0.038			
	169			0.072				
D10Mit20	169			0.013		0.111		0.079
	173	0.227		0.257	0.074	0.278	0.188	0.053
	177	0.068	0.100	0.270	0.148	0.111		0.105
	181		0.150	0.395	0.426	0.167	0.021	0.105
	185	0.341	0.100	0.026	0.259	0.111	0.104	0.211
	189	0.364	0.500	0.007	0.037		0.167	0.158
	193		0.150		0.019	0.222	0.313	0.158
	197				0.037			0.053
	201			0.033			0.208	0.079
D15Mit16	121					0.334	0.063	0.053
	125			0.092	0.019	0.278		0.053
	129	0.227	0.300	0.230	0.130	0.111	0.063	0.026
	133	0.727	0.100	0.204	0.019	0.056	0.542	0.079
	137	0.023	0.150	0.329	0.185	0.111		0.079
	141			0.020	0.130		0.146	0.079
	145		0.400	0.125	0.333	0.111	0.188	0.184
	149				0.130			0.132
D15Mit16	153	0.023	0.050		0.056			0.184
	157							0.079
	161							0.053
34	*198*						*0.021*	
	202		0.050	0.013	0.019		0.292	0.105
	206		0.050	0.026	0.019		0.042	0.079
	210	0.977	0.100	0.270	0.130	0.167	0.083	0.079
	241			0.138	0.278	0.444	0.042	0.211
	218	0.023	0.550	0.112	0.389	0.223	0.375	0.316
	222		0.250	0.408	0.093			0.079
	226			0.020	0.019	0.167	0.146	0.079
	230			0.013	0.056			0.053
105	125	0.455			0.019	0.056		0.053
	130	0.477	0.800	0.566	0.612	0.112	0.938	0.553
	135				0.019	0.611		0.053
	140				0.056		0.063	0.132
	145			0.414	0.148	0.222		0.158
	150	0.068	0.200	0.013	0.130			0.053
	155			0.007	0.019			
150	115		0.050		0.019			
	118	0.045						0.026
	121	0.955	0.350	0.691	0.371	0.556		0.184
	124		0.350	0.066	0.130	0.111	0.083	0.105
	127			0.026	0.075	0.056	0.083	0.132
	130		0.250	0.020	0.037	0.056	0.042	0.289
	133			0.013	0.278	0.111		0.053
	136				0.019			0.026
	139			0.099	0.037	0.111	0.792	0.132
	142			0.079	0.019			0.026
	145			0.007	0.019			
	148							0.026
Average allele no. ($\bar{N}$)		3.17	4.17	7.17	8.50	5.00	4.67	8.50

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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) R_{ST}, which is similar to Wright's F_{ST} except that its calculation is based on variance of allele size.

Gene flow was estimated by three measures: *N*m, M_R and "private allele" methods. *N*m was estimated by the formula $F_{ST} = 1/(1+4\alpha Nm)$. In this formula, F_{ST} was estimated as θ (Weir and Cockerham 1984) and $\alpha=(n / n-1)^2$, 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 $M_R = (ds -1)(1-R_{ST})/(4dsR_{ST})$ that is derived using Slakin's R_{ST}. *ds* 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 in 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_{IS}, F_{ST}, and F_{IT}) in the notation of Weir and Cockerham (1984) (i.e., *f*, θ, and *F*, respectively) were calculated to reveal population genetic subdivision

Table 2. General information, heterozygosity, and *F*-statistics for the six microsatellite loci applied in the Taiwanese house mouse. N is the number of alleles. Allele sizes are in base pairs. H_o is observed heterozygosity and H_e is unbiased estimate of expected heterozygosity (Nei, 1978). The calculation of *F*-statistics is based on the formulas in Weir and Cockerham (1984). Asterisks indicate the percentage of permutation-based values (*P*) greater than the values shown: *(0.05>*P*>0.01), **(0.01>*P*>0.001), ***(*P*<0.001)

Locus	Motif	N	Allele size range	Allele size interval	H_o	H_e	*f*	θ	*F*
D6Mit138	2+4	12	133–169	2 or 4	0.57	0.77	0.179***	0.136***	0.291***
D10Mit20	4	9	169–201	4	0.71	0.84	0.060	0.133***	0.185***
D15Mit16	4	11	121–161	4	0.71	0.84	0.047	0.142***	0.183***
34	4	9	198–230	4	0.60	0.81	0.102*	0.216***	0.296***
105	5	7	125–155	5	0.37	0.59	0.240***	0.200***	0.392***
150	3	12	115–148	3	0.45	0.69	0.158***	0.281***	0.395***
All loci					0.57	0.76	0.122***	0.182***	0.282***

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within Taiwanese house mice (Table 2). The population subdivision was inferred when either single-locus or multi-locus θ values were high and significant. The multi-locus *f* was significant, but the trend was not consistent across all loci. Single-locus *f* was significant in 4 out of 6 loci. Both multi-locus and single-locus *F* were significant, implying overall heterozygote deficiencies in the whole population.

Table 3 gives measures (both θ and R_{ST}) of population differentiation in a pairwise fashion. All the θ values are significant ($P<0.001$) 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 population 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 Taiwanese populations (mean θ=0.155). The same pattern was observed for R_{ST} estimates of subdivision.

Gene flow

The levels of gene flow were estimated by three methods: *Nm*, "private allele" estimate and M_R (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 (*N*m), 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 extraordinary and requires further interpretation in the context of historical human settlements in Taiwan.

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

Table 3. Measures of population differentiation for all pairwise combinations of house mouse populations in Taiwan. Both θ (=F_{ST}; above diagonal) and R_{ST} (below diagonal) are given

	GS	SP	LN	TN	GP	SF	JM
GS		0.393	0.243	0.298	0.353	0.444	0.291
SP	0.269		0.135	0.072	0.164	0.178	0.052
LN	0.241	0.130		0.082	0.123	0.241	0.105
TN	0.322	0.060	0.118		0.095	0.197	0.025
GP	0.282	0.202	0.068	0.222		0.267	0.075
SF	0.437	0.260	0.356	0.289	0.361		0.132
JM	0.350	0.054	0.222	0.026	0.285	0.195	

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

Estimate	Populations	
	Within Taiwan	Between Taiwan and Jin-men
*N*m	0.60	1.12
"Private allele"	2.43	3.45
M_R	0.45	0.58

DISCUSSION

Human mediated gene flow and genetic drift

The gene flow estimated here for *M. musculus castaneus* cannot be understood without considering the commensal habits of the species in relation to human settlements 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 barriers have been existing among the populations living in different 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 continued 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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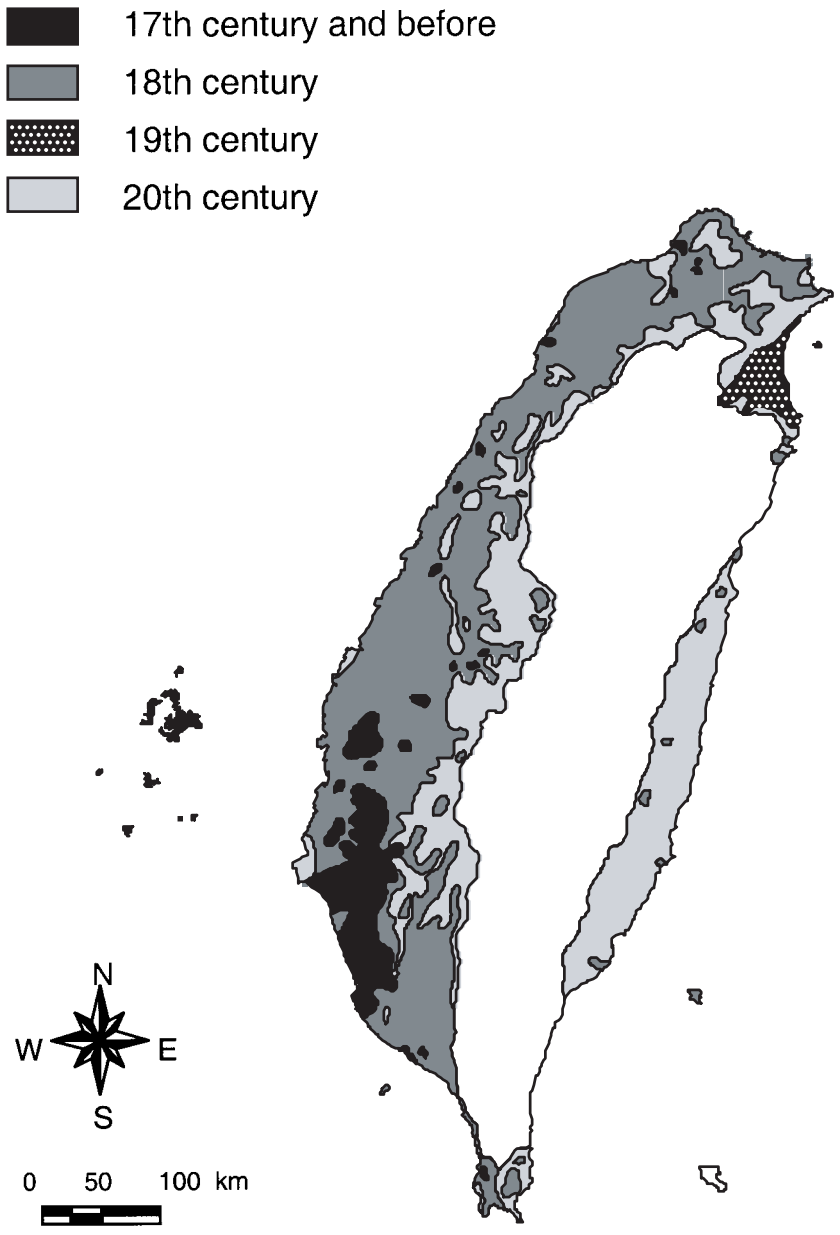


Fig. 2. Stages of land settlement in Taiwan from approximately 17th to 20th centuries.

est for all the populations studied, even though the island of Jin-men has an area of just 132 km^2. 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 θ 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 (Shoufeng; 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-

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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 (θ=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 θ (Table 2) but also all the θ 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_e > 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.

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