

Worker Size in the Formosan Subterranean Termite in Relation to Colony Breeding Structure as Inferred from Molecular Markers

CLAUDIA HUSSENER,¹ JANINE E. POWELL,² J. KENNETH GRACE,³
EDWARD L. VARGO,⁴ AND KENJI MATSUURA⁵

Environ. Entomol. 37(2): 400–408 (2008)

ABSTRACT The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is an invasive species that originated in China and has been introduced to Hawaii and the U.S. mainland. Colonies are headed either by a pair of reproductives (simple families) or by varying numbers of inbreeding reproductives (extended families), and therefore have variable degrees of inbreeding. Worker size also varies among colonies of Formosan termites. We tested whether variation in worker size can be explained by the breeding system. Workers were collected from colonies from three geographically separated populations (China, Hawaii, and Louisiana), and body weight and head size were measured. Microsatellite genotyping was used to establish whether colonies were simple or extended families and to determine the heterozygosity of workers and their degree of inbreeding relative to their colony (F_{IC} , sensitive to the number of reproductives). All Chinese colonies contained multiple inbreeding neotenic. In Hawaii, 37% of the colonies were simple families and 63% were extended families, both having considerable degrees of inbreeding. In Louisiana, 57% of the colonies were simple families, which were mostly headed by unrelated pairs, and 43% were extended families. In simple families, size and body weight of workers were not associated with F_{IC} or heterozygosity. In extended families of two populations, both size parameters were negatively correlated with F_{IC} ; however, heterozygosity was not associated with worker size in any of the populations. This suggests that the number of reproductives within colonies has a stronger influence on worker size than the individuals' genetic diversity in Formosan subterranean termite colonies.

KEY WORDS microsatellite genotyping, morphometry, breeding system, Isoptera, Rhinotermitidae

Effects of inbreeding have been generally considered negligible in subterranean termites (Bartz 1979, Hamilton 1972, Roisin 1999), because the life cycle of this group includes inbreeding among the colonies' offspring after the demise of the founding pair (Bartz 1979, Shellman-Reeve 1997). Colonies of subterranean termites, such as the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, generally begin as simple families headed by two primary (alate-derived) reproductives that pair after mating flights (King and Spink 1974, Su and Tamashiro 1987, Raina et al. 2003). Eventually, the primary king and/or queen will be replaced by varying numbers of neotenic (non-alate-derived reproductives) from within the colony; this replacement leads to extended fami-

lies with different degrees of inbreeding depending on the number of reproductives and the number of generations of inbreeding (Thorne et al. 1999, Bulmer et al. 2001, Vargo et al. 2003a, 2006, Husseneder et al. 2005, 2007).

In addition to variable breeding systems and degrees of inbreeding, there is considerable variation in worker size among colonies of *C. formosanus* (Su and La Fage 1984, Grace et al. 1995, Cornelius and Osbrink 2001). This variation has been attributed to a number of factors, including utilization of different host plants and seasonal changes (Waller 1988) and colony age and vigor (Shimizu 1962, Grace et al. 1995). The latter two factors might be confounded by the degree of inbreeding, because older colonies are believed to have gone through more inbreeding cycles than younger colonies. Husseneder et al. (2005) reported the only indication of a relationship between worker size and degree of inbreeding at the level of the colony: worker body weight within colonies located in New Orleans, LA, was negatively correlated with the degree of inbreeding in individuals relative to others in their colony (F_{IC}). Although these data are suggestive, a more detailed study of the relationship of breeding system and worker size is warranted.

¹ Corresponding author: Department of Entomology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803 (e-mail: chusseneder@agcenter.lsu.edu).

² Rocky Mountain Research Station, USDA Forest Service, Fort Collins, CO 80526.

³ Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, 3050 Maile Way, Honolulu, HI 96822.

⁴ Department of Entomology, Box 7613, North Carolina State University, Raleigh, NC 27695.

⁵ Laboratory of Insect Ecology, Graduate School of Environmental Science, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan.

In this study, we tested whether worker size is influenced by the breeding system of the colony, which was inferred from the genotypic make-up of the workers. We correlated body weight and head width of workers to a measure of inbreeding (F_{IC}) known to be highly sensitive to numbers of reproductives within colonies (Thorne et al. 1999, Bulmer et al. 2001, Vargo et al. 2006). F_{IC} is the coefficient of inbreeding of individuals compared with others in their colony and measures the heterozygote deficit in individuals using the colony as the reference population. As shown by computer simulations (Thorne et al. 1999, Bulmer et al. 2001), F_{IC} is expected to be strongly negative in outbred simple family colonies and extended families with a low number of reproductives. F_{IC} approaches zero with increasing numbers of neotenic reproductives in extended family colonies and becomes positive with assortative mating among multiple groups of reproductives within colonies or with mixing of individuals from different colonies. F_{IC} does not necessarily rise with increasing number of generations of inbreeding (Thorne et al. 1999, Vargo et al. 2006), but is highly sensitive to the number of functional reproductives within a colony and to possible assortative mating among reproductives (Thorne et al. 1999). F_{IC} does not entirely correspond to the classic notion of inbreeding, i.e., the lack of genetic diversity in offspring of related parents. To test if worker size is influenced by the degree of inbreeding in the classic sense of genetic variability, we also correlated the average head width and weight of workers to the mean level of heterozygosity within colonies measured by the proportion of heterozygous loci averaged over all individuals in each colony (Nei 1987). We also tested the possibilities that worker size variation among colonies could be caused by differences in the sex ratios of foragers or by differences in the proportions of early and late instars. Our results confirm a negative association between worker size and F_{IC} in extended family colonies, suggesting that the number of reproductives has a negative effect on the size of workers produced in the colony.

Materials and Methods

Collections. *Coptotermes formosanus* is an invasive species that has been introduced from its native range in southeast China (Kistner 1985) to many parts of the world. We studied three populations of the Formosan subterranean termite from its native (city of Guangzhou, Guangdong Province, China) and introduced range. The introduced range was represented by the Island of Oahu, HI, where this termite has been established since at least 1913 (Yates and Tamashiro 1999), and New Orleans, LA, where it was first detected in 1966 (Spink 1967).

Termite workers were collected from 13–19 sample sites from the three different populations. At least 50 workers were collected from each sample site, i.e., from inground monitoring stations (aggregation traps containing cut wood or naturally infested wood). Termites were collected in August 1999 from 13 sample

sites representing a population from the native range of *C. formosanus* on the campus of the Zhongshan University in the city of Guangzhou, Guangdong Province, China. Termites from 19 sample sites on the Island of Oahu, HI, representing the introduced range of *C. formosanus*, were collected between 1999 and 2000. The distance between the sample sites ranged between 120 m and 45 km (Husseneder and Grace 2001a, b). From the second introduced termite population in Louis Armstrong Park, New Orleans, LA, workers were collected from 14 sample sites representing the foraging areas of different colonies (Messenger et al. 2005, Husseneder et al. 2005) between 1999 and 2000. The termite population in Louis Armstrong Park has been the focus of previous studies of colony dynamics, behavior, and elimination (Messenger and Su 2005a, b, Messenger et al. 2005, Husseneder et al. 2003a, b, 2005). Because the effect of inbreeding on worker size within colonies was not the focus of this study, weight data (Messenger and Su 2005a) and genetic data (Husseneder et al. 2005) had been collected separately at different times under the assumption that the studied inground monitoring stations still contained the same colonies. Recent studies on termite colony dynamics in urban areas, however, revealed considerable movement of colonies (Aluko and Husseneder 2007). Therefore, the measurements of worker sizes were repeated using the same samples from which the genetic data were obtained. Data regarding the breeding system of the Louisiana colonies have been published (Husseneder et al. 2005) and are referenced in this study.

Body Weight and Head Width, Number of Antennal Segments, and Sex of Workers. Weight and head width were determined from 20 randomly selected workers per colony that have been preserved in 95% ethanol from all three populations. Weights of individual workers were determined after letting the ethanol evaporate for several minutes until the weight of the sample was stable. Head widths of workers were measured at the widest point of the head capsule using a dissecting scope ($\times 80$ magnification) with an ocular micrometer. Measurements of workers from China and Hawaii were performed at the University of Hawaii at Manoa (J. K. Grace's laboratory); workers from Louisiana were measured at the LSU Agricultural Center (C. Husseneder's laboratory). Units of measurement were standardized before each set of measurements by calibrating the ocular micrometer with a stage micrometer scale (1 U ranged between 0.014 mm for samples from China and Hawaii and 0.028 mm for samples from Louisiana). For the Louisiana population, the mean number of antennal segments, which reflects the predominance of early or late instars of workers (Higa 1981, Raina et al. 2004), was determined for 20 individuals from each colony's forager population and tested for correlation with size of workers and the breeding system of the colony (Spearman's rank correlation coefficient ρ ; SPSS, Chicago, IL). The sex of 20–25 late-instar workers was determined by K. Matsuura, who examined external abdominal characters under a stereomicroscope

Table 1. General statistics of the microsatellite loci used for genotyping of colonies from a native population (China) and two introduced populations of the Formosan subterranean termite

Locus	Allel size range (bp)	No. of alleles			Ho	He	Ho	He	Ho	He
		China	HI	LA	China		HI		LA	
Cf 4:1A2-4	173-197	6	5	4	0.63	0.54	0.56	0.50	0.57	0.61
Cf 4-4	218-251	11	5	3	0.69	0.68	0.40	0.39	0.21	0.54
Cf 10-4	146-176	7	4	2	0.75	0.57	0.57	0.53	0.29	0.51
Cf 12-4	140-191	7	4	4	0.49	0.52	0.33	0.33	0.57	0.52
Cf 8-4	231-249	7	—	—	0.65	0.50	—	—	—	—
Rf 6-1	163-172	—	3	2	—	—	0.37	0.35	0.36	0.30
Cf 4-10	233-245	5	—	3	0.58	0.50	—	—	0.71	0.59
Cf 10-5	278-314	11	—	3	0.73	0.66	—	—	0.71	0.64
Cf 4-9A	287-302	4	—	3	0.61	0.50	—	—	0.71	0.61
Mean		7.25	4.20	3.00	0.64	0.56	0.45	0.42	0.52	0.54
SD		2.55	0.84	0.76	0.08	0.07	0.11	0.09	0.20	0.11

Locus designations follow Vargo and Henderson (2000).

HI, Hawaii; LA, Louisiana; Ho, observed heterozygosity; He, expected heterozygosity.

(SZX7; Olympus, Tokyo, Japan) at $\times 40$ magnification with a digital imaging system (FLVFS-LS; Flovel, Tokyo, Japan). For females, the eighth sternite is reduced and the seventh sternite enlarges posteriorly, with an increasingly convex border, to overlap the eighth sternite. For males, the seventh sternite is not enlarged and sterna 8 and 9 are normally developed. For each colony, we tested whether the sex ratio (number of males/number of females) differed significantly from 1:1 (G -test; Sokal and Rohlf 1995) and whether the sex ratio was correlated to the average body weight of workers in the colony (Spearman's rank correlation coefficient ρ ; SPSS).

Microsatellite Genotyping. DNA was extracted from at least 20 individual workers per sample site using the DNeasy Tissue Kit (Qiagen, Valencia, CA). Individuals were scored at five (Hawaii) or eight microsatellite loci (China and Louisiana; Table 1). These loci, in connection with the polymerase chain reaction (PCR) conditions and genotype scoring procedures, were previously described by Vargo and Henderson (2000) and have been used in a number of population genetic studies of *C. formosanus* (Vargo et al. 2003a, b, 2006, Husseneder et al. 2005, 2006, 2007). Breeding systems of colonies from the Louisiana population have been published in a different context (Husseneder et al. 2005) but are referenced in this study. General descriptive statistics, such as numbers of alleles per locus, and observed versus expected heterozygosity were calculated for each colony and locus using the program FSTAT (Goudet 1995, 2001).

Colony Affiliation, Breeding System, F_{IC} , and Heterozygosity. To determine colony affiliation, groups of termites from different sample sites within the same population were tested for significant genetic differentiation using log-likelihood G -statistics (FSTAT; Goudet 2001). P values were obtained through permutations of the multilocus genotypes between each pair of samples, and standard Bonferroni corrections were applied. If genotypic frequencies of termite samples were significantly different, it was concluded that sample sites belonged to the foraging areas of different

colonies (Vargo et al. 2003a, 2006, Aluko and Husseneder 2007, Husseneder et al. 2005, 2007). We tested whether colonies were headed by a single pair of reproductives (simple family colonies) or multiple reproductives (extended family colonies) based on the worker genotypes (Vargo 2003a, b, Vargo et al. 2003a, 2006, Husseneder et al. 2005, 2007). Colonies were considered simple families if the genotypes of the workers were consistent with those expected for the offspring of a single pair of parents and if the frequencies of the observed genotypes did not differ significantly from the expected Mendelian ratios (G -test for goodness-of-fit summed across all loci). Colonies were considered extended families when they had more genotypes than possible for the offspring of a monogamous pair or the observed frequencies of the genotypes deviated significantly from those expected in simple families ($P < 0.05$, G -test).

To determine the degree of inbreeding within colonies, F_{IC} , i.e., the heterozygosity deficit of individuals relative to their colony, which in extended families is largely dependant on the number of reproductives (Thorne et al. 1999), was calculated for each colony by treating colonies as subpopulations (FSTAT). The breeding system was further characterized by comparing the coefficient of inbreeding of individuals relative to the total population (F_{IT}), the genetic differentiation between colonies (F_{CT}), and the within-colony relatedness (r) to the values predicted by simulations of a variety of possible reproductive systems for subterranean termites (Thorne et al. 1999, Bulmer et al. 2001). The 95% confidence intervals (CIs) were constructed by bootstrapping over loci. Values with overlapping CIs were considered as not significantly different.

From the worker genotypes present in each of the simple family colonies, the genotypes of the putative parents were inferred. To test if parents originated from the same colony, likelihood calculations were performed as implemented in KINSHIP 1.3.1 (Goodnight and Queller 1999; available at <http://www.gsoft-net.us/GSoft.html>). The null hypothesis assumed pairs originated from unrelated colonies ($r = 0$),

Table 2. Average weight, average head width, degrees of inbreeding within colonies (F_{IC}), and heterozygosity (H_o) in colonies of the Formosan subterranean termite in three different populations from the native (China) and the introduced range (Hawaii [HI] and Louisiana [LA])

China	Weight (mg)	Head (mm)	F_{IC}	H_o	HI	Weight (mg)	Head (mm)	F_{IC}	H_o	LA	Weight (mg)	Head (mm)	F_{IC}	H_o	m/f
1	3.21	1.39	-0.17	0.66	1	2.66	1.16	0.28*	0.29	1	1.82	1.16	-0.30	0.44	0.54
2	4.26	1.45	-0.36	0.56	2	3.37	1.26	-0.20	0.60	2	2.26	1.22	-0.50	0.62	0.71
3	2.47	1.34	-0.04	0.54	3	2.38	1.19	-0.01	0.42	3	2.55	1.18	-0.09	0.26	0.32
4	2.12	1.31	0.00	0.63	4	3.56	1.31	-0.20	0.52	4	2.07	1.17	-0.04	0.37	0.92
5	2.59	1.33	-0.04	0.64	5	3.38	1.38	-0.30	0.37	5	2.74	1.23	-0.26	0.69	0.25
6	3.05	1.42	-0.04	0.50	6	3.52	1.38	-0.32	0.48	6	3.59	1.26	-0.39	0.52	0.22
7	2.88	1.35	-0.16	0.64	7	3.07	1.29	-0.27	0.44	7	2.27	1.15	-0.22	0.56	0.44
8	2.61	1.35	-0.15	0.68	8	3.04	1.15	-0.23	0.49	8	2.51	1.23	-0.34	0.56	0.64
9	1.98	1.31	-0.04	0.60	9	2.47	1.12	0.13	0.61	9	3.06	1.23	-0.50	0.71	0.71
10	2.87	1.34	-0.15	0.38	10	2.58	1.16	-0.02	0.37	10	1.85	1.12	-0.14	0.48	1.63
11	3.98	1.42	-0.31	0.85	11	2.46	1.21	0.11	0.40	11	2.27	1.25	-0.44	0.52	0.62
12	3.69	1.41	-0.38	0.71	12	3.02	1.22	0.16*	0.51	12	1.89	1.18	-0.07	0.29	1.33
					13	2.62	1.25	-0.05	0.41	13	2.36	1.21	-0.23	0.48	3.40
					14	2.61	1.17	0.05	0.32	14	1.61	1.21	-0.11	0.36	0.77
					15	3.67	1.21	-0.29	0.60						
					16	2.85	1.26	0.06	0.29						
					17	1.94	1.18	-0.11	0.61						
					18	2.20	1.14	-0.04	0.33						
					19	2.47	1.12	0.09	0.53						
Mean simple family	—	—	—	—		2.95	1.22	-0.09	0.44		2.34	1.21	-0.27	0.51	1.06
SD	—	—	—	—		0.59	0.06	0.14	0.13		0.61	0.05	0.16	0.11	1.04
Mean extended family	2.98	1.37	-0.14	0.62		2.77	1.22	-0.10	0.46		2.35	1.19	-0.28	0.47	0.66
SD	0.71	0.05	0.14	0.12		0.45	0.09	0.16	0.10		0.46	0.03	0.16	0.17	0.36
Mean all	2.98	1.37	-0.14	0.62		2.84	1.22	-0.09	0.45		2.35	1.20	-0.28	0.49	0.89
SD all	0.71	0.05	0.14	0.12		0.50	0.08	0.16	0.11		0.53	0.04	0.16	0.14	0.82

Sex ratios (m/f) among workers were determined in the Louisiana population only.

Simple family colonies are shown with light gray background; extended families shown with darker gray background. H_o is the average proportion of heterozygote loci across all sampled individuals in each colony. Means for F_{IC} were calculated according to Queller and Goodnight (1989). SD values were calculated from the standard errors derived from jackknifing over loci (1000 replications). Colonies in the Hawaiian population with significantly positive F_{IC} values were excluded from calculations of means and from correlations (Figure 1).

whereas the alternate hypothesis assumed that pairs originated from the same colony (r = the average nest mate relatedness within all colonies of a given population). Significance at the level of 5% was determined by log likelihood tests using 10,000 series of pairs created at random.

Because F_{IC} values represent a relative measure of heterozygote deficit within colonies that is highly sensitive to the number of effective reproductives, we also tested whether size of workers is influenced by their actual genetic diversity, calculated directly from the degree of heterozygosity of individuals. The level of heterozygosity within colonies was measured by the proportion of heterozygous loci of each individual in each colony (Nei 1987). F_{IC} values and heterozygosity were tested for significant correlation with body weight and head width of workers in simple family colonies and extended family colonies in each of the three populations (Spearman's rank correlation coefficient ρ ; SPSS).

Results

General characteristics of the microsatellite loci, such as allele sizes, allele numbers, and observed and expected heterozygosity are presented in Table 1. Mean observed heterozygosity across all loci and colonies was not significantly different from the expected heterozygosity in the introduced populations (Hawaii: $P = 0.07$; Louisiana: $P > 0.20$); the native population from China showed a slight heterozygote ex-

cess ($P = 0.03$, Wilcoxon signed ranks test). Genetic diversity measured by the numbers of alleles per locus was significantly higher in the native population from China than in the introduced populations from Hawaii and Louisiana (China versus Hawaii: $P = 0.02$; China versus Louisiana: $P < 0.001$); allele numbers in the Hawaiian population were higher than in the Louisiana population with marginal significance ($P = 0.05$, Mann-Whitney- U test).

Termites from all 19 sample sites on the island of Oahu, HI, and all 14 sample sites within Louis Armstrong Park, New Orleans, LA, were genetically differentiated at the 5% level and thus belonged to independent colonies ($P < 0.001$; 3,420 permutations for samples from Hawaii and 1,820 permutations for samples from New Orleans; FSTAT). Genotypic frequencies of termites from two sample sites within the population from China were not significantly different ($P = 0.14$), indicating that these termites belonged to the same colony. Morphometric and genotypic data of termites from these two sample sites were therefore combined.

Body weights of workers ranged from an average across colonies of 2.35 ± 0.53 (SD) mg in the New Orleans population to 2.84 ± 0.50 mg in the Hawaii population and 2.98 ± 0.71 mg in the China population (Table 2). Worker weights were significantly different between the three different populations ($\chi^2 = 8.97$, $df = 2$, $P = 0.01$, Kruskal-Wallis test). Mean worker head widths in colonies ranged from 1.20 ± 0.04 mm in the New Orleans population to 1.22 ± 0.07 mm in

the Hawaiian population and 1.37 ± 0.05 mm in the Chinese population (Table 2). Head widths differed significantly among the three populations ($\chi^2 = 25.01$, $df = 2$, $P < 0.001$, Kruskal-Wallis test). Worker weight was positively correlated with head width in each of the three populations (Louisiana: Spearman's $\rho = 0.65$, $P = 0.012$; Hawaii: $\rho = 0.73$, $P < 0.001$; China: $\rho = 0.94$, $P < 0.001$).

The number of antennal segments of workers from the foraging populations of the 14 colonies from Louisiana ranged from 10 to 15. No significant difference was found between the number of antennal segment of the foraging individuals from different colonies ($P = 0.17$, Kruskal-Wallis test). The average number of antennal segments of workers in each colony did not correlate significantly to the average head width (Spearman's $\rho = 0.11$, $P > 0.20$) or weight of the individuals ($\rho = 0.50$, $P = 0.07$). Also, the mean number of antennal segments in each colony was not correlated to F_{IC} or heterozygosity ($P > 0.20$ in both cases, $n = 14$ colonies).

The male to female ratio of the workers varied widely among colonies of the Louisiana population and ranged from strong female bias (0.22) to strong male bias (3.40; Table 2). However, only in 4 of the 14 colonies (29%) did the sex ratio differ significantly from 1:1 (G -test, Sokal and Rohlf 1995). The sex ratio within colonies was not significantly correlated to the average body weight of workers (Spearman's $\rho = -0.54$, $P = 0.07$) and the average head width of workers ($\rho = -0.37$, $P = 0.19$, $n = 14$).

The proportion of simple and extended family colonies and the degrees of inbreeding varied among the three populations. All 12 colonies (100%) from the native population in China were extended families headed by inbreeding neotenic reproductives. Hierarchical F -statistics showed moderate inbreeding at the level of the population ($F_{IT} = 0.18$, 95% CI = 0.10–0.24, not overlapping zero), clear genetic differentiation among colonies ($F_{CT} = 0.28$, 95% CI = 0.21–0.31), a moderate degree of inbreeding within colonies ($F_{IC} = -0.14$, 95% CI = -0.23 to -0.05), and a degree of relatedness not significantly different from 0.50 ($r = 0.47$, 95% CI = 0.40–0.53). Comparisons to the previously published computer models (Thorne et al. 1999, Bulmer et al. 2001, Vargo et al. 2003a, 2006) suggest that most of the extended family colonies in the native population were headed by a low number of neotenics (on the order of five or less), which had been inbreeding for fewer than three generations.

Of the 19 colonies collected from the Island of Oahu, HI, 7 colonies (37%) were simple families and 12 colonies (63%) were extended families. The genetic distance among colonies in this introduced population were large regardless of the breeding system of the colonies (simple families: $F_{CT} = 0.42$, 95% CI = 0.30–0.55; extended families: $F_{CT} = 0.33$, 95% CI = 0.20–0.42). Simple families and extended families showed high degrees of inbreeding at the population level indicated by F_{IT} values significantly greater than zero (simple families: $F_{IT} = 0.38$, 95% CI = 0.25–0.52; ex-

tended families: $F_{IT} = 0.29$, 95% CI = 0.15–0.42). Simple and extended families also showed considerable inbreeding within colonies (simple families: $F_{IC} = -0.09$, 95% CI = -0.14 to -0.02 ; $r = 0.62$, 95% CI = 0.49–0.72; extended families: $F_{IC} = -0.10$, 95% CI = -0.10 –0.03; $r = 0.51$, 95% CI = 0.35–0.60, FSTAT). Comparison to the computer models (Thorne et al. 1999, Bulmer et al. 2001, Vargo et al. 2003a, 2006) suggest that the majority of simple families were headed by inbreeding related pairs, either nestmate alates or pairs of neotenics. A more detailed analysis using KINSHIP 1.3.1 (Goodnight and Queller 1999) revealed that five (71%) of the seven simple families were headed by putative nestmates. Extended families were most likely headed by a moderately high number of neotenics (on the order of <10) inbreeding for up to three generations. Significantly positive F_{IC} values in two of the extended families indicate assortative mating of groups of reproductives within colonies or mixing of different colonies at the same foraging site (Table 2); these F_{IC} values were excluded from correlations, because they are caused by a Wahlund effect and do not accurately reflect inbreeding and numbers of reproductives within colonies (Goudet 2001).

The breeding systems of the 14 colonies in Louis Armstrong Park, New Orleans, LA, have been published separately by Husseneder et al. (2005). Eight colonies (47%) were simple families; all but one (88%) were headed by outbred unrelated pairs of reproductives. Six colonies (53%) were extended families all headed by a low number of neotenic reproductives (as low as three as indicated by the strongly negative F_{IC} values; Table 2). The sex ratio of the workers was not significantly different between simple and extended families ($P > 0.20$, two-tailed Mann-Whitney U test). Furthermore, sex ratios of workers were not correlated to the degree of heterozygosity or inbreeding (F_{IC}) within colonies (Spearman's $\rho = -0.28$, $P > 0.20$ and $\rho = 0.32$, $P > 0.20$, respectively).

No significant differences could be detected between simple and extended families in worker body weight, head size, F_{IC} , and degree of heterozygosity in the populations of Hawaii and Louisiana (Table 2; $P > 0.20$ in all categories, two-tailed Mann-Whitney U test). The Chinese population contained only extended families, and therefore, no similar comparison was possible.

When simple and extended families were analyzed across all populations, worker size in simple family colonies was not correlated to F_{IC} (weight, Spearman's $\rho = -0.21$, $P > 0.20$; head size, $\rho = -0.39$, $P = 0.16$) or heterozygosity (weight, $\rho = 0.37$, $P = 0.18$; head size, $\rho = 0.44$, $P = 0.10$, $n = 15$). F_{IC} and heterozygosity were correlated in simple families, where the number of reproductives is always two ($\rho = -0.85$, $P < 0.001$), but not in extended families ($P > 0.20$, $n = 28$), where F_{IC} is influenced by the number of reproductives (Thorne et al. 1999). In extended family colonies, F_{IC} showed significant correlations with the size-related parameters (F_{IC} and weight, $\rho = -0.58$, $P = 0.001$; F_{IC}

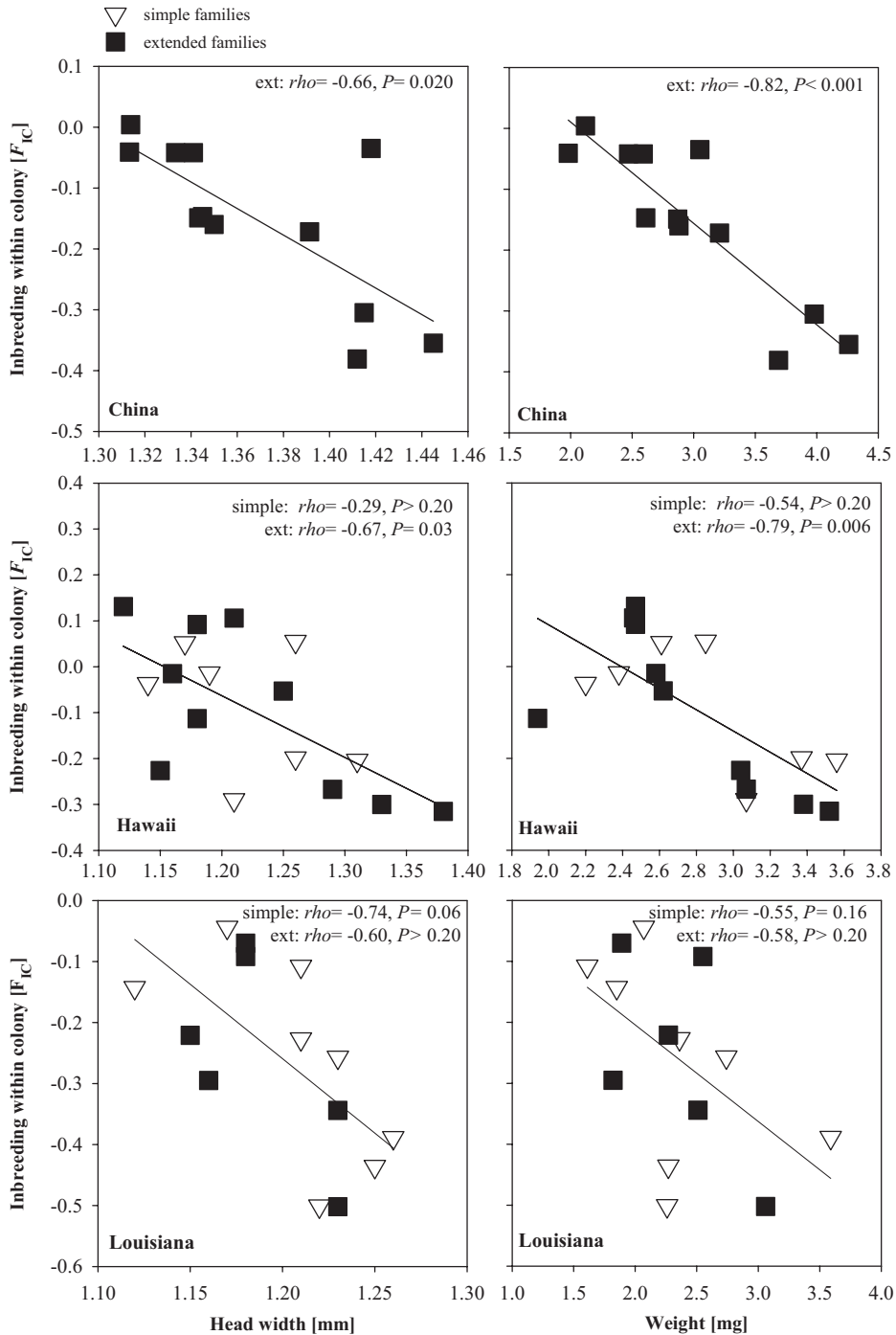


Fig. 1. Correlations of head width and body weight of workers with the degree of inbreeding (F_{IC}) within simple and extended family colonies in three populations (China, Hawaii, Louisiana) of the Formosan subterranean termite.

and head size, $\rho = -0.38, P = 0.044$). Heterozygosity in extended family colonies was not correlated to head size ($\rho = 0.43, P = 0.085$) and weight ($\rho = 0.23, P > 0.20, n = 28$).

Figure 1 shows correlations of F_{IC} and head size and weight of workers for simple and extended families in

each population separately. In extended families, F_{IC} was negatively correlated to both weight and head size in the populations of Hawaii and China. In the Louisiana population, the correlations were not significant, probably because of the small sample size of extended family colonies ($n = 6$).

Discussion

We found a negative correlation between the size of workers (measured in head width and body weight) and the number of reproductives (inferred from the coefficient of inbreeding of workers relative to their colony mates, F_{IC}) in extended family colonies of the Formosan subterranean termite. We tested several possible factors that could explain this correlation. First, we were able to rule out possible differences in worker size caused by variation among colonies in the predominant proportion of early or late instars among the foragers. We found no significant difference in the mean number of antennal segments of the workers from different colonies. Also, the average number of antennal segments was not correlated to the average head width or weight of workers in colonies.

Second, we were able to exclude possible differences among colonies caused by variation in sex ratios of worker. In *C. formosanus*, male alates had significantly higher inbreeding levels than females, which suggests that males originated from inbred extended family colonies headed by neotenic, whereas females were more likely to be produced by outbred simple family colonies (Husseneder et al. 2006). Similarly, the sex ratio in orphaned colonies headed by neotenic in *C. lacteus* Froggatt and *C. acinaciformis* Froggatt was strongly biased toward males (Lenz and Runko 1993, Roisin and Lenz 2002). Male alates of the Formosan subterranean termite have smaller heads and lower body weight than females (C.H. and D.M. Simms, unpublished data). If the same trends are true for workers, inbred colonies would produce more males, and their foraging population would thus have a lower average body size and weight. However, the majority of studied colonies had a worker sex ratio not significantly different from equality, and the sex ratio was not correlated with F_{IC} or heterozygosity. Thus, the sex ratio in the colony cannot explain the correlation of worker size and F_{IC} .

Third, inbred individuals (born to closely related parents) may show reduced fitness, e.g., in terms of size, weight, survival, parasite load, and adult reproduction (Amos et al. 2001). This phenomenon is known as inbreeding depression and is believed to be a result of heterozygote deficit, which leads to decreased genetic diversity (less disease resistance) and increased expression of recessive deleterious genes in homozygotes (Blouin and Blouin 1988). In social spiders, for example, morphological traits, such as growth rate, juvenile body mass, and adult offspring body size, were significantly reduced by inbreeding (Bilde et al. 2005). Similarly, crickets from inbred lines were smaller than those from outbred lines and took longer to develop (Rantala and Roff 2005). Indications that some form of inbreeding depression may be operating in subterranean termites have been previously reported. Fei and Henderson (2003) found lower lifetime reproductive success of colonies raised from siblings in *C. formosanus*; DeHeer and Vargo (2006)

inferred lower survival rate of incipient colonies originating from siblings in *R. flavipes*.

Although the parental relatedness in simple family colonies in our study spanned the whole spectrum from outbred unrelated pairs to putative nest mate pairs, and colonies showed a wide range of worker sizes (Table 2), the average degree of heterozygosity within colonies was not correlated with head size or body weight of workers in simple families. Thus, the parental relatedness alone, which determines the degree of heterozygosity within these colonies, did not explain the size differences in workers from different simple family colonies.

In simple families, the correlation between the proportion of heterozygote loci of the individuals and F_{IC} at the colony level was significant. In extended families, this correlation weakens, because F_{IC} is also highly sensitive to the effective numbers of reproductives within extended family colonies. F_{IC} is strongly negative for a pair of inbreeding neotenic (-0.14) and increases with the number of neotenic reproducing within the colony until it approaches zero (Thorne et al. 1999, Bulmer et al. 2001). F_{IC} values were significantly correlated with size of workers in extended families in the two populations with sufficient numbers of extended families. The Louisiana population contained only six extended families, and number of reproductives was low in these colonies as indicated by the highly negative F_{IC} values, which may explain the lack of correlation.

The correlation of body weight and head width of workers with the parameter F_{IC} in extended families suggests that the difference in worker size among colonies can be explained by the varying number of inbreeding reproductives in colonies of the Formosan subterranean termite. A similar relationship between the number of reproductives and worker size has been reported in other species. For example, workers from polygynous colonies in *Formica selysi* Bondroit (Schwander et al. 2005) and *Solenopsis invicta* Buren (Goodisman and Ross 1996, Colby et al. 2007) are, on average, smaller than workers from monogynous colonies. Similar to our findings, queen number and worker size were negatively correlated in polygynous colonies of *S. invicta* (Goodisman and Ross 1996).

The reasons why a larger number of reproductives should produce smaller workers remain unknown. However, it is plausible to assume that worker size and body weight should be constrained by availability of nutrients and costs of acquiring food. A colony with many reproductives can potentially produce offspring at a faster rate and in higher numbers than a colony with few reproductives (Goodisman and Ross 1996). If resources are limited or an increased need for food results in a higher cost of acquisition (e.g., expanding and maintaining the network of foraging tunnels), the payoff for a high number of workers might be reduced size caused by lower larval nutrition.

The influence of worker size on colony fitness has not been explored in detail, and data are still ambiguous. Campora and Grace (2001) reported that larger workers are initially more effective in building tunnels

and would thus increase the foraging ability of a colony. In contrast, larger worker size has been suggested to be a sign of age and decline of colonies (Shimizu 1962, Grace et al. 1995). Increased worker size in older colonies would be in agreement with our result, i.e., higher numbers of reproductives in extended family colonies lead to smaller worker sizes, if older colonies would be headed on the average by fewer neotenic; unfortunately, to date there are no data available on the number of effective reproductives in relation to colony age. However, the decrease in colony population size correlated with an increase in worker body size in aging colonies noted by Grace et al. (1995) is suggestive of a decrease in the number of active neotenic.

Acknowledgments

We thank Drs. R. J. Woodrow and M. Messenger for help with field collections in Hawaii and Louisiana; K. Funes, S. Garner, T. Juba, and D. Simms for technical assistance; and L. Foil for valuable comments on an earlier draft of the manuscript. This work was funded by grants from the USDA Tropical/Subtropical Agriculture Research (HAW00987-1011S) programs and USDA-ARS Specific Cooperative Agreements 58-6435-8-107, 58-6435-2-0023, and 58-6615-4-237). This paper was approved for publication by the Director, Louisiana Agricultural Experiment Station, as Manuscript 07-26-0332.

References Cited

- Aluko, G. K., and C. Husseneder. 2007. Colony dynamics of the Formosan subterranean termite, *Coptotermes formosanus*, in a frequently disturbed landscape. *J. Econ. Entomol.* 100: 1037-1046.
- Amos, W., J. W. Wilmer, K. Fullard, T. M. Burg, J. P. Croxall, D. Bloch, and T. Coulson. 2001. The influence of parental relatedness on reproductive success. *Proc. R. Soc. Lond. B.* 268: 2021-2027.
- Bartz, S. H. 1979. Evolution of eusociality in termites. *Proc. Natl. Acad. Sci. U.S.A.* 76: 5764-5768.
- Bilde, T., Y. Lubin, D. Smith, J. M. Schneider, and A. A. Maklakov. 2005. The transition to social inbred mating systems in spiders: role of inbreeding tolerance in a sub-social predecessor. *Evolution* 59: 160-174.
- Blouin, S. F., and M. Blouin. 1988. Inbreeding avoidance behaviors. *TREE*. 3: 230-233.
- Bulmer, M. S., E. S. Adams, and J.F.A. Traniello. 2001. Variation in colony structure in the subterranean termite *Reticulitermes flavipes*. *Behav. Ecol. Sociobiol.* 49:236-243.
- Campora, C. E., and J. K. Grace. 2001. Tunnel orientation and search pattern sequence of the Formosan subterranean termite (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 94: 1193-1199.
- Colby, D., L. Inmon, and L. Foil. 2007. Red imported fire ant (Hymenoptera: Formicidae) worker head widths as an indicator of social form in Louisiana. *J. Entomol. Sci.* 42: 20-27.
- Cornelius, M. L., and W.L.A. Osbrink. 2001. Tunneling behavior, foraging tenacity, and wood consumption rates of Formosan and Eastern subterranean termites (Isoptera: Rhinotermitidae) in laboratory bioassays. *Sociobiology* 37: 79-94.
- DeHeer, C. J., and E. L. Vargo. 2006. An indirect test of inbreeding depression in the termites *Reticulitermes flavipes* and *Reticulitermes virginicus*. *Behav. Ecol. Sociobiol.* 59: 753-761.
- Fei, H. X., and G. Henderson. 2003. Comparative study of incipient colony development in the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera, Rhinotermitidae). *Insect Soc.* 50: 226-233.
- Goodisman, M.A.D., and K. G. Ross. 1996. Relationship of queen number and worker size in polygyne colonies of the fire ant *Solenopsis invicta*. *Insect. Soc.* 43: 303-307.
- Goodnight, K. F., and D. C. Queller. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Mol. Ecol.* 8: 1231-1234.
- Goudet, J. 1995. FSTAT (Version 1.2): a computer program to calculate F-statistics. *J. Hered.* 86: 485-486.
- Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3) (<http://www.unil.ch/izea/software/fstat.html>).
- Grace, J. K., R. T. Yamamoto, and M. Tamashiro. 1995. Relationship of individual worker mass and population decline in a Formosan subterranean termite colony (Isoptera: Rhinotermitidae). *Environ. Entomol.* 24: 1258-1262.
- Hamilton, W. D. 1972. Altruism and related phenomena, mainly in social insects. *Annu. Rev. Ecol. Syst.* 3: 193-232.
- Higa, S. Y. 1981. Flight, colony foundation, and development of the gonads of the primary reproductives of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. PhD dissertation, University of Hawaii, Honolulu, HI.
- Husseneder, C., and J. K. Grace. 2001a. Evaluation of DNA fingerprinting, aggression tests and morphometry as tools for colony delineation of the Formosan subterranean termite. *J. Insect Behav.* 14: 173-186.
- Husseneder, C., and J. K. Grace. 2001b. Similarity is relative: hierarchy of genetic similarities in the Formosan subterranean termite (Isoptera: Rhinotermitidae) in Hawaii. *Environ. Entomol.* 30: 262-266.
- Husseneder, C., J. K. Grace, M. T. Messenger, E. L. Vargo, and N.-Y. Su. 2003a. Describing the spatial and social organization of Formosan subterranean termite colonies in Armstrong Park, New Orleans. *Sociobiology* 41: 61-65.
- Husseneder, C., E. L. Vargo, and J. K. Grace. 2003b. Molecular genetic methods: new approaches to termite biology, pp. 358-370. *In* B. Goodell, T. P. Schultz, and D. D. Nicholas (eds.), Wood deterioration and preservation: advances in our changing world. American Chemical Society, Washington, DC.
- Husseneder, C., M. T. Messenger, N.-Y. Su, J. K. Grace, and E. L. Vargo. 2005. Colony social organization and population genetic structure of an introduced population of the Formosan subterranean termite from New Orleans, Louisiana. *J. Econ. Entomol.* 98: 1421-1434.
- Husseneder, C., D. M. Simms, and D. R. Ring. 2006. Genetic diversity and genotypic differentiation between the sexes in swarm aggregations decrease inbreeding in the Formosan subterranean termite. *Insect Soc.* 53: 212-219.
- Husseneder, C., D. M. Simms, and C. Riegel. 2007. Evaluation of treatment success and patterns of reinfestation of the Formosan subterranean termite. *J. Econ. Entomol.* 100: 1370-1380.
- King, E. G., and W. T. Spink. 1974. Laboratory studies on the biology of the Formosan subterranean termite with primary emphasis on young colony development. *Ann. Entomol. Soc. Am.* 67: 953-958.
- Kistner, D. H. 1985. A new genus and species of termitophilous *Aleocharinae* from mainland China associated

- with *Coptotermes formosanus* and its zoogeographical significance (Coleoptera: Staphylinidae). *Sociobiology* 10: 93–104.
- Lenz, M., and S. Runko. 1993. Long-term impact of orphaning on field colonies of *Coptotermes lacteus* (Froggatt) (Isoptera: Rhinotermitidae). *Insect Soc.* 40: 439–456.
- Messenger, M. T., and N.-Y. Su. 2005a. Colony characteristics and seasonal activity of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in Louis Armstrong Park, New Orleans, Louisiana. *J. Entomol. Sci.* 40: 268–279.
- Messenger, M. T., and N.-Y. Su. 2005b. Agonistic behavior between colonies of the Formosan subterranean termite from Louis Armstrong Park, New Orleans, Louisiana. *Sociobiology* 45: 1–15.
- Messenger, M. T., N.-Y. Su, C. Husseneder, and J. K. Grace. 2005. Elimination and reinvasion studies with *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in Louisiana. *J. Econ. Entomol.* 98: 916–929.
- Nei, M. 1987. *Molecular evolutionary genetics*. Columbia University Press, New York.
- Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. *Evolution* 43: 258–275.
- Raina, A. K., J. M. Bland, J. C. Dickens, Y. I. Park, and B. Hollister. 2003. Premating behavior of dealates of the Formosan subterranean termite and evidence for the presence of a contact sex pheromone. *J. Insect Behav.* 16: 233–245.
- Raina, A. K., W. Osbrink, and Y. I. Park. 2004. Nymphs of the Formosan subterranean termite (Isoptera: Rhinotermitidae): aspects of formation and transformation. *Ann. Entomol. Soc. Am.* 97: 757–764.
- Rantala, M. J., and D. A. Roff. 2005. Analysis of the importance of genotypic variation, metabolic rate, morphology, sex and developmental time on immune function in the cricket, *Gryllus firmus*. *J. Evol. Biol.* 19:834–843.
- Roisin, Y. 1999. Philopatric reproduction, a prime mover in the evolution of termite sociality? *Insect Soc.* 46: 297–305.
- Roisin, Y., and M. Lenz. 2002. Origin of male-biased sex allocation in orphaned colonies of the termite, *Coptotermes lacteus*. *Behav. Ecol. Sociobiol.* 51:472–479.
- Schwander, T., H. Rosset, and M. Chapuisat. 2005. Division of labour and worker size polymorphism in ant colonies: the impact of social and genetic factors. *Behav. Ecol. Sociobiol.* 59: 215–221.
- Shellman-Reeve, J. S. 1997. The spectrum of eusociality in termites, pp. 52–93. *In* J. C. Choe and B. J. Crespi (eds.), *Social behavior in insects and arachnids*. Cambridge University Press, Cambridge, United Kingdom.
- Shimizu, K. 1962. Analytical studies on the vitality of colonies of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. I. Analysis of the strength of vitality. *Bull. Fac. Agric. Univ. Miyazaki* 8: 106–110.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry: the principles and practice of statistics in biological research*, 3rd ed. W.H. Freeman & Company, New York.
- Spink, W. T. 1967. The Formosan subterranean termite in Louisiana. *Louisiana State Univ. Circ.* 89: 1–12.
- Su, N.-Y., and J. P. La Fage. 1984. Differences in survival and feeding-activity among colonies of the Formosan subterranean termite (Isoptera: Rhinotermitidae). *Z. Angew. Entomol.* 97: 134–138.
- Su, N.-Y., and M. Tamashiro. 1987. An overview of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the world, pp. 3–14. *In* M. Tamashiro and N.-Y. Su (eds.), *Biology and control of the Formosan subterranean termite*. University of Hawaii, Honolulu, HI.
- Thorne, B. L., J.F.A. Traniello, E. S. Adams, and M. Bulmer. 1999. Reproductive dynamics and colony structure of subterranean termites of the genus *Reticulitermes* (Isoptera Rhinotermitidae): a review of the evidence from behavioral, ecological and genetic studies. *Ethol. Ecol. Evol.* 11: 149–169.
- Vargo, E. L. 2003a. Hierarchical analysis of colony and population genetic structure in the eastern subterranean termite, *Reticulitermes flavipes*, using two classes of molecular markers. *Evolution* 57: 2805–2818.
- Vargo, E. L. 2003b. Genetic structure of *Reticulitermes flavipes* and *R. virginicus* (Isoptera: Rhinotermitidae) colonies in an urban habitat and tracking of colonies following treatment with hexaflumuron bait. *Environ. Entomol.* 32: 1271–1282.
- Vargo, E. L., and G. Henderson. 2000. Identification of polymorphic microsatellite loci in the Formosan subterranean termite *Coptotermes formosanus* Shiraki. *Mol. Ecol.* 9: 1935–1938.
- Vargo, E. L., C. Husseneder, and J. K. Grace. 2003a. Colony and population genetic structure of the Formosan subterranean termite, *Coptotermes formosanus*, in Japan. *Mol. Ecol.* 12: 2599–2608.
- Vargo, E. L., C. Husseneder, J. K. Grace, G. Henderson, and D. Ring. 2003b. Colony and population genetic structure of Formosan subterranean termites from Hawaii and Louisiana. *Sociobiology* 41: 67–69.
- Vargo, E. L., C. Husseneder, D. Woodson, M. G. Waldvogel, and J. K. Grace. 2006. Genetic analysis of colony and population structure of three introduced populations of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the continental United States. *Environ. Entomol.* 35: 151–166.
- Waller, D.A. 1988. Size variation in *Coptotermes formosanus* Shiraki (Rhinotermitidae): consequences of host use. *Am. Midl. Nat.* 119: 436–440.
- Yates, J. R., and M. Tamashiro. 1999. *The Formosan subterranean termite in Hawaii*. University of Hawaii, Honolulu, HI.

Received 27 June 2007; accepted 11 November 2007.